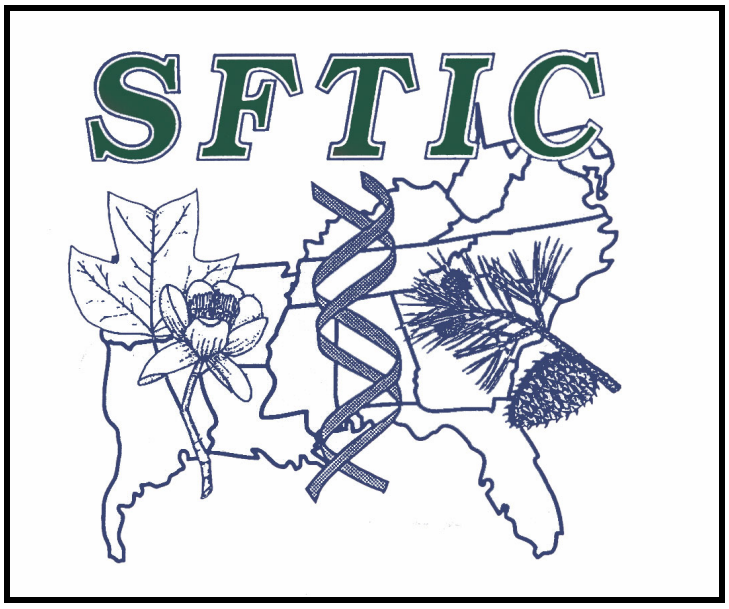
PROCEEDINGS

27th Southern Forest Tree Improvement Conference



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THE SOUTHERN FOREST TREE
IMPROVEMENT COMMITTEE

Oklahoma State University
Stillwater, Oklahoma USA
June 24-27, 2003

Proceedings of the 27th Southern Forest Tree Improvement Conference

Edited by Craig R. McKinley
Oklahoma State University

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Proceedings of the 27th Southern Forest Tree Improvement Conference

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Sponsored Publication No. 49 of the
Southern Forest Tree Improvement Committee

FOREWORD

In cooperation with the Southern Forest Tree Improvement Committee, Oklahoma State University was pleased to host the 27th Biennial Southern Forest Tree Improvement Conference (SFTIC). The North American Quantitative Forest Genetics Group met on the OSU campus prior to the SFTIC meeting.

For more than 50 years, professional foresters, researchers, managers, educators, and technical foresters involved with all aspects of tree improvement and forest productivity have been attending the SFTIC conferences. The 2003 Conference had as its theme "Tree Improvement and Forest Sustainability" and again provided an outstanding forum for discussion of forest genetics and tree improvement. There were a total of 40 contributed presentations, two invited presentations, and 13 posters. There were 94 registered participants.

Three awards were presented for outstanding contributions to the SFTIC meeting:

a) The **Tony Squillace Award** is given for the best oral presentation based on content, style and use of visual aids. This year's award, which includes a $200 check, was presented to Scott Merkle for *Light Quality Effects on Germination and Conversion of Southern Pine Somatic Embryos.*

b) The **Bruce Zobel Award** is given for the best oral presentation by a student. The award this year went to Kevin Potter for *Genetic Variation in Fraser Fir Mortality Due to Phytophthora Root Rot.* A $200 check was also provided to Kevin as part of this award.

c) The **Belle Baruch Foundation Award** includes a $100 check and is given for the best poster. This year's award went to Wei Tang and Ron Newton for *High Efficiency Transformation of Loblolly Pine (Pinus taeda L.) Using Green Fluorescent Protein as a Vital Screenable Marker.*

SFTIC Committee extends it congratulations to each of these individuals.

The SFTIC Committee would also like to acknowledge the staff of the OSU Agricultural Conference Services for their efforts in coordinating a successful meeting. The staff of the Wes Watkins Center, as well as members of the OSU Forestry Department and Kiamichi Forest Research Station are also to be recognized for their many contributions.

The 27th SFTIC Program Committee:

John Adams	Scott Merkle
Early McCall	Randy Rousseau
Craig McKinley	Mike Stine
Steve McKeand	ChuckTauer

27TH SOUTHERN FOREST TREE IMPROVEMENT CONFERENCE

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27TH SOUTHERN FOREST TREE IMPROVEMENT CONFERENCE

Papers and Poster Abstracts

Pioneering Tree Improvement in Oklahoma

Clark W. Lantz[1] and Craig R. McKinley[2]

Abstract:--The pioneering tree improvement work in Oklahoma started in 1965 when Clayton Posey moved from Auburn University to Oklahoma State University. Clayton was hired by Glen Durrell (Department Head) to fill a teaching/research position in the Department of Forestry. As a native Oklahoman, Clayton recognized the need to start some long-term studies with the economically important timber species in the state. Fortunately he had access to McIntire-Stennis funds which he used to initiate studies with loblolly pine (*Pinus taeda*) shortleaf pine (*Pinus echinata*) and eastern cottonwood (*Populus deltoides*).

Tree selection started in 1966 and concurrently the Kiamichi Field Station was transferred to the Forestry Department from Horticulture. In typical Oklahoma fashion a strong spirit of cooperation emerged with Dierks Lumber Company (soon to be acquired by Weyerhaeuser), Herron Lumber Company, Oklahoma Forestry Division, and the Tiak District of the Ouachita National Forest all assisting with the program. The cooperative spirit was formalized in 1980 when the Oklahoma Forestry Division officially joined the Western Gulf Forest Tree Improvement Program.

SPECIES TRIALS:

The Oklahoma State Forestry Department was founded in 1946, just after World War II. A number of species trials were established in the early days, including plantations of loblolly pine near Lake Carl Blackwell, possibly planted by Mike Afanasiev. These loblolly plantings survived and grew well and eventually produced extensive stands of progeny. Unfortunately there are no known records of the original sources planted. Later plantings of loblolly seedlings from Oklahoma sources survived and grew well in the Payne County environment (Lantz 1977).

TREE IMPROVEMENT PIONEERS

The real tree improvement pioneer at OSU was Clayton Posey. Clayton completed his Ph. D. under Bruce Zobel at N. C. State University and moved to Auburn University in the early 1960's. Glenn Durrell (Department Head at OSU) hired Clayton to fill a new teaching/research position in 1965. When Clayton arrived in Stillwater he immediately saw the need to initiate long-term genetic studies with the three major commercial species in Oklahoma: loblolly and shortleaf pine, and cottonwood. Fortunately McIntire-Stennis funds were available and these were used to initiate the long-term studies.

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[1] Reforestation Consultant, Granbury, TX and [2] Head, Department of Forestry, Oklahoma State University, Stillwater OK.

KIAMICHI STATION

The Kiamichi Station in Idabel was transferred from the Horticulture Department to Forestry in 1966-7. When the local people learned of the change they protested vigorously at the thought of their free veggies and peaches about to be replaced by rows of pine trees. The university prevailed, and soon there were pine trees all over with only a token few peach trees surviving.

Fortunately, Julius Canant and Truman Byrne, the resident horticultural technicians, were transferred to forestry along with their expertise in propagation and growing plants.

Clayton engineered a cooperative study with the USDA Forest Service, Southern Station, on hybrids between loblolly and slash pine. This study was planted just east of the station, on the north side of the highway. Reliable testimonials from former graduate students verified Clayton's dedication to the job. He was known for working students far into the night as they planted seedlings with the aid of headlights on the pickup trucks.

TREE SELECTION

Empirical evidence suggested that the pine tree improvement efforts be concentrated on 3 separate breeding programs: loblolly pine; low elevation shortleaf pine (below 1,000 feet in elevation) and mountain shortleaf (above 1,000 feet). Tree selections were started in natural stands in 1967 with the able assistance of the Oklahoma Forestry Division, Weyerhaeuser Company, U. S. Forest Service, Herron Industries, and several other private landowners. The N. C. State University grading system of five comparison trees in natural, even-aged stands was used.

GRAFTING

Field grafting started in 1967 for the three pine orchards. In later years vacant positions within the orchards were filled-in with transplants from a seedbed grafting area.

COTTONWOOD

Tree selection with cottonwood presented unique problems due to the general lack of even-aged stands and the common occurrence of vegetative reproduction. In most cases candidate trees were evaluated on their own merit, followed by planting in clonal banks via cuttings. These individuals were evaluated later by means of clonal tests.

JARI FLORESTAL

In the late 1960's Daniel Ludwig, a multi-millionaire shipping entrepreneur, started a new venture in the Amazon basin of Brazil on the banks of the Jari River. The project included cattle breeding, rice farming and plantation forestry. Ludwig recruited Clayton Posey to manage the forestry enterprise and Clayton left OSU in 1969. Close liaison continued with the Jari project, with Nat Walker and J. L. Albert working as consultants.

The 1974 OSU Forestry summer camp was held at the Jari site, Monte Dorado, with 40 students and 4 faculty attending.

NEW FACULTY MEMBERS

Roy Stonecypher replaced Posey in 1969. Roy had been working on the loblolly pine heritability study in Bainbridge GA for International Paper Company. Roy was also a Ph. D. graduate of NC State, and brought important quantitative skills to OSU.

Clark Lantz left NC State in the spring of 1970 to accept a new teaching/research position at OSU. Since Ted Silker was due to embark on a sabbatical Clark was elected to pinch-hit with Ted's courses, notably courses in Silviculture, Protection, and senior seminars. Roy and Clark shared the research projects with Roy working on the cottonwood and Clark on the pine.

Weyerhaeuser hired Roy in the early 1970's to be their primary quantitative geneticist in Centralia WA. Fortunately Floyd Bridgwater had studied under Roy long enough to handle the required quantitative studies when Roy left.

With Roy's departure, Clark assumed responsibilities for teaching the Forest Genetics and Regeneration course, the tree improvement research projects, and the seed orchard operations at Idabel.

GRADUATE STUDENTS

In the early 1970's Jerry Abbott started work on an MS project concerned with hybridization between loblolly and shortleaf in southeastern Oklahoma and Larry Miller started his MS work on crossing cottonwood in the greenhouse. A bit later, Cary Osterhaus started working on the Sarkey's Study, involving the conversion of Cross-Timbers sites to pine plantations.

NEW KIAMICHI STATION SUPERINTENDENT

The operation of the Kiamichi Station was critical to the maintenance and progress of the tree improvement programs. Ben Smith, a 1972 OSU Forestry graduate and retired Lt Commander-US Navy, had decided to move to Alaska and set-up a gold mining business, dredging shallow streams. Fortunately Clark was able to convince him to stay in Oklahoma and take the position of station superintendent. Ben had wide-reaching skills as a mechanic, plumber, electrician, and carpenter, and he was particularly good at improvising solutions to thorny problems. In those days federal surplus equipment was available to the state forestry organizations and Ben was a master at adapting much of that equipment to seed orchard use.

SEED PRODUCTION

The first improved seed from the Kiamichi seed orchards was harvested in 1972. Sufficient seed was collected from the loblolly orchard to plant an open-pollinated progeny test in February, 1975 (Smith and Tauer, 1982). The test plantation was planted in 4 reps of 12, 10-tree row plots. Information from additional open pollinated tests was used to rogue the loblolly orchard in 1982.

SARKEY'S STUDY

The Sarkey's study, located near Lamar, OK in Hughes County was originally set-up by Ted Silker to assess herbicides as a means of converting cross-timbers sites to pine plantations. Cary Osterhaus became interested in the study and his M. S. work was designed to compare survival and growth of loblolly, shortleaf, Virginia pine (*Pinus virginiana*) and *Pinus brutia* on the herbicide-treated site. Both loblolly and Virginia pines performed well on the Cross-Timbers site (Osterhaus and Lantz, 1978). Unfortunately an overzealous Agronomy faculty member set a "prescribed" fire on adjacent plots which completely destroyed the forestry study

MORE FACULTY CHANGES

Clark Lantz left OSU in December 1975 to join the U.S. Forest Service as Nursery/Tree Improvement Specialist in the Southern Regional office in Atlanta. Cary Osterhaus filled-in with teaching and research duties until he joined the Bureau of Land Management in Oregon. Chuck Tauer, a Ph. D. graduate of the University of Minnesota accepted the position in 1976.

WESTERN GULF FOREST TREE IMPROVEMENT PROGRAM

Chuck Tauer was instrumental in securing membership in the Western Gulf cooperative in 1980, a move that had been attempted previously without success. Through the 1970's the Oklahoma Forestry Division had assumed increasing responsibility for the tree improvement program and they now became an official member of the cooperative. Cooperative membership opened a number of doors for the state program: sharing reproductive material within the cooperative, design and analysis of progeny tests, advanced generation breeding plans, and improved communications within the forestry community. These have all paid big dividends in the last few years for the Oklahoma tree improvement program.

CONCLUSIONS

- Oklahoma students, staff and faculty worked hard to build a first rate tree improvement program.
- The spirit of cooperation seems to be a natural instinct for native Oklahomans.

This is what made the tree improvement program work when OSU, Oklahoma Forestry Division, Weyerhaeuser, U. S. Forest Service, Herron Industries, and numerous other private landowners all worked together for a common goal.

- Ben Smith, Julius Canant, and Truman Byrne always gave 200% at the Kiamichi Station.

- Students such as Floyd Bridgwater, Keith Lynch, Jimmie Buxton, Craig McKinley, Rex McCullough, Jerry Abbott, Larry Miller, and Cary Osterhaus put in long hours to keep the program moving.

- Strong support was always available from Al Myatt, Greg Huffman, Tom Smith, Kurt Atkinson and other members of the Oklahoma Forestry Division.

- Glen Durrell and Nat Walker of the OSU Forestry Department had the foresight to realize the potential of a state tree improvement program.

- Joining the Western Gulf Forest Tree Improvement Program was a major step forward

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Sustainable Forest Management, Forest Certification, Tree Improvement, and Forest Biotechnology

Frederick Cubbage[1]

INTRODUCTION

Sustainable forestry has become a widely accepted paradigm for forest management and protection since the UNCED conference on sustainable development in 1992. Since then, several international agreements have supported Sustainable Forest Management (SFM) and many forest certification approaches have been developed. This paper reviews the concept of SFM and forest certification as related to tree improvement and forest biotechnology. It covers general principles from the Montreal Process agreement, which the U.S. is a signatory member, and the Forest Stewardship Council (FSC) and Sustainable Forestry Initiative (SFI) certification systems.

A commonly accepted principle of sustainable forestry is that we must balance the growth and removals of forests over the long run to be sustainable. Furthermore, many people believe forest plantations are crucial in ensuring sustained yield of wood supply, and that forest plantations can help preserve some of the remaining natural forests in the world (i.e., Sedjo and Botkin 1997, World Wildlife Fund 2003).

World forest and plantation data from UN FAO and from our research were analysed in a paper by Siry et al. (2003), which is drawn on here for summary purposes. According to the Forest Resource Assessment (FRA) 2000 (FAO 2001), the world's forest cover amounts to nearly 3.9 billion ha. Forest plantations consist of forests that are artificially created and one or a few species, and often have greater yields than native forests. FAO (2001) estimates that there are 187 million ha (5%) in forest plantations. Drawing on additional surveys and our research, we estimate that there were about 204 million ha of planted forests by 2002.

Fast grown industrial plantations would be comprised of forests with growth rates of greater than 5 cu m/ha/yr and rotations of less than 30 years. This area would comprise 40 million ha, or 1.0% of the world's forests. However, they provide about 25% of the world's industrial fiber supply. In the United States, planted forests comprise about 18 million ha, with 14.4 million ha in the South (Smith et al. 2001). Thus 36% of the world's fast grown forests are in the U.S. South—in fact more than any other region in the world. Almost half of the softwood timber harvests in the South now come from pine plantations.

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The South also contributes about 15% to 20% of the world's industrial wood fiber supply—again the largest share of any region in the world (FAO 2002). At the end of 2002, about 121 million ha of forests were certified worldwide. This area amounts to only 3% of global forest area, but the influence of these systems on setting standards for forest management and affecting tree improvement and forest biotechnology is much greater that the small area covered might suggest.

SUSTAINABLE FOREST MANAGEMENT CRITERIA AND INDICATORS

According to the Brundtland Report in 1987, sustainable development is "…development that meets the needs of the present without compromising the ability of future generations to meet their needs." Sustainable forest management is an extension of the sustainable development and sustained yield principles. It includes sustainable ecosystems, communities, and economies, as well as commodity and noncommodity, market and nonmarket goods and services. It infers that society seeks to provide enhanced forest management and protection for diverse values. It can be implemented through measurement and monitoring of the status of forest and social conditions, improved forestry research and innovation, and application of new technology in adaptive forest management practices. These approaches will help sustain, enhance, and restore forests and their innumerable economic, environmental, and social values.

Montreal Process Criteria and Indicators

World leaders at the United Nations Conference on Environment and Development (UNCED) in 1992—termed the Earth Summit in Rio de Janeiro—developed a non-binding Statement of Forest Principles that consisted of 17 points outlining guidelines and means for protecting the world's forests. Since then, countries throughout the world have developed regional and international criteria and indicators that can measure and monitor success in achieving sustainable forest management (SFM). SFM criteria are large-scale reflections of major public values; indicators are means for measuring forest conditions and tracking subsequent changes. The "C&I", as they are termed, are tools to assess forest conditions and sustainability, not performance standards for certifying management.

Of the nine criteria and indicator initiatives in the world, the Montreal Process (2003) is geographically the largest, encompassing most of the world's temperate and boreal forests, and 60% of all of the world's forests (http://www.mpci.org). The broad Montreal criteria [indicators] encompass: (1) conservation of biological diversity [indicators 1-9]; (2) maintenance of productive capacity of productive ecosystems [10-14]; (3) maintenance of forest ecosystem health and vitality [15-17]; (4) conservation and maintenance of soil and water resources [18-25]; (5) maintenance of forest contribution to carbon cycles [26-28]; (6) maintenance and enhancement of long-term socio-economic benefits to meet the needs of societies [29-47]; and (7) development of legal, institutional, and economic framework for forest conservation and sustainable management [48-67]. The first approximation report for SFM C&I under the Montreal

Process was made in Seoul, South Korea in1997, and the current national plans will be summarized at the IUFRO meeting in Quebec City, Canada in September 2003.

Selected SFM C&I for Tree Improvement and Forest Biotech

Several SFM C&I are particularly relevant for tree improvement and forest biotechnology. A few salient components of the Montreal Process C&I follow. Criterion 1, the conservation of biologic diversity, specifically lists broad categories of ecosystem diversity, species diversity, and genetic diversity. The include indicators (6) number of forest-dependent species; (7) status of species of risk of not maintaining viable breeding populations; (8) number of species that occupy small portion of their former range; and (9) population levels of representative species from diverse habitats monitored across their ranges.

Criterion 2, maintenance of productive capacity, addresses measurement and monitoring of area, growing stock, removals, etc. of native and exotic species, compared to volume determined to be sustainable. These indicators (10-14) are extensions of the classic sustained yield/sustainable forestry principles to include broader timber, nontimber, and nonmarket forest outputs. Criterion 3, maintenance of forest ecosystem health and vitality, includes indicator numbers (15) area and percent of forests affected by pathogens, fire, land clearing, etc., (16) area and percent affected by air pollution, and (17) area with diminished biologic components. Tree improvement and forest biotechnology also can obviously contribute to Criterion 5, maintenance of forest contribution to global carbon cycles.

SFM Criterion 6, socio-economic benefits, includes relevant indicators (29) volume and value of wood products and (38) value of investment…including planted forests. Criterion 7 addresses the legal, institutional, and economic framework that supports SFM, and the capacity to conduct and apply research. Relevant indicators include (63) scientific understanding of ecosystems and (65) new technologies and the capacity to assess the socioeconomic consequences associated with the introduction of new technologies. These both encourage research, and suggest needs to monitor social effects of that research, such as with genetically modified organisms.

FOREST CERTIFICATION

As indicated, forest certification also has many components that are relevant to tree improvement and forest biotechnology programs, in both forestry research and in forest operations. The principles and objectives of forest certification are often quite similar to those of SFM. However, while SFM is aimed at measuring, monitoring, and tracking the status of forest at a national and global level, forest certification is more directly aimed at measuring, monitoring, auditing, and improving forest practices at the forest level. In the United States, the Sustainable Forestry Initiative (SFI), which was initiated by the forest industry, is the dominant certification system. The Forest Stewardship Council (FSC), which was initiated by environmental nongovernment organizations, is less prevalent in

the U.S., but remains a benchmark for "green" certification. Relevant components for each program are paraphrased below.

Sustainable Forestry Initiative

The SFI Objectives (Sustainable Forestry Initiative 2002) state that the program participants must: (1) broaden practice of sustainable forestry, such as with written policies, funding for research, recreation and education, and maintenance of sustainable harvest levels; (2) ensure long-term forest productivity and reforestation, protect forests from fire, disease, etc; (3) protect water quality with Best Management Practices (BMPs), exceed state water laws, provide BMP training; (4) manage quality and distribution of wildlife habitat and biological diversity; and (5) manage visual impact of forest operations.

The SFI Objectives also require that program participants must: (6) protect sites with ecologic, historic, geologic significance; (7) minimize waste and utilize wood efficiently; (8) cooperate with forest landowners, wood producers, consulting foresters, logging and forestry associations to use of BMPs; (9) publicly report progress; (10) provide for public participation; and (11) promote continual improvement, monitor, measure, and report progress.

SFI objectives are implemented through specific core indicators that all program participants must follow, and other indicators that participants may elect to use to document their compliance with the sustainable forestry objectives. Program participants must demonstrate their compliance with these objectives and indicators through third person certification audits of their written documentation and field practices.

Selected 2003 SFI Objectives and Indicators relevant for tree improvement and forest biotech include the following (Sustainable Forestry Initiative 2002). Section 4.1.1, Objective 1 states that program participants must broaden the implementation of sustainable forestry, using the best scientific information available. Germane specific core indicators require that: "*Program Participants* shall (individually, through cooperative efforts or through associations) provide funding for...": (1) "... forest research to improve the health, *productivity*, and management of all forests." (Performance Measure 4.1.1.1.2, Core Indicator 1); and (2) "...research to improve the science and understanding of wildlife management at *stand* or *landscape* levels, ecosystem functions and the *conservation* of *biological diversity*." (Performance Measure 4.1.4.1.2, Core Indicator 1). The core indicators for each of these components require "1. Current financial or in-kind support of research to address..." each of the relevant subjects.

Program component 4.1.2 Objective 2 requires participants to ensure long-term forest productivity and conservation of forest resources to promote reforestation, soil conservation, afforestation, and other measures. Core indicator 2 requires designation of all management units for either natural or artificial regeneration. Core indicator 5 of this objective requires that planting of exotic species is minimized, and core indicator 6 requires research documentation is available that exotic tree species planted operationally

pose minimal risk. An "other" indicator, number 4.1.2, 7, requires that genetically improved stock is deployed appropriately to achieve reforestation requirements. Core indicator 4.1.2.1.3 states that chemicals must applied using appropriate BMPs, which would of course apply to nurseries and to plantations.

SFI Objective 4 states that participants must manage the quality of wildlife habitats and contribute to conservation of biological diversity by implementing stand- and landscape-level measures. Objective 5 requires management of the visual impact of harvesting and other forest operations. All of these first five SFI objectives relate to sustainable forestry practices in various means; the last six relate more to social, utilization, procurement, and reporting goals.

Forest Stewardship Council

The FSC principles focus more on social issues in the first few components, and then address ecological issues. The individual principles cover (Forest Stewardship Council 2003): (1) compliance with laws & FSC principles, (2) tenure and use rights and responsibilities, (3) indigenous people's rights, (4) community relations and worker's rights, (5) multiple benefits from the forest, (6) environmental impact (biodiversity), (7) management plans, (8) monitoring and assessment, (9) maintenance of high conservation value forests, and (10) plantations.

Selected FSC standards for tree improvement and forest biotech include the following. Standard 5.6 states that the rate of harvest of forest products shall not exceed levels which can be permanently sustained. Standard 6.3.a. covers forest regeneration and succession. Subcomponent 6.3.a.2 requires certified owners to maintain or restore forests to natural conditions to the extent possible. Section 6.3.a.4 mandates that owners retain live trees and native vegetation when they employ even aged management.

FSC addresses tree improvement and forest biotechnology very specifically. Key FSC standards related to tree improvement and forest biotech includes section 6.3.b. genetic, species, and ecosystem diversity. This includes 6.3.b.1, select trees for harvest, retention, and planting to maintain genetic diversity and species diversity of residual stand. Standard 6.3.b.2 requires that diverse native habitats be maintained; and 6.3.b.3 requires use of locally adapted seed of known provenance be used for artificial regeneration.

Standard 6.6 requires development and adoption of environmentally friendly non-chemical methods of pest management. Standard 6.8 requires that use of biological control agents shall be documented, minimized, monitored, and strictly controlled. Furthermore, it states that use of genetically modified organisms shall be prohibited. This includes a statement of: "Applicability Note: Genetically improved mechanisms (e.g., …Mendelian crossed) are not considered to be GMOs and may be used. The prohibition of GMOs applies to all organisms including trees." In addition, standard 6.8.a states that exotic predators used only as part of IPM strategy if other methods ineffective.

The FSC standards for tree improvement and forest biotechnology also limit plantations, especially of exotic species. They state that: 6.9 The use of exotic species shall be carefully controlled and actively monitored to avoid adverse ecological impacts; 6.9.a. that they should be contingent on peer-reviewed scientific evidence that any species in question is non-invasive and does not diminish biodiversity…use must be actively monitored; 6.9.b. owners must use control measures for invasive plants. Furthermore, they mandate that: 6.10 Forest conversion to plantations or non-forest uses shall not occur, except for (a) when it occurs as a limited portion of FMU; (b) does not occur in high conservation value forests; and (c) provides long term conservation benefits.

The U.S. SmartWood (2001)/FSC plantation standards, which we used in certifying our NC State College Forests in North Carolina, state that owners must manage their forest plantations per Principles and Criteria 1 through 9; that plantations must complement management of, and reduce pressures on, and promote restoration and conservation of natural forests; that plantation management objectives be clearly stated; that wildlife corridors, streamside zones, different ages and rotations must be employed; and that there must be a diversity in size, species, and distribution of planted and natural forests. Native species are preferred over exotic and plantations must be managed as part of the forest to restore natural cover. Soils in plantations must maintain their structure and fertility and cannot be degraded. Managers must minimize pests, diseases, fire, and pesticides, and assess on- and off-site ecological and social impacts and local access and use. Plantations converted from natural forests after November 1994 normally shall not qualify for certification, unless the manager/owner is not responsible directly or indirectly for conversion. However, typical southern forests regenerated from old farm fields are not considered “natural”, so this may not be daunting as it appears.

TREE IMPROVEMENT AND FOREST BIOTECHNOLOGY

Tree improvement and forest biotechnology offer related scientific means to increase forest productivity, achieve sustained timber yields, and perhaps enhance forest biodiversity and conservation of multiple values. Tree improvement provides classical approaches to achieve better timber production. It has achieved substantial gains through generations of tree selection and breeding. Li et al. (1999) found that tree improvement in the South has improved yields 7% to 12% in the first generation, and 13% to 21% in he second generation. More than one billion seedlings are produced each year, with about 40% being used on forest industry lands and 60% sold to nonindustrial private forest (NIPF) owners or timber management organizations (McKeand et al. 2003).

Tree improvement seeks to identify and improve several important tree attributes, including growth rates, disease and pest resistance, climate change and adaptability, tree form and wood fiber quality, straightness, branch number and size, and taper. Improved fiber characteristics for processing ease, lignin content, fibril angle, specific gravity, and wood density also are sought. Improved forest genetics and intensive silviculture can help achieve SFM High-yield plantations can produce at least triple the volume of natural stands in the South. Enhanced tree improvement can offer more gains, by site. Tree improvement has been widely accepted by environmental organizations, foresters,

and certifiers, if natural forests are actually reserved or remain in low intensity management.

In theory, forest biotechnology can identify and map important genes, manipulate and insert desired traits in cells (transgenes), and create genetically modified organisms (GMOs). Biotechnology is a promising opportunity in plantation forestry. It possesses the same productivity advantages of tree improvement, plus others, but has more substantial drawbacks as well. It could provide higher yields, better wood quality, lower risk associated with pests and pest management, as well as offer engineered genetic diversity at the cell, stand, ecosystem, all much faster than waiting for generations of tree breeding and testing and plantation establishment. However, the research and development costs and risks are much higher, and it thus will have higher seedling costs, at least in the short run. There also are major technical challenges, social issues, environmental risks, and market acceptance questions (Lucier et al. 2002, Lindgren 2003).

CONCLUSIONS

I have covered forests and plantations, Sustainable Forest Management, forest certification, tree improvement, and forest biotechnology. What is impact of first three subjects on last two? What is the interaction among all factors?

Plantations provide about 25% of world industrial wood fiber. Furthermore, plantations provide the basis for and target of most of our tree improvement and forest biotechnology efforts. Genetically improved trees and wood, achieved through traditional tree improvement programs, are generally accepted for industry and NIPF private lands.

Sustainable Forest Management criteria and indicators for temperate and other forests have been developed in various international agreements, including the Montreal Process for U.S. and other non-European temperate forests. SFM mandates national measuring and monitoring of progress toward sustainable forestry for a broad range of environmental, economic, and social goods and services. SFM is informative, but not prescriptive. It is largely the focus of governments and policy experts, and perhaps forestry researchers and a small number of forest practitioners. SFM may encourage research support for sustainable forestry. But it is not a strong tool for advocacy, regulation, or encouraging public or private forestry investment per se.

Forest certification, which mandates and audits standards of forestry practice at the stand or ownership level, has potential for a much larger impact of forest management, tree improvement, and forest biotechnology. The SFI specifically requires that program participants demonstrate that they conduct or support forestry research in health and productivity, water quality, and wildlife and biodiversity. SFI clearly encourages use of plantations, tree improvement, and forest management, and infers that forest biotech would be acceptable. With appropriate safeguards, exotics are legitimate under SFI, although there are not many exotic timber species being planted in the U.S. yet. SFI

might offer specific opportunities in tree, stand, or ecosystem biodiversity for applying the science of tree improvement or forest biotechnology.

Forest certification by FSC requires that managers favor natural stands and biodiversity. FSC allows plantations and tree improvement with fairly extensive strictures to protect natural stands and ecosystems. It explicitly proscribes the use of GMOs. FSC has been very flexible in decisions, allowing a large number of forests with exotic plantations to be certified if they have a large natural stand/reserve component as well. It does require refereed science to justify the use exotics and ensure that they do not cause any environmental harm.

Tree improvement is a very practical tool to achieve SFM. It has contributed to large realized gains in forest productivity already, and has more potential. It is largely taken for granted by academic administrators and federal forestry research programs. However, it can achieve continual gains without the use of transgenic GMOs. Forest biotechnology can be used to identify desirable traits, and traditional selection and breeding can be used to implement production of the most desirable families of trees. This probably remains most cost effective strategy, and fits well with forest certification. Surely tree improvement and breeding can use more funding by academia, government, and industry. However, it does have a significant marketing and packaging problem, seeming to be too staid for new research programs and new funding.

Forest biotechnology possesses more promise, more charisma, more financial support, and more problems. The perhaps distant promise of forest biotech is that of designer trees that are perfect for specific wood, paper, or environmental remediation applications, with known genetic diversity at the tree, stand, or ecosystem level. Given that even well supported agriculture applications have been limited to modest herbicide resistance or Bt disease insertions (Lindgren 2003), the promise of complex wood quality or growth improvements seems distant, however. The production economics and costs and returns for forest GMOs are daunting, and the social acceptance may be more challenging. FSC forest certification prohibits the use of GMOs for trees or for IPM, and several wood and paper retailers have or are considering adopting this policy. On the other hand, perhaps some of the outstanding recent medical breakthroughs, such as RNAi, can be duplicated in forestry at a much lower cost using similar technology. Maybe medicine and agriculture will pave the way for much less expensive subsequent forestry applications.

Increased government research in forest biotech is necessary, but costly, and the payoff will be distant. We still need to map the genomes of model tree species, discover molecular controls of key processes, assess ecological issues and opportunities, and understand risk management. We must link our forest biotechnology programs with traditional tree improvement, silviculture, and forest management, and vice versa. One avenue for this could be to use forest biotech to identify desirable characteristics, as described before, and then use vegetative propagation and/or somatic embryogenesis to rapidly ramp up and develop container stock for planting of superior trees. Perhaps this would avoid the clear social and certification antipathy for transgenics and GMOs, and still allow rapid implementation of the best science at reasonable costs.

In conclusion, tree improvement can enhance sustainable forestry by enhancing forest plantations and sustained yield of timber. It does increase timber yields, enhance insect and disease resistance, improve tree form and wood quality, and impart other benefits. Tree improvement requires continuous management attention to strategic breeding decisions and tactical operational implementation. It provides reasonable market returns to private and public investments. Forest biotechnology and GMOs offer more promise for dramatic breakthroughs in timber productivity and biodiversity conservation if successful. For example, biotech in medical applications and in the stock market has seen its most dramatic gains ever in 2002 and 2003. Biotech must be pursued with care in forestry so we do not waste scarce resources on extremely distant payoffs. We should capitalize on advances in medicine and agriculture, apply them well in forestry, answer pressing social and market questions, and integrate biotech with existing tree improvement and forest management research and development programs. With such advances, tree improvement and forest biotechnology can help us achieve the widely accepted paradigm of Sustainable Forest Management that promotes economic, ecological, and social benefits for this and future generations.

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Conservation Efforts for *Pinus maximinoi* in Mesoamerica and Its Potential as a Hybrid with *Pinus taeda* in South America

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Abstract

Seed samples were collected from 25 populations of *Pinus maximinoi* from Guerrero, Mexico to central Nicaragua and established in provenance tests in Brazil, Colombia and South Africa by the CAMCORE Cooperative. The trials were assessed for volume production at 3, 5 and 8 years of age. Subsets of 13 and 5 provenances were assessed using RAPD and allozyme markers, respectively, to determine patterns of genetic diversity and mating systems in natural stands. RAPD analyses indicated significant differences among provenances in percent polymorphism and observed heterozygosity. Geographical location of the population in Mesoamerica greatly influenced genetic diversity, with populations from Mexico and Guatemala exhibiting more diversity than those from Honduras and Nicaragua. Observed heterozygosity patterns detected in the RAPD analysis correlated reasonably well with provenance performance in Brazil ($r = 0.53$, $p = 0.06$), Colombia ($r = 0.48$, $p = 0.10$) and South Africa ($r = 0.43$, $p = 0.14$). Allozyme assessment showed *P. maximinoi* to be polymorphic for 22 of the 25 loci analyzed with an average of 2.86 alleles per polymorphic locus. There was also evidence of inbreeding in the *P. maximinoi* populations. Provenances selected in trials for good volume production were generally the most genetically diverse based on biochemical and molecular marker assessment. Because of this relationship and the socio-economic needs of local people, *in situ* conservation programs for *P. maximinoi* in Mesoamerica should be based on securing the gene resources of populations that performed the best in well-replicated, international field trials.

Pinus maximinoi has grown much faster than *P. taeda* in field trials established subtropical areas of Brazil through 14 years of age. Its juvenile wood properties are as good as or better than *P. taeda*. Specifically, its juvenile wood has a lower latewood percentage than found for the southern pines in the region, resulting in greater wood uniformity and stability. Hybrid crosses between *P. taeda* and *P. maximinoi* appear to be successful. The presentation discusses the opportunities of using *P. maximinoi* either as a pure species or as a hybrid with *P. taeda* in subtropical regions of South America. Sound conservation efforts now will ensure that breeding material is available in the future.

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Third-Cycle Breeding Strategy for Slash Pine by the Cooperative Forest Genetics Research Program

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Abstract: The Cooperative Forest Genetics Research Program (CFGRP) third-cycle slash pine tree improvement program will span 11 years (2003-2013), involving a breeding population of 360 third-cycle selections and 20 full-sib (FS) progeny tests with a total of 75,000 measurement trees. Of the 360 selections, two-thirds are forward selections (selections made in 1400 second-cycle full-sib selection plots) and one-third are backward selections (excellent first- and second-cycle selections brought forward into the third cycle). Selections will be top-grafted into sexually-mature, insect-protected, seed orchard trees where third-cycle breeding can begin as soon as one year after grafting. The main breeding population is divided into ten sublines with approximately 36 selections per subline. The top 6 selections from each of the 10 sublines comprise an elite population (6 per subline x 10 sublines = 60 elite selections). The ten sublines are divided into two superlines (the orange and blue superlines). Each superline has 5 sublines containing a total 180 selections of which 30 comprise the elite population for that superline.

The breeding for the main and elite populations is scheduled for three breeding seasons (2004-2006) and consists entirely of FS matings planted in replicated tests with single tree plots in incomplete block designs. For the elite population, a circular mating design will create 75 crosses in each of the 2 superlines (5 crosses for each of the 30 selections) for a total of 150 total elite FS families (75 crosses/superline x 2 superlines). Ten third-cycle elite FS progeny tests will be planted with each test having 3,000 measure trees (150 families/test x 20 blocks/test) for a total of 30,000 FS seedlings. For the main population, the circular mating design will attempt 72 crosses within each of the 10 sublines (averaging 4 crosses per selection) for a total of 720 main population crosses (72 crosses/subline x 10 sublines). Ten third-cycle main population FS progeny tests will be planted with each test containing FS progeny from half of the sublines (2 series with 5 tests for the orange subline and 5 for the blue). Assuming that only 600 of the 720 attempted FS families are successful, each test will contain 4,500 measure trees (300 families/test x 15 block/test of single tree plots) for a total of 45,000 FS progeny planted in main population tests (2 series * 5 tests/series * 4500 trees/test).

Keywords: Breeding strategies, *Pinus elliottii* var *elliottii*, third-cycle, selections, top grafting, breeding, progeny testing

INTRODUCTION

The Cooperative Forest Genetics Research Program (CFGRP), founded in November of 1953 (Perry and Wu, 1955), includes the University of Florida's School of Forest Resources and Conservation, forest industries and state agencies as cooperators.

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Our mission is to develop genetically-improved varieties of southern pines for reforestation of harvested timberlands in the lower coastal plain of the southeastern United States.

Throughout the 50 years of the CFGRP slash pine tree improvement program, several aspects have remained consistent: 1) Members of the CFGRP (industries and state forestry agencies from Florida and Georgia) conduct the selection, breeding and testing on their timberlands and provide policy and strategic direction; 2) CFGRP staff at the University of Florida provides strategic planning, technical support, supportive research, data management and analysis; 3) The program employs a single breeding unit aimed at creating broadly-adapted improved varieties for the entire slash pine native range in the lower coastal plain; and 4) The two most important traits are volume growth and fusiform rust resistance (*Cronartium fusiforme* sp. *fusiforme*).

Many other factors have changed over the 50 years of slash pine breeding: 1) The number of members and their names (only two of the original 10 members remain after 50 years, Rayonier Inc. and International Paper Company); 2) Breeding strategies; 3) Selection methods; 4) Genetic test designs; and 5) Data analysis methods. The purposes of this paper are to summarize the first 2 cycles of slash pine breeding by the CFGRP and detail the strategy for the third-cycle. We compare and contrast the breeding, selection and testing strategies and methods used in each of the three cycles.

FIRST-CYCLE SLASH PINE BREEDING PROGRAM (1953-1986)

The first breeding cycle for slash pine spanned 34 years with the selection phase beginning in 1953 and terminating in 1986 with the first second-cycle selections. Selections were grafted into first-cycle seed orchards from 1955-1982. Breeding began in 1965 and was completed in 1985. Open-pollinated (OP) tests were established between 1959-1985 and full-sib (FS) tests were established between 1978-1986.

First-cycle Slash Pine Selected Population

The first-cycle selected population included 3969 selections made in natural stands throughout the native range of slash pine between 1953 and1980 (Table 1). Of these 3969 selections, 2516 were tested in progeny tests and/or subsequently used for breeding. Of the 2516, approximately 2000 first-cycle selections were high-intensity selections for growth and form using the comparison tree method in which candidate trees were graded against dominant neighbors for various form (straightness, branch diameter, branch angle, pruning, crown diameter etc.) and volume characteristics. Candidate trees were also free of disease; however, most stands were low in disease incidence, so selection intensities for rust and pitch canker resistance were very low.

During the 1960's and early 70's, it became apparent that fusiform rust resistance was a very important trait for our tree improvement program. So, beginning in 1972 a concentrated effort was made to introduce an acceptable level of rust resistance into the selected population. As a result, the other 500 first-cycle selections were dominant, rust-

free trees selected in high hazard, 10-year-old or older plantations in which at least 70% of the trees were infected with fusiform rust. This resulted in immediate gains for fusiform rust resistance (Goddard *et al*. 1975).

	Cycle 1	Cycle 2	Cycle 3
Cycle Duration			
Start (year)	1953	1987	2003
Finish (year)	1986	2002	2013
Years (#)	34	16	11
Size			
Selected Population (#)	3969	1051	500
Breeding Population (#)	2516	1017	360
Effective Number (N_e)	2516	665	NA
Types of Selections			
Backward (#)	2016	396	180
Forward (#)	0	330	320
Infusions (#)	500	291	0
Population Structure			
Elite Population?	No	Yes, 75 selns	Yes, 60 selns
Main Population:			
Sublines (#)	1	24	10
Selections/Subline (#)	2,516	42	50
Genetic Gain			
Volume (%)	9	15	23
Rust (R50)	45	39	31

Table 1: Selected and breeding populations for 3 cycles of slash pine tree improvement by the CFGRP. Selected populations include all selections made by the cooperative, while breeding populations include only those selections used in breeding and/or testing. Effective size of the breeding population was calculated from Falconer and Mackay (1996). Infusions are untested material added to the breeding population. Genetic gain is estimated as the mean BLP-predicted breeding value of all selections in the breeding population. Individual-tree volume gains are percentage (at 15 years) above unimproved slash pine. R50 values are rust incidence of improved material relative to an unimproved incidence of 50% of the trees infected. Figures for Cycle 3 were estimated in 2003 when approximately 3/4 of the selections had been made with 1/4 still to be made in 2004.

Genetic gains for the 2516 members of the first-cycle breeding population (9% for volume and an R50 of 45%) are a weighted average of the gains from the 2000 high-intensity selections for growth and form (10% for volume and little gain in rust resistance with R50=48%) and the 500 rust-free selections in high hazard stands (5% volume gain and R50=35%). The R50 value of 45% means that seedling offspring obtained from random matings among the 2516 first-cycle selections are expected to incur rust incidence of 45% when 50% of unimproved slash pine trees are infected with stem or branch galls.

First-cycle Slash Pine Breeding and Testing

In the first cycle of slash pine tree improvement, progeny testing and breeding were conducted in tandem. Progeny testing using orchard OP seed was done first to rank selections with OP field trials being planted mainly in the 1960s and 1970s as the first-cycle grafted orchards reached seed production. Then, in the 1970s and 1980s, the results of the OP trials were used to identify better parents (approximately 1000 of the first-cycle selections) to include in control-pollinated breeding to produce FS families for field trials in which second-cycle selections were made. The tandem approach, progeny testing followed by breeding, resulted in good genetic gain, but lengthened the first cycle to 34 years.

In total, 363 first-cycle OP progeny tests were planted by CFGRP cooperators in Florida, Georgia, Alabama and Mississippi to rank 2516 parents for volume growth and rust resistance (Table 2). Each test contained 10 to 150 OP families (typically 20 to 40 families), and the field design was randomized complete block (RCB) with 6 to 10 blocks established with row plots containing 6 to 10 trees per plot. In total, the 363 OP tests contained approximately 580,000 total measure trees.

All first-cycle breeding was conducted in a breeding population that was unstructured in any way (no sublines, no elite population and no multiple populations). The most common mating design utilized was 6-parent disconnected diallels, but some were disconnected factorials in which pollen from 4 rust-free selections was used as males with 6 original selections used as females. As a result of control-pollinated breeding, 277 first-cycle genetic tests containing over 2700 FS families were planted by CFGRP cooperators in Florida, Georgia, Alabama and Mississippi (Table 2). The purposes of these tests were to rank the parents and make second-cycle forward selections. The field design of these tests was RCB with row plots. A typical test has 20-30 FS families, 6-10 blocks and 6-10 trees per plot for an average of 1600 measure trees per test. In total, more than 440,000 test trees were planted as a result of the first-cycle FS breeding efforts.

Site preparation and test maintenance (weed control, fertilization, mowing) were minimal in first-cycle tests resulting in relatively low h^2 for volume (0.09) and rust (0.21), while G x E interaction was relatively high with type B genetic correlations of 0.6 for volume and 0.7 for rust (Table 2).

First-Cycle Propagation Population and Deployment

In 1955, CFGRP members began establishing first-cycle, grafted seed orchards. Each member's seed orchard contained that company's 40 to 200 selections (*i.e.*, selections were not interchanged among members). Approximately 50 orchards were established by members in this manner. For many years (1960s and 1970s) the main deployment method was to use bulk seed collections obtained from these OP first-cycle, slash pine seed orchards. Expected genetic gains in harvest yields at 20 years from the operational plantations established with bulk collections from unrogued, first-cycle seed orchards

averaged 7.6% and 12.9% on low hazard and high hazard fusiform rust sites, respectively (Figure 2). The actual gains varied among the 50 orchards.

	Cycle 1	Cycle 2	Cycle 3
OP and PM Tests			
Test Sites (#)	363	16	0
Purpose	Rank parents	Rank parents	NA
Mating Design	OP from orchards	PM in clone bank	NA
Field Design	RCB, row plots	RCB+IBD, STP	NA
Families (#)	2,500	315	NA
Trees (#)	580,000	54,400	NA
Full-sib Tests			
Test Sites (#)	277	10	20
Purposes	Rank parents and Make selections	Make selections	Rank parents and Make selections
Mating Design	Disconnected diallels and factorials	Stratified in sublines	Circular diallels in sublines
Field Design	RCB, row plots	Unreplicated blocks	IBD, STP
Families (#)	2700	1407	750
Trees (#)	440,000	105,000	75,000
Heritability (h^2)			
Volume	0.09	0.31	NA
Rust	0.16	0.26	NA
G x E (r_b)			
Volume	0.6	0.8	NA
Rust	0.7	0.8	NA

Table 2. Characteristics of breeding and genetic testing for 3 cycles of slash pine tree improvement by the CFGRP. Abbreviations are: G x E= genotype x environment interaction; h^2=individual, pooled-site heritability; IBD=incomplete block design; OP=open-pollinated; PM=pollen-mix pollinated; RCB=randomized complete block; STP=single tree plots; and rb=type B genetic correlation of family performance for the same trait across sites.

As OP progeny test data became available, seed orchard clones were ranked and poor performing clones were removed (rogued) from the orchards. Average estimated gains from members' rogued seed orchards rose to 12.8% and 17.9% in 20-year harvest yield on low and high hazard sites, respectively (Figure 1). This is a gain of slightly more than 5% from roguing seed orchards based on OP progeny test information and varied among orchards depending on the quality and quantity of progeny test data available to rank the clones in each orchard. This seed was the main source of operational seed from 1980 to 1995 (White and Duryea 1997).

Some members also chose to graft new seed orchards containing only the highest ranking first-cycle parents based on the OP progeny test data. These 1.5 generation orchards required large-scale exchange of scion material among members, because most members had originally selected only a few of the high ranking parents. Average estimated gains in harvest yield from members' 1.5 generation seed orchards were 17.1% and 22.5% in low and high rust hazard areas (Figure 1). These seed orchards have been a main source of operational seed between 1985 and the present time. It is now common for members to collect seed by clone and deploy OP families operationally (McKeand *et al*. 2003).

SECOND-CYCLE SLASH PINE BREEDING PROGRAM (1987-2002)

The second-cycle slash pine selection, breeding and test establishment spanned 16 years (1987-2002). The selection phase began in 1987 and was completed in 1990. Selections were grafted into clone banks for breeding between 1988 and 1990. Breeding began in 1993 and was completed in 2001. Two series of polymix (PM) tests were established to rank many, but not all, of the parents in the selected population: Series I in 1998 and Series II in 2001. As part of the complementary mating design, FS breeding was conducted simultaneously with the PM breeding and 10 sites (1 per member) were established with unreplicated block plots of FS families for making third-cycle forward selections. A total of 1407 FS families were planted in unreplicated plots between 1995 and 1999 with third-cycle selections beginning in 2003. The second-cycle tree improvement program for slash pine is outlined below and detailed in White *et al*. (1993).

Second-Cycle Slash Pine Selected Population

The second-cycle slash pine total population size was 1051; however, thirty-four selections are for research purposes only and are in a non-breeding or testing subline (subline Q). Therefore the second-cycle breeding and testing population size was 1017 with an effective population size of 665 (Table 1). Our objectives for the selected population were to maximize genetic gain, while maintaining a broad genetic base. Potential candidates available for inclusion in the selected population were: 1) 2500 original first-generation selections (backward selections); 2) 440,000 trees from 2700 different FS families generated from crosses among first-cycle selections (forward selections); and 3) A number of promising, but generally untested, materials from a range of sources (infusions).

All forward and backward candidates were ranked according to their predicted genetic worth for volume and R50 using best linear prediction (Hodge et. al. 1989). Then, 3 selection indexes were developed: 1) Growth and rust combined; 2) Most weight on volume; and 3) Most weight on rust resistance. The best candidates identified by these three indices were selected with a maximum 7 relatives permitted for top ranking selections (to maximize gain) and fewer relatives for less-superior selections (to maintain genetic diversity. For each forward selection, a computer generated selection form was printed and CFGRP members visited the full-sib tests to make forward selections. This selection process was completed in 1990.

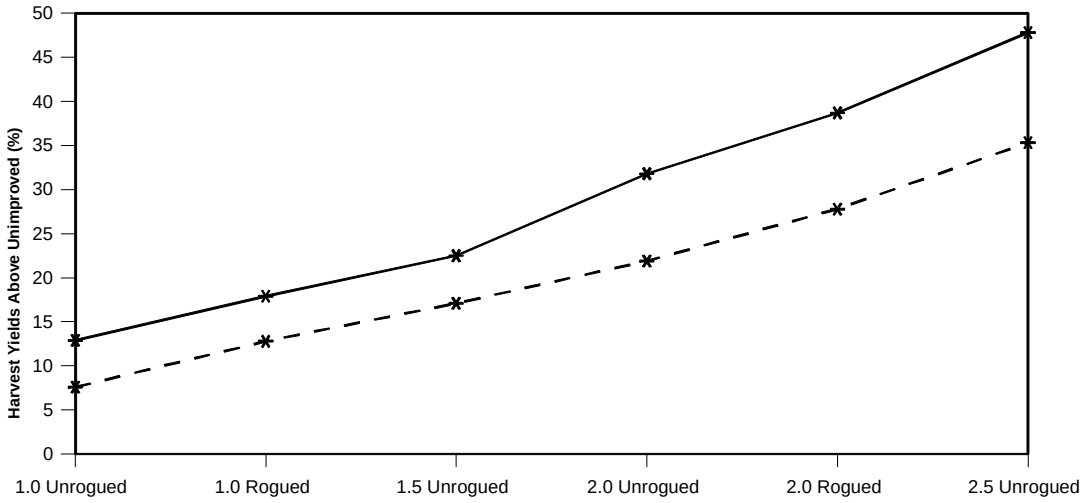

Figure 1. Estimated genetic gains in harvest yield from slash pine seed orchards when material is planted in low (lower dashed line) and high (upper solid line) rust hazard areas. First and second cycle gains are estimated from actual orchard compositions of members' orchards, while 2.5 orchard gains are mean breeding values of the top 20 available selections. These gains in harvest yield combine the effects of volume growth and rust resistance and are projected to a 20-year pulpwood rotation.

Of the 1017 selections, 396 were backward selections, 330 were forward selections and 291 were infusions (Table 1). The selected population was structured in several ways to both control relatedness (within sublines and superlines) and to focus increased effort on more superior selections in the elite population and upper stratum of the main population. First, the selected population was divided into 2 superlines, orange and blue, each one consisting of an elite population and a main population of 12 sublines (Figure 2). Each subline contained approximately 42 selections which were divided into three stratum based on genetic worth (I, II, III, with selections of the highest quality in stratum I). All related selections were assigned to the same subline and each CFGRP member was given one or two sublines to breed. All forward selections, infusions and some backward selections were grafted into breeding clone banks (1988 to 90). All forward selections were established in at least two members' clone banks.

Compared to unimproved slash pine, the mean genetic gain (predicted as an average of the BLP-breeding values) for this population of 1017 selections was 15% for 15-year individual-tree volume and R50=39%. These second-cycle gains indicate a 6% increase in volume breeding value and a 6% reduction in R50 compared to the entire first-cycle population (Table 1). However, the second-cycle gains are decreased by inclusion of 291 untested infusions, most of which were assumed to have volume gains of 10% or lower and R50=40 or higher.

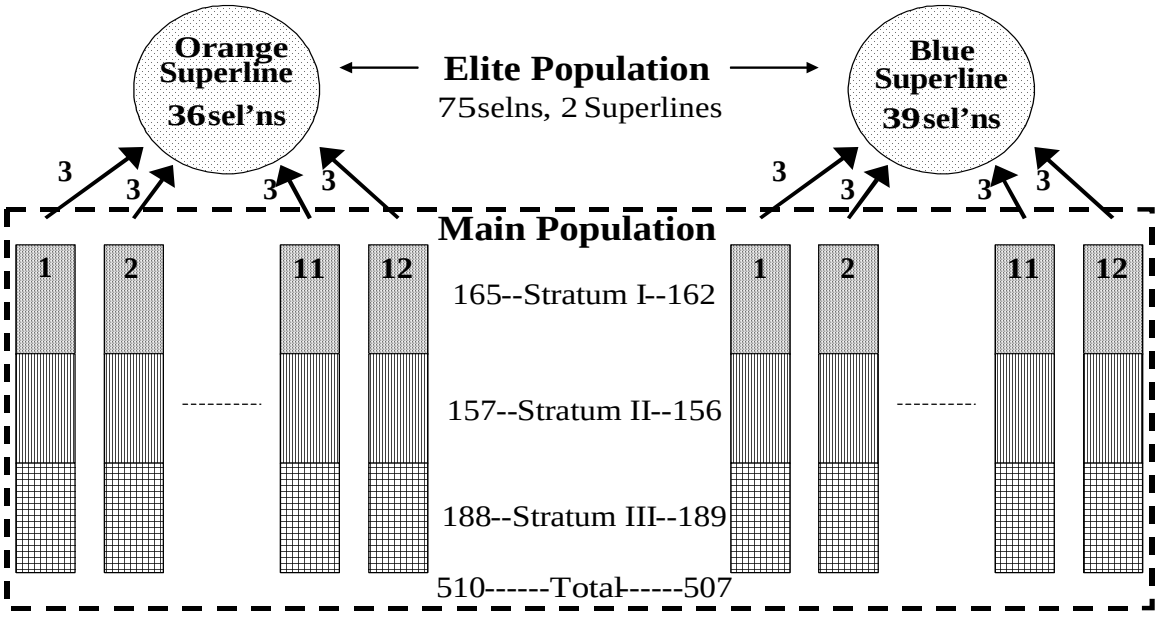

Figure 2. Breeding population for second-cycle slash pine. Selections were divided into 2 superlines, orange and blue, each one consisting of an elite population and a main population of 12 sublines. All relatedness was confined with sublines and superlines. Each subline was divided into three strata based on genetic quality (I, II, and III with selections of the highest quality in I).

Second-Cycle Slash Pine Breeding and Testing

The breeding and testing strategy for the second cycle employed complementary mating designs (van Buijtenen 1976, van Buijtenen and Lowe 1979): 1) Polymix (PM) crosses planted in randomized and replicated progeny tests to rank parents; and 2) Full-sib (FS) crosses planted in unreplicated block plots of 50 to 100 trees per family from which to make third-cycle forward selections.

Of the 1017 selections, 315 poorly-tested forward and backward selections were crossed with a single pollen mix and planted in PM tests. Infusions and selections that were well tested in the first cycle were not included in the PM testing, but rather used only in the FS breeding. The test design was an RCB with 20 blocks and single tree plots (STPs). A total of sixteen tests were established in two series of 8 tests. Series I was planted in 1998 and the 5 year measurements were taken in 2002. Series II was planted in 2002 and the first year measurements were taken in 2003. The total number of measure trees in these 16 tests are 54,400 (Table 2). Narrow sense heritability from these trials for volume at age 5 years is triple the heritability for the same trait from the first-cycle OP trials (Table 2) and we attribute this both to smaller size of replication with STPs and better site preparation, weed control and fertilization of the second-cycle trials.

The FS breeding phase began in 1993 simultaneously with the PM breeding. In total, 1407 families were created as part of the breeding in the main and elite populations. No specific mating design was followed; rather, more crosses were made with better selections contained in the elite and top stratum of the main breeding population (Lindgren 1986, Lindgren and Matheson 1986). In the main breeding population (Figure 2), all FS breeding was done within each of the 24 sublines, crossing selections together according to their strata: I X I, I X II, I X III, II X II (there are no crosses among strata II x III or III x III).

The 1407 FS families were established by CFGRP members in unreplicated block plots, with a total of 105,000 measure trees across the 10 sites (1 site per member with their FS block plots). These are the selection plots in which the third-cycle, forward selections are being made in 2003 and 2004.

Second-Cycle Propagation Population and Deployment

From 1988 to 1998, CFGRP members established 15 second-cycle, grafted seed orchards using a mixture of tested, first-cycle selections (backwards) and newly-selected second-cycle selections (forwards). The most common field design employed a systematic, repeating design that maximized distance between related clones (Hodge and White 1993). Clones selected for each seed orchard depended on the weight that each member put on volume growth and rust resistance. In total 194 (out of 1017) selections were grafted into one or more orchards, but the number utilized in several orchards is closer to 60. A typical second-cycle CFGRP seed orchard ranges from 5 to 50 acres (mean= 23) and started with 22-93 clones (mean=39). Many of these orchards are now in full seed production and are providing substantial quantities of operational slash pine seed. Most members collect seed by clone and operationally deploy OP families (McKeand *et al*. 2003).

Average genetic gains in plantations established from the 15 unrogued second-cycle seed orchards (based on bulk collection and no pollen contamination) are 22% and 32% for total harvest yield at age 20 on low and high hazard rust sites, respectively (Figure 1). Many members are currently roguing their second-cycle seed orchards, and while actual results from the roguing are not yet available, a simulated roguing leaving the best 20 clones in each of the second-cycle orchards increases expected gain in harvest yield approximately 6% above the unrogued orchards (Figure 1). Furthermore, a simulated 2.5 cycle orchard including only the best 20 selections, now that PM tests have provided rankings, would produce expected gains still higher (35% and 48%) gains in harvest yield in low and high hazard rust sites, respectively.

THIRD-CYCLE SLASH PINE BREEDING PROGRAM (2003-2013)

The CFGRP third-cycle slash pine selection, breeding and testing will span11 years (2003-2013) beginning with the third-cycle selection phase in the winters of 2003 and 2004. Selections will be top grafted into seed orchards in the winters of 2003 to 2005. Breeding is planned from 2004 to 2006. No OP or PM tests are planned in the third-

cycle. Rather, FS families from breeding the 360 selections in the breeding population will be planted in 20 total field tests in 2008 with fourth-cycle forward selections from these tests planned in 2014.

Third-Cycle Slash Pine Selected Population

Approximately 500 third-cycle selections will be made during the winters of 2003 and 2004 with two-thirds being forward selections (selections made in the 1400 second-cycle, unreplicated FS selection plots) and one-third being backward selections (excellent first- and second-cycle selections brought forward into the third cycle). All forward selections and some backward selections will be top-grafted into sexually-mature, insect-protected, seed orchard trees where third-cycle breeding can begin as soon as one year after grafting. The third-cycle main population is divided into ten sublines with approximately 50 selections per subline (Figure 3). The 24 second-cycle sublines (Figure 2) were condensed to 10 for the third cycle and all related selections are assigned to the same subline. The top 6 selections from each of the 10 sublines make up an elite population (6 per subline x 10 sublines = 60 elite selections). The ten sublines are divided into two superlines (the orange and blue superlines). Each superline has 5 sublines containing a total 250 selections (50 selection/subline x 5 sublines = 250 selections, of which 30 comprise the elite population for that superline).

The PM Series II trials from the second-cycle program along with FS unreplicated selection plots younger than 5 years in 2004 were not considered available for the third-cycle program. Rather, we decided to move ahead rapidly with the third-cycle selection phase in 2003-2004. Then, any excellent backward selections identified by the second-cycle PM tests and any excellent forward selections from the younger FS selection plots will be included in the fourth-cycle selected population (*i.e.*, these will skip a cycle).

Genetic gains for the third-cycle selected population of 500 selections are anticipated to be 23% for individual tree volume with R50=31% (Table 2). These are the mean values of the 419 selections made in 2003 with still another 81left for making in 2004. These gains are considerably higher than the mean for the second-cycle breeding population due to: 1) Few untested infusions being included; 2) Heritabilities from the PM trials are high, so excellent FS families were identified in which to make third-cycle selections; and 3) Unreplicated FS family selection plots were generally well maintained with 50 to 100 trees of the same family (meaning that within-family resulted in appreciable gain).

Third-Cycle Slash Pine Breeding and Testing

Starting with 500 third-cycle selections, we anticipate that only 360 will comprise the breeding population due to losses during grafting, flowering and breeding (Table 2). Crossing for the main and elite populations is scheduled for three breeding seasons (2004-2006) and consists entirely of full-sib (FS) matings planted in replicated tests with single tree plots in incomplete block designs (IBD). We have opted for this strategy instead of complementary mating designs, because all trees planted are used for both ranking parents and making forward selections (as opposed to CMD in which the PM

tests provide only information, not selections). A further advantage is that rankings will be available for a large number of well-tested FS families which benefits those CFGRP members deploying FS families operationally (such as through control mass pollination).

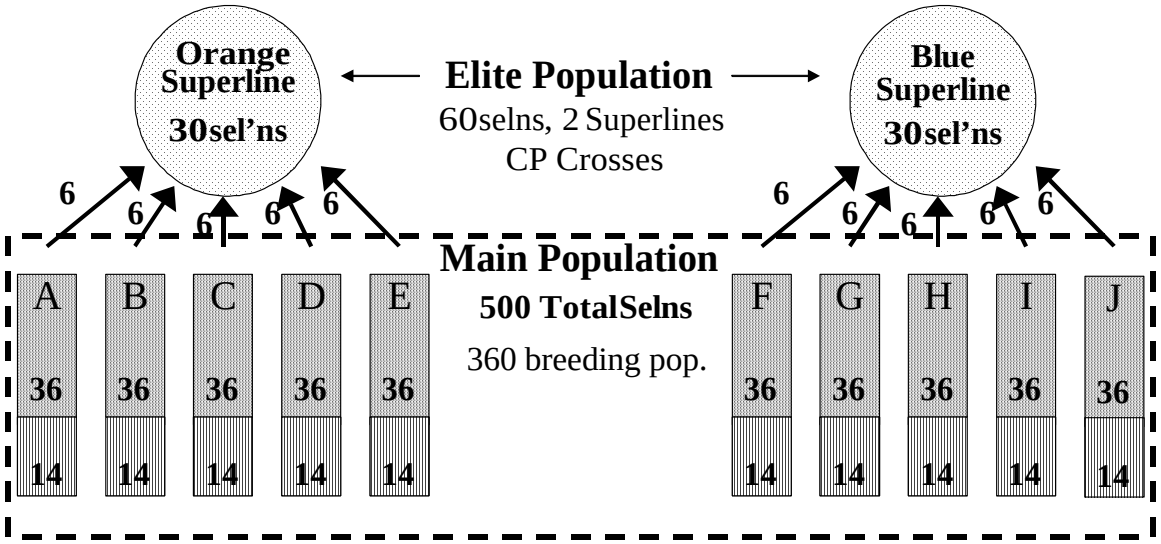

Figure 3. Third-cycle selected and breeding populations of slash pine. The 500 selections are divided into 10 sublines of 50 selections each. The top 6 selections in each subline are also in the elite population of 60 selections which is divided into 2 superlines, orange and blue. Assuming that 14 selections in each subline do not survive top grafting or do not flower, then the breeding population will consist of 360 selections (36 in each of 10 sublines).

For the elite population (Figure 3), a circular mating design will create 75 crosses in each of the 2 superlines (5 crosses for each of the 30 selections) for a total of 150 total elite FS families. Ten third-cycle elite FS progeny tests will be planted with each test having 3,000 measure trees (150 families/test x 20 replications/test) for a total of 30,000 FS seedlings in third-cycle elite tests.

For the main population, the circular mating design will attempt 72 crosses in each of the 10 sublines (averaging 4 crosses for each of the 36 selections) for a total of 720 main population FS families (72 crosses/subline x 10 sublines). All crosses in the main population will be made within a subline. Ten third-cycle main population FS progeny tests will be planted, with each test containing FS progeny from half of the sublines (5 tests containing FS families from the orange sublines and 5 tests for the blue sublines). Assuming that only 600 of the 720 attempted FS families are successful, each test will contain 4,500 measure trees (300 families/test x 15 blocks/test of single tree plots in an IDB) for a total of 45,000 FS progeny planted in main population tests (2 series x 5 tests/series x 4500 trees/test).

Tests of elite and main population FS families are scheduled for planting in 2008 with 6-year measurements in 2013 and fourth-cycle selections beginning in 2014. Counting the tests for both the main and elite populations, there are a total of 20 tests containing 750 FS families with 75,000 measure trees planned for the third cycle. This compares favorably with nearly 160,000 measure trees in second-cycle tests (summing PM and FS tests from Table 2) and over a million measure trees in genetic tests of the first cycle.

Third-Cycle Propagation Population and Deployment

Once all third-cycle selections are made (2004), third-cycle seed orchards will be developed using the very best of these selections. Clones selected for each seed orchard will again depend on the weights that each member wants to assign to volume growth and rust resistance. These would be orchards containing both untested, forward selections and some tested backward selections also.

CONCLUSIONS

It is valuable to reflect on breeding, testing and analytical strategies and how these have changed over the 50 years and 3 cycles of slash pine tree improvement. New developments by many people have resulted in changes in breeding strategies and methods including: 1) Structuring of the breeding population into sublines and superlines; 2) Increasing focus on elite material; 3) Use of overlapping generations (*e.g.*, forward and backward selections); 4) Evolution of mating designs away from disconnected and complementary mating designs to interconnected full-sib designs; and 5) Top-grafting to obviate clone banks and reduce the duration of a breeding cycle by several years.

In genetic testing, recognition of the importance of single tree plots and incomplete block designs coupled with quality installation, maintenance and measurement have all contributed to increased precision of the trials (higher heritabilities and larger gains from selection). These developments allow substantial reduction in the number of test sites and measure trees in genetic tests, because each site is providing more information. In the CFGRP slash pine program, the number of trees in genetic tests has decreased steadily with no anticipated loss in genetic gain per cycle (Table 2).

It is only through the sustained efforts of the people involved and members of the CFGRP that this cooperative has maintained its long term tree improvement program for slash pine. Their commitment to this program for half a century (1953-2003) has produced ever-increasing genetic gains from slash pine seed orchards (Figure 1), an achievement that all members can be proud of!

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Responsiveness of Diverse Families of Loblolly Pine to Fertilization: Eight-Year Results from SETRES-2

S. E. McKeand, J. E. Grissom, R. Rubilar, and H. L. Allen[1]

Keywords: Genotype by environment, *Pinus taeda*, Provenance

Loblolly pine is by far the most important forest tree species in the South, with over 1 billion seedlings planted annually by forest industry and non-industrial private forest landowners (McKeand et al. 2003). Genetic gains from tree improvement programs have been large, since geographic and within-provenance variation for growth and adaptive traits in loblolly pine is very large. General trends in productivity variation are that families from southern and eastern coastal sources grow faster than families from northern, western, and interior sources. Contrasting the response to nutrient stress of two very different provenances of loblolly pine such as from the "Lost Pines" region of Texas and the Atlantic Coastal Plain may give us insight into the adaptive significance of different ecophysiological traits.

Previous work indicates that the Lost Pines Texas (LPT) sources are generally more stable across environments, while productivity of eastern sources depends more on the environment. Eastern sources were very responsive to environmental enhancement, since productivity was high on the better sites, but very low on the droughty sites. In this report, we describe a study designed to assess spatial and temporal variation in response of loblolly pine genotypes to environmental stress. Trees have completed eight growing seasons in the field under two different nutrient regimes, and variation in early growth is described.

MATERIALS AND METHODS

The study site is located in Scotland County, North Carolina adjacent to the U.S. Forest Service / N.C. State University SETRES (Southeast Tree Research and Education Site) study. The soil is very infertile and somewhat excessively drained. The existing 10-year-old loblolly pine stand was carefully removed and large block plots of different family-treatment combinations were established. Open-pollinated families from the North Carolina and South Carolina Coastal Plain and from the "Lost-Pines" area of Texas were included in the study. Five families from each provenance with average or slightly above average breeding values for volume were used. Seeds were sown in containers (10 in^3 RL Super Cells) in the greenhouse in June 1993, and seedlings were field-planted in November 1993.

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To facilitate the application of nutrients, a split-split-plot design was used with the two nutrient treatments as main plots, provenances as sub-plots, and families within provenances as sub-sub-plots. Each family plot consists of 100 measurement trees planted at 5' x 7' spacing. Buffer trees, 40' around each treatment plot, were planted at the same spacing to eliminate the influence of one nutrient treatment on another. The study was replicated across 9 blocks (10 blocks were originally established, but one was sacrificed after age 6) for a total of 18,000 measurement trees (9 blocks x 2 nutrient treatments x 2 provenances x 5 families per provenance x 100 trees per family plot). Fertilizer has been applied annually to maintain a balanced supply of all nutrients in the fertilized plots. Our goal has been to supply optimal levels of nutrients (including micros) each year to stimulate rapid growth.

All trees were measured annually (except for year 7) for height and starting in year 3 for breast height diameter. Individual tree volumes were calculated, and plot volumes were estimated as the sum of the individual tree volumes and converted to per hectare volumes. Analyses of variance were conducted on a family-plot-mean basis. Means and within family-plot standard deviations and coefficients of variation were calculated for height for each 100-tree family plot. Within family-plot standard deviations and coefficients of variation were also subjected to analyses of variance to determine if sub-sub-plot uniformity varied.

RESULTS AND DISCUSSION

Growth responses to fertilization were very large and significant each year (Figure 1). Height was 21%, 46%, 43%, 43%, 43%, 50%, and 66% greater in the fertilized plots for years one to eight, respectively (Figure 1). Volume differences at age 8 were even more dramatic (Control=709 ft^3/a, Fertilized=1829 ft^3/a), with the fertilized trees having 2.6 times more volume per acre than the controls. Although this is a well-drained site, from the results of the nutrition by irrigation study (SETRES) adjacent to this trial, we know that the primary limit to productivity is nutrition (Albaugh et al. 1998). The huge increase in productivity in the first eight growing seasons is possible since all potential nutrient limitations (i.e. more than just N and P) were ameliorated.

One of the most dramatic effects of the nutrition amendments has been the increase in uniformity within the 100-tree family plots. The average within-plot coefficient of variation for eighth-year height was 20.0% for the control plots and 10.1% for the fertilized plots. The within plot standard deviations for height were also significantly different and were 3.15 ft. for the control plots and 2.72 ft. for the taller fertilized plots. While increased uniformity typically results from nutritional amendments on very poor sites, the dramatic differences in uniformity were surprising.

As expected, the five families from the Atlantic Coastal Plain grew faster than the five Lost Pines Texas families (Figure 1). We anticipated that under the harsher environmental conditions in the control plots that the Texas families would perform relatively better. However, the ACP families were superior in both environments

(Control: LPT=675 ft^3/a, ACP=744 ft^3/a, Fertilized: LPT=1767 ft^3/a, ACP=1890 ft^3/a,), and the provenance by treatment interactions for height in all eight years were not close to being significant.

The provenance by treatment means for volume per acre at age eight are somewhat indicative of the greater responsiveness to nutritional amendments of the Atlantic Coastal Plain provenance compared to the Lost Pines Texas provenance. Although there was no provenance rank change in the two environments, the difference in the magnitude of the provenance means (greater in the fertilized plots) is similar to previous trials.

Families within provenances also differed for growth traits. The family means at age eight for the ACP families varied from 669ft^3/ac to 791ft^3/ac in the control plots and from 1648ft^3/ac to 2037ft^3/ac in the fertilized plots. The Texas families also differed in the control plots (631ft^3/ac to 709ft^3/ac) and in the fertilized plots (1674ft^3/ac to 1884ft^3/ac). The lack of rank change across the treatments both at the provenance and family level was surprising for height and volume. Given the magnitude of the imposed environmental differences and the young age of the trees, differential performance of the families in the two treatments was expected. This result reinforces the tenet of the stability of open-pollinated families of loblolly pine as well as the better responsiveness of the ACP provenance compared to the Lost Pines provenance.

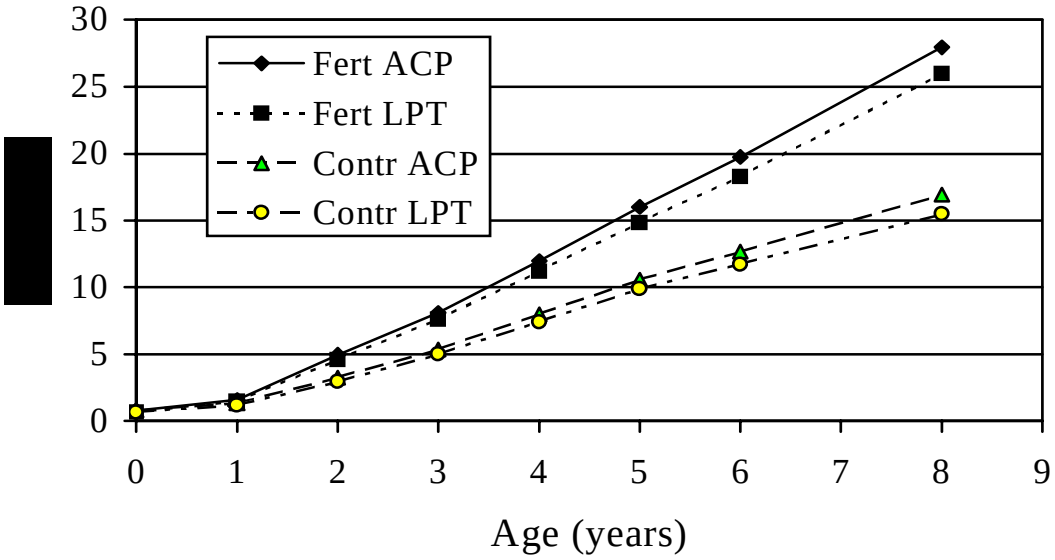

Figure 1. Mean tree heights during the first eight growing seasons in the field for trees from the Lost Pines Texas (LPT) and Atlantic Coastal Plain (ACP) provenances in the fertilized and control plots. Initial height of seedlings (age 0) at planting was measured in 1994.

ACKNOWLEDGEMENTS

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Dominance and Stand Structure Analyses of a GXE Interaction Trial

Rafael Rubilar, Steve McKeand, H. Lee Allen[1]

Keywords: Dominance, selection, genotype-environment interaction, *Pinus taeda*

Intra-specific competition of loblolly pine (*Pinus taeda*) is a key factor for individual tree development. Liu and Burkhart (1994) indicated that at the seedling stage environmental gradients dominate on tree growth, where intraspecific competition dominate later stages of the stand. However, little information has been provided on how genetic material and intensive silviculture interactions affect individual tree and stand performance. In addition, tree breeders have always been interested in early selection of future outstanding individual "dominant trees" to obtain maximum economic benefits and productivity of future stands (Bridgwater et al., 1985; Li et al., 1991). Our objective was to analyze how contrasting genotypes of loblolly pine, under contrasting nutrition treatments, differ in dominance and stand structure from planting until 8 years of age.

METHODS

At SETRES2 seedlings from five open-pollinated families (FAM) each of Atlantic Coastal Plain (ACP) and Lost Pines of Texas (LPT) provenances (PROV), were planted at 1.5m x 2m in 1993 and received two nutritional treatments (TRT) (CTRL=no fertilization vs. FERT=continuous fertilization) in a split-split plot layout. After removal of all trees with severe tip moth attack, snow damage or other significant damage, a dominance index was calculated at each age (DIage) for each tree based on the number of standard deviations (SD) from the average of its neighbors. DIage values of 0.1, 0.2, 0.4, 0.6, 0.8 and 1 were assigned if a tree was <-2SD, -2SD to <-1SD, -1SD to <0SD, 0SD to 1SD, >1SD to 2SD, and >2SD, respectively. In addition, an additive score at each age (SC_{age}) was calculated as the sum of previous DI_{age} indexes representing the history of a tree in the dominant or co-dominant classes.

For all dominant trees at year eight (DI_8 >= 0.5 or SC_8>4.0), correlations at sub-sub-plot level (families within replicates) were calculated for indexes (DI_{age}) and scores (SC_{age}) at ages 0 to 6 (DI_{0-6} or SC_{0-6}) with age 8 (DI_8 and SC_8). In order to evaluate the statistical differences among the correlation changes in time and the treatments applied, a split-plot design with repeated measurements analysis (RMA) was used (Gumpertz and Brownie, 1993), but blocks and families were considered as random components.

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RESULTS

Average dominance indexes ranged from 0.52(DI0) to 0.66 (DI8) and showed a reduction in variation with time. Average scores ranged from 0.52(SC0) to 4.95 (SC8) and showed a slight increase in variation with time.

Correlations for DI0-6 with DI8 showed a linear pattern of increasing correlation up to age 5 (Figure 1). This pattern indicated that the probability of dominance prediction from earlier ages increases, as trees get older. In contrast, correlations of DI0-6 with SC8 showed an early increase after age 2 that was maintained until age 5 on an asymptotic trend. A decrease, or lack of increase in correlation after year 5, was observed for both types of parameters and was probably associated with snow damage during that season.

Orthogonal contrasts for linear and quadratic degree polynomials using multivariate repeated measures analysis of DI0-6 with DI8 correlations indicated a strong linear effect ($p<0.01$) of age on TRT and TRT*FAM(PROV), and a strong quadratic effect on TRT (Table1, Figures 1 and 2). The same analyses for DI0-6 with SC8 correlations indicated a strong linear effect ($p<0.01$) of age on TRT and a strong quadratic effect of age on TRT*FAM(PROV) (Table1, Figure 1).

		DIageDI8		**DIageSC8**	
SOURCE	**DF**	**linear**	**quad**	**linear**	**quad**
Mean	1	**<.0001**	**<.0001**	**<.0001**	**<.0001**
REP	8	0.7283	0.0024	0.2990	0.0268
TRT	1	**<.0001**	**0.0510**	**0.0010**	0.1316
PROV	1	0.5763	0.1480	0.4651	0.9333
REP*TRT	8	0.8404	0.6119	0.9104	0.1644
REP*PROV	8	0.8559	0.1204	0.8572	0.0994
TRT*PROV	1	0.7526	0.8181	0.0704	0.2319
REP*TRT*PROV	8	0.6516	0.6937	0.9792	0.2395
FAMILY(PROV)	8	0.4314	0.4161	**0.0532**	0.3999
REP*FAMILY(PROV)	64	0.3684	**0.0413**	0.8922	0.0505
TRT*FAMILY(PROV)	8	**0.0094**	0.2251	0.4631	**0.0111**

Table 1.- Orthogonal contrasts for linear and quadratic degree polynomials using multivariate repeated measures analysis of $DIageDI_8$ and $DIageSC_8$correlations. Linear or quadratic (quad) degree polynomial contrast p-value for the age of the stand and treatment effects.

Analysis of dominance-indexes parameters showed that dominance prediction from earlier ages is feasible, and in all cases increases, as trees get older. Our results also indicate that individual tree dominances at early ages are not good predictors of dominance at age 8, and cumulative history (SC8) may be predicted from age 2.

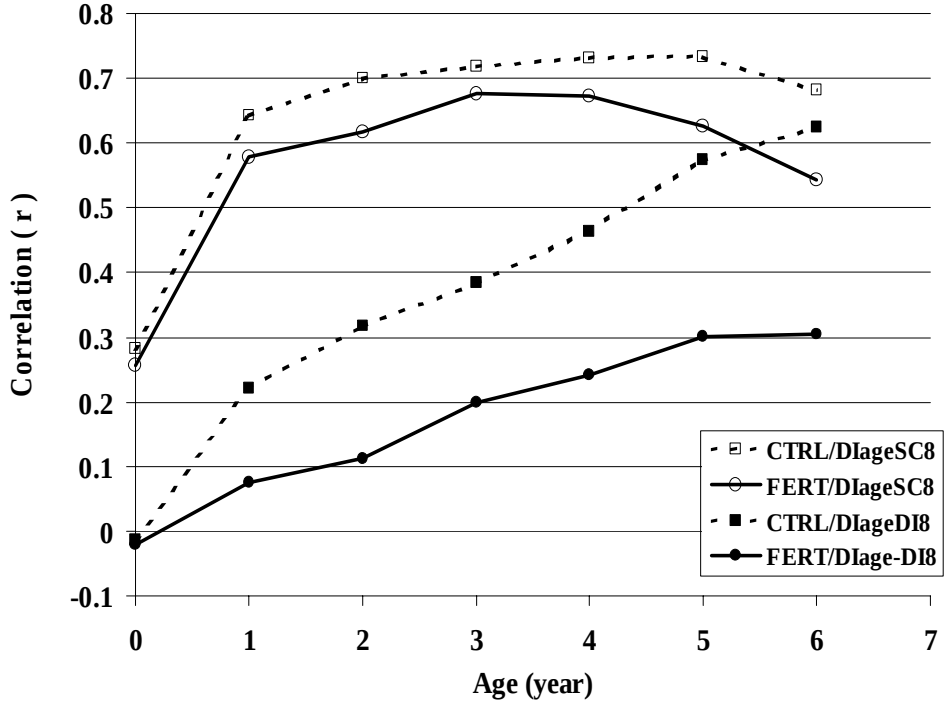

Figure 1.- Correlation of DI from ages 0 to 6 with DI8 and SC8 for each fertilization treatment.

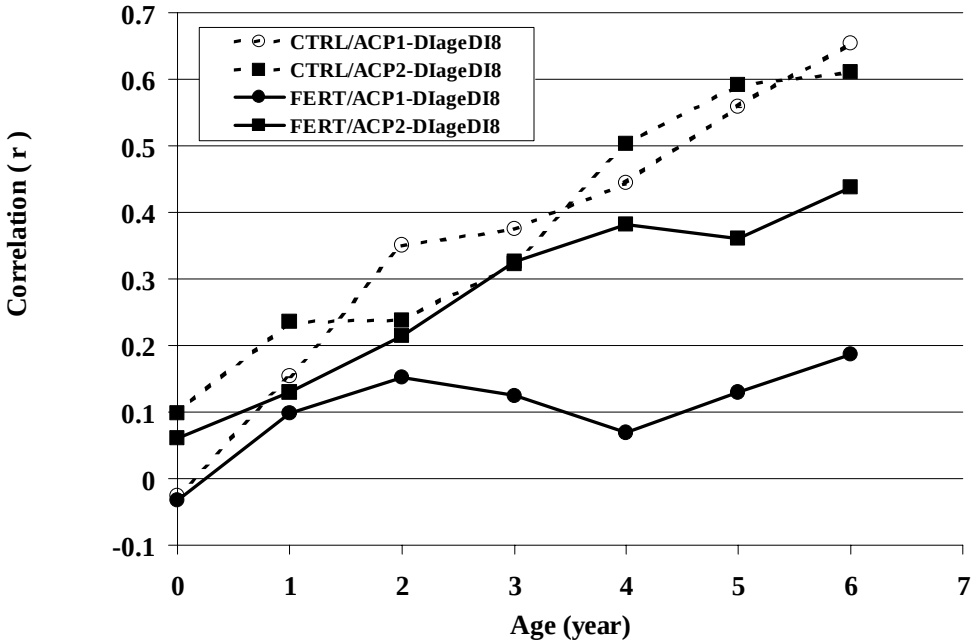

Figure 2.- Correlation of DI from ages 0 to 6 with DI8 for families ACP1 and ACP2.

Strong effects of TRT and no effects of PROV in dominance determination were not surprising considering the large nutritional effects in all other growth parameters of the stand (McKeand et al., 2000; Handest et al., 1999). An interesting result was the fact that lower correlations were obtained for fertilized trees compared to unfertilized trees. For fertilized trees dominance continues to change where no fertilized trees establish and maintain dominance at earlier ages.

Dominance at the family level changed under different nutritional conditions (Figure 2). A significant lower correlation across ages was observed under improved nutrition for ACP1 families compared to ACP2 families. On the other hand both families showed high correlations under no fertilization. Interestingly, only ACP fast growing families showed significant interactions under improved nutrition.

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Age-Five Results from the Cooperative Forest Genetics Research Program Slash Pine Polymix Trials

D. A. Huber[1], T. L. White[2] and G. L. Powell[3]

Abstract:--The Cooperative Forest Genetics Research Program (CFGRP) has completed polymix test installation for the second generation of slash pine (White, *et al.* 1993).The objective of this testing is to evaluate the general combining ability of advanced generation slash pine selections for rust resistance and individual tree volume. The eight tests comprising Polymix Series I were established in the winter of 1997-1998 and contain 139 polymix families. The field design for this test series was randomized complete block with twenty blocks of single-tree plots per location. Great care was taken in selection and silvicultural treatment of these sites. The resulting age-five single-tree heritabilities for rust resistance and individual tree volume were 0.26 and 0.30, respectively. These heritabilities are from twice to three times those realized from first generation tests at the same age. The differences between heritabilities across generations result from better silvicultural treatment which provided a more uniform environment and faster growth, and from a better field design which positioned the offspring more efficiently across the environment. Increases in the heritability of rust resistance are most probably due to the uniform environment for growth. The more uniform environment also affected the difference between filler and non-filler microsites making the types of microsite appear less different than was previously assumed.

Keywords: Heritability, rust resistance, volume, slash pine

INTRODUCTION

As part of a complementary mating and testing structure for the second cycle of slash pine, the CFGRP installed two series of polymix tests intended to evaluate the general combining ability of 359 advanced generation selections (van Buijtenen and Lowe 1979, White *et al.* 1993). The breeding population for the second cycle was divided into two superlines with approximately 30 elite selections and with 12 breeding groups per superline. Each breeding group, which contained about 40 individuals, was divided according to rust resistance and volume breeding values into three strata, classes. Class I contained the top one-third of the parents, while Class II was intermediate, and Class III was the bottom one-third composed primarily of infusions. inclusion of an individual in the polymix breeding was based on the following criteria: (1) Elite selections; (2) Class I forward selections; (3) Class I backward selections tested at fewer than six locations; (4) Class II backward selections tested at fewer than two locations; (5) Class II forward selections whose parents were, on average, tested at fewer than two locations; and (6) Poorly tested parents present in seed orchards (White *et al.* 1993).

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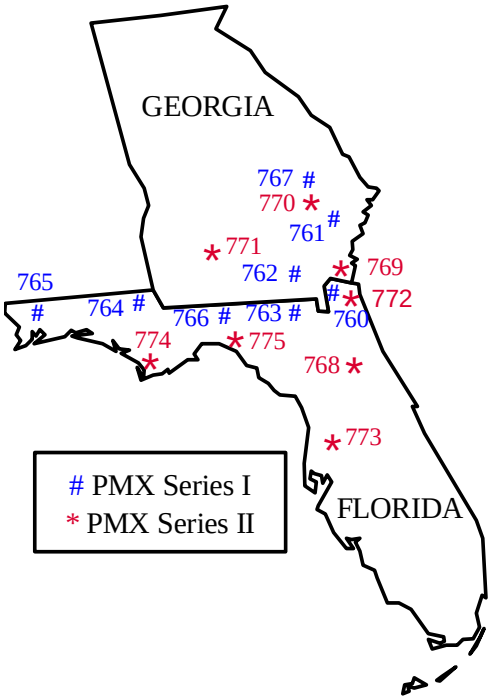

Figure 1. Locations of slash pine PMX tests in Series I and Series II, eight tests apiece.

Test sites were intended to be environments where slash pine would be planted operationally, The were reasonably uniform and were to be treated in a manner which enhanced seedling growth and site uniformity through reduced weed competition, nutrient amendment and soil preparation. The specific weed competition goals were no weed competition on the beds for the first three years and no woody competition in the test until the measurement schedule was completed. The general guidelines for fertilizer application were phosphorus application at planting, if needed, and a balanced fertilization at age three.

Analyses of the age-five data from the eight locations of Polymix Series I are reported here (Figure 1). The analyses were intended to (1) rank parents and predict breeding values for rust resistance and volume, (2) quantify the effect of stem-galled trees on the analysis of volume, and (3) quantify the effect of filler microsite on growth, rust resistance and survival.

MATERIAL AND METHODS

Seedlings were grown in greenhouses by two cooperators, Rayonier and International Paper, during the summer of 1997. The growers along with the cooperators for whom the seedlings were being grown randomized the seedlings into planting order during mid-summer. Seedlings completed the growing season with the growers. From December of 1997 through February of 1998, eight tests were planted as one each by the following cooperators: Florida Division of Forestry, Foley Timber and Land, Plum Creek, Georgia Forestry Commission, International Paper, Smurfit-Stone, Rayonier and Packaging Corporation. All sites were well-tilled and weed-free. Tillage ranged from disking on old-field sites to single, double or triple bedding on wet sites. Soil types varied from a sand with a deep spodic horizon to a sandy clay loam.

The nominal spacing was six feet along the bed and 10 feet between beds. Family identification was maintained for all planting positions, although some positions were designated as fillers because of an atypical microsite. There was no specific limitation on the number of planting spots designated as filler within a block. Sites were mapped by row and column as well as block and tree within block.

Weeds were primarily spot treated along the beds after planting and were controlled by mowing or directed spray between the beds. Fertilization was applied during the third growing season in the form of 500 kg of 10-10-10 with micro-elements per hectare.

Polymix Series I was measured in the fall of 2002 after the fifth growing season. The variables measured were height (m), diameter at breast height (cm) and status. Within the status code are levels indicating rust infected or healthy and, if infected, the extent of the infection (branch or stem). Volume in dm^3 was calculated from height and diameter as:

> Volume=$0.25\pi(10dbh)^2(1.4+.33((height/10)-1.4))$, which considers the stem a cylinder up to breast height and a cone thereafter (Lambeth *et al.* 1983).

Two traits were considered in the analyses, Volume and Rust (infected or not infected). For most volume analyses rust infected trees with stem galls were removed. Tests 762 and 765 were discarded from the rust analyses because of low incidence of the disease.

Analyses were run by site and then across sites for both traits with filler positions removed. Two additional analyses were performed: (1) stem rusted tree were included in the volume analyses and the results compared to those from volume with removal of stem rusted trees; and (2) the effect of filler microsite was assessed for height, survival, volume and rust by indicating filler and non-filler positions as a treatment. Mixed model analyses considering tests and block as fixed effects and family and its interactions as random effects were used throughout.

RESULTS AND DISCUSSION

Single Site Analyses

Least-square means for survival, volume and rust are given by test in Table 1. The age 5 survivals were excellent ranging from 84 to 97%. Test means for volume and rust proved more variable with values from 8.2 to 28.9 dm^3 for volume per tree and 6 to 61% for rust incidence. Single-site heritabilities (Table 1) for volume were all quite good for age 5 data and ranged from 0.16 to 0.62. Among sites where the rust infection level was greater than 20%, the single-site heritabilities ranged from 0.16 to 0.41 on the binary scale. There is little doubt that the PMX I Series is a vast improvement in test quality over first-generation slash pine progeny tests. The mean volume across the eight PMX I tests is 19.3 dm^3. This is the size equivalent in average volume of first-generation tests

between 7 and 8 years of age. The average single-site heritability for volume at age 5 is 0.37 compared to 0.11 for first generation tests (White *et al.* 1996). These results indicate the increased precision from the attention to detail implementing and caring for these tests.

Across Site Analyses

The enhancement in heritability is even more evident in the across-site analyses (Table 2). The across-site heritabilities for volume per tree without stem-galled trees were almost identical for the standardized and the non-standardized analyses (0.30), and the type B genetic correlations were 0.85 and 0.81, respectively. In the analysis of volume with stem-galled tree included, the heritability dropped to 0.22 and the type B genetic correlation dropped to 0.69. The across-site heritability estimate of 0.3 for volume per tree is almost three times that of average first-generation tests at any age (White *et al.* 1996). The removal of stem-galled trees from analyses raised the across-site heritability from 0.22 to 0.30 and the type B genetic correlation from 0.69 to 0.81. The heritability with stem-galled trees is three times that of the equivalent first- generation values.

Test	Survival %	Volume dm^3	h^2_b Volume	Rust	h^2_b Rust
760	93.1	15.3	0.29	44.6	0.31
761	90.4	16.4	0.16	28.8	0.27
762	97.0	21.8	0.38	6.0	0.03
763	84.1	16.7	0.47	47.7	0.38
764	88.0	20.4	0.23	60.6	0.36
765	97.3	25.8	0.49	8.2	0.04
766	88.7	28.9	0.62	49.5	0.41
767	91.8	8.2	0.30	23.5	0.16
Average	91.3	19.3	0.37	33.6	0.24

Table 1. Least-square means and single-site heritabilities for survival, volume per tree (dm^3) and rust by test for age 5 data from slash pine PMX Series I. Rusted trees with stem galls were removed from volume calculations.

Analysis	h^2_i	r_B
Non-standardized, no stem galls	0.30	0.81
Standardized, no stem galls	0.30	0.85
Non-standardized, stem galls	0.22	0.69

Table 2. Individual tree heritabilities (h^2_i) and type B genetic correlations (r_B) from three across-site analyses for volume (dm^3) in the eight slash pine PMX Series I trials. The three analyses are non-standardized and standardized volume without stem-galled tree and non-standardized volume with stem-galled trees. For standardized volume, the volume of each tree was divided by the volume phenotypic standard deviation for that site.

Trait	Filler	Non-filler
Height (m)	5.5	5.6
Volume (dm^3)	18.7*	19.3
Survival (%)	87.01	91.44
Rust Incidence (%)	30.09	37.82

*significant at α=0.05.

Table 3. Least-square means and significance levels for analyses of the effect of filler microsite versus non-filler microsite adjusted for genetic entry for height, volume, survival and rust incidence across the eight sites of PMX Series I. Site by microsite type interaction was never significant.

So, for across-site heritability for volume, better testing techniques tripled the first generation heritability and the elimination of stem-galled trees caused the value to quadruple. The primary reason that the removal of stem-galled trees raised the type B correlation and thus the heritability is that differential infection rates for families across sites were caused by different rust resistance levels for the parents and different rust hazards for sites. These differential rust infection levels for families affected the average growth rate for the family by site causing apparent genotype-by-environment interaction for growth rate. When stem-galled trees were removed from the analysis, GxE for growth rate virtually disappeared. The increases in heritability for this test series also apply to fusiform rust incidence. All of these single-site rust heritabilities are more than twice the average values reported by rust incidence level for the first-generation slash pine tests (White *et al.* 1996).

Filler Microsite Analyses

In the analysis for the effect of filler microsites, four variables were analyzed: height, volume, survival and rust incidence. Of these four variables only volume was significantly affected by filler microsite, Table 3. The trends for the effect of filler microsite were generally in the direction expected. When adjusted for genetic entry, the trees in filler microsites were shorter (5.5 vs 5.6 m), had lower survival (87% vs 91%) and less volume per tree (18.7 vs 19.3 dm^3) than non-filler microsite trees. The effect of filler versus non-filler microsites was only statistically significant for volume. While the filler effect apparently exists, the magnitudes of the differences in growth and survival between microsite types is not sufficient to discourage implementation of spacial statistical designs for which the designation of planting spots as fillers is extremely problematic.

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The Use of Site Factor Studies in Determining Environmental Variables That May be Important for Genotype by Environment Interaction

Arnulf Kanzler[1], Gary R. Hodge[2] and Lee Allen[2]

Abstract

The environmental variables important in determining genotype x environment interaction with *Pinus patula* in Southern Africa were investigated using a 'site factor study' approach. Several previous such studies with *P. patula* in the region have identified elevation, rainfall, aspect and some soil characteristics as important for the growth of the species as a whole. The objective of this study was to utilise the large number of progeny trials of *P. patula* available to further refine those variables that may be important in determining genotype x environment interaction. Top height was estimated utilising a minimum of 30 trees from the genetic checks present within each of 59 progeny trials planted across the region. A Site Index at base age 15 was derived for each site from tests aged between 4 and 10 years of age. This derived Site Index was then utilised as the dependent variable in a site factor study. Linear regression and correlation analysis was used to screen a large number of physical and climatic factors, as well as combinations of these, and relate them to tree growth as estimated by Site Index. Although the amount of variation explained by any single, or combination of variables was relatively small, several climatic factors were identified as being important in predicting some of the variation in Site Index. Many of the variables identified were associated with water availability and in particular specific seasons where water related issues could be constraining or impacting tree growth. Some of examples of these were factors related to precipitation and evaporative demand during the early spring growing season such as the October median precipitation and September evaporative demand. At this time of the year early growth may be constrained by a lack of available water after the winter dry season. In addition, many of these variables have been linked to genotype x environment interaction with *P. patula* in Southern Africa.

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An Evaluation of Height as an Early Selection Criterion for Volume and Predictor of Site Index Gain in the Western Gulf

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Abstract: -- Data from repeated periodic measures of height, diameter and volume from eleven loblolly pine progeny tests maintained as part of the Western Gulf Forest Tree Improvement Program (WGFTIP) were analyzed to 1) determine the potential of using early height, diameter, or volume as selection criteria for rotation-age volume, and 2) to develop a method of expressing height performance as predicted change in site index. Using family means, few differences in family mean correlations existed between 5-year traits with volume at 15 or 20 years, but they were slightly higher for volume than the other two traits. Gain efficiency estimates for all three traits at age 5 were similar, suggesting that the traits were equally efficient in predicting rotation volume. However, at age ten, per-acre volume was the better predictor of per-acre volume at later ages.
Predicted gains in breeding value for height, expressed as percent change in site index (SIBV), were estimated following published WGFTIP methodology for volume. Age-specific site index equations and coefficient of genetic prediction (CGP) estimates for height were developed using the 15-year data from the eleven progeny tests. Estimates of CGP for height at ages 5 and 10 with height at age 15 were 0.55 and 0.61, respectively. Correlation between parental breeding values for volume production and site index breeding values were high (r=0.80). Predicted genetic gain for site index provides additional information for decision-making. Uses and limitations of this gain information are demonstrated.

Keywords: early selection, height, site index gain, loblolly pine (*Pinus taeda* L.)

INTRODUCTION

Early selection is critical to the long-term efficiency of any tree improvement program. Early selection for volume at rotation may be based on any number of growth traits, including height, diameter, or volume. Early tree height has been recommended as the selection criterion for loblolly pine (*Pinus taeda* L.) volume at rotation age (McKeand 1988, Balocchi and others 1993.) Height has been shown to be a good predictor of rotation age volume and is less affected by competition than diameter (Foster 1986, Lambeth and others 1983), and has a higher heritability than diameter (Bridgwater and others 1983, Foster 1986). Furthermore, height is more correlated with tree-form traits in *P. radiata* (Burdon and others 1992) and wood density in *P. taeda* (Bridgwater and others 1983) than diameter.

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Li and others (1996), however, found that average family mean age-to-age genetic correlations showed that juvenile DBH and volume were significantly better predictors than height for 20-year volume.

The Western Gulf Forest Tree Improvement Cooperative (WGFTIP) has always used early per-acre volume as its primary selection criterion to support the breeding objective of improvement in per-acre volume at rotation. The practice of ranking and selecting parents based on volume performance is based on early program research which showed that the composite trait, volume per unit area, was as good a predictor of rotation-age volume as a selection index composed of height, diameter or survival. Use of the composite trait also allows performance differences to be examined that may result from differences in survival and/or growth rates. Family height performance expressed as a function of site index, however, would be more useful for input into growth and yield models.

This study was conducted to 1) determine the potential of using early measures of height, diameter or volume as selection criteria for rotation-age volume in loblolly pine in the western gulf region, and 2) to develop a method of assessing height performance as a predicted change in site index. The objectives of the early selection portion of this work are 1) to estimate heritabilities of early selection criteria (height, DBH and volume at ages five and ten), 2) to estimate heritabilities for the breeding goal (volume at age 15 and 20), 3) to estimate family mean correlations between selection criteria and the breeding goal and 4) to evaluate the implications of these parameters on the current selection criteria used for loblolly pine in the Western Gulf Forest Tree Improvement Program. The objectives of the height performance portion of the study were to 1) develop age-dependent site index equations using data for loblolly pine in the western gulf region and 2), to develop an expression for height gain information as a function of site index.

MATERIAL AND METHODS

1. Early selection

The data come from eleven progeny tests established and maintained by four WGFTIP members, International Paper Company (IPCo), Plum Creek Timber Company (PlCrk), Potlatch Corporation (PC) and Weyerhaeuser Company (Weyco). For all tests differences among families were statistically significant for height, diameter and volume at 5, 10, 15 and 20 years. Three of the tests have data through age 20, while the remaining eight have data through age 15. The tests have varying numbers of half-and full-sib families comprised of first-generation parents and were established at various spacings and may have been thinned prior to the age 20 measurements. Most tests are located in southern Arkansas (Table 1).

Test	Owner[1/]	Location (County,State)	# Parents/ Families	# Reps	# Trees per Plot	Spacing (feet)	5	10	15	20
065	PlCrk	Ashley, AR	12 / 47	10	10	8 x 8	√	√	√T[2/]	√
357	Weyco	Sevier, AR	12 / 34	12	5	8 x 10	√	√	√	
412	IPCo	Grant, LA	7 / 24	6	6	8 x 10	√	√	√	√
420	IPCo	Columbia, AR	9 / 44	6	6	10 x 10	√	√	√	√
422	IPCo	Columbia, AR	8 / 29	6	6	10 x 10	√	√	√	
458	IPCo	Ouachita, AR	8 / 29	6	6	10 x 10	√	√	√	
498	PC	Bradley, AR	8 / 20	12	6	8 x 8	√	√	√T	
501	PC	Bradley, AR	8 / 20	12	6	8 x 8	√	√	√T	
502	PC	Bradley, AR	8 / 20	12	6	8 x 8	√	√	√T	
518	PC	Cleveland,AR	8 / 19	12	6	8 x 8	√	√	√	
536	IPCo	Lafayette, AR	12 / 27	8	6	6 x 8	√	√	√	

[1/] see text for full owner name [2/]T=thinned

Table 1. Details of progeny tests used in this study.

Volumes at ages 5 and 10 were calculated using the standard formula for conic volumes with data for height in meters and diameter at breast height in centimeters. Volumes at ages 15 and 20 received a standard adjustment for taper change. Volumes at age 5 are expressed in cubic decimeters per planted tree. Volumes at older ages are expressed as cubic meters per-hectare-per-year. In thinned tests, volume removed during thinning is added back prior to calculating final volume production.

All tests were analyzed using SAS PROC GLM (SAS Institute 1990). Means separation was provided by Duncan's Multiple range test. The statistical model used for all analyses was:

$$\gamma_{ijk} = \mu + R_j + F_k + e_{jkl}$$

where: γ_{ijk} = overall mean of the ith tree in the jth replicate and the kth family;
R_j = fixed effect of the jth replicate;
F_k = random effect of the kth family, and
e_{jkl} = error.

Family mean variance components were estimated using the VARCOMP procedure in SAS. Total phenotypic variance (σ^2_T) was estimated as: $\sigma^2_T = \sigma^2_F + \sigma^2_E$

Family-mean heritabilities were estimated as: $h^2_f = \sigma^2_F / [\sigma^2_F + \sigma^2_E/k]$

where: k=mean for number of trees per plot.

Family-mean correlations were calculated as correlation coefficients. Gain efficiency was estimated assuming equal intensities of selection between mature and young ages, using family means as described in Falconer and Mackay (1996):

$$E_{gen} = h^{-1}_{fj} * r_F * h^{-1}_{fm}$$

where:
E_{gen} = gain efficiency per generation,
r_F = family-mean correlation between the juvenile and mature trait,
and h^{-1}_{fj}, h^{-1}_{fm} = square roots of family-mean heritabilities at early and mature ages, respectively.

2. Site index prediction

The eight progeny tests with measurement data through age 15 were used in this portion of the study. Coefficient of genetic prediction (CGP) estimates were obtained between juvenile and mature height using the following formula (Baradat 1976):

$$CGP_{JM} = Cov_A(J,M) / \sigma_{PJ} * \sigma_{PM,}$$

where: $Cov_A(J,M)$ = additive covariance between the juvenile and mature traits,
and $\sigma_{PJ,}$ σ_{PM} = phenotypic standard deviation at juvenile and mature ages.

Gain for height was determined using the methodology of Lowe and van Buijtenen (1991), whereby substitution, gain in site index (base age 25) can be expressed as:

$$Gain_{SI25\backslash J} = i_j * CGP * \sigma_{SI25.}$$

If gain is expressed as a deviation from 100 (i.e., mean value = 100), then predicted breeding value for site index can be expressed as:

$$PBV_{SI25\backslash J} = ((SI25 + i_j * CGP * \sigma_{SI25})/SI25) * 100.$$

where: SI25 = average site index (base age 25),
i_j = family deviation from the mean height in standard deviations or the standardized selection differential,
CGP = coefficient for genetic prediction for height between measurement age and age 15,
and σ_{SI25} = standard deviation about SI25 for family performance

Site index (base age 25) was estimated using equations specific to the western gulf coastal plain (Popham and others 1979) and the more general southwide equations (Golden and others 1981). The performance of the two equations was evaluated using data from several 25-year-old WGFTIP tests. The site index equations by Golden and others (1981) over-estimated the observed site index while those by Popham and others (1979) under-estimated the observed site index. It was decided that the site index equations by Popham and others (1979) would be used in this study because they made conservative estimates of site index at 25 years. Predicted site index for every measurement age was calculated for each test.

RESULTS AND DISCUSSION

1. Early selection

a. Heritability estimates.

Family-mean heritability estimates for height, diameter and volume ranged from 0.28 to 0.88 (Table 2). Age 5 heritability estimates were highest for height, followed by those for volume and diameter, which were identical. Age 10 heritability estimates were highest for diameter, followed by volume and then by height, which was the lowest. Family-mean heritability estimates for age-15 volume ranged from 0.45 to 0.88. Estimates for age-20 volume ranged from 0.49 to 0.81. Mean heritability estimates for all traits were highest at age 10.

Test	Ht05	DBH05	VOL05	Ht10	DBH10	VOL10	VOL15	VOL20
065	0.73	0.71	0.64	0.75	0.85	0.83	0.88	0.81
357	0.58	0.58	0.49	0.62	0.53	0.57	0.61	
412	0.66	0.54	0.58	0.60	0.66	0.57	0.73	0.78
420	0.54	0.57	0.57	0.70	0.76	0.70	0.70	0.49
422	0.51	0.38	0.39	0.63	0.58	0.60	0.61	
458	0.47	0.60	0.58	0.68	0.83	0.77	0.86	
498	0.63	0.61	0.65	0.70	0.87	0.83	0.78	
501	0.77	0.62	0.65	0.75	0.73	0.70	0.70	
502	0.79	0.75	0.78	0.76	0.82	0.84	0.78	
518	0.78	0.76	0.71	0.28	0.62	0.67	0.51	
536	0.79	0.73	0.79	0.82	0.80	0.79	0.53	
Mean	0.66	0.62	0.62	0.66	0.73	0.71	0.70	0.69

Table 2. Family-mean heritability estimates for height (Ht), Diameter (DBH) and volume (VOL) at each of the measurement ages for each test.

b. Family mean correlations.

Volume at five years had the highest correlation with volume at 15 years in eight out of eleven tests and on average was higher than the average correlations for either height or

diameter (Table 3a). Correlations of 10-year traits with 15-year volumes were highest for volume followed by diameter and then height.

(a)

Test	Ht05	DBH05	VOL05	Ht10	DBH10	VOL10
065	0.72	0.70	0.69	0.73	0.84	0.94
357	0.57	0.53	0.61	0.52	0.70	0.84
412	0.67	0.66	0.69	0.65	0.71	0.92
420	0.38	0.44	0.49	0.50	0.77	0.91
422	0.70	0.72	0.68	0.63	0.78	0.91
458	0.47	0.52	0.55	0.56	0.81	0.87
498	0.37	0.36	0.50	0.38	0.51	0.75
501	0.50	0.51	0.52	0.53	0.64	0.74
502	0.65	0.65	0.68	0.64	0.77	0.85
518	0.51	0.53	0.54	0.52	0.65	0.78
536	0.45	0.45	0.45	0.58	0.58	0.66
Mean	0.54	0.55	0.58	0.57	0.70	0.83

(b)

Test	Ht05	DBH05	VOL05	Ht10	DBH10	VOL10
065	0.64	0.61	0.60	0.65	0.79	0.87
412	0.67	0.65	0.69	0.65	0.70	0.89
420	0.30	0.36	0.41	0.44	0.72	0.84
Mean	0.53	0.54	0.57	0.58	0.73	0.87

Table 3. Family-mean correlations of height (Ht), diameter (DBH) and volume (VOL) at ages 5 and 10 with age15 (a) and age 20 volume (b).

Family-mean correlations of volume at 20 years with age 5 traits were not consistent across the three 20-year-old tests (Table 3b). Five-year volumes were highly correlated with 20-year volumes in tests 065 and 412, but had low correlation in test 420. Correlations in test 420 were low at age five for all traits. At age 10 the trend of the family mean correlations for the three traits was consistent across tests, with the exception of height in test 420.

For volume at age 15, across tests, no trait had consistently high efficiency of selection at 5 years (Table 4a). Both height and volume had the highest efficiencies in five out of eleven tests, although volume, on average, had a higher efficiency of selection at age 5 for 15-year volume than the other two traits. Volume at ten years had the highest efficiency for volume at 15 years in all tests. Efficiencies for selecting on 10-year traits were highest for volume when selecting for 15- or 20-year volume.

Efficiencies for selecting at age 5 for 20-year volumes were fairly similar for the three traits (Table 4b). Early selection for height at 5 years was an efficient predictor of volume at 20 years in tests 065 and 412, but not in 420 (Table 4b). Early selection for volume at 10 years was confirmed to be a superior predictor of volume at 20 years.

(a)

Test	Ht05	DBH05	VOL05	Ht10	DBH10	VOL10
065	0.66	0.63	0.59	0.67	0.83	0.91
357	0.56	0.53	0.55	0.23	0.65	0.81
412	0.64	0.57	0.61	0.59	0.68	0.81
420	0.34	0.39	0.44	0.50	0.80	0.90
422	0.64	0.57	0.54	0.64	0.76	0.90
458	0.34	0.43	0.45	0.50	0.79	0.83
498	0.33	0.31	0.45	0.36	0.53	0.77
501	0.53	0.48	0.50	0.55	0.65	0.74
502	0.65	0.64	0.68	0.63	0.80	0.88
518	0.63	0.65	0.65	0.39	0.72	0.89
536	0.55	0.53	0.55	0.71	0.70	0.81
Mean	0.53	0.52	0.55	0.52	0.72	0.84

(b)

Test	Ht05	DBH05	VOL05	Ht10	DBH10	VOL10
065	0.61	0.58	0.54	0.63	0.81	0.88
412	0.61	0.54	0.59	0.67	0.83	0.91
420	0.31	0.39	0.40	0.52	0.89	1.01
Mean	0.51	0.50	0.51	0.57	0.78	0.88

Table 4. Selection efficiencies at ages 5 and 10 compared to selection for volume at ages 15 (a) and 20 (b).

2. Site index prediction.

Coefficient of genetic prediction estimates for height for this dataset ranged from 0.22 to 0.90 (Table 5). Mean values, though comparing across different rotation ages, were similar those reported for volume by Lowe and van Buijtenen (1991).

Site index predictions for each measurement age in each test calculated from the equations of Popham and others (1979) are given in Table 6. Fifteen-year heights were the oldest measurement available and, therefore, assumed to provide the most accurate predictor if SI25. Compared to this standard, five-year heights poorly predicted site index. Ten-year heights predicted site index fairly well. Site index estimated from the 15-year measurements were used to derive predictive equations (Table 7).

The age-dependent site index equations and CGP estimates for height were incorporated into all loblolly progeny tests analyses programs and site index breeding values developed for all parents and families in tests measured in 2002-2003. In addition the oldest data from nearly every active WGFTIP progeny test was analyzed and site index breeding values (SIBV) calculated. For illustration SIBVs for the WGFTIP unimproved checklots were used as input into a growth and yield model (Harrison and Borders 1996) as implemented on the Texas Forest Service Timberland Stand Management website

(http://tfsfrd.tamu.edu/tdss/models/tis.asp). Predicted harvest volumes were calculated for a cutover, site prepared loblolly pine site of base site index

Test	Ht05-Ht15	Ht10-Ht15	Ht15-Ht15
065	0.69	0.73	0.82
357	0.57	0.62	0.66
412	0.62	0.67	0.77
420	0.48	0.42	0.59
422	0.56	0.67	0.82
458	0.43	0.77	0.90
536	0.72	0.76	0.61
498	0.33	0.53	0.55
501	0.64	0.69	0.62
502	0.66	0.62	0.70
518	0.31	0.22	0.30
Mean	0.55	0.61	0.67

Table 5. Coefficient of genetic prediction (CGP) estimates for height by progeny test.

60 established with 600 trees per acre and managed unthinned for 20 years. Percent change in volumes predicted for the unimproved checklots were calculated and compared to their volume breeding values. Results are presented in Table 8.

					Predicted SI25		
Test	N	Ht05	Ht10	Ht15	Age 05	Age 10	Age 15
065	461	2.24	7.55	13.11	7.60	14.39	17.37
357	359	3.54	8.58	13.15	11.94	16.36	17.44
412	114	2.95	7.31	10.47	9.28	14.20	13.86
420	197	3.64	10.30	14.68	12.39	19.67	19.44
422	150	3.48	9.81	15.39	11.74	18.74	20.38
458	150	3.93	10.36	13.43	13.32	19.78	17.79
498	190	5.13	12.33	16.66	17.48	23.32	21.97
501	192	5.11	12.49	16.92	17.35	23.86	22.41
502	190	4.81	12.31	16.68	16.38	23.35	21.99
518	179	4.89	10.34	14.53	16.63	19.66	19.20
536	216	3.44	8.95	12.34	11.68	17.10	16.34

Table 6. Height means (in meters) and predicted site index (base 25) using age 5, 10 and 15 mean heights.

Age	Regression	R^2
5	SI25 = 10.50 + 2.15 * HT05	0.58
10	SI25 = 5.62 + 1.33 * HT10	0.83
15	SI25 = -0.15 + 1.31 * HT15	1.00

Table 7. Site index predictive equations and coefficients of determination (R^2) developed from age 15 WGFTIP progeny test data.

Unimproved Checklot	No. of Obs.	SIBV (%)	No. of Obs.	BV Vol (%)	Predicted Vol (%)
NE TX	22	96.8	39	91.5	93.0
E TX	65	97.6	127	93.4	94.9
SE TX	31	99.0	46	97.4	97.9
N LA	31	95.2	37	90.1	89.7
LIV PAR	24	99.0	28	98.1	97.9
AR/OK	95	97.2	144	93.9	93.9
S MS	32	97.3	34	92.0	94.1
N MS	12	96.9	12	95.3	93.2

Table 8. Breeding values for volume (BV Vol) and site index (SIBV) for unimproved checks in WGFTIP tests and predicted volume breeding values derived from growth and yield model based on changes in SIBV.

CONCLUSIONS

1.Early Selection

Using family mean data, gain efficiency estimates for height, diameter and volume at age 5 were similar when selecting either age 15 or age 20 volumes, suggesting that the traits were equally efficient in predicting rotation volume. At age ten, per-acre volume was the better predictor of per-acre volume at later ages. These results support the current WGFTIP practice of ranking parents based on per-acre volume.

2. Site Index Predictions

Changes in harvest volume predicted from SIBV matched very closely with actual volume breeding values for each lot. On average the harvest volume breeding values based on changes in SIBV tended to be slightly higher than the actual values. This is due to the fact that predictions based on changes in SIBV are based on height alone compared

to the actual breeding values for volume, which factor in survival. For WGFTIP parents with SIBVs, correlation between volume and SIBVs were high (r=0.80).

Predicting genetic gain for site index using early heights is now operational. The additional information provided with site index breeding values can assist in selection and deployment. Take for example a case where two families have identical volume breeding values but different site index breeding values. The family with the higher SIBV is likely to have obtained this result with larger, but fewer individuals and should be favored for deployment on better sites. The family with an equal volume breeding value but a lower SIBV has better survival and should be favored on more difficult sites.

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Introgression between *Pinus taeda* L. and *Pinus echinata* Mill.

Jiwang Chen[1], C. G. Tauer[1], Guihua Bai[2], and Yinghua Huang[1]

Key words: *cp*DNA; introgression; microsatellite; nuclear ribosomal DNA; loblolly pine; shortleaf pine

INTRODUCTION

Loblolly pine (*Pinus taeda* L.) and shortleaf pine (*Pinus echinata* Mill.) have widely overlapping geographic ranges. Hybridization between the two species has interested tree breeders for a long time. Morphologically, the two pine species are different. The needles of loblolly pine are 6 to 9 inches long, usually with three yellow-green needles per fascicle; but shortleaf pine needles are 3 to 5 inches long, with two or three dark yellow-green slender and flexible needles per fascicle. Loblolly pine also has larger cones than shortleaf, as well as other differences, however, these characters offer limited help when the genotypes of the parents and their probable hybrids are compounded by environmental factors The limitations of morphological characters resulted in the identification of the allozyme marker IDH (*isocitrate dehydrogenase*) to identify hybrids (Huneycutt and Askew, 1989). The high frequency of IDH variation seen in natural shortleaf pine populations outside the natural range of loblolly pine (Rajiv *et al.*, 1997) suggests either profuse hybridization between the two species or that IDH is an unreliable marker. These data required us to look for new markers to confirm the identity of putative hybrids.

A more extensive study (relative to the study of Rajiv *et al.*, 1997), sampling a larger portion of shortleaf-loblolly pine sympatric population, was conducted to further explore the nature and extent of these hybrids in the native populations. We combined morphological traits, the allozyme marker (IDH), a codominant DNA nuclear marker, a paternally-inherited chloroplast DNA marker and SSR markers to explore natural hybridization between the two species.

MATERIALS AND METHODS

The sample population was defined as the pine stands of Montgomery County, Arkansas. Five trees in each of sixteen stands were sampled on a southeast to northwest transect across the county. The southeast stands are mixed loblolly and shortleaf pine, while the northwest stands are only shortleaf pine. Rajiv *et al*. (1997) showed that about sixteen percent of the individuals within a population near Mt. Ida are hybrids. Mt. Ida is the approximate central point of the transect we sampled, and a few miles north of any known stands of loblolly pine.

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In addition to this study population, parents of one controlled cross, shortleaf pine (Z15, seed parent) x loblolly pine (#631, pollen parent), and 20 artificially produced F1 hybrids from this cross were used to confirm the utility of our codominant DNA marker developed from the nuclear ribosomal internal transcribed spacer region.

All samples were measured for the number of needles per fascicle, needle length, fascicle sheath length and cone length. The mean values and the standard deviations of these traits for the eighty trees were calculated. Since these eighty trees were ultimately assigned into four groups of unequal size, a pseudo-t test was used to test for differences among them.

Total DNA was extracted from needles using the CTAB protocol. PCR was used to amplify the nuclear ITS-1 region. Agarose gel electrophoresis (2.0%) and ethidum bromide staining were used to reveal PCR-RFLP bands. PCR-RFLP analysis was also used to amplify the chloroplast *rbcL* region.

The IDH allozyme marker reported by Huneycutt and Askew (1989) to identify shortleaf loblolly pine hybrids was assayed for all of the individuals in the population identified as hybrids.

Eleven highly polymorphic genomic microsatellite markers were selected from http://forestry.tamu.edu/genetics/microsatellite_primers.html for use in this study. Microsatellite loci were selected based on their molecular size. Allele frequencies were determined by direct manual count.

Based on the morphological data and the PCR-RFLP analysis of ribosomal DNA ITS-1 marker, the eighty individuals in the study population were placed in four groups: pure shortleaf pine, pure loblolly pine, hybrids morphologically similar to loblolly pine and hybrids morphologically similar to shortleaf pine. All SSR data were then combined as four groups within one population and the genetic distance was calculated between the four groups. The relationship between groups was depicted by a dendrogram obtained from Nei's (1978) unbiased genetic distance using UPGMA (the unweighted pair group method with arithmatic mean). Genetic differentiation was estimated by Fst (Slatkin and Barton, 1989).

RESULTS

Mean values of the morphological data for the 80 trees as placed into four groups are shown in Table 1. The pseudo-t tests comparing each possible paring of the groups showed that the morphological data clearly distinguishes loblolly pine from shortleaf pine. Loblolly pine has longer needles, cones and fascicle sheaths, and essentially 3 needles per fascicle while shortleaf has an average of 2.3. These data also distinguish the pure species from the hybrids that are morphologically similar to the other parent, but do not allow identification of those hybrids morphologically similar to themselves. Since all the hybrids identified from the natural population are morphologically either similar to

shortleaf pine or loblolly pine, they could be easily misclassified as pure species without utilizing molecular marker data.

Trait	Mean value (standard deviation)			
	Group[a] (sample size)			
	L (16)	HL (2)	HS (8)	S (54)
Number of needles/fascicle	3.0 (0.17)	3.0 (0.00)	2.4 (0.3)	2.31(0.08)
Needle length (cm)	17.96 (5.23)	19.54 (0.28)	10.75(0.90)	10.17(6.64)
Cone length (cm)	6.94(4.53)	6.22(0.20)	4.84(0.6)	4.28(0.60)
Fascicle sheath length (mm)	1.92 (0.00)	1.91(0.18)	1.45(0.25)	1.30(0.50)

Table 1. Mean values for morphological characters of 80 samples from a natural mixed population of shortleaf and loblolly pine.

[a] Abbreviations: L; loblolly pine, HL; the putative hybrids morphologically similar to loblolly pine, HS; the putative hybrids morphologically similar to shortleaf pine, S; shortleaf pine.

The nuclear DNA internal transcribed spacer region produced a polymorphic pattern between the parental species. The artificial hybrids showed codominant restriction site patterns concordant with the patterns of the parental species. The diagnostic nuclear ribosomal DNA marker was used to screen the 80samples in the natural population, and ten hybrids were identified. Among the ten hybrids, two were morphologically similar to loblolly pine and the others were morphologically similar to shortleaf pine. The HindIII-digested PCR amplified *rbc*L chloroplast DNA fragment produced polymorphic patterns which showed two different patterns, two putative hybrids, morphologically similar to loblolly pine, showed the loblolly pine pattern, while the other putative hybrids, morphologically similar to shortleaf pine, showed the shortleaf pine pattern.

Since our data indicated that the hybrids identified with nuclear markers are morphologically similar to one parent or the other, and not intermediate as expected for an F1, these hybrids are most probably later generation backcrosses or intercrosses. As such, one would expect segregation at the IDH locus, resulting in some of these later generation hybrids being homozygous for one parent or the other at the IDH locus. Consequently, the IDH locus appears to be reliable in identifying hybrids of these two pine species, but that reliability does not extend to later generation hybrids. The same would be true for the nuclear marker we developed, but these markers used in combination should allow the identification of most of the naturally occurring hybrids between loblolly pine and shortleaf pine.

Genetic identity between loblolly pine and the loblolly-like hybrids was 0.9370 and 0.9742 between shortleaf pine and the shortleaf-like hybrids. Based on Nei's (1978) genetic distance, the phenetic relationship among the four groups was drawn. This

dendrogram indicates that the loblolly-like hybrids share one clade with loblolly pine, while the shortleaf-like hybrids share another clade with shortleaf pine.

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Development of Differential Screening Panels for Slash Pine-Fusiform Rust Reaction Types

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Abstract

Understanding the genetic basis for disease resistance is crucial to ensure a successful breeding and deployment strategy for slash pine. A complementary genetic interaction model has been hypothesized for the pine-fusiform rust pathosystem. In such a model, resistance is due to a major gene effect that confers resistance to some strains of pathogens, but not others. If this model for the pine-fusiform rust pathosystem is correct, it will be necessary to identify the genes for resistance in order to develop successful strategies. Some resistance genes are dispersed throughout the genome and can be identified by genetic mapping. However, resistance genes may be clustered in the host and it may be possible to identify them only by empirically determining their differential interaction with different pathogen strains. In the latter case, it will be necessary to develop a panel of slash pine parents that can be used to screen for presence/absence of avirulence among collections of the pathogen and a panel of pathogen strains that can be used to screen for presence/absence of resistance genes in the host. The feasibility of developing such screening panels was tested by evaluating 43 slash pine (*Pinus elliottii*, Engelm. var *elliottii*) parents with 8 single-urediniospore cultures of *Cronartium quercuum* f. sp. *fusiforme*. The slash pine parents were selected to include a range of resistance levels based on their performance in an earlier screening trial. Each parent was control-pollinated with a pollen mix collected from 10 trees known to be highly susceptible to bulk inocula. The 8 inocula were derived from eight single-urediniospore rust cultures obtained from galls from across the southeastern USA. Examination of the reaction data under the assumption of a gene-for-gene host:pathogen system allowed us to infer the presence of at least 8 putative genotypes for resistance in the host and pathogenicity in the fungus, suggesting that screening panels can be developed with current propagation technologies.

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Time Trend and Genetic Difference of Rust Infection in a Diallel Loblolly Pine Population Across Four Tests

Hua Li, Henry Amerson and Bailian Li [1]

Abstract

Loblolly pines from a six-parent half diallel mating were planted in a randomized complete block field design at four test sites. Fusiform rust infection (gall presence / absence) was recorded annually through age 8. A Bayesian model with logistic regression was used to estimate genetic parameters and variance components for the half-diallel cross design with binary data. The percentage of rust infection on each site was low in the first two years, and then increased dramatically at age 3 with little change in percentage galled thereafter. Large genetics differences among parents were found for rust infection. The rank of infection for the 6 parents was consistent over time and across sites even though infection rates were significantly different among test sites. No significant genotype by environmental interaction was found. A time trend showed that additive genetic variance increased from age 3 to 4 and remained stable through age 8. For genetic control in rust infection among families, the parental general combining ability (GCA) due to additive genetic effect was much more important than specific combining ability (SCA) of full-sib combination due to non-additive genetic effect. Results supported selection for rust infection at age 6 or earlier.

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On the Origin of Fusiform Rust Resistance in Loblolly Pine

R. C. Schmidtling, C. D. Nelson and T. Kubisiak[1]

Abstract

Studies of geographic variation in loblolly pine (*Pinus taeda* L.) have shown that seed sources from the western (generally west of the Mississippi River) and the northeastern part of the natural distribution are relatively resistant to fusiform rust, and those from elsewhere are susceptible. The greatest incidence of infection, on the other hand, is in the center of the distribution, exactly where the frequency of resistant genotypes is low. One might expect that the frequency of resistant genotypes would be higher where the disease is more prevalent, due to natural selection. It has been proposed that (1) fusiform rust resistance in loblolly pine in the west originates from hybridization with shortleaf pine. It is well known that shortleaf is resistant to fusiform rust, and it is also known that natural hybrids between the two species exist, and they seem to be more common in the west. (2) In the northeastern loblolly, it has been proposed that hybridization with pond pine is the source of resistance. Once again, natural hybridization between loblolly and pond pine is known to exist in the northeast, but not much is known about the relative resistance of pond pine to fusiform rust. Allozyme data was used to refute hypothesis (1) and cortical monoterpene data was used to refute hypothesis (2). A hypothesis is proposed involving selection during the Pleistocene to explain the present pattern of resistance and the development of a gene-for-gene pathosystem.

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The Efficacy of Breeding for Brown Spot Disease Resistance in Longleaf Pine

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Abstract:-- The study objective was to determine whether selection for brown spot disease (caused by *Scirrhia acicola* (Dearn.) Siggers) resistance in longleaf pine (*Pinus palustris* Mill.) is beneficial for areas where brown spot is not present. Two groups of selections, comprising those that performed (survival and growth) well in the presence of brown spot disease and those that performed well in its absence, were selected. These selections were made in tests planted on the Harrison Experimental Forest (HEF) in southeast Mississippi. Within selection groups, the selections were mated in a partial diallel and their progeny were planted in replicated tests on two sites at the HEF. At one site, all trees were sprayed with a fungicide to protect the trees from brown spot disease, while at the other site no protection was provided. Brown spot infection was assessed one year after planting, and survival and height were assessed at years 1, 2, 3, 4, and 7. Overall, survival was significantly lower and disease incidence higher at the unsprayed site. At 7 years, survival at the unsprayed site was 73% for families selected in the presence of brown spot and 59% for the families selected in the absence of brown spot. Brown spot infection was significantly lower in the families selected in the presence of brown spot when planted at the unsprayed site, indicating that selection for brown spot resistance was effective. At 7 years, families selected in the presence of brown spot were significantly taller at the unsprayed site, but were significantly shorter at the sprayed site. Thus, selection for brown spot resistance is beneficial for those areas where brown spot disease is present, but not for areas where brown spot is controlled or absent.

Keywords: Brown spot needle blight, disease resistance, *Pinus palustris*.

INTRODUCTION

Despite longleaf pine (*Pinus palustris* Mill.) being more resistant to all the major insects and diseases that affect other southern pines, its wide planting and regeneration is limited by brown spot disease caused by *Scirrhia acicola* (Dearn.) Siggers. Brown spot attacks seedlings in the nursery and young seedlings in the field during the grass stage, causing mortality, delaying the initiation of rapid growth and generally leading to reduced growth at maturity (Boyer 1990). Infected seedlings grow slowly and may remain in the grass stage for 10 years or more, while non-infected seedlings remain in the grass stage for only one or two years (Boyer 1990; Phelps and others 1978). Once out of the grass stage, longleaf pine is no longer susceptible to brown-spot and has many desirable traits such as excellent stem form and good wood qualities that make it a highly valued species.

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Brown spot can be controlled by proper silvicultural practices, but breeding for disease resistance offers a more permanent solution and may be economical in controlling the disease. Genetic improvement will not only improve resistance to brown spot but also increase survival and growth (Snyder and others 1977). The limited information on breeding for brown spot resistance in longleaf pine suggests that selection for brown spot resistance can be effective. For example, Snyder and Allen (1968) found that provenances differed in resistance to brown spot disease, and Byram and Lowe (1985) and Snyder and Derr (1972) found high heritability estimates for brown spot disease resistance. The study objective was to determine if selection for resistance is beneficial for those areas where brown spot is not present.

MATERIALS AND METHODS

Treatments

Two groups of tress that performed well for survival and height growth were selected in tests planted on the Harrison Experimental Forest (HEF) in southeast Mississippi. Family plus within-family selection was applied to obtain (1) nine unrelated trees that performed well in the absence of brown spot disease and (2) nine unrelated trees that performed well in the presence of brown spot. The first group was selected in tests sprayed with a fungicide to protect the trees from brown spot disease while the second group was selected in unsprayed tests. Both groups were selected from tests on the HEF; however the second group included family information for performance (survival and height) in tests in Alexandria, Louisiana as well as the HEF (see Lott and others 2001 and Synder and Kais unpublished[1] for details).

Each of the nine trees was control pollinated with from one to four different trees (partial diallel) within the two selection groups giving a total of 18 full-sib families per group. The set of 18 families within each group is hereafter referred to as a treatment, where treatment 1 (T1) is families whose parents were selected in the absence of brown spot disease and treatment 2 (T2) is families whose parents were selected in the presence of brown spot. Two open-pollinated families (one known to be resistant and one susceptible to brown spot) were added to each treatment at each site to serve as controls. The susceptible control was similar to the material commonly used for planting in southeast Mississippi.

Field tests

Seeds were sown in a greenhouse at the HEF in 1982. Two field tests were established in 1984 on the HEF. At one site (S1) all trees were sprayed with Bordeaux fungicide according to label to protect the trees from brown spot disease while at the other site (S2)

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[1] Study Plan FS-SO-4153(1401)-3.45. Control pollinating to determine efficient methods of breeding longleaf pine for brown-spot resistance. On file at the Southern Institute of Forest Genetics, Saucier, MS.

no protection against brown spot was provided. The field test design was a randomized complete block using 24 replications of single-tree plots at each site. Seedlings were evaluated for brown spot infection in the field one year after planting. Brown spot infection was scored visually for each seedling as a proportion of total needle tissue showing signs of brown spot disease. Survival and total height were assessed at ages 1, 2, 3, 4 and 7 years after planting.

Analyses

To test the significance of site, treatment and family, and their interactions, data were pooled across sites and analysed using the general linear model (GLM) procedure of SAS version 6.03 (SAS Institute, Inc. 1985). The following linear model was used for the analyses:

$$Y_{ijklm} = \mu + S_i + R_{j(i)} + T_k + ST_{ik} + F_{l(k)} + SF_{il} + \varepsilon_{ijklm} \qquad [1]$$

where:

Y_{ijklm} = is the observation on the m^{th} tree in the i^{th} site in the j^{th} replicate in the k^{th} treatment and l^{th} family,
μ = overall mean,
S_i = random effect of the i^{th} site,
$R_{j(i)}$ = random effect of the j^{th} replicate within site,
T_k = random effect of the k^{th} treatment,
$F_{l(k)}$ = random effect of the l^{th} family within treatment,
ST_{ik} = random interaction effect of the site and treatment,
SF_{il} = random interaction effect of the site and family, and
ε_{ijklm} = residual.

All means reported are least squares means (SAS Institute, Inc. 1985) adjusted for missing values. Survival data were converted to 0,1 (dead, alive) scale prior to analysis. To determine if early assessments were good predictors of later assessments and to determine relationships between different traits family mean correlations were estimated as product-moment correlations using PROC CORR procedure in SAS (SAS Institute, Inc. 1985).

RESULTS

Survival

Sites, treatments and families differed significantly in survival at all ages ($P < 0.05$), except sites at ages 3 and 4 years, and treatments at age 7 (Table 1). Interactions between site and treatment, and site and family were only significant at ages older than 3 years.

Survival at age 1 year was high (> 80%) at both sites. Survival progressively declined with time at the unsprayed site (S2). At 7 years, survival declined to 66% at the

unsprayed site, but was unchanged at the sprayed site (S1), suggesting that spraying against brown spot improves survival especially when considering older ages.
At 7 years, survival at the unsprayed site was 73% for families selected in the presence of brown spot and 59% for the families selected in the absence of brown spot (S2-T2 vs. S2-T1, Figure 1). This represents a 24% improvement in survival due to both selecting parents and planting their progeny in the presence of brown spot disease. In contrast, families derived from parents selected in the absence of brown spot had better survival in the sprayed test, supporting the significant site x treatment interaction. The advantage of planting resistant families in unsprayed test was quite apparent at age 7 years (Figure 1).

Brown spot infection

Significant differences ($P < 0.05$) were observed for brown spot infection among sites, treatments and families at age 1 year (Table 1). Brown spot infections were much lower at the sprayed site (S1; 2%) than the unsprayed site (S2; 43%), indicating that fungicide treatment offers effective protection against brown spot disease. The absolute levels of brown spot infections at the unsprayed site were higher in families whose parents were not specifically selected for brown spot resistance (T1; 51%) than in families whose parents were selected for resistance (T2; 34%) (Figure 2). The realized gain in disease resistance at this site is 41%, indicating that breeding for resistance to brown spot disease is effective. Realized gain in resistance estimated at 4 years using data from a previous study (Lott and others 2001) was in close agreement with findings in this study (realized gain was 38% at Alexandria and 47% at the HEF).

Height growth

Sites, treatments and families differed significantly in height growth at all ages ($P < 0.05$), except for treatment at ages 2, 3, and 7 years (Table 1). Interactions between site and treatment, and site and family were significant at all ages ($P < 0.05$). Trees planted at the sprayed site (S1) grew better than those at the unsprayed site (S2) at ages older than 1 year. At 7 years of age, trees at the sprayed site averaged 6 meters in height while those at the unsprayed site were only 3.8 meters in height.

At 7 years, families from parents selected in the presence of brown spot (T2) were significantly taller at the unsprayed site (S2), but were significantly shorter at the sprayed site (S1) than families selected in the absence of brown spot (T1) (Figure 3). At 7 years of age, families selected in the presence of brown spot averaged 4.4 meters in height while those selected in the absence of brown spot were 3.4 meters in height at the unsprayed site, indicating a realized gain of 29.4%. In contrast, at the sprayed site, families selected in the presence of brown spot averaged 5.6 meters in height while those selected in the absence of brown spot were 6.4 meters in height at 7 years. This indicates that there is a 12.5% cost for breeding for resistance if planting on a site where brown spot is not present. Thus, selection for brown spot resistance is beneficial for those areas where brown spot disease is present, but not for areas where brown spot is controlled or absent. Our findings support those of Snyder and Derr (1972) and Snyder and Bey (1978) who reported a poor relationship between family height growth on sprayed and

unsprayed sites. The realized gain in height (57%) was lower than that predicted using data from a previous study (178%) reported by Lott and others (2001).

Age	Source of variance	DF	Survival	HT ($x10^{-2}$)	BS ($x10^{-3}$)
1 year	Site (S)	1	1.69***	33.36***	542.88***
	Rep (R)	46	0.23***	0.49**	0.48*
	Treatment (T)	1	1.67***	5.41***	25.4***
	Family (F(T))	35	0.35***	1.01***	2.23***
	S*T	1	0.23ns	17.75***	24.27***
	S*F(T)	35	0.10ns	0.58**	1.71***
	Residual	1447	0.11	0.27	0.36
2 years	Site (S)	1	0.89**	224.06***	-
	Rep (R)	46	0.27***	15.50**	-
	Treatment (T)	1	2.14***	1.12ns	-
	Family (F(T))	35	0.33***	39.43***	-
	S*T	1	0.13ns	552.53***	-
	S*F(T)	35	0.11ns	20.60***	-
	Residual	1447	0.12	7.92	-
3 years	Site (S)	1	0.28ns	65.58***	-
	Rep (R)	46	0.30***	27.79**	-
	Treatment (T)	1	1.45***	0.43ns	-
	Family (F(T))	35	0.37***	58.19***	-
	S* T	1	0.25ns	20.49***	-
	S*F(T)	35	0.15ns	40.34***	-
	Residual	1447	0.13	0.33	-
4 years	Site (S)	1	0.12ns	238.61***	-
	Rep (R)	46	0.39***	1.38**	-
	Treatment (T)	1	1.44**	6.44**	-
	Family (F(T))	35	0.38***	4.31***	-
	S* T	1	0.57*	80.58***	-
	S*F(T)	35	0.21*	3.04***	-
	Residual	1447	0.14	0.86	-
7 years	Site (S)	1	5.19***	925.84***	-
	Rep (R)	46	0.33**	4.54**	-
	Treatment (T)	1	0.17ns	4.67ns	-
	Family (F(T))	35	0.48***	16.89***	-
	S* T	1	4.90***	166.11***	-
	S*F(T)	35	0.36***	12.82***	-
	Residual	1447	0.17	2.82	-

Table 1. Mean squares for analysis of variance for survival (0,1 scale), brown spot (% infected needles), height (HT, cm for ages 1 and 2 and m for ages 3, 4 and 7) assessed at two sites on the Harrison Experimental Forest. Note: ns, *, **, *** = not significant at $P < 0.05$, significant at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

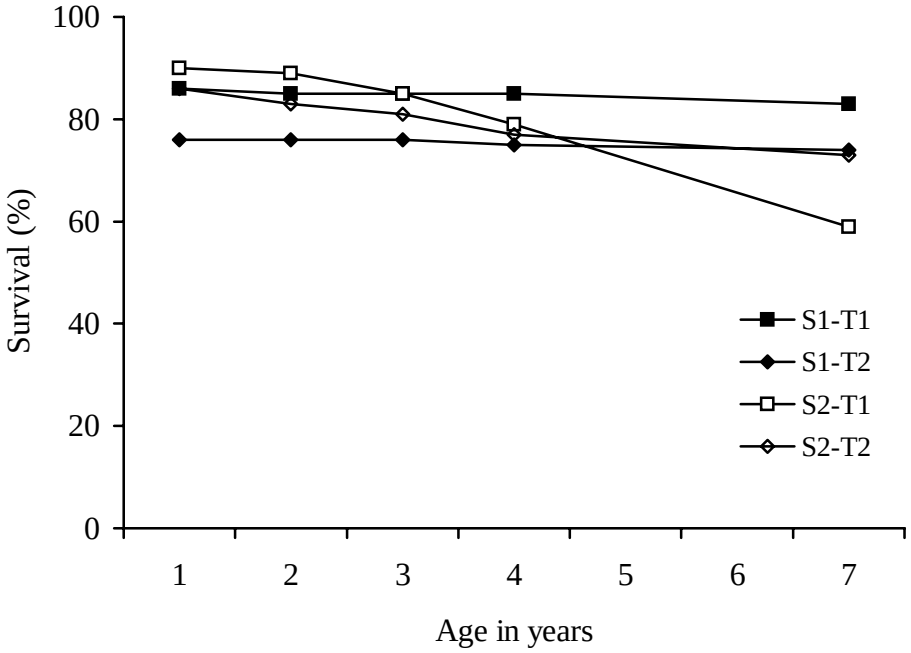

Figure 1. Average survival of two treatments (parents selected in sprayed tests T1 or unsprayed tests T2) at two sites (sprayed test S1 and unsprayed test S2).

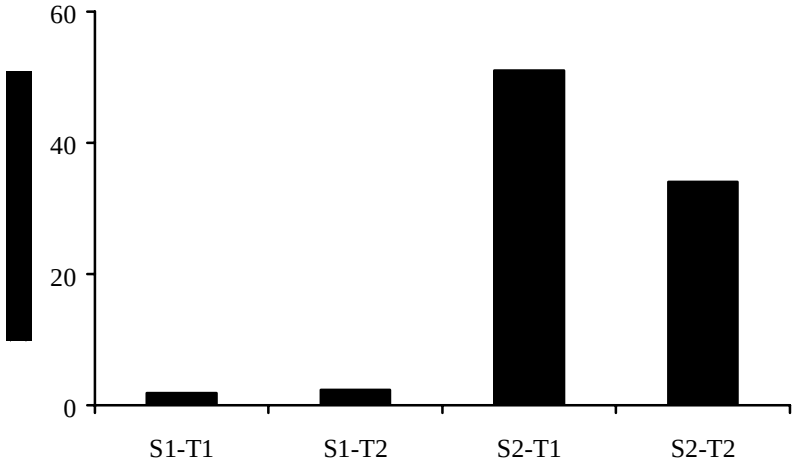

Figure 2. Brown spot infection of two treatments (parents selected in sprayed tests T1 or unsprayed tests T2) at two sites (sprayed S1 or unsprayed S2).

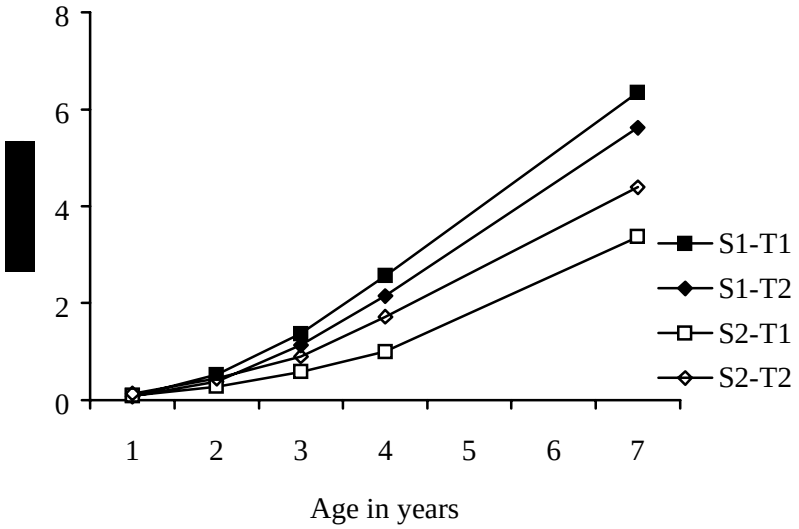

Figure 3. Height growth of two treatments (parents selected in sprayed tests T1 or unsprayed tests T2) at two sites (sprayed S1 or unsprayed S2).

Family mean correlations

Survival assessments at different ages were moderate to highly correlated at the phenotypic level ($r_p \geq 0.68$) (Table 3), indicating that the families that had good survival at the early ages had good survival in the field at older ages. Brown spot infection at one year and survival were negatively correlated, and as age difference between brown spot and survival assessments increased so did the negative correlation.

Height assessments at ages 2 years and older were also highly correlated at the phenotypic level ($r_p \geq 0.69$) (Table 4), but correlation between ages 1 and 7 year heights was low ($r_p = 0.34$) (Table 4). Thus, early assessments of height at ages greater than 1 year may be good indicators of height at older ages. Our results are consistent with those of Lott and others (2001) who found a high correlation between 2 and 10-year heights ($r_p = 0.76$).

	Surv1*	Surv2	Surv3	Surv4	Surv7
Surv2	0.95				
Surv3	0.90	0.95			
Surv4	0.82	0.86	0.90		
Surv7	0.68	0.72	0.74	0.79	
BS1	-0.07	-0.15	-0.25	-0.33	-0.58

Table 3. Phenotypic correlations between survival and brown spot infection *Surv = survival, BS = brown spot, HT = height, number is age in years.

	HT1*	HT2	HT3	HT4	HT7
HT2	0.69				
HT3	0.53	0.91			
HT4	0.47	0.84	0.95		
HT7	0.34	0.69	0.84	0.92	
BS1	-0.08	-0.49	-0.67	-0.74	-0.79

Table 4. Phenotypic correlations between brown spot infection and height *HT = height, BS = brown spot, HT = height, number is age in years.

CONCLUSION

Survival, resistance to brown spot infection and height growth at the unsprayed site were higher in the families selected in the presence of brown spot than those selected in the absence of brown spot. Conversely, when families were planted at the fungicide-sprayed site, height growth was higher in the families selected in the absence of brown spot than those selected in its presence. Thus, selection for resistance is not beneficial for those areas where brown spot is not present, but is beneficial for those areas at which brown spot is present. For effective breeding, selections should therefore be made in a representative environment because selections made in one selection environment are not necessarily the best in an alternative environment.

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Genetic Variation in Fraser Fir Mortality Due to Phytophthora Root Rot

Kevin M. Potter[1] and John Frampton[2]

Fraser fir (*Abies fraseri* (Pursh.) Poir.), native to a small number of isolated ridgetops in North Carolina, Tennessee, and Virginia, is widely grown in the Southern Appalachians for the fresh-cut Christmas tree market. This species is the primary Christmas tree grown in North Carolina, which accounts for more than 12 percent of real Christmas trees produced in the United States and ranks second in the total number of trees harvested and first in the dollars made per tree. In 1999, for example, 1,600 North Carolina growers sold 3.7 million trees worth a reported value of more than $92 million (N.C. Department of Agriculture and Consumer Services 2003).

Phytophthora root rot presents a serious economic limitation to Christmas tree growers, with the majority of root rot damage caused by *Phytophthora cinnamomi* Rands. In 1997 and 1998, a survey of 58 Fraser fir field sites in North Carolina found an average Phytophthora root rot incidence of 9 percent, with *P. cinnamomi* accounting for 91 percent of the Phytophthora isolates recovered (Benson and Grand 2000). Phytophthora root rot in Fraser fir typically occurs in sites with poorly drained soils (Grand and Lapp 1974, Kuhlman and Hendrix 1963). Determining whether Phythophthora root rot resistance exists in Fraser fir, and then selectively breeding to increase that resistance, would be highly useful to Christmas tree growers in the Southern Appalachians. This is especially true given that Fraser fir growers have typically used unimproved and mostly unselected seed sources (Arnold *et al.* 1994).

Species that occur in disjunct subpopulations are expected to have high inter-population genetic divergence resulting from genetic drift, reduced gene flow, elevated inbreeding, and differing selection pressures experienced among isolated populations (Young *et al.* 1996). Such variation in Fraser fir natural subpopulations could be associated with varying amounts of resistance to Phytophthora. While resistance to the pathogen has not been established in a genetic field test setting, recent field tests have indicated significant genetic variability within the species for other traits, specifically growth and form characteristics (Arnold *et al.* 1994, Jett *et al.* 1993, and Li *et al.* 1988). A recent greenhouse Phytophthora inoculation of Fraser fir seedlings found slight differences among seed sources, with highest occurring in the Roan Mountain source, the lowest in the Mount Mitchell source, and an intermediate amount occurring in the Richland Balsam source (Frampton and Benson 2003, in press).

In 2000, a progeny test of three-year-old Fraser fir seedlings in Avery County, North Carolina, became infested by *P. cinnamomi*. We analyzed seedling mortality at the test

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site with three objectives: 1) quantifying the genetic variation within the six major Fraser fir subpopulations and 94 open-pollinated families in the study for potential Phytophthora root rot resistance, 2) estimating genetic parameters for root rot mortality; and 3) exploring the feasibility of using a Geographic Information System (GIS) neighborhood analysis as a tool for separating potential genetic resistance to Phytophthora from uneven exposure to the pathogen in a field setting. Specifically, neighborhood analyses generated by the GIS package ArcView (ESRI 1999) were used to generate data on the proportion of dead trees within a given distance of each tree in a field test; that information was then used as a covariate in the genetic analyses of variance.

The differences in mortality among the six sources of Fraser fir were small in the third year following root rot exposure, ranging from 38.4 percent for the Roan Mountain source to 45.8 percent for the Mount Mitchell source. A greater amount of variability existed among the 94 families, from a low of 21.4 percent mortality to a high of 62.1 percent.

We used the statistics package ASReml (Gilmour *et al*. 2002) to generate variance terms and heritability estimates for mortality, because ASReml could both logistically transform the binomial mortality data and conduct a residual maximum likelihood (REML) analysis. In our mixed linear model, seed source, source-block interaction, and family within source were considered random effects, and blocks were treated as fixed effects.

Family within source was the only variance component significantly different from zero, and accounted for about 5 percent of the variation in the mortality data. When the populations were analyzed separately, family variance was significant in only the Balsam Mountains, Mount Mitchell, and Roan Mountain sources. Across all populations, narrow-sense family mean heritability was 0.63 and individual heritability was 0.20.

The results from these analyses, and from future analyses of additional mortality at the site, will be compared to results from a greenhouse inoculation test in progress during the summer of 2003. In this test, Fraser fir from 120 families and all six seed sources are being inoculated with a single *P. cinnamomi* isolate.

Our GIS analysis generated information on the proportion of dead trees near each individual in the plot. When we used this data as a covariate term in our genetic analysis of variance, the results did not produce different variance estimates and genetic parameters than without the use of the GIS-generated neighborhood mortality data. This may indicate that the single-tree non-contiguous plot design for the field study successfully reduced differences in *Phytophthora* exposure among Fraser fir families. The neighborhood analysis approach, however, may result in more precise genetic parameter estimates in other experimental designs.

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Cooperative Second-Generation Breeding and Testing of Coastal Douglas-fir in the US Pacific Northwest

Keith J. S. Jayawickrama[1]

Abstract

Forest tree improvement got underway in the Pacific Northwest of the USA in the 1950s. Graft incompatibility became evident by the early 1960s and dampened enthusiasm for grafted clonal orchards for this species A different approach, the IFA-PNW "Progressive Tree Improvement System" was launched in 1966. The emphasis here was on forming local cooperatives to share costs, and on progeny testing large numbers of trees using wind-pollinated seed in small testing zones. This phase ran from 1967 till 1993, during which over 26,000 first-generation parents were tested in 109 breeding units (between local cooperatives, the USDI Bureau of Land Management, the USDA Forest Service and WA Department of Natural Resources), with over 3 million progeny test trees planted.
A second-generation breeding and testing strategy was developed between 1996 and 1997:

- Adjacent first-generation testing programs could merge to share genetic material for breeding and testing. Breeding population size for any merged programs should be at least 300 selections. Within a breeding population, breeding groups of 20-30 selections each would be used to manage inbreeding and create multiple populations. The top 10-20 percent of selections within a breeding population could also be assigned an elite population.
- A minimum of nine breeding groups would be needed within a given testing zone. Each new testing zone was to use all of the families from "local" breeding groups and only the most elite selections from breeding groups originating further away from the testing zone.
- Each selection would be used in at least two crosses. The committee proposed using three types of tests, each with a specific purpose and design: family-ranking/selection tests; long-term stability tests; and adaptability-screening tests.

Breeding and testing is in various stages of completion for seven different programs, which would lead to nine or 10 testing zones. The rule of thumb in choosing selections (first-generation parents, forward selections from open-pollinated progeny tests and from full-sib orchards) crossed to form the second-generation populations has been a 1 in 10 between-family selection intensity. Most selections were based on age-15 height. Where available information on stem sinuosity, forking, ramicorn branches and wood specific gravity were also considered. Between five and six tests have been established per testing zone, with 20 trees planted per family per site in single-tree plots. Trials established to date have contained from 143 to 283 full-sib crosses. All tests are fenced to protect the seedlings from browse. Tests will probably be measured twice, around seven and 12 years from seed (or when the trees are 15 and 30 feet tall respectively).

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Complementary Pairs Interaction of Resistance Genes and Avirulent Genes in Loblolly Pine: Fusiform Rust Pathosystem Using Diallel Data

Hua Li, Henry Amerson and Bailian Li[1]

Abstract

Loblolly pine (*Pinus taeda* L.) seedlings from 12 full-sib families were obtained from a 6-parent half diallel mating design. These seedlings were challenged using two inocula of the fusiforn rust fungus(*Cronartium quercuum f.sp. fusiforme*) at extremely high spore density (250,000sp/ml ~ 300,000sp/ml) with replications in the greenhouse (concentrated basidiospore spray method). Each basidiospore inoculum originated from a mixed gall collection of aeciospores obtained from field-infected trees. Presence or absence of rust galls was recorded at four and half months & nine months after inoculation and infection percentages were calculated. The rust infection rates were high for every full-sib family and were above 90% for most of full-sib families for both inocula. However, the infection in family of 28-321 by 28-301progeny was relatively lower. The analysis showed that the rust infection of 28-321 by 28-301progeny was not significant different from 75% for both inocula. Two pairs of complementary genes could explain the observed percent infection levels in this diallel based on a gene-for-gene hypothesis. The 75% infection in the full-sib family of 28-321 by 28-301 may be explained by epistasis between two resistance genes. The putative genotypes of host parents and virulence compositions of mixed inocula were postulated. A bulk-segregrant analysis based on phenotype (gall vs no gall) was used to search for dominant molecular markers associated with the potential resistance genes in the host parents. Few candidate marker polymorphisms were observed between the gall vs no gall bulks and none of the candidates co-segregated with phenotype when tested across the progeny set.

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Evaluation of an Open-Nucleus Model for Forest Tree Breeding

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Keywords: assortative mating, nucleus breeding, breeding strategy.

INTRODUCTION

"Nucleus breeding" refers to hierarchical structuring of a breeding population. The top level in the hierarchy is designated as the nucleus (elite) tier. The nucleus can be "closed" (no gene flow into nucleus) or "open" in which case there is gene flow into the nucleus from tiers of lower hierarchy (Roden 1994). The open-nucleus breeding concept was first used by sheep breeders in Australia and New Zealand in the mid 60's (del-Bosque González 1989). The genetic advantage of an open nucleus comes from the assortment of mates in a group sense (Shepherd and Kinghorn 1992). The concept was adopted into forest tree breeding (Cotterill *et al.* 1989) and has been continuously considered in tree breeding programs throughout the world.

The objective of this study was to investigate if there is any genetic advantage of the open-nucleus system compared to the individual assortment of mates (positive assortative mating) across the entire breeding population. The evaluation focus of our study was the gain and diversity in a seed orchard production population (the breeder's target).

METHODS

A stochastic simulation model was developed to represent open-nucleus breeding in a multi-generation forest tree breeding program. A breeding population of 48 founders was managed in three different ways through five cycles of breeding and selection. In the first alternative there was no hierarchical structure and the population was randomly mated (**RM**) to produce the recruitment population, serving as the source of candidates for "forward selection" of breeding parents in the next cycle breeding population. Individual positive assortment of mates (**PAM**) across the entire breeding population was conducted in the second alternative. In the third approach, the top-ranking parents in each cycle were allocated into a nucleus tier and the remaining parents to the main tier (**NB**). In this latter alternative, random mating took place within both tiers.

The recruitment population was composed of 24 families, each having 100 seedlings that were each clonally replicated by 10 ramets. Group-merit selection (Lindgren and Mullin 1997) was employed to select the next cycle's breeding population considering both gain and diversity. A production population (seed orchard) was a selected subset of the 6 top-ranking parents in the breeding population in each cycle. The alternatives were compared over 5 cycles considering seed orchard gain and diversity (group coancestry).

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It is often suggested to allocate more effort to better parents. Such an approach was evaluated by performing double-pair mating in the nucleus and single-pair mating in the main tier at a fixed level of total testing resources. The mating was either random within both tiers (**NB-RM**) or positive assortative in the nucleus and random in the main tier (**NB-PAM**). These open-nucleus strategies were compared with the individual assortment of mates in the whole breeding population with the top ranking third of parents each involved in 3 crosses, the lower thirds in 2 crosses and the poorest third in only one cross (**PAM 3-2-1**).

RESULTS

Positive assortative mating enhanced the additive genetic variance in the breeding population when the restriction on diversity was moderate to high (situations closer to balanced selection within families). Though the assortative mating did not significantly alter the additive variance in the breeding population when the diversity was not considered (strong family selection), the average population additive effect increased, as there was potential for more extreme genotypes in crosses among parents with top breeding values. Average inbreeding in the breeding population was higher with positive assortative mating than with random mating. This is because trees with similar breeding values are on average expected to be more related with each other than with other trees in the breeding population. All of these impacts of parental assortment on the breeding population structure were more pronounced in magnitude at the individual level of assortment (**PAM**) compared to assortment in a group sense (**NB**).

Individual assortment of mates (**PAM**) across the whole breeding population delivered more genetic gain in the seed orchard compared to the open-nucleus alternative (**NB**) at any target level of diversity (Figure 1). Optimum nucleus sizes varied depending on what was considered the target population, what was the desired level of diversity in that population and upon other factors. Generally, larger nucleus sizes resulted in more seed orchard gain when diversity was considered as important.

Open-nucleus breeding with more effort allocated to better parents (**NB-RM**) was also inferior to the individual assortment of mates (**PAM 3-2-1**) (Figure 2). Nucleus breeding with positive assortative mating in the nucleus and random mating in the main tier (**NB-PAM**) resulted in lower seed orchard diversity in later cycles under balanced within-family selection and was still inferior to the PAM 3-2-1 alternative.

The conclusion of this study is that when seed orchard gain and diversity are considered simultaneously, individual parental assortment across the whole breeding population can result in more gain than open-nucleus breeding. This conclusion holds for balanced distribution of testing effort and also for cases when more effort is allocated to better parents.

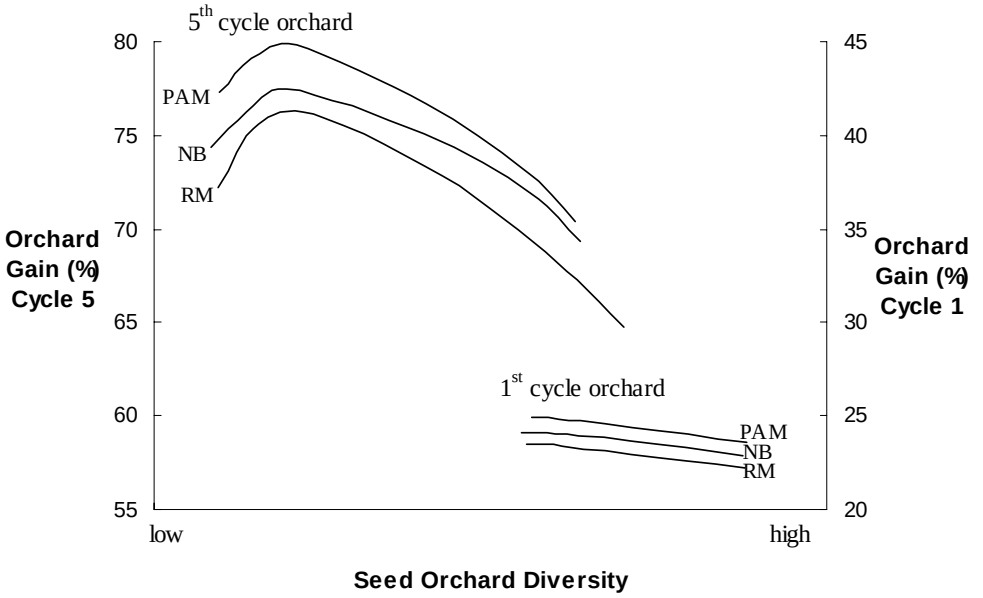

Figure 1. Genetic gain and diversity in the 1st and 5th cycle seed orchards under balanced distribution of testing effort. Alternatives are: random mating **RM**, nucleus breeding **NB**, and positive assortative mating **PAM**.

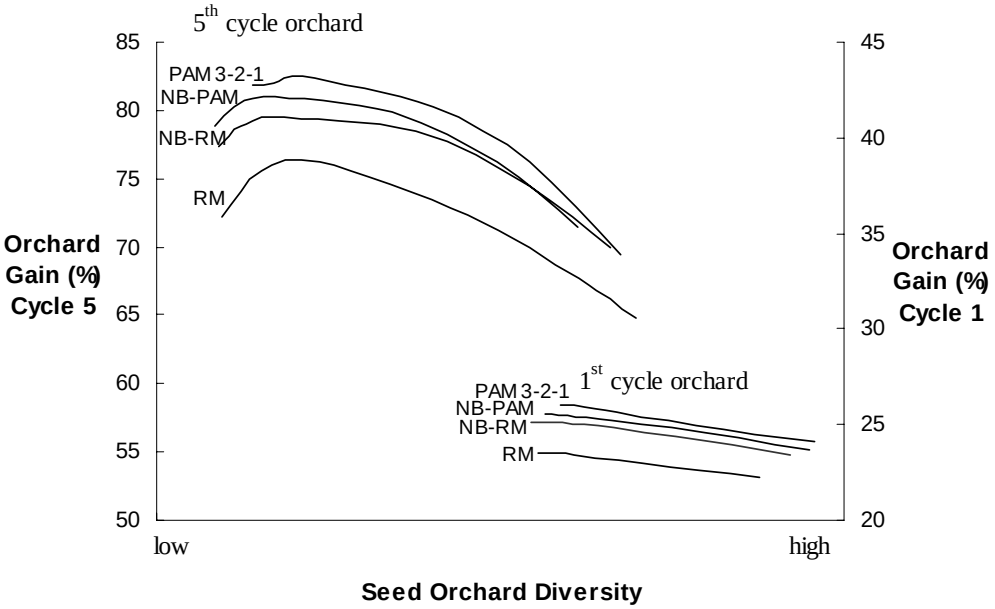

Figure 2. Genetic gain and diversity in the 1st and 5th cycle seed orchards when more resources were allocated to higher-ranking parents. Alternatives are: random mating **RM**, nucleus breeding with random mating in nucleus **NB-RM**, nucleus breeding with positive assortative mating in nucleus **NB-PAM**, and positive assortative mating **PAM 3-2-1**.

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Implementation of Third–Cycle Loblolly Pine Breeding Plan – N. C. State University-Industry Cooperative Tree Improvement Program

Bailian Li[1]

Abstract

The N. C. State University-Industry Cooperative Tree improvement Program has officially completed two cycles of loblolly pine breeding and testing program. As the third cycle selections are being completed, an efficient, cost effective, detailed breeding plan for third-cycle breeding is being finalized to ensure both the short- and long-term benefits for Cooperative members. While a general framework of the third cycle breeding plan has been suggested by a breeding and testing task force (Task Force Report 1992 and McKeand and Bridgwater 1998), only recently the detailed implementation plan has been finalized. Based on the results of research simulation and practical considerations of workload and logistical arrangements, an implementation plan for the third cycle breeding is outlined. Three general test zones will be used for the cooperative loblolly pine breeding program, i.e., 1) Northern: Virginia and northern NC, 2) Coastal: Atlantic coastal plain and Lower Gulf, and 3) Piedmont: Piedmont regions of SC and GA and Upper Gulf. A common pollen mix will be used within each zone for polycross breeding and evaluating 3rd cycle selections across sites within the zone. The mainline breeding population will be structured as sublines to manage genetic variation and inbreeding for long-term breeding. A complementary design of polycross and a modified diallel (smart diallel) will be used for mainline breeding. The polycross will be used to estimate breeding values to rank selections and followed by the smart diallel mating to provide the progeny for within-family selection. Polycross tests will be established with a single-tree plot design on different sites across the range within each zone, while smart diallel crosses will be planted in full-sib family blocks for within family selection. The best selections will be selected and bred intensively in an elite breeding population within each zone for short-term genetic gain.

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Variation in Estimation of Genetic Parameters from Small Disconnected Diallel Mating

Fikret Isik and Bailian Li[1]

Abstract

Diallel mating designs are widely used in plant improvement programs to estimate additive and dominance genetic variances, and to provide a base population for advanced selection. It is very common to limit the number of parents in a diallel small (4-8) in order to keep the number of crosses in a manageable size and complete the diallel breeding within a short time period. Many such diallels are created in a breeding program, and often are disconnected. Thus there are generally large sampling errors among diallels for genetic variance components. In this study, we examined variation in estimates of variance components and heritabilities of two loblolly pine breeding populations. Over 100 diallels (105) from the Coastal breeding population and and 114 from Piedmont breeding populations were examined for the distribution of these parameters. Frequency distribution of GCA variance and narrow-sense heritability showed an approximate normal distribution, while SCA variance showed a considerable skewness and displayed a significant departure from normality. Genetic parameter estimates fluctuated considerably from one diallel to another as expected due to the effect of the small population size. Individual heritability ranged from 0.0 to 0.62 in the Coastal breeding population and from 0.0 to 0.52 in the Piedmont population. Some theoretical considerations regarding to the optimum sample size to estimate reliable genetic parameters from diallel tests were addressed.

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Age-Age Correlations In *Pinus Taeda* In Brazil

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Keywords**:** quantitative genetics, heritability, genetic parameters

INTRODUCTION

A series of four progeny tests of *Pinus taeda* in southern Brazil in the states of Santa Catarina and Paraná was established in 1986 by Rigesa Celulose, Papel e Embalagens Ltda., and served as an excellent opportunity to examine age-age correlations.

METHODS

The genetic material used in the trials was 30 full-sib families created from two six-parent disconnected diallels. The planting design was 6 replications of 6-tree row-plots established at a 2.5 × 3 m spacing. The tests were measured nearly every year from age 1 to age 15. There were some inconsistencies from year to year: at ages 1 and 2, diameters were not measured; each test was measured for DBH at either age 5 or 6, and heights were not measured at those ages; only the Bishop test was measured at age 7. Data from each replication in each trial was standardized to a common mean and variance. The diallel mating design was written into a matrix using a SAS PROC IML routine, and this matrix was then used within SAS PROC MIXED to estimate genetic parameters. For each age and growth trait, estimates were calculated for heritability (h^2), proportion of dominance (d^2), and Type B additive genetic (r_{Bg}) and Type B dominance genetic correlations (r_{Bd}), which express genotype x environment interaction.

RESULTS

Survival in all four trials averaged above 90% at age 15 years. Growth was also quite good, with average height growth of 6 m at age 4 years, 17 m at age 10, and 24 m at age 15 years. All genetic parameter estimates were very similar for all growth traits at all ages. This made it reasonable to use parameters for one trait, e.g., diameter, to serve as proxies for another trait, e.g. volume, in examining age-age relationships. Heritability estimates for all growth traits were quite high, around 0.60. Dominance variance was moderately low relative to additive variance, which is consistent with estimates for *P. taeda* in the southeast United States (Balocchi et al. 1993). Third, there is essentially zero additive genotype x environment interaction (i.e., $r_{Bg} \approx 1.00$). Dominance genotype x environment interaction is also low.

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Heritability increased rapidly to a maximum of $h^2 = 0.71$ at age 4, and by age 7 has stabilized at $h^2 = 0.63$ which was maintained until age 15. Age-age correlations (additive) were modeled using a Lambeth relationship. The results indicated that indirect selection at age 4 was 95% as efficient as selection at age 15, and even age 3 selection was 90% as efficient as selection at age 15. For these 12 parents, the GCA ranks at age 3 were almost identical with the age 15 ranks, with the only changes being a swap of positions 4 and 5, and of positions 6 and 7. It is possible that both the high heritability estimates and the high age-age correlations may be due to a "tail-heavy" distribution of parents in this particular sample of 12 parental genotypes: For example, at age 8 the GCA estimates were 23.1, 13.4, 12.5, 9.0, 6.0, 3.4, -1.2, -3.7, -5.4, -9.3, -20.8, -26.9 (units are % gains in individual tree volume). In this case, ¼ of the parents have GCA estimates in the tails of the distribution (i.e., $|GCA| > 20$). This will inflate the additive variance and heritability estimates, and may inflate correlations. However, previous results from another large *P. taeda* data set (10 tests, 83 parents and 310 crosses) established by Igaras Papeis e Embalagens in southern Brazil (Hodge et al. 1999) also indicated high age 3-15 correlations with $r_g = 0.89$, compared to $r_g = 0.91$ in this study.

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Genetic Variation in Stone Pine Half-Sib Progenies

Ioan Blada[1]

Abstract:--Total height, annual height growth, root collar diameter, total number of branches and total number of buds around the leader bud were recorded at age 6.

The experimental material was included 136 half-sib families originating from stone pine natural populations from the Carpathian Mountains. Population samples were included in a randomized complete block experiment with four replications and ten seedlings per family per replication. Highly significant ($p<0.001$) family variation for all traits was detected. Very high family heritabilities were estimated for total height (h_f^2 =0 .968), root collar diameter (h_f^2 = 0.938) and total number of branches (h_f^2 = 0.966). Genetic correlations between total and annual height growth and root collar diameter were high or very high, ranging between 0.804 and 0.969. These correlations indicated favorable conditions for obtaining substantial genetic gain for a combination of these traits. By selecting the best 30 to 45 families, genetic gains in total height growth and diameter between 28.8 % and 23.4 % and between 18.8 % and 15.3 %, respectively, could be achieved. Suggestions for a breeding strategy are made.

Keywords: *Pinus cembra*, half-sib progeny, growth traits, genetic variance, heritability, genetic gain.

INTRODUCTION

Stone pine (*Pinus cembra* L.) is naturally distributed at high elevations of the Alps and Carpathians, including the Tatra Mountains. (Georgescu & Ionescu 1932; Beldie 1941; Critchfield & Little 1966; Sauermoser 1994; Konak 1994). The species is important from the following points of view: ecological (Holzer 1972; Frey 1994); silvicultural (Frey 1994; Holzer 1994; Blada 1996); industrial (Holzer, 1972; Contini & Lavarelo 1982); genetic (Bingham 1972; Holzer 1975; Blada 1994); landscaping, tourism and other recreation functions (Gordon 1994; Blada 1997 b)

Although stone pine is a very slow-growing species, it is of particular importance for forestry in the Carpathian subalpine zone. Due to a lack of improved planting material, a genetic improvement program including intra- and interspecific crosses was developed in Romania (Blada 1990). Improvement of growth traits was the main objective of the program.

This paper reports nursery-stage variation among 136 stone pine half-sib progenies.

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MATERIALS AND METHODS

Initial material and nursery progeny test

Open pollinated seeds were collected from 136 stone pine trees growing in several natural populations from the Carpathian and Alps Mountains. Only the availability of cones per tree was taken into account in parent selection. To reduce the likelihood of relatedness, the trees were separated by a minimum of 50 meters. In September 1991, two seeds were sown per polyethylene pot (22 cm x 18 cm) filled with spruce humus. After sowing, the seeded pots were placed in nursery beds where they were arranged in a randomized complete block design The second seedling, if present, was removed in the second year of growth. A 10-seedling row plot in each of four blocks represented each of the 136 families. As stone pine is a very slow-growing species, the seedlings were grown in the initial pots throughout the six-year nursery testing period.

Traits measured

Five traits (Table 1) were measured in the autumn of 1995 when the plants were six years old. Plot means comprised the basic data for statistical analysis.

Traits	Units	Symbols
Total height growth	cm	H.6
Annual height growth	cm	h.6
Root collar diameter	mm	RCD.6
Total branches	No.	TNB.6
Total buds around the terminal bud	No.	TNBAL.6

Table 1. Traits measured at age six.

Statistical analysis

Two-way ANOVA based on plot means was performed. The following mathematical model was applied:

$$X_{ik} = m + a_i + b_k + e_{ik} \qquad (1)$$

where: X_{ik} = plot average from the *i-th* open-pollinated family in the *k-th* replication; m = the general mean of the whole experiment; a_i = the random effect of the *i-th* half-sib progeny (i = 1,2…I); b_k = the effect of the *k-th* replication (k = 1,2…K); e_{ik} = the random error. Replications and families were considered to be random effects. Variance components of the random effects were estimated by equating mean squares to expected mean squares. Standard errors (SE) of the variance components were computed with the

formula given by Anderson & Bancroft (1952). Genetic coefficients of variation (GCV) were calculated with the formula:

$$GCV = (\sqrt{\ } \acute{o}^2_f / x)\ 100 \qquad (2)$$

where: $\acute{o}^2_f$ =the family genetic variance ; x = the population mean
Narrow-sense family heritabilities (h^2_f) were calculated as:

$$h^2_f = \acute{o}^2_f / (\acute{o}^2_f + \acute{o}^2_e / k) \qquad (3)$$

The confidence intervals (95%) were estimated for heritability by Knapp's et al. (1985) formulas. Genetic gain (ÄG) was calculated by Falconer's (1981) formula:

$$ÄG = ih^2_f \acute{o}_{Ph1} \qquad (4)$$

where: i = intensity of selection taken from Becker (1984), and $\acute{o}_{Ph1}$ = phenotypic standard deviation.

RESULTS AND DISCUSSIONS

Genetic variation

Highly significant ($p<0.001$) differences among the 136 families were found in all traits (Table 2, row 2). As a full table of family means cannot be presented here, Table 3 shows family means of 10 best and 10 poorest families for each trait, indicating the magnitude of family variation. Large variation among family means was found. For total height growth and root collar diameter, the poorest groups had averages (X_2) of 13.4 cm and 7.7 mm, respectively; while the averages of the best groups (X_1) were 29.6 cm and 13.0 mm, respectively, i. e. a difference (D_1) of 120.7 % and 69.3 %. At the same time, the difference between the two groups of families was 152.6 % in total number of branches and 120.3 % in total number of buds around the leader. Differences (D_2) between the top group (X_1) and the test mean (X) were smaller but still significant (Table 3, last line). As expected, differences among individual families were much greater. For example, at six years of age, the worst family was 11.5 cm tall, while the best family measured 35.4 cm, i.e. a difference of 204.3%. The genetic coefficients of variation for total height, annual height growth, root collar diameter and total number of branches was 22.0%, 25.8%, 14.6% and 26.3%, respectively (Table 2, last line). Thus, it has been demonstrated that stone pine half-sib families posses considerable genetic variation in the analysed traits, suggesting that selection for improvement will be effective.

Variance components

Analyses of the trait data yielded estimates of variance components and their standard errors presented in the lower part of Table 2. At age 6, the family additive genetic variance was 88% for total height growth, 80 % for annual height growth, 79 % for root

Source	DF	Mean squares of the traits				
		H.6	h.6	RCD.6	TNB.6	TNBAL.6
Replications (r)	3	5.3533	5.9100	5.3800	0.6767	0.7933
Families (f)	135	76.5636***	19.0947***	8.7253***	12.4602***	2.1853***
Error (E)	405	2.4094	1.1166	0.5438	0.4252	0.2839
Components						
$ó_f^2$ ± SE		18.5386 (88) ± 2.3131	4.4945 (80) ± 0.5771	2.0454 (79) ± 0.2637	3.0088 (88) ± 0.3764	0.4754 (63) ± 0.0662
$ó_e^2$ ± SE		2.4094 (12) ± 0.1689	1.1166 (20) ± 0.0783	0.5438 (21) ± 0.0381	0.4252 (12) ± 0.0298	0.2839 (37) ± 0.0199
$ó^2_{Ph} = ó_f^2 + ó_e^2$		20.9480	5.6111	2.5892	3.4340	0.7593
$ó^2_{Ph1} = ó_f^2 + ó_e^2/k$		19.1409	4.7737	2.1814	3.1151	0.5464
$ó_{Ph1} = ?ó^2_{Ph1}$		4.3750	2.1849	1.4770	1.7650	0.7392
GCV (%)		22.0	25.8	14.6	26.3	20.3

Table 2. ANOVA, variance components ($ó^2$) (percents in brackets), standard errors (SE) and genetic coefficient of variation (GCV) for 136 open-pollinated stone pine families.

Family[1]	H.6	h.6	RCD.6	TNB.6	TNBAL.6
1	35.0	16.3	15.6	13.0	5.6
2	34.6	15.3	13.8	11.5	5.3
3	31.8	14.8	13.6	10.7	5.1
4	30.6	13.6	13.0	10.1	4.9
5	30.4	13.6	12.7	10.0	4.9
6	28.1	13.4	12.5	10.0	4.9
7	27.6	11.7	12.4	9.7	4.8
8	27.3	11.4	12.3	9.6	4.7
9	25.7	11.1	12.2	9.6	4.7
10	25.2	11.0	12.2	9.4	4.6
X_1	29.6	13.2	13.0	10.4	4.9
127	14.2	5.5	8.0	4.3	2.5
128	14.1	5.5	8.0	4.3	2.4
129	14.0	5.4	7.9	4.3	2.4
130	13.9	5.3	7.9	4.3	2.4
131	13.7	5.1	7.9	4.2	2.3
132	13.7	4.8	7.8	4.2	2.3
133	13.5	4.6	7.6	4.1	2.3
134	13.1	4.4	7.6	4.0	2.2
135	12.7	4.2	7.2	3.8	1.9
136	11.5	4.1	7.2	3.5	1.8
X_2	13.4	4.9	7.7	4.1	2.2
X	19.6	8.2	9.8	6.6	3.5
D1(%)	120.7	170.6	69.3	152.6	120.3
D2(%)	51.3	61.8	32.9	56.5	43.3

Table 3. Ranking of the 10 best and the 10 poorest stone pine families based on nursery performance

Legend:

D1 = differences (%) between mean of the best (X_1) and the poorest (X_2) group of the 10 families;

D2 = differences (%) between mean of the best group (X_1) and the test mean (X);

[1)] The best and the poorest families were not the same for every trait.

collar diameter and 88 % for total number of branches. Therefore, a significant contribution of genetic variance was noted not only for total height but for the other traits as well.

The data suggest that additive genetic control is high in all traits. Rather than relying on a constructed F-test, Snyder & Namkoong (1978) recommended that the magnitude of a variance component be compared with its standard error. The variance component is deemed to be important if it is estimated to have a standard error less than half the magnitude of the component. In this experiment, the variance components for all traits had standard errors about seven times lower than the estimated components themselves (Table 2, line 5). This indicates that the genetic variances and heritabilities were reliable and that a selective breeding program using additive variation will be effective in improving any tested trait.

In summary, because the amount of variation was considerable an improvement program with stone pine would be practical.

Genetic and phenotypic correlations

Highly significant ($p<0.001$) phenotypic correlations were found among all but two traits (Table 4). Genetic correlations among growth characteristics, i.e. total and annual height growth and root collar diameter were high or very high ranging between 0.804 and 0.969. These correlations suggest that selection for one trait should lead to strong positive indirect responses in the others. Both total height and diameter at root collar were moderately and highly associated with the total number of branches, with genetic correlations ranging from 0.571 to 0.713. Phenotypic correlations for the same traits were highly significant ($p<0.001$) and positive. Selection for total height growth or growth in root collar diameter should lead to an indirect increase in the total number of branches, but this is a negative feature of trees because an increased number of branches means lower wood quality. Therefore, reduction in the incidence of the total number of branches in the next generation is likely to be achieved most readily by selection against this trait. Consequently, the breeder should act towards breaking this undesirable positive correlation and to select in favour of fast-growing trees with a small number of branches. Similar genetic correlations have been reported for the full-sib family test (Blada 1999).

Heritability

Table 5 presents a summary of the heritability estimates and their 95% confidence intervals for the open-pollinated families. All estimates of heritability in this study may be somewhat upwardly biased because the experiment was restricted to a single nursery where family-site interactions were not accounted for. Since the additive genetic variance of all analysed traits was high, family narrow-sense heritabilities were also high, ranging between 0.870 and 0.968. The heritability estimate for root collar diameter was 0.938 and was about the same magnitude as the corresponding estimates for growth in height. Similarly, high estimates were obtained for annual height growth, total number of branches and total number of buds around the leader bud. High heritability has also been

observed in the *P. cembra* full-sib progeny test carried out in the same nursery (Blada 1999). All estimates calculated here fell within the 95% confidence interval suggesting their reliability.

Traits	h.6	RDC.6	TNB.6	TNBAL.6
H.6	0.969 0.937***	0.881 0.830***	0.571 0.559***	0.703 0.622***
h.6		0.804 0.783***	0.422 0.432***	0.660 0.638***
RCD.6			0.713 0.674***	0.715 0.622***
TNB.6				0.354 0.333***

Table 4. Genetic correlations (upper line) and phenotypic correlations (lower line) (Df = 134). *** $p < 0.001$

Traits	h_f^2(CI)	ÄG (%) when selecting best 30,35 40 and 45 families out of 136 tested			
		30	35	40	45
H.6	0.968 (0.960 - 0.975)	28.8	26.8	25.1	23.4
h.6	0.941 (0.922 - 0.951)	33.4	31.1	29.1	27.2
RCD.6	0.938 (0.922 - 0.951)	18.8	17.6	16.4	15.3
TNB.6	0.966 (0.958 - 0.973)	34.4	32.1	30.0	28.0
TNBAL.6	0.870 (0.838 - 0.898)	25.2	23.5	22.0	20.5

Table 5. Family narrow-sense heritability estimates (h_f^2) with 95 % confidence interval (CI) and expected genetic gain (ÄG) at family level.

In conclusion, all analysed traits were under strong genetic control and thus, appeared quite amenable to genetic selection.

Expected genetic gain

Table 5 presents estimates of gain, as a percentage of the nursery test mean, which might be expected in growth and other traits after one generation of selection. If the best 30, 35,

40 or 45 of the 136 families were selected and used in a planting program, genetic gains in total height growth and diameter at root collar of 28.8%, 26.8%, 25.1% and 23.4% and 18.8%, 17.6%, 16.4% and 15.3%, respectively could be expected. Similar genetic progress can be made in the other traits tested. This genetic gain could result in substantial returns if large planting programs are developed. These results suggest that growth improvement through family selection in slow-growing stone pine is possible. Increased use of stone pine may lead to a situation where fast-growing trees become commercially valuable.

Implications for breeding

As stated earlier, stone pine is a very slow-growing species. However, according to our previous report (Blada 1999) and to the nursery test results presented in this study, growth traits of stone pine are under strong genetic control. Consequently, selection on the basis of progeny performance in the nursery test could provide substantial improvement in diameter, height and total number of branches. Improving height and diameter growth in stone pine is the main objective to be achieved. Therefore, action will concentrate on the production of improved seed for operational planting, based on the results acquired from this six-year nursery test of 136 half-sib progenies. This population was divided into two equal parts. One part is already planted in field trails to be used for estimation of genetic variation including juvenile-mature genetic correlations. The other half of the population will be used for seed orchard development according to Zobel & Talbert (1984) recommendations.

After field testing the breeding strategy will be improved according to the new estimated genetic parameters. The objective is to incorporate the early testing procedures into an operational improvement program.

Two types of production seed orchards are planned:

(i) A clonal seed orchard will be developed using as ortets the best 45 female trees; selection was based on the height and diameter performances of their open-pollinated families (Figure 1). The commercial F_1 seedlings will be planted in regions to which they are adapted, e.g., on sites relatively similar to those of the wild female parents. Taking into account our previous observations (Blada 1990, unpublished data), this clonal seed orchard will have the first seed crop about seven years after grafting.

(ii) A seedling seed orchard will be established by planting the fastest growing seedlings selected from the best 45 families out of the 136 tested, i.e., the best offspring of the trees planted in the clonal seed orchards. This is a first generation seed orchard. To maintain a large genetic base, 80 seedlings of each family will be planted at 3 x 3 m, requiring an area of 3.2 ha. The first commercial F_2 crop is expected to be available about 20 years after planting.

By planting improved material from the two types of seed orchards, significant genetic gains should be obtained. In estimating these gains, one can use the procedures indicated by Namkoong et al. (1966); and Shelbourne (1992).

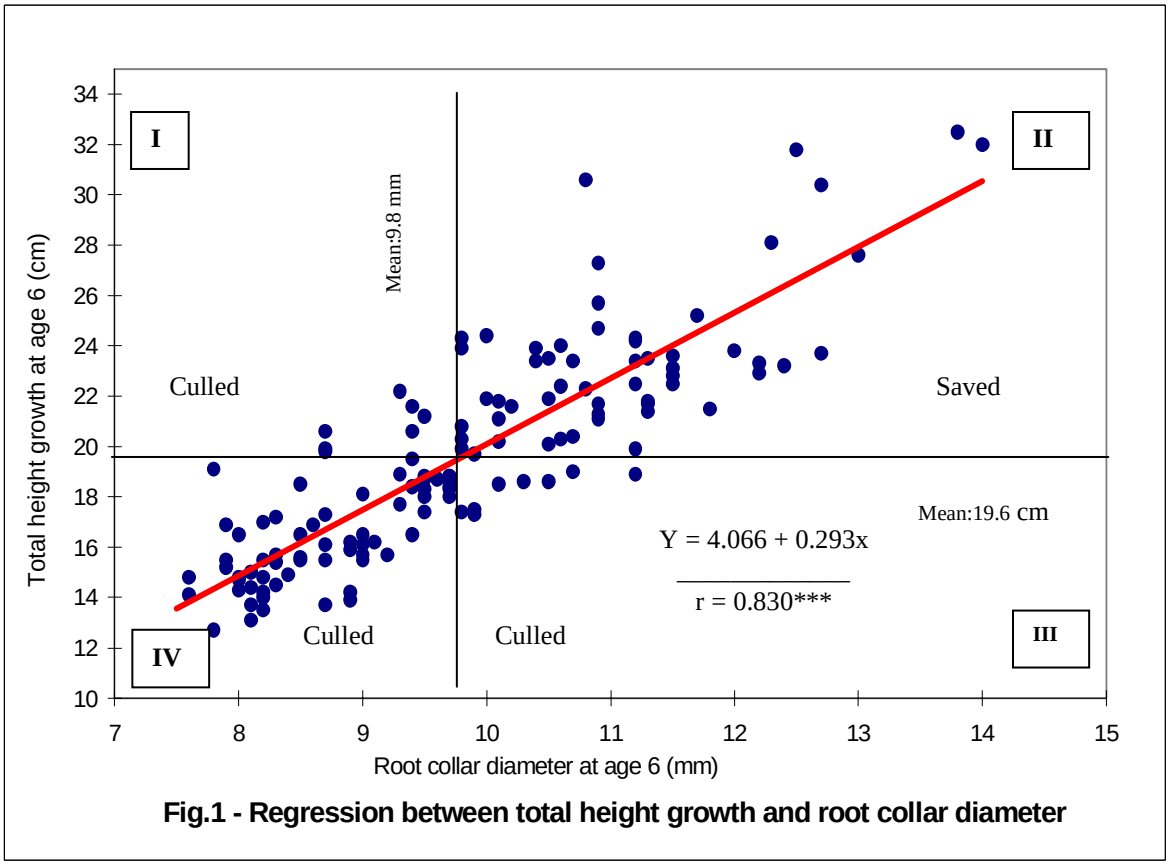

Fig.1 - Regression between total height growth and root collar diameter

If the best 45 tested progenies are used directly in operational planting, a genetic gain in total height of 23.4% could be expected (Table 5, row 1).

The provenance test (Blada 1997a) demonstrated that the improvement of growth in height and diameter by provenance selection is also possible even if the species is a very slow growing one.

It should be noted that our decision to utilize early selection to develop production seed orchards after only six years of testing was encouraged by similar work reported by others. For example, Lambeth et al. (1983) suggested that most selection in loblolly pine (*Pinus taeda* L.) is currently carried out between ages five and ten years. Also, Lowe & Van Buijtenen (1989); and Bridgwater & McKeand (1997) were in favour of early selection.

CONCLUSIONS

Although stone pine is a very slow-growing species, high genetic variation among half-sib families in growth traits and total number of branches was found.

The additive genetic variation detected in this breeding population can be incorporated into an operational program or as a base for an advanced breeding population.

Genetic and phenotypic correlations suggest that correlated responses for growth traits and total number of branches should be obtained through indirect selection.

The high variability of the material and comparatively high heritability estimates showed that consistent genetic gain in growth and total number of branches is possible.

Results of this experiment indicated that early height growth could be used as an early testing trait for stone pine; consequently, early evaluation trials will permit making crosses among the best parent trees after only a few years of testing.

By using results of this early testing, corroborated with previous results, an operational improvement program for stone pine was developed.

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Top Grafting Longleaf x Slash Pine F_1 Hybrids on Mature Longleaf and Slash Pine Interstocks

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Abstract: -- Top grafting is used to accelerate the breeding cycle of loblolly pine (*Pinus taeda* L). Scions, collected from seedlings as young as 1 year from seed, are grafted onto mature interstocks of the same or a related species. Male and female strobili production often begins 1 or 2 years after grafting, thus potentially decreasing the generation time by several years over conventional accelerated breeding methods. We are interested in applying top grafting to our breeding program involving interspecific hybrids, in particular longleaf x slash pine (*Pinus palustris* Mill. x *Pinus elliottii* Englem. var. *elliottii*) hybrids and their backcross generations. Towards this end, we grafted scions from 16 longleaf x slash pine F_1 selections onto two longleaf and two slash pine interstock trees of different genotype. The F_1 selections were 6 years old and none showed any signs of strobili development prior to, or during the experiment. A total of 100 grafts were made on to each interstock species. Scion survival after the second year was significantly higher on slash (72%) pine interstocks than on longleaf pine (18%). However there were no differences in male or female strobili production per living scion between the interstock species. Scions grew longer and produced more branches on slash pine interstocks than they did on longleaf. Given the relatively poor survival of the scions grafted onto longleaf interstocks and the reasonably good strobili production of scions grafted onto slash, we recommend using slash pine as the interstock species for top grafting longleaf x slash F_1 hybrids.

Keywords: *Pinus palustris*, *Pinus elliottii*, accelerated breeding, interspecific hybrids, grass stage

INTRODUCTION

Longleaf pine possesses many desirable qualities such as excellent bole form, high naval stores content, moderate to high wood specific gravity, and fusiform rust resistance (USDA 1965). Despite these qualities, an extended phase of juvenile development referred to as the grass stage has limited longleaf pine's use in artificial regeneration programs (Schmidtling and White 1989). The grass stage greatly increases the opportunity for brown spot needle blight infection caused by the fungus *Scirrhia acicola* (Dearn.) Siggers (1944). This disease can greatly prolong the grass stage and, if severe enough, can kill seedlings. Increased seedling mortality, when compared to the other southern pines and the unpredictability of the duration of the grass stage, make planting longleaf pine a risky investment under intensive management systems. Inter-specific hybrids of longleaf pine have shown promise for addressing the problem of delayed

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height growth, but pedigree advancement has been greatly hindered by the long juvenile period characteristic of pines.

Top grafting is a technique whereby juvenile scion material is grafted into reproductively mature trees and is being widely used to accelerate the breeding cycle of loblolly pines in seed orchards. This technique has been successfully used to promote early initiation of strobili in loblolly pine (Bramlett and Burris 1995, 1998; McKeand and Raley 2000). Gooding and others (1999) demonstrated that loblolly pine could be top grafted successfully onto loblolly or slash pine interstocks without a penalty in graft success or strobili production. It was also noted that scions grafted onto slash pine grew faster, making bagging (for control pollination) slightly easier and faster. By top grafting, advanced generation selections can be made and grafted in the spring. These grafts often produce strobili that can be pollinated the next spring, and produce cones with a good yield of sound seed in the fall of the following year, thus shortening the breeding cycle to as few as 5 or 6 years.

Our overall goal is to apply top grafting to help accelerate our breeding program involving interspecific hybrids of longleaf x slash pine and their backcross progenies. The specific objectives of this research were to: 1) determine which pine species might serve as a better source of interstock for top grafting longleaf x slash pine F_1 scion material; and 2) test for genotype differences among the F_1 trees used as a source of scion material.

MATERIALS AND METHODS

Scions were collected during the week of March 7, 2000, from 16 different longleaf pine x slash pine F_1 hybrids growing in a 6 year-old test planting located on the Harrison Experimental Forest (HEF) in southeast Mississippi. The trees were selected from five different full-sib families. Prior to and during the course of the study there was no evidence of strobili development on any of the F_1 trees. After collection, the scions were divided into two groups and kept moist and cool (4^o C) until grafting. In order to accommodate all the grafts, two slash pine trees and two longleaf pine trees (each of different genotype) located in a grafted seed orchard on the HEF were used. The interstock trees were identified as being reproductively mature based on prior observations of cone production, although the age and height of the longleaf interstocks were considerably older and taller, respectively (~30 years and 56.8 feet vs. ~15 years and 49 feet). Scion material was grafted into the upper crown of the interstock trees using a standard cleft and wax grafting method (White and others 1983). All grafting was performed by a single technician (LML) during the week of March 14, 2000. A total of 100 grafts were made onto each of the interstock species.

The following metrics were recorded at both 1 and 2 years post-grafting: scion survival, diameter of the scion approximately 5 cm above the union, length of the scion, number of first order branches, number of female strobili, number of male strobili, and the number of first year cones. Data were analyzed using the PROC MEANS and PROC GLM

procedures in SAS version 8 (SAS 1999). All significance tests were performed at $p<0.05$.

RESULTS AND DISCUSSION

After one year, there were significant differences in survival based upon interstock species with 77% of the scions grafted onto slash pine interstock alive compared to only 20% of those grafted onto longleaf pine (Figure 1). A few additional scions were lost (died and missing tags) over the course of the second year, but these additional losses were not considerable (year two survival percentages were 72% and 18%, respectively). Although we tested only two interstock trees, the specific tree of the interstock species was found to have a significant effect on graft survival. This was especially apparent for longleaf pine. Approximately 40% of the scions grafted onto one of the longleaf pine interstock trees (17 of 42 grafts) were alive after 1 year whereas only 5% of those grafted onto the other (3 of 58 grafts) survived. Differences in scion survival between the slash pine interstocks were not apparent (83% versus 70%).

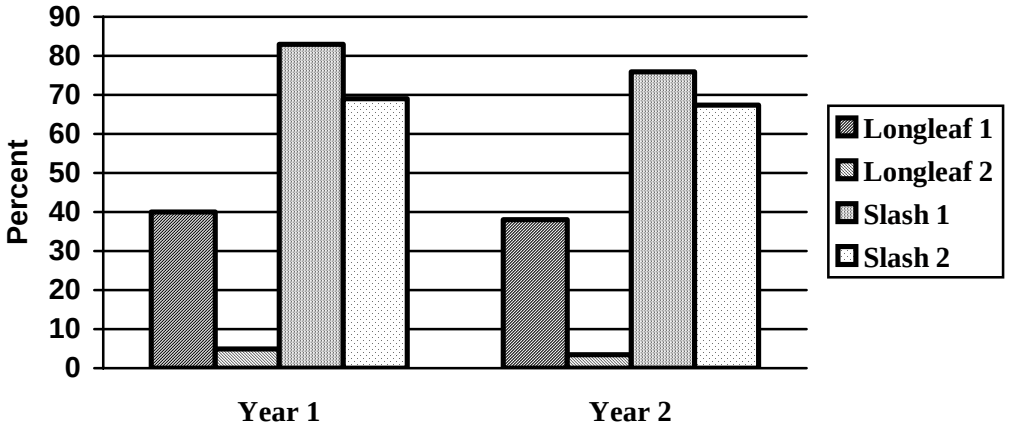

Figure 1. Percent survival of scions grafted onto four different interstocks after 1 and 2 years.

Scion material was collected from a total of 16 different interspecific F_1 trees. The number of scions collected from each F_1 tree ranged from as few as 6 to as many as 20, averaging 12.5. Differences in graft survival were observed among the F_1 genotypes from which scions were collected. The percentage of live scions per F_1 genotype after one year ranged from 0.0% to 87.5%, averaging 41.3% and is shown by interstock species in Figure 2. No significant interstock by F_1 genotype interactions were observed, however it is important to note than only 5 of 16 F_1 genotypes survived on longleaf pine interstocks while 15 of 16 survived on slash pine.

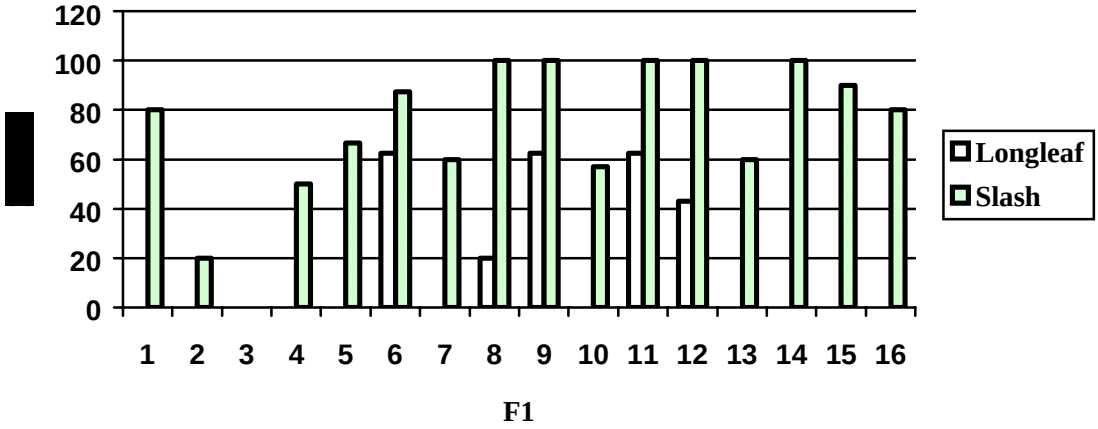

Figure 2. Percent survival after 1 year for scions collected from16 different longleaf pine x slash pine F_1 genotypes.

Of those grafts that survived the first year, the length and diameter was significantly greater for those grafted onto slash pine versus those grafted onto longleaf pine. After the first growing season, the scions on slash pine were nearly twice as long as those on longleaf pine (average 58 cm versus 31 cm, respectively). This increased growth continued through the second growing season (average 83 cm versus 42 cm, respectively). The diameter of the scions after the first and second years was also significantly greater on slash pine than on longleaf pine (second year average 2.1 cm versus 1.5 cm, respectively). These results are similar to those reported by Gooding and others (1999) where it was noted that scions grafted onto slash pine grew faster than those grafted onto loblolly pine. The number of branches per living scion in year 2 was also significantly greater for grafts on slash pine (4.2 branches) than for those on longleaf pine (2.1 branches).

Interstock species were not significantly different in terms of the average number of pollen clusters produced per living graft after either the first or second year. In the first year, longleaf pine produced 0.25 pollen clusters per living graft and slash pine produced an average of 0.20. In the second year, the numbers of pollen clusters per living graft increased to 1.71 for longleaf pine and to 2.95 for slash pine. There was however, a significant difference between interstock species in terms of the average number of female strobili produced per living graft. In the first year, the surviving scions on longleaf pine produced on average significantly more female strobili (18 strobili per 20 grafts) than did those on slash pine (29 strobili per 75 grafts). However, this difference was not significant after the second year (20 strobili per 19 grafts for longleaf pine versus 79 strobili per 75 grafts for slash). Thus, slash pine interstock did better in terms of survival and growth of the scion, but by the second year, there was little difference between interstock species in terms of male and female strobili production per living graft.

Although slash pine was found to provide higher graft survival rates in this study, further investigation among and within interstock species is warranted. McKeand and Raley (2000) found significant differences among interstock genotypes of loblolly pine in the number of strobili produced and in this study, examining only two trees per species, we were unable to adequately test this hypothesis. Additionally, the branches on our longleaf pine interstock trees were generally larger in diameter when compared to the F_1 scions. This made aligning the scion and branch difficult, and may have contributed to the lower survival rates on the longleaf interstocks. Finally, it is also likely that the developmental and physiological status of the interstock trees play a critical role in graft success. In the present study, the longleaf pine interstocks were about 15 years older and 7.8 feet taller than the slash and all grafting was completed at the same time of the year. Both age- and height-of-interstock and time-of-year effects need to be evaluated further to insure an efficient implementation of top grafting in longleaf x slash pine backcross breeding.

In conclusion, given the poor survival of the scions grafted onto longleaf pine interstocks and the relatively good survival and strobili production of scions grafted onto slash pine, we recommend slash pine as the interstock species for top grafting longleaf x slash F_1 hybrids. Though, as backcross generations advance, and the scions become more like the recurrent species, we may need to reevaluate the choice of interstock species. By employing the top grafting technique a breeder should be able to substantially reduce generation time, possibly to as few as 5 or 6 years.

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Genetic and Phenotypic Variability for Constitutive Oleoresin Flow in Loblolly Pine

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In loblolly pine, *Pinus taeda* L., flow of oleoresin at penetration sites is considered to be a major component of defense against attack by the southern pine beetle (SPB) *Dendroctonus frontalis* Zimm. Trees with copious amounts of constitutive or preformed oleoresin appear to be most able to prevent or impede colonization by this destructive insect pest of southern pines.

Prior research has revealed that flow of constitutive oleoresin, referred to hereafter as resin flow, is influenced by both genetic and environmental factors (Lorio 1986, Lorio et al. 1990, Nebeker et al. 1988, Nebeker et al. 1990). Little is known, however, about levels of genetic variability for resin yielding capacity existing in populations of loblolly pine, and genetic associations between resin flow and growth traits have not been investigated. In view of questions regarding the feasibility of breeding for resistance to attack by SPB and interest in the evolutionary biology of traits associated with insect resistance in conifers, we studied components of genetic variation and covariation for resin flow and growth traits in a population of this host species.

Data were collected for resin yield, total height and DBH on 10- and 11- year old trees in progeny trials located near Pensacola, Florida. These tests were established in 1989 and 1990 as part of the loblolly pine breeding program being conducted by International Paper Company in cooperation with the N. C. State University — Industry Cooperative Tree Improvement Program. Trees included in our investigation were from a subset of the plots in these trials and constitute a progeny sample from 72 full-sib families. This sample of families resulted from intercrossing 48 parents in a disconnected diallel mating design composed of six-parent, balanced partial diallel units. In this design, each parent tree was mated to three others. Field plot layout for the experiment followed a replications-in-blocks experimental design. Data from 1131 trees were available for analysis. Two resin flow measurements were taken. The first at a time in late summer when latewood is normally produced (summer resin flow), and the second at a time in the following spring during earlywood formation (spring resin flow). Resin yields were determined from samples obtained from wounds made at breast height using a modification of the sampling procedure described in Strom et al. (2000). Weight of the resin collected during the 24 hours following wounding was used as a measure of resin flow.

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Mean values for the growth variables indicate that trees in the experiment had good growth. The 11-year old trees had a mean height of 12.6 m and mean DBH of 18.9 cm. Corresponding values for the 10-year old trees were 12.1 m and 17.7 cm. Spring ($\bar{x}$ = 1.88 g) and summer ($\bar{x}$ = 1.72 g) resin flow did not differ greatly in the 11-year old trees. A somewhat larger difference was found in the 10-year old cohort, which had a mean of 1.92 g for spring resin flow as opposed to 1.28 g for summer resin flow.

Genetic components of variance made up a significant portion of the phenotypic variance observed in both the resin flow and growth traits. Except for spring resin flow, dominance variance was small and inconsequential compared to additive variance. Dominance variance for spring flow was roughly half that of the additive component indicating that differences in nonadditive genetic effects contributed substantially to variation in this trait.

Estimates of individual tree heritabilities were moderately large for both the resin flow and growth traits. Values obtained for the resin flow traits were $h^2 = 0.44$ for spring flow and $h^2 = 0.59$ for summer flow. For the growth variables, estimates were $h^2 = 0.48$ for height, $h^2 = 0.49$ for DBH and $h^2 = 0.53$ for volume. While these values are consistent with estimates for comparable growth traits in several loblolly pine experiments (Gwaze et al. 1997, Hodge et al. 1999, Gwaze et al. 2001), they are higher than values reported for most tests of similar age in southern United States (Bridgwater et al. 1983, Foster 1986, Balocchi et al. 1993, Xiang 2000, Gwaze et al. 2001). In contrast to results for heritability, estimates for additive and dominance coefficients of variation for the resin flow traits were greater than those for the growth traits. These findings suggest that the resin flow traits exhibited higher genetic variance.

Additive genetic correlation estimates between the resin flow and growth traits were positive and of moderate size. Values for trait pairs involving summer resin flow were $r_A = 0.67$ for height, $r_A = 0.58$ for DBH and $r_A = 0.59$ for volume. Similar but slightly lower estimates were obtained for spring flow. Phenotypic correlation estimates for these character combinations while positive, were somewhat smaller than those for the additive genetic correlations.

In conclusion, our results demonstrate that substantial genetic variation exists for resin flow in the loblolly population we studied. This suggests that directional selection to improve resin flow should be successful. Moreover, since genetic correlations between resin flow and tree growth traits appear to be positive and sizeable, it should be possible in this population to concurrently improve both through breeding.

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TREEPLAN® - A Genetic Evaluation System for Forest Trees

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Abstract: -- The TREEPLAN® genetic evaluation system is designed specifically for the efficient and accurate prediction of breeding and other genetic values in trees. TREEPLAN® uses the preferred statistical method of best linear unbiased prediction (BLUP) using an individual tree additive genetic effect. Although BLUP methods are well developed theoretically, other software is suitable only for breeding value estimation and prediction on small and/or highly structured (balanced) data sets. Packages such as ASREML and SAS have hardware and software limitations that make them unsuitable for routine prediction on large data sets with complex pedigree structures and overlapping generations. TREEPLAN® fits a reduced individual tree model for purposes of efficiency. TREEPLAN® can model multiple genetic groups, handle clonal data, fit multi-trait models with more than 50 traits, accommodate heterogeneous variances, fit site specific statistical and genetic models, and also weights information across environments (accounts for genotype by environment interaction) and time (allows for age:age correlations).

The Southern Tree Breeding Association (STBA) is routinely using TREEPLAN® for genetic evaluation in Australian tree improvement programs for *Pinus radiata, Eucalyptus globulus* and *E. nitens.* TREEPLAN® has allowed data across generations and years to be combined in a multi-trait analysis to produce single lists of breeding values for each trait and environment combination. TREEPLAN® is easy to use and has the 'industrial strength' to handle large amounts of unbalanced data with the complex pedigree structures that are usually associated with national or regional tree improvement programs. TREEPLAN® is fully integrated with a web based data management system that efficiently handles data and pedigree information. The analytical power and flexibility of the TREEPLAN® system has made routine genetic evaluation in trees a straightforward process.

INTRODUCTION

The total plantation estate in Australia is 1.63 million hectares (National Plantation Inventory 2003). The Southern Tree Breeding Association (STBA) runs the national breeding cooperatives for *Pinus radiata* and *Eucalyptus globulus.* These two species comprise about two-thirds of the national estate, and are mostly used for solid wood products and pulp and paper production.

Tree improvement programs fundamentally consist of (i) defining a breeding objective,

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(ii) mating among parents, (iii) testing offspring in field trials, (iv) analysing performance data and genetic evaluation, (v) selecting trees for deployment and further breeding with elite parents. In general, tree breeders have been proficient at handling the biological aspects of tree breeding and trial establishment. However, they have often failed to optimise in a timely manner genetic evaluation using pedigree and correlated performance information. That is, it is relatively easy to plant and assess trees in progeny trials to generate data. However, it is often much more difficult to process the data collected in an efficient and comprehensive manner. As a consequence, the STBA, like many other tree improvement programs, had access to many records (unprocessed data) from research and breeding trials that did not meet the usual restrictive requirements of a simple analysis.

Historically, tree breeding has emphasised experimental design features (replicates, plots and incomplete blocks in increasingly complex designs) in trees to account for local environmental effects, compared to more complete modelling of the genetic components. Single-generation, single-site and single-trait mixed models have thus been the norm in tree breeding. This has allowed the use of straightforward methods of analysis, including best linear prediction (BLP), without a numerator relationship matrix. Family models have largely been used with a second stage to predict within-family values.

The STBA adopted the individual tree additive genetic model (ITM) BLUP in its tree improvement programs during the 1990s (Jarvis *et al*. 1995). However, its application was limited to relatively small and uncomplicated data sets until the development of the TREEPLAN® system. The application of such a model occurred later in tree breeding, and is much less common than in animal breeding. This situation has arisen because breeding programs for trees are usually in their early generations, with simple shallow pedigrees, and trees are evaluated in large designed trials. Families are often the result of open-pollination, such that simpler family models are possible for the prediction of parental breeding values. Unlike animals, trees are often not subject to culling, so that data sets are more balanced. The magnitude of genotype by environment interactions (GxE) is often unknown, except in a large environmental range. The number and type of traits measured is usually limited, but is rapidly evolving as wood quality traits assume greater importance.

Modern tree improvement programs demand a greater use of BLUP to predict genetic values for several reasons. Breeding programs are progressing and now span several generations. Individual programs with different samples of the same base population are being consolidated into larger cooperatives. It is important to account for the effects of selection over time. Many programs are now making the transition to overlapping generations, where a proportion of all breeding activities is performed each year, and all families are not tested at all test sites at the same time. Finally, there is a need for integrating all data between trees and between traits, making it easier for selection and to monitor the genetic progress of breeding programs.

Currently, the STBA is collecting performance data in trials on third-generation progeny in *P. radiata* and second-generation progeny in *E. globulus*. In the past, breeding values

were estimated using BLP for *P. radiata* (White *et al.* 1992 ab) and BLUP for *E. globulus* (Jarvis *et al*. 1995). Due to a lack of suitable BLUP software, multiple and independent lists of breeding values made it difficult to compare trees for genetic merit across a population. Despite the existence of good genetic linkage, pedigrees were too complex to be accommodated. Large quantities of data were also excluded because trial assessments were incomplete or done at different ages. That is, the data were 'messy' or did not fully satisfy other restrictive requirements of 'balance'.

This inefficient use of data and information is clearly undesirable, particularly for large national breeding cooperatives. In order to overcome this weakness, the STBA designed TREEPLAN® to apply 'industrial strength' individual tree model BLUP on a program wide basis. Although the STBA and AGBU initially developed the TREEPLAN® system for use in the Australian tree improvement programs for *P. radiata* and *E. globulus*, it has been designed with flexibility for much wider application.

This paper discusses some of the key features of TREEPLAN® and its routine application of BLUP in forestry.

The Genetic and Statistical Models

The statistical approach used in TREEPLAN is designed for maximal efficiency as it includes all the design effects used in simpler analyses, but can incorporate all of the data that has been collected in a single analysis – combining different traits and across all pedigrees. It fits a linear mixed model of the form:

$$\mathbf{y} = \mathbf{Wf} + \mathbf{Xr} + \mathbf{Yu} + \mathbf{Zs} + \mathbf{e}$$

where: y is the vector of observations on one or more traits; f is the vector of fixed site and design effects, with its incidence matrix W; r is the vector of random design effects, with its incidence matrix X; u is the vector of random additive genetic effects (breeding values) with its incidence matrix Y; s is the vector of random specific combining effects (SCA) with its incidence matrix Z; and e is the vector of residuals.

The estimates of the fixed and random design and genetic effects are obtained by solving the mixed model equations (MME's) (Henderson 1984) using Gauss-Seidel iteration:

$$\begin{bmatrix} W'\mathbf{R}^{-1}W & W'\mathbf{R}^{-1}\mathbf{X} & W'\mathbf{R}^{-1}Y & W'\mathbf{R}^{-1}Z \\ X'\mathbf{R}^{-1}W & \mathbf{X}'\mathbf{R}^{-1}\mathbf{X}+\left[I\otimes\mathbf{G}_r\right]^{-1} & \mathbf{X}'\mathbf{R}^{-1}\mathbf{Y} & \mathbf{X}'\mathbf{R}^{-1}\mathbf{Z} \\ Y'\mathbf{R}^{-1}W & Y'\mathbf{R}^{-1}X & Y'\mathbf{R}^{-1}Y+\left[A\otimes\mathbf{G}_a\right]^{-1} & Y'\mathbf{R}^{-1}\mathbf{Z} \\ \mathbf{Z}'\mathbf{R}^{-1}W & \mathbf{Z}'\mathbf{R}^{-1}\mathbf{X} & \mathbf{Z}'\mathbf{R}^{-1}Y & \mathbf{Z}'\mathbf{R}^{-1}\mathbf{Z}+\left[I\otimes\mathbf{G}_s\right]^{-1} \end{bmatrix} \begin{bmatrix} \hat{\mathbf{f}} \\ \hat{r} \\ \hat{u} \\ \hat{s} \end{bmatrix} = \begin{bmatrix} W'\mathbf{R}^{-1}\mathbf{y} \\ \mathbf{X}'\mathbf{R}^{-1}\mathbf{y} \\ Y'\mathbf{R}^{-1}\mathbf{y} \\ \mathbf{Z}'\mathbf{R}^{-1}\mathbf{y} \end{bmatrix}$$

where, the new terms represent variance-covariance matrices of the error (R), random design effects (G_r), additive genetic effects (G_a), and specific combining effects (G_s) and the relationships between the additive genetic effects (A, the additive (or numerator) relationship matrix) and independent random effects (I), and $\otimes$ is the Kronecker product.

This model offers substantial advantages over the models usually used in forest genetic trial analysis. Breeding values (and other genetic effects) are estimated for all traits, for all trees in the pedigree – both parents and offspring, in a single analysis. Where a trait has not been measured on a tree then the best prediction is made of its breeding value using information from relatives and from traits correlated at the genetic, design or error levels. If there is no such information, then the estimate is at the population mean, but the variance of the estimates grows as the amount of information, and thus its reliability, increases. The use of correlated traits allows correction for the effect of selection in measurement, as long as the data used for selection is included. The solutions give the highest correlation between true and estimated values, provided that the variances and covariances are known. This is a substantial improvement over BLP, where the fixed effects are assumed to be known. The mixed model equations are extremely robust, and can be readily extended to more complex models.

The model uses the A matrix to track the proportion of genes in common between trees in the pedigree and gives solutions for all of them without any secondary process of the data in what has been called an individual tree model (ITM). It easily handles half-sib and full-sib pedigrees, and simple rules have been worked out (Henderson 1976) to create the inverse that is used in the MME's. The matrix can be modified for the types of pedigrees that are common in forest genetic trials: fixed provenance or selected parentage (such as seed orchard) effects (Quaas 1988), partial selfing (Dutkowski and Gilmour 2001), and even pollen mixes (Perez-Enciso and Fernando 1992).

The software uses an equivalent gametic model for computational efficiency in the prediction of breeding values for trees without offspring (the majority).

$$y_i = \mu_i + \frac{u_f + u_m}{2} + s_j + \phi_i + e_i$$

where: μ_I is the mean, y_i, s_j and e_i are as defined above, u_f and u_m represent the breeding values for the tree's female and male parents respectively, and ϕ_i represents Mendelian sampling in the formation of the tree's genotype. That is, $.5u_f + .5u_m$ represent "average" gametes from each parent, and ϕ_i represents the deviation from the average of the gametes received by the progeny. The genotypic and gametic models are equivalent models, in that the solutions to the unknowns will be exactly the same for both models. Their combined use is called a "reduced" individual tree model.

Trait Mapping to Selection Criteria

In theory, the MME's can handle all data by treating each measurement on each site as a separate trait, as long as all the variances and correlations are known. In practice, however, such and approach is computationally infeasible, not all variances and correlations are known and dealing with output would be very confusing to the breeder, because of the many traits. The mapping of multiple measured traits to a smaller meaningful number of selection criteria (SC) traits is a feature of TREEPLAN®. This allows a reduction (consolidation) in the number of traits for which breeding values are predicted in a multi-trait analysis. This mapping gives TREEPLAN® its flexibility and ease of use as the breeder can easily define the SC traits of interest. The mapping allows

us to consolidate data with different forms and scales of measurement, different ages and different sites, as long as it can be realistically assumed that all the measurements have a sufficiently high correlation to be treated as one. For example, if diameter at breast height (DBH) is measured between ages 3 to 12 years, then a sensible strategy is to propose three SC traits: DBH ≤4 yrs, DBH 5–8 yrs and DBH 9-12 years. We recommend only mapping traits displaying significant genetic variance in a single-site analysis.

Heterogeneous Variances

Breeding programs collect data from trials spread across a diverse range of site types and age classes. Some traits are or have been assessed using different protocols. For example, growth may have been measured as tree height, stem diameter or tree volume; and stem form using several scales with different levels of precision. The variance of performance traits such as growth usually increases with size, growth rate and age of trees. A linear transformation of the data such that the phenotypic variance is unity is an approach often used in plant and animal breeding to make variances homogeneous. A disadvantage of this approach for tree breeding is that a constant heritability would need to be assumed across all sites, despite some sites being more homogeneous. Tree breeders also have the benefit of large designed trials that provide good estimates of variances and spatial variability (replication and blocking), genetic and residual variances and correlations specific to each site. TREEPLAN takes advantage of the availability of these estimates to overcome these problems by: (i) transforming the data for each trait to unit additive variance on a site by site basis; and (ii) using the within site error (to allow for different heritabilities) and significant design factor (eg. rep, plot and incomplete block) variances in the BLUP analysis.

Genotype x Environment Interaction

As well as age differences, geographical location and/or site type are other possible criteria for proposing new SC traits out of the one generic trait such as growth. For example, it may be necessary to partition the SC trait, DBH ≤4 yrs, further in a multi-site run, according to province, state or soil type. GxE interaction is where different environments induce different kinds of genetic variance to be displayed. That is, GxE may result in a change of ranking of genotypes across environments. However, GxE due to scale effects is effectively removed by data transformation (standardisation). Flexibility in mapping of traits in TREEPLAN accommodates specific geographical and environmental combinations by creating environmental subclasses.

In practice, the best method to handle GxE is to consider the same character measured in two different environments as two different but correlated traits (Falconer and Mackay 1994). A trait measured at different locations can be considered biologically the same SC trait when the genetic correlation is high (for example, ≥0.8). A breeder can either define different production environments or ignore GxE (effectively selecting for general adaptation) if environmental effects are not repeatable. Past studies to quantify the magnitude and nature of GxE in Australia for *P. radiata* and *E. globulus* have been based

on limited data sets. Studies with more extensive data sets are currently under way to estimate across site correlations and better define the target production environments.

Genetic Groups

In forestry, parents of first-generation progeny are typically trees from native stands (or plantations) sampled from many different geographical regions that represent different provenances or races. Because provenances are quite genetically distinct it is important to assume that $E(g) \neq 0$, where g is the vector of genetic values. Male parents are usually unknown and female parents are assumed to be unrelated. Seeds from the female parents (founders) are collected from various localities spread across a wide geographical area. Thus, it is reasonable to consider that progeny are from more than one genetically divergent sub-population. TREEPLAN relates all foundation parents on the basis of their original provenance to genetic groups. In practise, data sets are likely to be far more complex. For example, a male parent (pollen) might be identified as belonging to a particular population, such as, a routine or an improved population. Founders introduced from another unrelated breeding program might also constitute a different genetic group. The modified mixed model equations of Quaas (1988) are used to derive solutions to g.

Clonal Data

Individual trees can be replicated using various forms of vegetative propagation. Clonal tests are common in *P. radiata* and are also used in some Eucalypt breeding programs. TREEPLAN currently treats clones as the same individual and matches unique clone identities to a single genotype. Clonal replication can improve the precision of breeding values. Versions of TREEPLAN currently being developed will be capable of predicting genetic values, including additive and non-additive genetic effects, for individual clones, recognising the potential for somaclonal variation and propagation effects. This functionality is particularly important for deployment of clones.

Partial Selfing in Open Pollinated Seed

Trees can be partially self-fertile, generating pedigrees where two progeny may be selfed sibs (both progeny result from selfing), a selfed sib and an outcrossed sib, full-sibs or half-sibs. In the *E. globulus* breeding program most progeny tested in the first-generation are derived from open-pollinated seed collected from founder trees in native forest stands. Until many more second-generation progeny (from controlled pollination crosses) are included in the analysis, the accuracy of breeding value prediction is dependent on how well the relationship coefficients between sibs of open-pollinated trees can be defined. Dutkowski (2001) has outlined simple rules to modify the NRM when a selfing rate in native stands is assumed. These rules can be further extended to account for the equilibrium level of inbreeding in the stand and the level of coancestry in the trees local to the female parent from which seeds were collected. Sparse stands of trees are expected to have a higher level of inbreeding among the progeny than dense stands. This functionality is currently being implemented in TREEPLAN .

Running TREEPLAN®

An efficient data management system is critical for accessing data and pedigree information to produce breeding values quickly. The TREEPLAN® analytical system is fully integrated with a modern data management system (STBA-DMS) which operates via a web based interface. TREEPLAN® can be run independently of the STBA-DMS, but its interactive nature makes the process of genetic evaluation far more straightforward and efficient. It also facilitates data entry and analysis from various locations. The STBA-DMS is mainly designed for storage and retrieval of tree data for the purposes of genetic evaluation. It is flexible and accommodates different species of trees. User access is restricted and data is password protected to the level of traits within trials. This allows us to easily complete multiple TREEPLAN® runs for the membership, firstly using only generic data, but then also including data for traits belonging to a restricted group of clients. This provides the flexibility needed in large cooperative tree improvement programs to satisfy individual client needs and produce customised breeding values.

TREEPLAN® extracts genetic parameters, data and run specifications from the STBA-DMS. Making changes to specifications for a new TREEPLAN® run is a simple process. That is, it is a straightforward process to include (exclude) new trials and/or more traits in a multi-trait BLUP analysis. As new trials are assessed, the data is validated and entered. Multi-variate analyses are first done on a trial by trial basis using ASREML and the variances and correlations for all significant design and random genetic components are stored in the STBA-DMS. The system is designed to regularly update breeding values. That is, as quickly as a trait is measured, data entered and single site analysis completed, TREEPLAN® is then run with the complete database.

Genetic Evaluation in *E. globulus* and *P. radiata*

TREEPLAN® is being used routinely to predict genetic (breeding and deployment) values for trees included in the *E. globulus* and *P. radiata* databases. As new trials and traits are assessed, the data is entered into the database, analyses are done on a single site basis and parameters estimated, TREEPLAN® is run, and breeding values for all trees in the specified population are updated. Table 1 lists details of data sets used in recent runs of TREEPLAN®.

Pinus radiata. Breeding values were predicted for 117,778 genotypes (different trees) in the population. This included trials from the southern States of Australia (Powell *et al.* 2002). The inclusion of many (hundreds) outstanding historical first- and second-generation trials yet to be entered in the database, will be done as resources are made available. At this stage, breeding values are predicted for Selection Criteria targeting the different production regions defined in the National Plantation Inventory for Australia (Wood *et al.* 2001). Selection Criteria traits for growth include: six production regions by four age classes (0-5 yrs, 6-12 yrs, 13-24 yrs and >24 years). Branch angle, branch quality, branch size and stem straightness comprise the form traits. Basic density (0-12

yrs and >13 years) and Spiral Grain (0-6 yrs and ≥6 years) constitute wood quality traits. Data for disease and pest resistance/tolerance traits will be incorporated with time.

Eucalyptus globulus. Beeding values were predicted for 174,369 genotypes in the population. This included trials from South Australia, Tasmania, Victoria and Western Australia (Pilbeam *et al*. 2002). A rolling front is used with some breeding, assessment and selection activities done on an annual basis. Prediction of breeding values is a dynamic process, such that TREEPLAN® breeding values are updated regularly as traits are measured, data compiled and validated. At this stage, breeding values for growth are predicted in four production regions by three age classes (0-4 yrs, 5-8 yrs and 9-12 years). Basic density, by two age classes, and pilodyn penetration comprise quality traits. Data for pest and disease resistances (defoliation), kraft pulp yield, NIRA pulp and cellulose content, collapse, shrinkage and tree form traits will be incorporated with time. Trees in the CSIRO collections (Gardner and Crawford 1987, 1988) will be used to establish a baseline for monitoring genetic improvement over time.

	Species	
	Pinus radiata	***Eucalyptus globulus***
Generations	**3**	**2**
Trials included in Analysis	**68**	**87**
Number of Selection Criteria Traits Analysed	**19**	**10**
Genetic (founder) Groups fitted	**12**	**25**
Families	**3033**	**1550**
Genotypes included in Analysis	**117,778**	**174,369**

Table 1. Data sets used in recent runs of TREEPLAN® for *P. radiata* and *E. globulus.*

Future Enhancements

In partnership with the Forest and Wood Products Research and Development Corporation (FWPRDC), STBA and AGBU plan to develop Version 2 of TREEPLAN®. Additional features will include: (1) Better modelling of intra-site environmental variation using spatial and competition models, (2) Incorporation of information at the DNA level (markers and candidate genes), (3) Modelling of dominance and epistatic effects to allow for the full exploitation of these non-additive genetic effects in clonal deployment populations, and (4) Development of a clearer understanding of GxE to better target different production environments.

CONCLUSIONS

Tree breeding programs have evolved to the stage where the adoption of BLUP is required to maximise return on investment through breeding. TREEPLAN® is a genetic evaluation system that facilitates the routine application of individual tree model BLUP

to forest tree data. TREEPLAN® can model multiple genetic groups, handle clonal data, fit multi-trait models with more than 50 traits, accommodate heterogeneous variances, fit site specific statistical and genetic models, and weight information to account for age-age correlations and genotype by environment interaction. TREEPLAN® has allowed data across generations and years to be combined in multi-trait analyses to produce breeding values for each trait and environment combination of interest on a program basis. TREEPLAN® is easy to use and has the 'industrial strength' and speed to handle large amounts of unbalanced data with complex pedigree structures. TREEPLAN® is fully integrated with a web based data management system that efficiently handles data and pedigree information. The TREEPLAN® system is being used routinely to update breeding values in the Australian tree improvement programs for *P. radiata* and *E. globulus*. TREEPLAN® also facilitates the adoption of efficient rolling front breeding programs with overlapping generations.

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A New Model for Moving Forward with Marker-based Selection

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Abstract

Increasing the frequency of favorable alleles in populations is the goal of tree breeding based on an additive genetic model. Conceptually, marker based selection (MBS) combined with accelerated breeding offers a more efficient means to this end by reducing the generation interval and increasing genetic gain per generation. Yet, while DNA markers for qualitative and quantitative traits have been identified and reported, neither these nor other genetic markers have been widely accepted in southern pine breeding programs. Some of the reasons for not using MBS include the following: marker development and genotyping costs are too high, QTL effects appear too small and have not been demonstrated in independent families, and the requirement of multiple populations, traits and environment complicate matters such that there is no clear path forward.

To address these theoretical and operational issues we are proposing two general strategies for MBS-accelerated southern pine breeding. One strategy applies QTL detection and marker selection to established breeding resources, while the other generates breeding populations structured to make optimal use of DNA marker information in an operational breeding context. Both strategies depend on performing multiple generations of selection prior to progeny testing. This is in contrast to the normal mode of operation in which selections are progeny tested each generation. We suggest that these strategies offer much flexibility with respect to moving selections to production populations and therefore should offer additional genetic gain at intermediate time points.

Options for population structure and family sizes and for phenotypic and genotypic data requirements of each strategy will be discussed. Half-sib family designs are utilized in both strategies to capture linkage-disequilibrium of the constructed populations and to infer linkage phase relationships of marker and QTL alleles in individual families. Selective genotyping using highly informative DNA markers are required to reduce genotyping costs, while BLUP analyses are proposed to improve phenotypic data quality. Accelerated breeding options include top grafting or the use of a short generation pine species, such as Virginia pine, as a model species.

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Genetic Variation of Juvenile Wood Properties in a Loblolly Pine Diallel Test

Robert Sykes, Fikret Isik, Bailian Li, John Kadla and H-m. Chang[1]

Abstract

The reduced rotation age for loblolly pine plantations has resulted in an increased percentage of juvenile wood. Juvenile wood has lower wood density, shorter tracheid length and higher lignin content than mature wood. The increased use of juvenile wood has reduced yields and increased pulping costs for the pulp and paper industry. If significant genetic variation in juvenile wood properties can be found, breeders may be able to improve juvenile wood properties to reduce pulping losses. Genetic variation in several wood quality traits of loblolly pine (*Pinus taeda* L.) was investigated for 14 full-sib families generated by a 6-parent half-diallel mating design. Wood samples of 12 mm increment cores were collected from 11-year-old trees from one test site. Earlywood and latewood of ring three (juvenile wood) and ring eight (transition wood) for each increment core were analyzed for alpha cellulose content (ACY), average fiber length (FLW), coarseness (COA), and lignin content (LIG). Ring three and ring eight had significant differences in ACY, FLW, and COA, but not for LIG. Latewood of both rings had higher ACY, FLW, and COA than earlywood. Transition wood had significantly higher ACY, FLW, and COA, but lower LIG than juvenile wood. Families differed significantly for ACY, FLW, and COA, but not for LIG. In general, additive genetic effects explained greater percentages of family variation than dominance genetic effects. Genetic variation increased from juvenile to transition wood. While weak individual and family heritabilities were found for ACY, FLW, and COA for juvenile wood, heritability estimates for transition wood were moderate, indicating the potential for improving these juvenile traits.

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Cone and Seed Insect Pest Research: The Role of the Southwide Studies

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Abstract: A reliable supply of genetically improved pine seed is critical to the success of production forestry. The most significant environmental threat to the ability to meet this demand (over 100,000 pounds per year) is insect predation. Cone and seed insect pests can easily destroy half the potential orchard crop, and there have been instances in which 90% of the harvest was lost. Effective insect control is dependent on continued availability of pesticides, both because the economic loss threshold is low, and because alternative control methods have not been successful. Because seed orchards are a minor use, there is limited support from pesticide manufacturers for either efficacy testing or continued product registration. The tree improvement community has responded to this challenge by developing a collaborative working arrangement between entomologists and seed orchard managers that has resulted in a series of southwide efficacy studies. These studies, which have now included evaluations of Guthion®, Asana®, Capture®, and Imidan®, were coordinated through the Seed Orchard Pest Management Subcommittee, a working group of the Southern Forest Tree Improvement Committee.

Southwide studies are the culmination of a multi-step process in which promising pesticide formulations and rates are first identified by USDA Forest Service entomologists through small-scale testing, typically with hydraulic spray applications to single trees. This method of application, while allowing for accurate treatment evaluations, does not reflect operational conditions. It is therefore necessary to evaluate the most promising treatments under operational conditions with aerial applications on large treatment blocks. Results from both published and unpublished studies have underscored the strengths and weaknesses of these large-scale tests. Efficacy studies are difficult to implement and have substantial direct and indirect costs to the participants. Seedbug control is easy to achieve. Coneworm control, however, is much more difficult both to achieve and to accurately document. Interpretation of composite traits such as the number of good seed produced per initial flower can lead to erroneous conclusions when efficacy is primarily due to seedbug control. Despite these deficiencies, southwide studies will continue to be needed to validate cone and seed insect control under operational conditions. Studies that will be needed in the future are discussed.

Keywords: Seed orchards, Coneworms, Seedbugs, Pesticide efficacy studies

INTRODUCTION

Commercial forestry in the southeastern United States is based on plantation management with approximately 2.62 million acres planted in 1998 (Moulton and Hernandez 2000).

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Annual plantation establishment on this scale requires a dependable source of genetically improved seed, most of which is supplied from seed orchards that currently have fifty-years of investment in breeding and progeny testing behind them. Seedlings produced from this source currently exceed the growth rate of woods run seed sources by 20 to 30 percent (Byram et al. 2000, Li et al. 1999, 2000). The differential between seed orchard seed and woods run seed will continue to increase as seed orchards incorporate additional genetic gains from tree improvement programs and new methods of capturing gain such as control mass pollination are implemented. Seed may easily exceed an imputed value of $100-$150 per pound per percent genetic improvement based on the discounted value of future wood production (calculated by the method of van Buijtenen 1984). Mature orchards may be expected to yield in excess of 60 pounds of seed per acre per year, this seed may exceed 20 percent improvement in growth rate, and the reproductive biology of pines results in two and sometime three crops being present simultaneously. With these assumptions, the value of the seed orchard crop may easily exceed $240,000 per acre. Therefore, seed orchard seed is an extremely valuable crop assessed either in terms of sunk costs or in potential growth gains.

Seed yields are notoriously variable, being influenced by both biotic and abiotic factors. One of the most serious causes of seed loss is insect predation, which can result in up to 90% crop damage in a given season (Fatzinger et al. 1980, Hodge et al. 1992). Effective pest management is, therefore, an essential part of seed orchard management. Because economic thresholds are low and alternative non-insecticide based methods have yet to be proven effective, the seed orchard manager is forced to rely on pesticides. This has resulted in what Mangini et al. (*in press*) have referred to as the 'registration dilemma'. Conifer seed orchards are a minor use crop with probably no more than 8,000 to 10,000 acres nationwide and with probably less than 6,000 acres under active production management in any given year (Byram and Lowe 1998). A recent survey showed that only 11,625 pounds active ingredient (ai) of pesticide were applied to southern pine seed orchards in 1999 (unpublished data). Because the total amount of pesticide applied is small compared to most agronomic crops, pesticide manufactures have shown only limited support for efficacy testing or product registration. The tree improvement community has responded to this challenge by developing a collaborative working arrangement between entomologists and seed orchard managers that has resulted in a series of southwide efficacy studies coordinated through the Seed Orchard Pest Management Subcommittee (SOPM), a working group of the southern Forest Tree Improvement Committee.

SOUTHWIDE EFFICACY STUDIES

The southwide studies are the culmination of a multi-step process in which promising pesticide formulations and rates are first identified, candidates are then evaluated in small pilot-scale experiments, and then the results are validated by large operational level tests. Federal and state entomologists have traditionally done the initial screening and pilot-scale experiments. Operational validation has been done by collaboration between entomologists, chemical company representatives, and seed orchard mangers.

The first step in this process, the identification of promising pesticides, has been most productive when it has concentrated on treatments that have been shown to be effective on similar groups of insect pests. By screening chemicals that are already registered for other crops, the likelihood of obtaining an additional registration for conifer seed orchards is enhanced. This is so because these products already have the extremely expensive environmental fate studies required by the Environmental Protection Agency (EPA) and pesticide companies can more easily be persuaded to pursue registration for other uses. This strategy takes advantage of the fact that the SOPM subcommittee successfully lobbied the EPA to classify seed orchards as a terrestrial nonfood crop rather than having orchards grouped with forest sites (J.W. Taylor, *personal communication*). This makes it possible to screen chemicals registered for such crops as cotton rather than being restricted to the much smaller number of pesticides labeled for forestry.

Once candidate pesticides are identified, efficacy screening in seed orchards is required, because effectiveness on the insect pests of row crops in no way guarantees that these chemicals will work in seed orchards. The number and variety of insects causing damage in seed orchards differs from those in row crops. The major pests have life cycles with specialized developmental stages that may be inaccessible to control methods (e.g. dormant coneworm larvae inside cones). Finally, the three dimensional aspect of the cone crop where trees sometimes exceed 80 feet in height makes obtaining adequate coverage difficult. This last problem is probably only shared with one other commodity group: pecan growers. Pilot-scale screening has traditionally been carried out by a small but dedicated number of state and federal entomologists.

After a handful of promising chemicals and rates have been identified, they must be tested under operational application conditions. Treatment blocks have typically had a minimum size of five acres, which limit the number of treatments in any one orchard to no more than four. The only group that has the resources to do this has been the members of the tree improvement cooperatives who actively manage large production seed orchards.

Rationale Behind the Studies

The southwide studies are necessary because research methods used to evaluate pesticide efficacies differ markedly from operational application techniques. Research applications are typically made on single trees with high volume hydraulic sprayers while operational applications are most often made aerially to large acreages. High-volume hydraulic sprayers give good coverage with large volumes of spray and large droplet sizes. Typical treatments may call for spray to runoff or spray until the foliage is thoroughly wet. Aerial applications use much smaller volumes, typically 10 gallons of spray per treated acre. This is only 0.029 fluid ounces of solution per square foot of flat surface area. The problem of obtaining adequate coverage on the pine foliage and cones with aerial applications is particularly difficult since the target (needles and cones) is dispersed vertically, sometimes as much as 80 feet. Furthermore, needles and cones are not flat

surfaces. Actual needle area may exceed projected area by more than a factor of three (Johnson 1984, Murthy and Dougherty 1997).

Operational applications have benefits that may offset some of the difficulties inherent in low volume aerial applications. These benefits could possibly result in better control than obtained with the pilot-scale studies. Aerial applications come from the top down placing the coverage in the crown where the majority of crop is located. Most significantly, aerial applications treat large acreages, which may be both a benefit and a drawback. Treating large areas may reduce insect pressures that are exerted by mobile pests from adjacent untreated trees in the single-tree treatment paradigm used in pilot scale studies. On the other hand, it may also reduce the presence of beneficial insects that would otherwise move back onto treated trees from adjacent untreated areas. Detrimental impacts on beneficial insects, which as a group tend to be very mobile, are frequently overlooked in single-tree treatments. Finally, large-scale applications are the only way to calculate cost/benefit ratios for various application alternatives.

Successes

The tree improvement community has now participated in five southwide studies since 1991. These efforts have been supported by the donations of pesticide application costs and personnel and equipment for test installation and evaluation by 19 organizations in a combined total of 32 orchards (Table 1). These studies have included evaluations of Guthion®, Asana®, Capture®, and Imidan®. Some of these studies have been extremely useful in obtaining and maintaining registration as well as refining application rates.

The Capture® study (Lowe et al. 1994) compared applications of Capture® and Guthion®, at the then legal rate of 3 lbs ai/ac, to an untreated control. Treatment with either Guthion® or Capture® were both effective, resulting in more seeds per cone, more sound seeds per cone and less seedbug damage. The beneficial impact of the pesticide treatments was most dramatic when the synthetic trait, the number of sound seeds produced per first-year conelet was analyzed. This trait incorporated conelet survival, cone survival, seed per cone and percent sound seed. This study resulted in Capture® receiving 24C registration for conifer seed orchards in all of the southern states with the exception of North Carolina which already had an alternative chemical with an emergency use registration for cone and seed insect control. The major draw back of the study noted by the authors was that there were very low coneworm populations in the year of the study and very little coneworm damage occurred. Coneworm damage was significantly reduced from 7.6% in the control to 4.2% in the Guthion® treatment and 5.6% in the Capture® treatment (unpublished data). To show statistical significance at these low levels, the control must have been real. Most of the benefits evident in this study; however, could be attributed to the control of seedbugs.

Cooperator	Capture Efficacy 1991	Guthion Rate 1992	Asana Timing 1993	Asana Rate 2001	Imidan/ Capture 2002
Boise Cascade					1
Bowater, Inc.	1	1			
Champion International		2	1		
Chesapeake			1		
Container Corp of America		1			
Deltic Farm and Timber		1			
Florida Division of Forestry				1	
Georgia Forestry Comm.	1				
Georgia Pacific Corp.	2				
International Paper				2	
Mississippi Forestry Comm.		2		1	
North Carolina Division of Forest Resources					1
Plum Creek Corporation					2
Potlatch Corporation	1				
Scott Paper Company			1		
Temple-Inland Forest	2	1		1	
USDA Forest Service	2				
Westvaco		1			
Weyerhaeuser Company			1	1	
Total	9	9	4	6	4

Table 1. Participants in several of the southwide efficacy trials. The numbers of orchards are shown in the table.

The Guthion® rate study (Mangini et al. 1998) was a tremendous undertaking because of the number of rate comparisons included. This required an incomplete block design necessitating the use of a large number of orchards. This study compared rates in 0.5 lbs. ai/ac increments from 1.0 to 3.0 lbs. ai/ac. First-year conelet survival, second-year cone survival, sound seeds per cone and the synthetic trait of sound seeds produced per first-year conelet improved at nearly every rate of Guthion®. Furthermore, there was no linear relationship between protection level and pesticide application rate. This study was used successfully to keep Guthion® registered for pine seed orchards by showing that application rates could be cut in half from 3.0 to 1.5 lbs. ai/ac. Once again, the apparent level of coneworm damage was low, and while whole-tree counts of healthy and coneworm damaged cones were tallied, these data were not included in the study report.

A recently completed rate study for Asana® (manuscript in preparation), compared three rates of pesticide to an untreated control. This study compared rates of 0.03, 0.10 and 0.19 lbs ai/ac/application rates to an untreated control. Any pesticide application reduced damage directly attributed to seedbugs (Figure 1). Only the high rate; however, reduced

coneworm damage even in this year with relatively high levels of coneworm damage present. This study will be used to justify keeping the current application rates, which are 1.9 times higher than the next currently labeled use (control of peachtree borer and filbertworm in almonds and filberts).

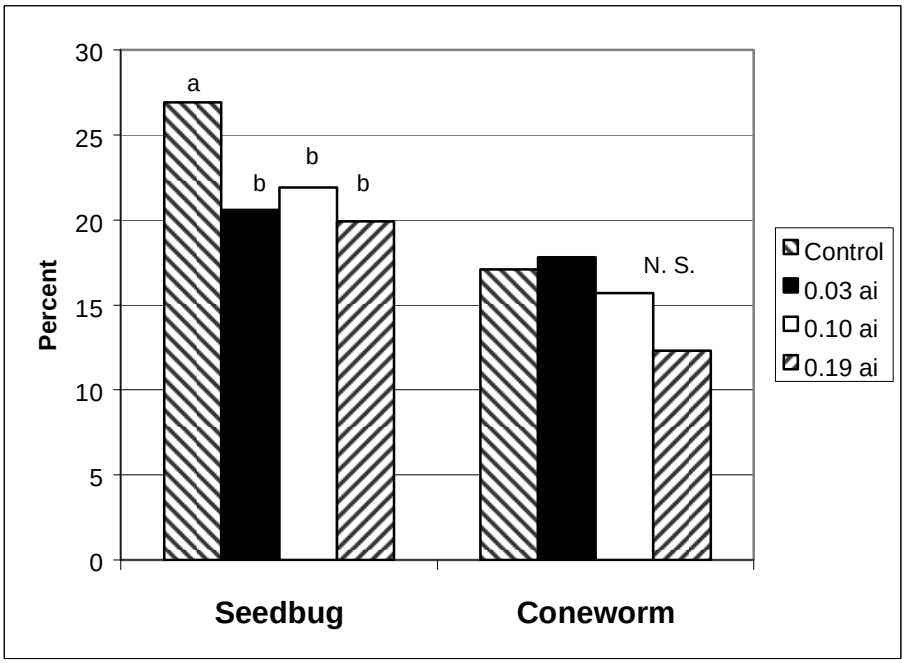

Figure 1. Results from the Asana® rate study showing A) the percent seed damaged by seed bugs as determined by radiographic analysis of seed extracted from healthy cones and B) the percentage of total cones collected damaged by coneworms. (from Byram et al. 2002)

Failures

Not all southwide studies have given clear answers despite the participants' considerable investments in time and resources and careful study implementation. This can happen for a number of reasons. First, insect pressure may be limited making it impossible to judge the differences between treatments. Secondly, because of the size of the treatment blocks, it has been necessary to consider orchards as replications. Therefore, the statistical precision of these tests is low; consequently, small, but operationally important, differences are difficult to detect. Finally, management histories between orchards differ. Protection programs in prior years to the installation of the southwide studies have varied from none to intensive. Cone collection histories have also varied with some orchards having been completely harvested in past years while other orchards have been inactive for a number of years. This can make it difficult for the collection crews making whole tree cone counts to correctly divide the cone crop into the current years healthy and coneworm damaged categories.

Lessons Learned

The tree improvement community has learned a number of lessons in implementing these extensive studies that will guide similar efforts in the future. Several points refer to the application and data collection protocols that have been standard in all of the southwide studies (for more detail see Lowe et al. 1994 and Mangini et al. 1998). Following is a partial list:

1. The spray protocol originally set up for the southwide studies generally works. This includes large treatment blocks (minimum size of five acres) separated by untreated buffers. Examination of damage caused by the easily controlled seedbugs occurring in adjacent blocks indicates that spray drift between treatments is seldom significant (unpublished data).

2. Adequate set up is necessary. Prior to several studies, entomologists worked with applicators to calibrate spray equipment to ensure proper application rate, spray pattern and droplet size. In several instances, applicators were using equipment that was either incorrectly calibrated or worn. To the applicator's credit, help correcting these situations has always been well received. This experience; however, emphasizes the need to periodically verify the proper use of application equipment in all operational programs.

3. Seedbug control is both easy to obtain and to document. Several indexes in the data collected verify seedbug control. These include tallies of first-year conelet survival, tallies of damaged ovules from dissected conelets, per-cone seed yield, and damage revealed by radiography of mature seeds. Almost all studies have shown that seedbugs are controlled by a variety of chemicals and rates and can be controlled by lower rates than those required to control coneworms.

4. Coneworm control is much harder to estimate, and therefore conclusions are less certain. Unlike seedbug damage, which can be estimated several ways, there is only one measure of coneworm damage – the damaged cones themselves. The protocol requires that all the current year's cones are collected on the sample trees and that these cones be divided into healthy and coneworm-damaged categories. This protocol can give spurious results for a number of reasons. First, some coneworm damage is missed as the cones are no longer present at the time of collection, or they are so damaged that they are destroyed in the collection process. Early-season coneworm attacks cause the small cones to become fragile. They fall off early, the cone collectors overlook them, or they crumble apart when collected. Secondly, when orchards have not been completely picked in previous years, some old cones are invariably included in the total. As a result of these factors, the tree improvement community is probably underestimating the damage done by coneworms both operationally and in these studies.

5. It follows that conclusions based on synthetic traits such as overall flower to seed yields may overemphasize the benefits attributable to seedbug control while

underestimating the damage done by coneworms. As seedbugs are easier to control, this can lead to pesticide recommendations that are less than optimal.

6. There is considerable indirect opportunity cost to the participating orchards. Because treatments are designed to include a range of management outcomes, untreated controls are needed for comparison, and untreated buffers must surround each treatment, most of the orchard will be unprotected or under-protected.

7. Inadequately supported studies are seldom worth doing. Statistically rigorous studies are required for submission of the data to chemical companies and the EPA. Studies with small numbers of orchards cause problems in two ways. First, meaningful differences are always difficult to detect when few replications result in small degrees of freedom in the analysis of variance. Secondly, there is no operational backup for situations in which mistakes are made and treatments are invalidated. With small numbers of orchards, any miscommunication between contract spray crews, mechanical failures or any number of failures at one orchard can jeopardize the efforts of all concerned. Fortunately, in practice this has rarely happened.

Future Needs

Despite all the difficulties, costs, and limitations to southwide studies, it is likely that the tree improvement community will need to continue their support for these efforts. The primary reason for this is that these are the only studies that can verify operational effectiveness of proposed control methods. Among the needs that have been identified for the near future is the efficacy of the southwide study protocol itself. Is coneworm damage being correctly evaluated? Are large treatment blocks necessary? Hanula et al. (2002) holds out some hope that large blocks may not be needed. Data collected in an operational spray block next to an area with a designed experiment showed that single-tree treatments may be adequate predictors of control. Ironically, it will probably require a southwide study to show if this is true.

Spray volumes and droplet sizes required by current labels have been challenged. Early work showed that 10 gallons of solution was required to obtain adequate coverage in conifer seed orchards (Barry et al. 1982). This quantity of solution is difficult for most applicators to apply in a single pass resulting in the need for multiple trips across the orchard. Since this spray volume was decided on, a new generation of chemicals with much longer residuals and new types of nozzles with smaller droplet sizes have become available. Because of the unique dynamics of large area treatments, a southwide study may well be needed to resolve this issue.

When new pesticides become available, southwide studies are the only way to verify operational effectiveness. Current examples are Warrior® and Mimic®, which have proven effective in single-tree treatments. They most likely will also be effective in area-wide applications, but at what rates and at what intervals?

Integrated pest management (IPM) systems will be necessary to reduce the reliance on chemical controls. By their very nature, many IPM methods can only be effective when applied to large areas and therefore will require southwide studies for their evaluation. As an example, non-chemical control methods such as mating disruption will only be effective if they disrupt populations over large neighborhoods. Multiple control methods with different methods of action also are likely to require evaluation over large areas. Hanula et al. (2002) has shown that a combination of trapping and spray timing may be adequate to control *D. amatella*, but how this effects other important pests in an operational setting over several orchards in regions with different weather regimes remains to be resolved.

CONCLUSIONS

Southwide studies have been successful in efficacy testing of pesticide treatments and have resulted in the registration of new chemicals and the refining of application rates for older chemicals. This would not have been possible without the single-tree treatment research that first identified likely candidates for operational trials. The southwide studies; however, remain one of the most important tools for verifying operational effectiveness over the many different conditions encountered on a regional basis.

Despite the value of the seed crop and the importance of having a dependable supply of seed, consolidation in the industry and the implementation of cost cutting measures make it more difficult to do this type of expensive and risky research. Failure to invest in these kinds of studies; however, would be extremely short sighted as no one else in the pest control community has any interest in supporting research for such a unique minor-use market.

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Performance of Nuttall Oak (*Quercus texana* Buckl.) Provenances in the Western Gulf Region

D. P. Gwaze[2], T. D. Byram[2] and E. M. Raley[1]

Abstract:-- Three series of three tests each of Nuttall oak (*Quercus texana* Buckl. formally *Q. nuttallii* Palmer) were established by the Western Gulf Forest Tree Improvement Program at three locations: Desha and Lonoke Counties in Arkansas and Sharkey County in Mississippi. The three series included 28-42 different half-sib families from throughout the natural range of Nuttall oak. Families were arbitrarily divided into provenances based on the river basin in which they originated. Significant provenance differences were found for survival in all series. Provenance differences for growth were highly significant for series 2 and 3, but not for series 1. The Red River provenance had the best growth performance in series 3 tests, but it was not represented in the other two test series. The best provenance in series 2, the Ouachita River provenance, ranked second in series 3 tests. The Western provenance performed well in series 1 and 3 tests but had poorer performance in series 2 tests. The interactions between site and provenance were significant for all growth traits in all series. Family-mean heritability estimates, however, were high ranging from 0.72-0.96 for height and 0.22-0.95 for diameter. There were good families from all sources indicating that family selection will be effective in this species.

Keywords: Provenance variation, heritability, genotype x environment interactions, Nuttall oak, *Quercus texana* Buckl.

INTRODUCTION

Nuttall oak (*Quercus texana* Buckl. formally Q. *nuttallii* Palmer) is a member of the red oak group. It has a restricted natural range on bottomlands of the Gulf Coastal Plain of the southern US from Alabama to southern Texas, north in the Mississippi Valley to Arkansas, southeastern Missouri and western Tennessee (Figure 1, Filer 1990). It is the most tolerant of the red oak species to the heavy, poorly drained, alluvial clay soils. Nuttall oak is an important species because it produces high quality sawtimber on poorly drained sites and because it is beneficial to wildlife, producing large acorn crops at young ages. Nuttall oak is currently favored for bottomland planting and restoration because it exhibits good survival on a range of sites and is fast growing (Ducks Unlimited 2001). Like most oaks, it is shade intolerant. Previous studies have mainly focused on natural regeneration, direct seeding, and comparison of growth performance of Nuttall oak with other species (Bonner 1966, Johnson 1975, Krinard and Johnson 1981).

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There is no genetic information on this species. Considerable provenance variation has been reported in other red oaks (*Q. rubra*; Russell and Dawson 1995, *Q. nigra*; Adams

1989), implying provenance variation might also exist in Nuttall oak. Knowledge of such genetic variation, if it does exists in this species, would be important for selecting the best provenances for reforestation and to form the base population for a tree improvement program.

This report is based on three series of Nuttall oak provenance tests established between 1994 and 1997 by members of the Western Gulf Forest Tree Improvement Program – Hardwood Cooperative. The primary objective of the tests was to improve the Nuttall oak for restoration of marginal croplands and for wildlife management through testing and selection. Each test series include samples from throughout the range of Nuttall oak, providing opportunity to determine provenance and within-provenance variation in the species. This paper reports on 5 and 7-year survival and growth results for provenances and families of Nuttall oak established in three series of tests established in Mississippi and Arkansas. Presence of genotype by environment interactions was determined and heritabilities estimated.

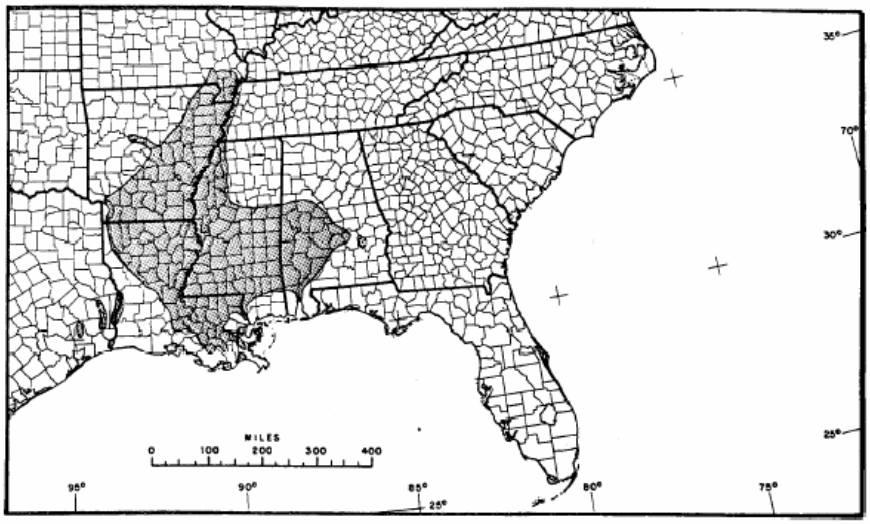

Figure 1. Natural distribution of *Quercus texana* Buckl. (formerly Q. *nuttallii* Palmer) (Filer 1990).

MATERIALS AND METHODS

Three series of three tests each were established at three locations: Desha and Lonoke Counties in Arkansas and Sharkey County in Mississippi (Figure 2, Table 2). Series 1 was established in 1994, series 2 in 1995 and series 3 in 1997. The three series included 28-42 different half-sib families collected from throughout the natural range of Nuttall oak. Families were arbitrarily divided into provenances based on the river basin in which

they originated. The provenances were Black-White, Ouachita, Mississippi, Red, Tallahatchie-Yalobusha Rivers and the sixth provenance (Western Region) originated in the western fringe of the main natural range of the species (Figure 2, Table 1).

The test designs were the same at each location, a randomized complete block design replicated ten times with four-tree row plots for families. Spacing was 2.4 x 2.4 m in all tests. All tests were assessed at 5 years with the exception of the Desha County test in series 2, which was measured at age 7, for survival, height (HT, m) and diameter (DBH, cm). Height and diameter were used to calculate volume of each tree using the following cone volume equation:

$$Volume\ (dm^3) = HT \bullet DBH^2 \bullet 0.02618.$$

Provenance number	Provenance Name	**State**	**Counties/Parishes**
1	Western Region	Texas Louisiana	Liberty (1,3), Smith (2), Tyler (3) Beauregard (3)
2	Black-White Rivers	Arkansas	Arkansas (2), Clay (2), Monroe (2), Prairie (2), Randolph (2,3), Woodruff (3)
3	Ouachita River	Arkansas	Clark (3), Union (1,2,3)
4	Mississippi River	Arkansas Louisiana Mississippi	Mississippi (1,2) Chicot (1), Franklin (3), Richland (3), Tensas (3) Bolivar (1), Issaquene (1), Washington (1)
5	Red River	Louisiana	Bienville (3), Bossier (3), Caddo (3)
6	Tallahatchie- Yalobusha Rivers	Mississippi	Leflore (1), Quitman (1), Grenada (1), Tallahatchie (1), Union (1)

Table 1. Details of seed origin of Nuttall oak provenances in the three series. Series number is in parenthesis.

Plot means were used for all analyses. Analyses were carried out for survival, height, diameter and volume for each series separately. In each series data at each age were pooled across the tests. Using the SAS PROC GLM procedure (SAS Institute 1989), analyses of variance (ANOVAs) were used to test for significant differences among sites, families, provenances, and replications.

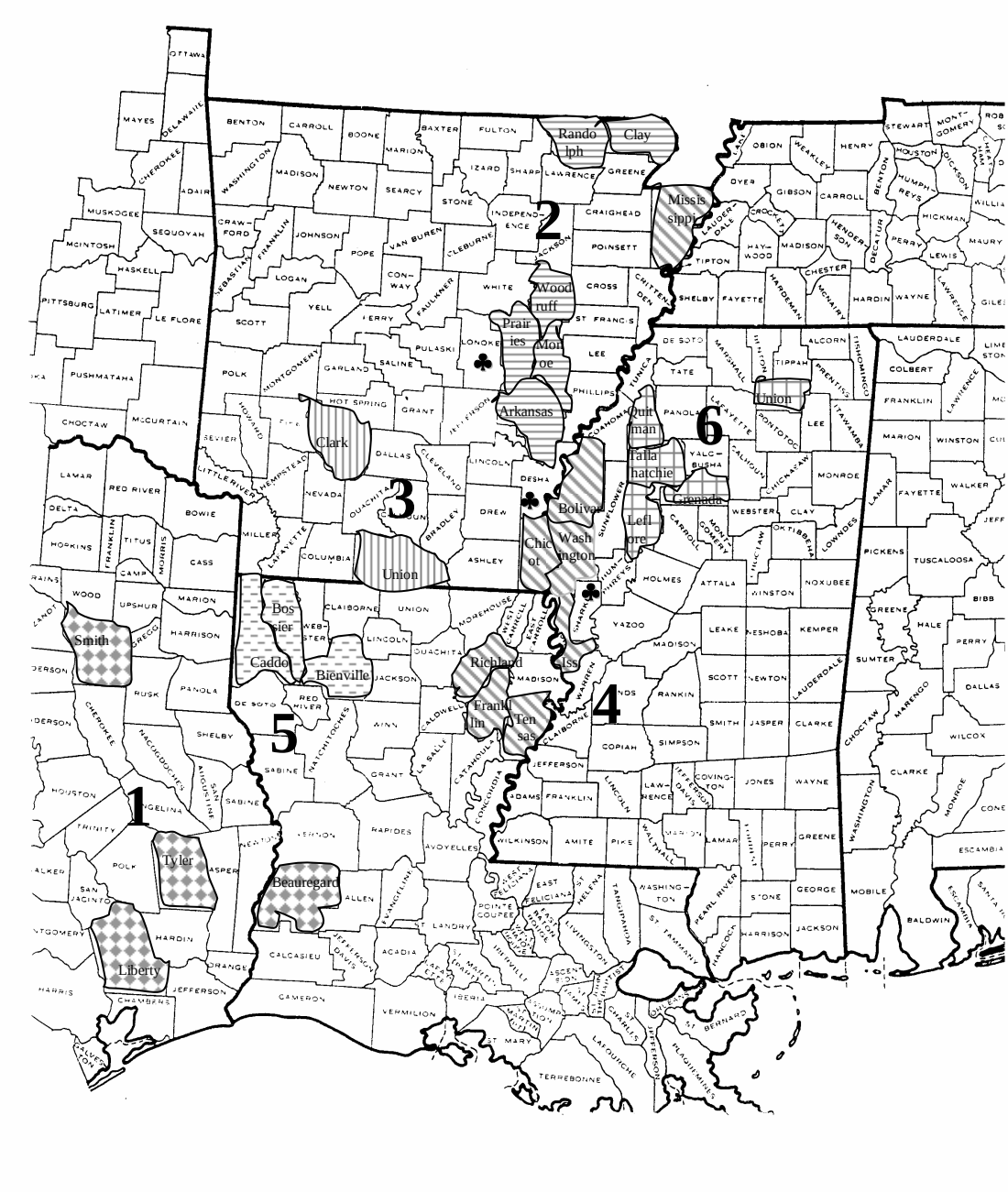

Figure 2. County/Parish locations of families used in the study are shaded. Western Region (Provenance 1), Black-White Rivers (Provenance 2), Ouachita River (Provenance 3), Mississippi River (Provenance 4) Red River (Provenance 5) and Tallahatchie-Yalobusha Rivers (Provenance 6). The test locations are marked by ♣.

The interactions between site and provenance, replication and provenance, and site and family were also tested. The following linear model was used for the pooled analysis across sites in each series:

$$Y_{ijklm} = \mu + S_i + R_{j(i)} + P_k + F_{l(k)} + SP_{ik} + SF_{il(k)} + e_{ijklm}$$

where Y_{ijklm} is the observation on the m^{th} plot of the l^{th} family of the k^{th} provenance in the j^{th} replication in the i^{th} site, μ is the population mean, S_i is the random variable for site, $R_{j(i)}$ is the random variable for replication nested within site, P_j is the fixed effect of provenance, $F_{l(k)}$ is the random variable for family nested within provenance, SP_{ik} is the random interaction site by provenance, $SF_{il(k)}$ is the random interaction site by family nested within provenance, e_{ijklm} is the error term.

Where significant differences were detected among provenances and among sites in the pooled data, Duncan's Multiple Range Test was used to compare means. Variance components were estimated using the VARCOMP procedure in SAS (SAS Institute 1985). Heritability estimates were determined using family variances for individual trees and at the family mean level. Since single-site heritability estimates are biased upwards because of genotype-environment interaction, only unbiased heritability was estimated using data pooled across the sites as

$$h^2_{F(P)} = 4 \times \sigma^2_{F(P)} / [\sigma^2_{F(P)} + \sigma^2_{SF(P)} + \sigma^2_e],$$

for individual tree heritability and family – mean heritability as

$$h^2_{F(P)} = \sigma^2_{F(P)} / [\sigma^2_{F(P)} + \sigma^2_{SF(P)} / s + \sigma^2_e / nrs]$$

where:

n = mean for number of trees per plot, and
r =number of replicates.
s =number of sites.

Test Number	**Cooperator**	**Location**		Mean Rainfall (mm)
		County	State	
1	Arkansas Forestry Commission	Lonoke	Arkansas	1041-1143
2	Mississippi Forestry Commission	Sharkey	Mississippi	1168-1170
3	Potlatch	Desha	Arkansas	1168-1170

Table 2. Details of field tests of Nuttall Oak provenances in the USA for all series.

Genotype by environment interaction (GxE) was estimated at both the provenance and family level using analysis of variance. The family mean correlations among traits were estimated as product-moment correlations using PROC CORR in SAS (SAS 1985).

RESULTS AND DISCUSSION

Survival

There were significant differences among provenances for survival at age 5 years in all three series, except for the Desha test in series 2 ($P < 0.05$) (Table 3). The provenance differences were probably not operationally meaningful as survival per provenance was generally good, ranging from 80.1% to 88.4% in series 1, 83.6 to 96.9% in series 2, and 91.7 to 96.8% in series 3 (Table 4). The Western sources tended to have slightly poorer survival in all series. Families within provenances were also significantly different for all three test series with the exception of the Desha test for series 2 ($P<0.05$) (Table 3). Survival was generally high at all sites for all series, apart from a rather low survival at Desha site (75.4%) in series 1. Sites were not significantly different ($P > 0.05$) for survival for series 3 (range: 91.7 – 93.6%), but were significantly different for series 1 (range: 75.4 – 96.3%) and series 2 (range: 89.8 – 97.4%) (Table 5). The excellent survival of Nuttall oak provenances in this study supports the results from previous studies that indicated that survival was 85% on Sharkey clay soil at two years (Krinard and Kennedy 1987), and those of Wittwer (1991) that showed a 78% survival for bottomland oaks planted in eastern Oklahoma at three years of age. However, poor survival due to drought observed at a test established in Angelina County in Texas (data not presented), suggests that even in this well adapted species survival can be low if conditions are not favorable.

Growth

There were significant differences among provenances ($P < 0.05$) for height, diameter and volume in series 2 and 3, and height in series 1 (Table 3). Families within provenances were significantly different for all growth traits in series 2 and 3, but not for volume in series 1.

In series 1, provenances were not statistically different for volume with similar volume growth performance at 5 years (range: 0.195- 0.247 dm^3). In series 2, the Ouachita River provenance had the best volume growth (0.184 dm^3) and the Black-White River provenance the poorest at 5 years (0.097 dm^3). In series 3, the Red River provenance had the best volume growth performance (0.314 dm^3), and Black-White River and the Mississippi River provenances (0.184 and 0.185 dm^3, respectively) performed the poorest in volume growth at 5 years (Table 4).

Individual site analysis (data not presented) indicated that local seed sources either performed equal to or poorer than some distant seed sources. For example in series 3, both local and distant seed sources had similar volume growth at the Lonoke site, but at Sharkey site, the local seed sources, Mississippi River provenance, were outperformed by

the more distant seed sources, Red River provenance. Similarly in series 3, the distant provenances, Western Region and Red River provenances, outperformed the more local seed sources at Sharkey and Desha sites.

Source of variance	DF	Survival (%)	Height (m)	DBH (cm)	Volume ($dm^3.tree^{-1}$)
Series 1 (age 5 years)					
Site (S)	1	41174.1***	14.63***	108.70***	6.97***
Replication (Site) (R (S))	17	1939.1***	1.10***	4.46***	0.91***
P	3	3477.6***	0.47*	0.55ns	0.19ns
SXP	6	196.8ns	0.68**	1.41***	0.71*
PXR(S)	81	319.9ns	0.17ns	0.35ns	0.17ns
Family (F(P))	40	561.0*	0.47***	0.84***	0.36ns
SXF(P)	74	439.3ns	0.19ns	0.37ns	0.39ns
Residual	1004	360.7	0.16	0.34	0.18
Series 2 (age 5 years)					
Site (S)	1	4210.1***	43.31***	135.15***	3.85***
Replication (Site) (R (S))	18	334.4**	0.39***	1.44***	0.15***
P	3	2994.7***	0.50***	1.53***	0.10**
SXP	3	389.9*	0.48***	0.64*	0.08**
PXR(S)	54	156.6ns	0.06ns	0.20ns	0.02ns
F(P)	24	928.4***	0.30***	0.49***	0.05***
SXF(P)	24	186.3ns	0.12*	0.39**	0.04***
Residual	425	143.3	0.06	0.17	0.02
Series 2 (age 7 years)					
Replication (R)	9	788.5***	5.33***	8.21***	58.20***
Provenance (P)	3	217.4ns	4.01***	10.89***	34.85***
PXR	27	199.1ns	0.61ns	1.12ns	5.92ns
F(P)	23	162.4ns	2.22***	5.44***	22.49***
Residual	195	163.3	0.43	0.95	4.48
Series 3 (age 5 years)					
Site (S)	1	100.8ns	14.01	91.09***	1.00***
Replication (Site) (R (S))	16	1101.7***	1.04***	1.69***	0.13***
P	4	611.9**	3.64***	5.75***	0.60***
SXP	8	139.9ns	1.09***	1.59***	0.17***
PXR(S)	104	136.8ns	0.11ns	0.15ns	0.01ns
F(P)	38	549.1***	0.97***	1.15***	0.15***
SXF(P)	66	160.6***	0.14ns	0.19ns	0.04*
Residual	897	173.6	0.13	0.15	0.03

Table 3. Mean squares for analysis of variance for height, diameter and volume at 5 and 7 years for three series of Nuttall oak provenance tests $^{+.+}$ ns, *, **, *** = Not significant, significant at P $\leq$ 5%, 1%, and 0.1%.

Provenance (River)	Survival (%)	Height (m)	Dbh (cm)	Volume ($dm^3.tree^{-1}$)
Series 1 (age 5 years)				
1. Western	80.1c	2.10ab	1.31a	0.247a
3. Ouachita	88.4a	2.14a	1.42a	0.195a
4. Mississippi	86.4ab	2.06b	1.34a	0.219a
6. Tallahatchie-Yalobusha Rivers	85.5b	2.04b	1.30a	0.231a
Series 2 (age 5 years				
1. Western	83.6b	1.83b	0.99bc	0.138b
2. Black-White	94.6a	1.80b	0.88c	0.097c
3. Ouachita	96.9a	1.99a	1.21a	0.184a
4. Mississippi	94.2a	1.86b	1.03b	0.131bc
Series 2 (age 7 years)				
1. Western	90.0a	4.60c	4.87c	4.36c
2. Black-White	96.0a	5.24b	6.09b	6.12b
3. Ouachita	96.3a	5.66a	6.59a	7.34a
4. Mississippi	96.4a	5.10b	6.00b	5.52b
Series 3 (age 5 years)				
1. Western	91.1bc	2.25a	2.00b	0.279b
2. Black-White	91.7c	1.92d	1.71c	0.184c
3. Ouachita	94.4b	2.13b	1.94b	0.254b
4. Mississippi	96.8a	2.01c	1.65c	0.185c
5. Red	93.1bc	2.24a	2.11a	0.314a

Table 4. Nuttall oak provenance means across three sites for survival, height, dbh and volume at age 5 and 7 years^{+}. $^{+}$ Means within a column with different letters differ at the 5% level of significance on a Duncan's Multiple Range Test.

Unfortunately, provenance performance was not consistent across series making it difficult to draw conclusions. The instability of provenance performance across the different series may be due to the fact that the provenances were arbitrarily divided and may not reflect true biological differences. A case in point is the Mississippi County, Arkansas sources which originated at the same latitude as the Black-White River, yet the selections were grouped with the Mississippi River provenance which was generally more southern. The instability of provenances may have also resulted from differences in the number and origin of families in each series. For example, the Western Region provenance in series 2 was comprised of families from Smith County only, but in series 3 it was comprised of families from Liberty and Tyler Counties in Texas and Beauregard Parish, Louisiana. Similarly, Mississippi River provenance in series 2 was comprised of

families from Mississippi County, Arkansas only, while in series 3 it includes families originating in Franklin, Richland and Tensas Parishes in Louisiana.

By comparing data from age 7 measurements of series 2 with age 5 measurements from series 1 and 3 tests located on adjacent sites in Desha County, it would appear that Nuttall oak is capable of rapid growth between 5 and 7 years. At 5 years provenances were on average 2.20 m in height, and at 7 years they averaged 5.21 m in height, an increment of more than 100% (Table 5). Similarly for volume, provenances averaged 0.280 dm^3 at 5 years and 6.014 dm^3 at 7 years, an increment of more than 20 fold. The slow growth of Nuttall oak at 5 years appears to support the observations that Nuttall oak seedlings allocate much of their growth to their root systems in the first few years and exhibit slow early growth (Taylor and Golden 2002). The results at 7 years suggest that Nuttall oak is fast growing once established.

Site	Survival (%)	Height (m)	Dbh (cm)	Volume ($dm^3.tree^{-1}$)
Series 1 (age 5 years)				
Lonoke	96.3a	2.23a	1.63a	0.236b
Sharkey	81.5b	1.78b	0.72b	0.094c
Desha	75.4c	2.23a	1.67a	0.360a
Series 2 (age 5 years)				
Lonoke	89.8b	2.18a	1.58a	0.214a
Sharkey	97.4a	1.47b	0.29b	0.009b
Series 2 (age 7 years)				
Desha	95.6	5.21	6.02	6.014
Series 3 (age 5 years)				
Lonoke	93.6a	2.38a	1.66b	0.247b
Sharkey	93.5a	1.97c	2.55a	0.349a
Desha	91.7a	2.15b	1.62b	0.199c

Table 5. Site means for survival, height dbh and volume at age 5 and 7 years[+].

[+] Means within a column with different superscripts differ at the 5% level of significance on a Duncan's Multiple Range Test.

Genotype x environment interactions

Provenance by site interactions for survival were not significant ($P > 0.05$) for all series, except series 2 (Table 3). Site by family interactions for survival were not significant for

series 1 and 2. The interactions between site and provenance were significant ($P < 0.05$) for all growth traits indicating the presence of genotype by environment interaction.

Heritabilities and trait-trait correlations

The family-mean heritability estimates and individual-tree heritability estimates were very high for all traits (Table 6), suggesting that phenotypic variation observed was due to family and individual tree effects. Generally, there were good families from all provenances. For example in series 1, the top 10 families in volume growth were composed of 5 from Western Region, 2 from Ouachita River, 2 from Tallahatchie-Yalobusha Rivers and 1 from Mississippi River provenances. This suggests that family selection will be effective in this species. This suggests that significant gains can be made through selection of the best families and the best individuals. Correlations among growth traits were moderate to high, suggesting selecting on one trait will result in an increase in the other traits. Correlations between survival with growth traits were weak for series 1 and 3, and moderate for the Desha test in series 2.

Trait	h_i	h_F	HT	DBH	VOL
Series 1 (age 5 years)					
SUR	0.04	0.41	0.13**	0.09**	-
HT	0.14	0.83		0.81**	-
DBH	0.18	0.80			-
VOL	-	-			
Series 2 (age 5 years)					
SUR	0.80	0.90	0ns	-0.01ns	0.11*
HT	0.52	0.72		0.92**	0.80**
DBH	0.08	0.22			0.90**
VOL					
Series 2 (age 7 years)					
SUR	-	-	0.51**	0.52**	0.46**
HT	-	-		0.93**	0.92**
DBH	-	-			0.94**
VOL	-	-			
Series 3 (age 5 years)					
SUR	0.33	0.81	0.16**	0.17**	0.19**
HT	0.85	0.96		0.44**	0.67**
DBH	0.56	0.95			0.83**
VOL	0.47	0.91			

Table 6. Individual tree heritability, family-mean heritability and phenotypic correlations for survival, height, diameter and volume for Nuttall oak at age 5 and 7 years[+]. [+]ns, *, **, = Not significant, significant at P $\leq$ 5%, and 1%

CONCLUSION

Nuttall oak provenances had excellent survival and growth confirming it is a good choice for planting and restoring bottomlands. The provenance and family-within provenance variation and estimates of heritability indicate that genetic improvement of Nuttall oak would be successful. Provenance effects were inconsistent, but it would appear that seed collected toward the center of the range (northern Louisiana or southern Arkansas) should be favored when purchasing wild seed. The better performing provenances tended to be from the Ouachita and Red River basins while the poorer provenances tended to be from the Black-White and Mississippi drainages. The Western sources tended to have lightly poorer survival. It must be emphasized that these conclusions are based on arbitrary provenance divisions with limited numbers of families per provenance. As with other outcrossing species there was significant tree to tree variation as evidenced by relatively high heritabilities. Therefore tree improvement and orchard establishment programs should concentrate on identifying the best individuals regardless of provenance.

ACKNOWLEDGEMENTS

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Early Performance and Genetic Parameters for Atlantic Coastal and Piedmont Loblolly Pine and Their Hybrids in the Piedmont

Paraskevi Alizoti, Bailian Li and Steve McKeand[1]

Abstract

Atlantic Coastal (C) and Piedmont (P) loblolly pine (*Pinus taeda* L.) sources and their hybrids were assessed at four years of age for height and survival in 15 test sites across five Piedmont regions (Piedmont, Upper Gulf, Blue Ridge, North East and Cold Area). Two intra-provenance (CxC and PxP) and two inter-provenance (CxP and PxC) populations were generated. Twenty polymix families represented each population. Main objectives of the study were to: (1) determine whether the inter-provenance hybrids can combine the growth of Atlantic Coastal and the cold hardiness of Piedmont sources when planted in Piedmont regions, (2) characterize the genetic architecture among and within populations across and within Piedmont regions, and (3) evaluate the stability of performance of families within populations across different environments.

The performance of inter-provenance hybrids was intermediate to that of the parental populations. When compared to the Piedmont population, which is the commonly planted source in Piedmont regions because of its cold hardiness, CxP inter-provenance hybrids exhibited significantly better height growth, with superiority ranging among 0.16% to 5.81% for height. Survival differences among populations within Piedmont regions were not significant at this age, except in the Cold Area, where significantly higher survival was found for the Piedmont population.

There were large family differences within populations for growth and survival. Genetic control for growth traits varied among populations, with stronger additive genetic control for CxP hybrids. Considerable variation was also detected for family performance for growth and stability across sites. The CxC and CxP populations were more responsive to site quality increase (measured by the test means), with a higher percentage of families having regression slopes larger than 1.0.

This early evaluation showed some promise for using loblolly pine hybrids as planting stock in the Piedmont region. With additional testing for cold hardiness, there is a potential to combine the growth of Atlantic Coastal and the adaptation to cold of Piedmont sources for planting in Piedmont regions. The CxP hybrids may perform well in milder Piedmont environments, while PxC could be more suitable for more inland and north Piedmont regions. Long-term monitoring of population performance and survival is essential, as prolonged exposure to adverse climatic conditions will provide more confidence about the results.

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Cone and Seed Insect Pest Research: The Seed Source Comparisons in 100 Tests in Arkansas and Oklahoma

Lou Anne Dill and Clem Lambeth[1]

Keywords: Seed source, regression

INTRODUCTION

Weyerhaeuser Company has planted non-local seed sources of loblolly pine in southwest Arkansas and southeast Oklahoma for nearly 30 years. In an effort to further study the benefits and risks of using nonlocal material, seed sources were compared in 100 tests (ages 4 to 22 years) planted in the region between 1974 and 1994. Many of these trials are progeny tests of nonlocal seed sources that have local seed source check lots included. They form the foundation of a land race of seed sources from east of the Mississippi River.

METHODS

Mean volume per live tree, volume per planted tree, survival, site index, sweep and ice damage of nonlocal seed sources and unimproved local seed source were evaluated by running regressions against Arkansas/Oklahoma improved material for each of these traits. Slopes and intercepts of these plots were used as indicators of general performance trends. There were 100 tests with measurement ages of 4 to 22 years.

Seed sources:

Arkansas/Oklahoma Improved (AR/OK Imp)	Seed orchard mix of 10 parents
Arkansas/Oklahoma Unimproved (AR/OK)	Unimproved check lot
North Louisiana (N LA)	Unimproved check lot
North Mississippi (N MS)	Combination of two unimproved check lots
North Carolina Improved (NC Imp)	Combination of improved parents

Traits:

- Site Index (ft – based on 25 years)
- Sweep - % trees with sweep less than 4cm (this is the deviation from a straight edge in the first 4m of the stem)
- Ice Damage - % trees with ice damage (this damage is mostly from the 2000 ice storm, probably the worst storm in recorded history for this area)
- % Survival (after establishment through latest measurement)
- Volume per Live Tree (dm^3)
- Volume per Planted Tree (dead = 0 volume)

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Early mortality was dropped when it was available. "Mortality the first year after planting is affected by several factors, which may or may not be provenance-related: e.g., seedling conditioning in the nursery, planting quality, insect damage, and moisture stress. Generally, when overall conditions are good for survival, provenance effects are small."(Lambeth, *et al.* 1984)

RESULTS

Results showed the North Carolina material was slightly straighter than the local seed source (Figure 1) but had slightly lower survival (Figure 2) (on a few sites) and slightly higher ice damage (Figure 3). Site Index (Figure 4), volume per live tree (Graphs 5 and 6), and even, volume per planted tree (Graphs 7 and 8) were clearly superior to the Arkansas/Oklahoma material. However, gain for volume per planted tree, which is a reflection of growth and survival, was lower than that for volume per live tree (Table 1).

Tests < 10-Years-Old		Tests > 10-Years-Old	
Volume Live Tree	Volume Planted Tree	Volume Live Tree	Volume Planted Tree
1.32	1.31	1.24	1.17

Table 1. Regression slopes of NC improved seed source on Arkansas/Oklahoma improved seed source in trials in SW AR and SE OK

CONCLUSIONS

The best overall performance was registered by the North Carolina coastal seed source, which has been the favored source from commercial regeneration for a number of years.

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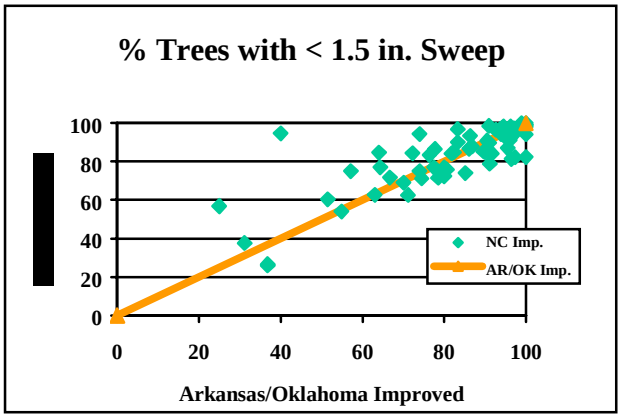

Figure 1. Regression of Sweep of NC improved seed source to the local AR/OK improved seed source in trials in southwest Arkansas and southeast Oklahoma.

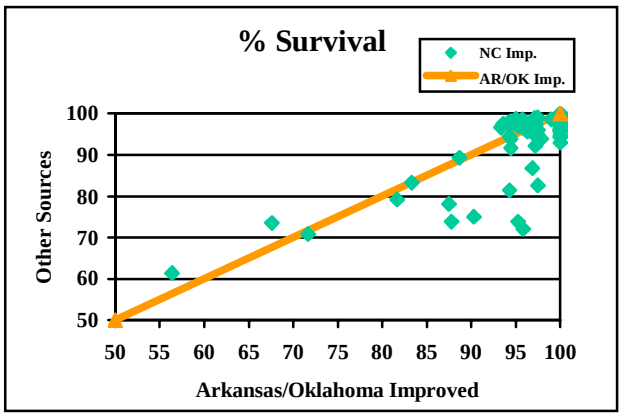

Figure 2. Regression of Survival of NC improved seed source to the local AR/OK improved seed source in trials in southwest Arkansas and southeast Oklahoma.

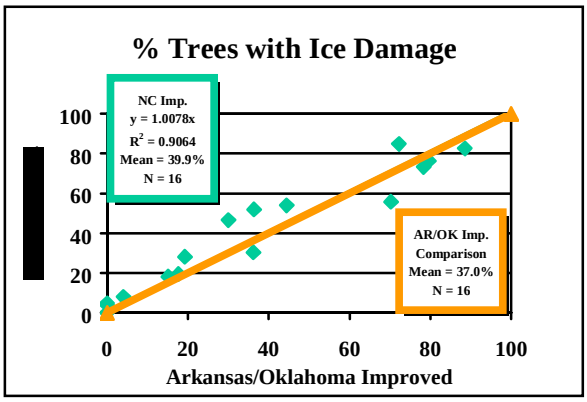

Figure 3. Regression of Ice Damage of NC improved seed source to the local AR/OK improved seed source in trials in southwest Arkansas and southeast Oklahoma.

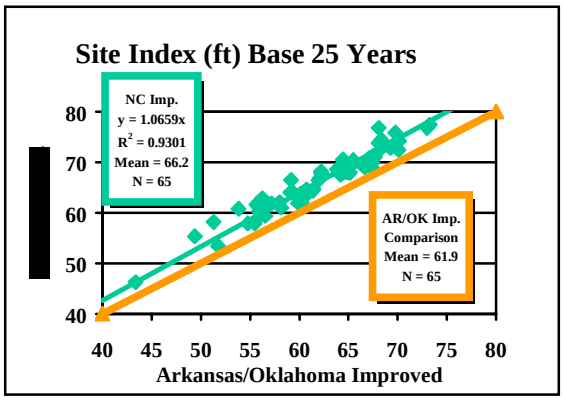

Figure 4. Regression of Site Index of NC improved seed source to the local AR/OK improved seed source in trials in southwest Arkansas and southeast Oklahoma.

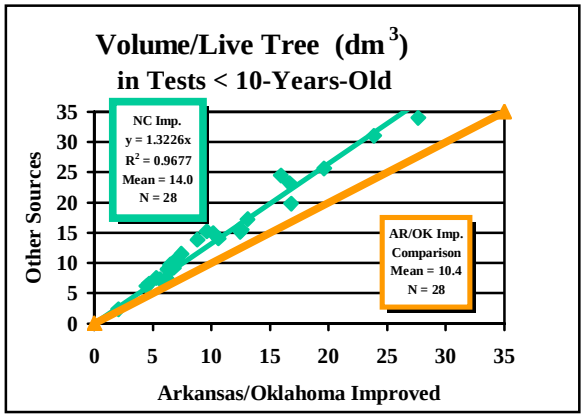

Figure 5. Regression of Volume/Live Tree of NC improved seed source to the local AR/OK improved seed source in trials in southwest Arkansas and southeast Oklahoma.

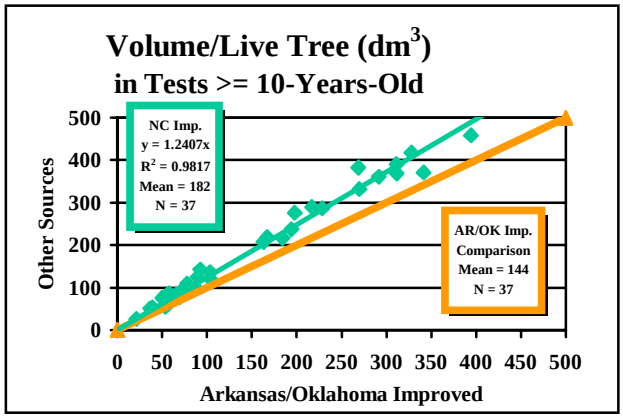

Figure 6. Regression of Volume/Live Tree of NC improved seed source to the local AR/OK improved seed source in trials in southwest Arkansas and southeast Oklahoma.

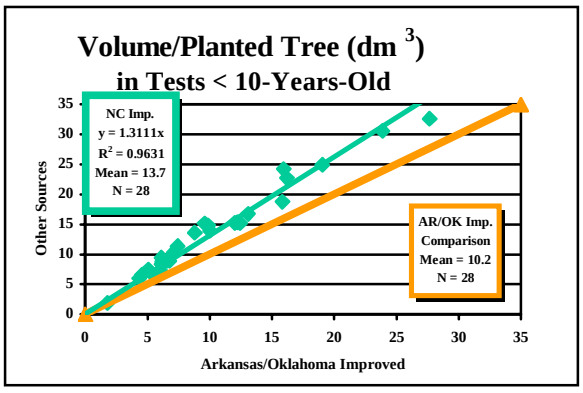

Figure 7. Regression of Volume/Planted Tree of NC improved seed source to the local AR/OK improved seed source in trials in southwest Arkansas and southeast Oklahoma.

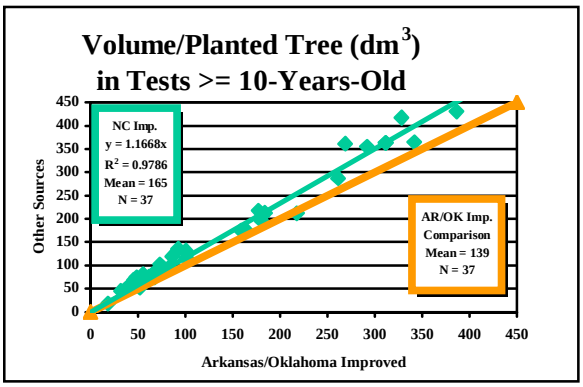

Figure 8. Regression of Volume/Planted Tree of NC improved seed source to the local AR/OK improved seed source in trials in southwest Arkansas and southeast Oklahoma.

Validation of Predicted Breeding Values for Slash Pine (*Pinus elliottii* var. *elliottii*) Using Field Trials Planted in Large Block Plots

Rodrigo Vergara[1,2], Timothy L. White[1], Dudley A. Huber[1], Barry D. Shiver[3], and Donald L. Rockwood[1]

Abstract: Predicted breeding values were validated using realized gains estimated from large-rectangular-plot field trials from the first generation breeding population of slash pine (*Pinus elliottii* var. *elliottii* Engelm.) in the Southeast. These 43 realized gain trials consisted of three types: 1) rust resistant and rust susceptible material growing in high rust hazard sites in the Best Management Practices study (5 trials), 2) material selected for growth by the Cooperative Forest Genetics Research Program at the University of Florida (19 trials), and 3) Improved and unimproved material established by the Plantation Management Research Cooperative at the University of Georgia (19 trials). All trials contained slash pine seedlots collected from unrogued or lightly rogued first generation seed orchards. Multiple regression analyses were conducted to validate predicted breeding values calculated for each seedlot considering pollen background. Observed realized gains for each seedlot were used as the dependent variable, while site variables (site index and rust hazard) along with the predicted breeding values were used as independent variables. BLP values predicted for rust resistance were reasonably accurate, and most of the known variation in rust incidence was accounted for by the predicted breeding values. Conversely, validation of BLP-predicted volume breeding values was difficult due to excessive noise in the data for individual tree volume and stand yield. The use of highly replicated medium-size rectangular plots is suggested to overcome this problem of imprecise field data from realized gain trials.

Keywords: Tree improvement, realized gains, breeding values, validation, block plots, slash pine, *Pinus elliottii*.

INTRODUCTION

The underlying breeding value of an individual is the sum of the average effects of the alleles that it carries (Falconer and Mackay 1996). Breeding values are predicted from genetic trails containing an observed sample of offspring from a given parent using the mean value of that progeny (Falconer and Mackay 1996). Thus, progeny tests provide estimations of how future offspring will perform under operational conditions (White and Hodge 1987). The estimation of breeding values is an important and useful tool in tree improvement programs, where having accurate and precise predictions is fundamental in

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making reliable decisions to maximize genetic gains (White and Hodge 1987, White and Hodge 1988, Hodge and White 1992).

The Cooperative Forest Genetics Research Program (CFGRP) at the University of Florida planted many open-pollinated (OP) and control-pollinated (CP) progeny tests in the southeastern USA in order to predict breeding values of their first-generation selections of slash pine (*Pinus elliottii* var. *elliottii* Engelm.). In 1995, volume and rust resistance breeding values for 2,491 first-generation selections were predicted using best linear prediction (BLP) and using data from about 500 OP and CP progeny tests planted from the 1960's to 1990, generally in randomized complete block designs with row plots (White *et al*. 1996).

Because volume breeding values are meant to accurately predict genetic gains for a given progeny above unimproved controls under operational conditions, it is desirable to validate these predictions using a large set of realized gain trials established with large rectangular plots. Unlike row-plot or single-tree-plot trials, large rectangular-plot trials resemble operational planting conditions where all entries compete evenly across time (Foster 1989, Lambeth *et al*. 1994). However, rectangular plots have low statistical precision (Dhakal *et al*. 1996), and many trials are needed for reliable validation of *a priori* expectations.

Slash pine breeding value predictions have not been extensively validated using large rectangular plots. However, several studies have compared average realized and expected gains, especially in rust resistance. Comparisons of realized gain against conventional predicted values (Sohn and Goddard 1979), against 1988 BLP breeding values (Hodge *et al*. 1993) and against 1995 BLP breeding values (Lopez-Upton *et al*. 2000, Vergara *et al*. in review, Vergara *et al*. in preparation) have been conducted. One in-depth validation study in slash pine using data from 175 CP row-plot and single-tree-plot trials (Dhakal 1995) found that 1988 BLP breeding values in rust resistance and volume over-estimated the realized gains by 31% and 47%, respectively. These findings were used to adjust the 1995 BLP predictions (White *et al*. 1996).

The aim of this study is to validate the 1995 BLP breeding values for rust resistance and volumes using observed rust incidence and stand yield realized gains estimated for 187 seedlot-trial combinations from a total of 43 field trials established with large block plots. The specific objectives were to: 1) Validate the accuracy of rust resistance breeding value predictions (R50s) values); 2) Validate the accuracy of individual tree volume breeding value predictions (BVVs); and 3) Assess the impact of rust hazard and site index on the accuracy of breeding values.

MATERIALS AND METHODS

Realized Genetic Gain Trials

The database for validating slash pine BLP predicted breeding values included realized gain trials planted in the Southeast with large rectangular plots of genetically improved

material and unimproved controls. The 43 trials were planted between 1977 and 1987 by the Best Management Practices study (5 BMP trials), CFGRP (19 trials), and the Plantation Management Research Cooperative at the University of Georgia (19 PMRC trials). All trials contained first-generation several types of OP slash pine progenies from unrogued or lightly rogued seed orchards (Table 1). Experimental design, age, location, site index, and rust hazard information of each trial are described by Vergara *et al*. (in review) for the BMP trials and by Vergara *et al*. (in preparation) for the CFGRP and PMRC trials.

Type of Progenies	# improved seedlots/trial	Unimproved controls	# Trials	# Reps	BreedingValue[1]	
					R50	BVV
Single OP-families (CFGRP trials)	19-22	UF checklot	3	3	43.9	12.3
	10-12	UF checklot	3	3-4	42.3	11.6
	9-10	None	4	2-4	42.4[2]	6.1[2]
Bulk seed orchard mixtures (CFGRP trials)	1-3	UF checklot and/or others	9	4-10	48.3	9.1
Mixture of 6 rust-resistant families (BMP trials)	1	Rust susceptible mixture	5	3	31.4	2.5
Mixture of 6 families + 1 OP-family (PMRC trials)	2	Unimproved bulk seedlot	19	1	39.3	15.3

Table 1. Slash pine progenies in 43 realized gain trials used to validate the 1995 BLP breeding values.

[1]Adjusted BLP-breeding values (White *et al*. 1996) in percentage, averaged across all seedlots from a specific type taking into account pollen background (R50=rust incidence breeding value and BVV=volume breeding value). [2]Value adjusted to the difference between each family and the breeding value of the family taken as the unimproved control.

Calculation of Realized Genetic Gains

Plot-level data were used to obtain least square means (LSM) for each improved seedlot (*I*) in each trial. Each record also had the LSM for unimproved value (*U*) averaged across all the controls in the trial. The final database included 187 records containing LSM for percentage rust incidence (RUST), average volume of living trees (TREEVOL), and mean annual increment (MAI, extrapolated to a per-area basis) for both improved and unimproved material. To compute RUST, data from the youngest measurement age were used, since the effect of mortality could bias the RUST estimations (Anderson *et al*. 1986, Schmidt and Allen 1997). For TREEVOL and MAI, data from the measurement age closest to rotation age were used in each trial. Additionally, the trial's rust hazard (RHAZ, estimated as RUST from the unimproved control(s)) and site index (SI, at 25 years as estimated from the data for the unimproved control(s)) were included in the final database (Vergara 2003).

Percentage of realized gain in rust resistance was calculated as the incidence on each improved seedlot, *I*, adjusted to the rust hazard level in each site, as follows. RUST on *I* was transformed to adjusted incidence (I50) using the hypothesis of proportional resistance (Hodge *et al*. 1993) through the equation I50=(*I**50)/rust hazard, where *I* was average RUST on the improved seedlot and rust hazard was the RUST value on *U*, the unimproved control. Thus, I50=(*I*/*U*)*50, where I50 was the realized rust incidence adjusted to an environment in which unimproved material would have 50% rust incidence. Therefore, an I50 smaller than 50% means positive realized gain in rust resistance. I50s were estimated only for sites with a rust hazard greater than 15% (Lopez-Upton *et al*. 1999), because low rust incidence levels have small variances (White and Hodge 1987) and because the proportional resistance hypothesis is not applicable on low-rust-hazard sites (Hodge *et al*. 1993).

Realized gains for TREEVOL and MAI were estimated as percentage gains (G_TREEVOL or G_MAI=((*I*-*U*)/*U*)*100, respectively), and as the non-percentage difference between improved and unimproved material (DIF_TREEVOL or DIF_MAI= *I*-*U* in m^3 and m^3 ha^{-1} $year^{-1}$, respectively).

Predicted Breeding Values

The first-generation R50s and BVVs currently used in slash pine were predicted in 1995 by the CFGRP using about 500 OP and CP progeny tests planted from the 1960's to 1990 (White *et al*. 1996). R50s were expressed as the predicted percentage of infection when unimproved material would have a 50% rust incidence (50% rust hazard environment); consequently, low values of R50 indicate high rust resistance. BVVs were expressed in percentage superiority above unimproved material.

To assign the correct predicted breeding value for every seedlot used in the validation, it was necessary to consider that improved seedlots (*I*) such as single families, family mixtures, or bulk-seed-orchard seedlots were obtained from wind-pollinated first-generation seed orchards. Thus, the seedlot's predicted breeding values were affected by pollen from other clones in the orchard and/or by pollen from external unselected populations. When reliable information was available, the seed orchard pollen contribution was calculated by averaging breeding values across the clones that were present at the time that the seed was collected, weighted according to the number of ramets of each clone. When information was not available, R50=50% and BVV=10% were used for the male contribution to the seedlot.

Pollen coming from external sources was considered to be undomesticated with R50=50% and BVV=0%, the same as unimproved controls (*U*). This contaminating pollen also affects the adjusted breeding value of an orchard wind-pollinated seedlot. Pollen contamination in fully productive seed orchards may be between 5% and 50% with values commonly from 20-40% in different pine species (Wang *et al*. 1960, Friedman and Adams 1985, El-Kassaby *et al*. 1989, Lai and Chen 1997). Thus, 30% pollen contamination was assumed in this study, and the computation of all adjusted breeding values reflected this value. For example, for seed from a single mother with

R50=35% and BVV=19%, in the seed orchard, the adjusted R50 would be 35% x 0.5 (mother contribution) + 50% x 0.5 x 0.7 (pollen from the seed orchard) + 50% x 0.5 x 0.3 (pollen from external sources) = 42.5%. Likewise, the adjusted BVV would be 19% x 0.5 (mother contribution) + 10% x 0.5 x 0.7 (pollen from the seed orchard) + 0% x 0.5 x 0.3 (pollen from external sources) = 13%, assuming an orchard in which the clones averaged R50=50% and BVV=10%. Henceforth, the adjusted R50s and BVVs for single-family, mixed-family, and bulkseed orchard collections are denoted simply as R50s and BVVs, respectively.

Validation of Breeding Values Using Regression Analysis

If parental BLP-breeding values are precise and accurate, offspring performance should be directly predictable by the adjusted breeding values of each seedlot evaluated in realized gain trials (White and Hodge 1989, Mrode 1996). To validate current breeding values predicted by the CFGRP (White *et al*. 1996) and to examine the influence of site index and rust hazard on the realized gains, multiple regression analyses (Rawlings *et al*. 1998) were conducted with PROC GLM in SAS (SAS 1990). Realized rust infection in percentage (I50), TREEVOL realized gain in percentage (G_TREEVOL), and MAI realized gain in percentage (G_MAI) were regressed against BVV, R50, SI, RHAZ, and the two, three, and four way interactions among those variables. Also, variables DIF_TREEVOL (TREEVOL *I*-*U* difference in m^3) and DIF_MAI (MAI *I*-*U* difference in m^3 ha^{-1} $year^{-1}$) were regressed against all variables except BVV, which was replaced by predicted deviation in volume between *I* and *U* in m^3, (DEV using age 15 data with mean individual volume = 0.108 m^3, White *et al*. 1996). Each regression using growth-related variables as dependent variables had 187 observations, one for each seedlot-trial combination from 43 trials with 146 single-family lots, 24 mixed families, and 17 bulk seed orchard collections. Regressions using I50 as dependent variable had only 140 observations, with 122 single-family lots, 10 mixed families, and 8 bulk seed orchard collections, because seedlots growing in trials with rust hazard <15% were not used in the analysis.

"Backwards elimination" stepwise regression (Rawlings *et al*. 1998) was used to choose the most parsimonious model as follows: 1) Start with the full model (including all main effects and their interactions) and drop non-significant effects of the highest order interactions; 2) Run the reduced model dropping all non-significant terms; and 3) Continue this process until all terms were significant and the model had a reasonable biological interpretation. Type III sums of squares were utilized to define non-significant effects with $p>0.1$. Adjusted R^2 (adj-R^2) was used to compare models with different number of parameters (Rawlings *et al*. 1998).

RESULTS AND DISCUSSION

Validation of R50s

The regression analysis relating the observed I50 values for each seedlot to their predicted R50s and other site factors provided a final model that included R50, BVV,

RHAZ, and the interaction BVV x RHAZ with an adj-R^2=0.386. However, BVV and BVV x RHAZ effects were not biologically interpretable, meaning that graphical analyses did not reveal any logical or important trends. So, a new backwards elimination round was conducted starting with a full model excluding BVV. The new final model I50=1.169*R50-0.1839*RHAZ was the most parsimonious explanation for I50 with an adj-R^2=0.361, similar to that obtained for the model including the BVV effects. In this new model, the general intercept was not significantly different from zero (p=0.3135). Therefore, it was dropped and the intercept changed across different levels of rust hazard, although having the same slope among those levels (Figure 1a).

In the final model, R50s alone did not adequately predict observed I50s. Rather, RHAZ was required to predict R50 in different rust hazard environments (Figure 1a). Also, the slope of 1.169 was significantly different from the expected value of 1.000 if R50s unbiasedly predict field rust resistance expressed as I50. So, the accuracy of predicting field performance by BLP R50 values is compromised by rust hazard of the site and a slight over prediction of I50 by R50. Interestingly, the most accurate predictions occurred when R50 values were near the same rust hazard level. For example, R50s between 15 and 30% predicted accurately the I50 values at rust hazard = 20% but were biased at 60%. Similarly, R50s between 60 and 70% were accurate in predicting I50s at rust hazard = 60% but biased at 20% (Figure 1b). I50 values are calculated by adjusting a given rust incidence to a 50% rust hazard environment using the hypothesis of proportional resistance (Hodge *et al*. 1993), and the accuracy of R50 predictions rests on the reliability of this hypothesis. Therefore, these results are evidence that rust resistance is not completely proportional across different rust hazard environments. The difference between predicted and realized rust incidence values (R50=31.4% and I50=21.9%) found by Vergara *et al.* (in review) in the BMP trials could be explained in part by this bias, where the R50=31.4% might be biased in predicting the I50, under predicting rust resistance by approximately 3%, since the average rust hazard was 43% in the BMP trials (Figure 1).

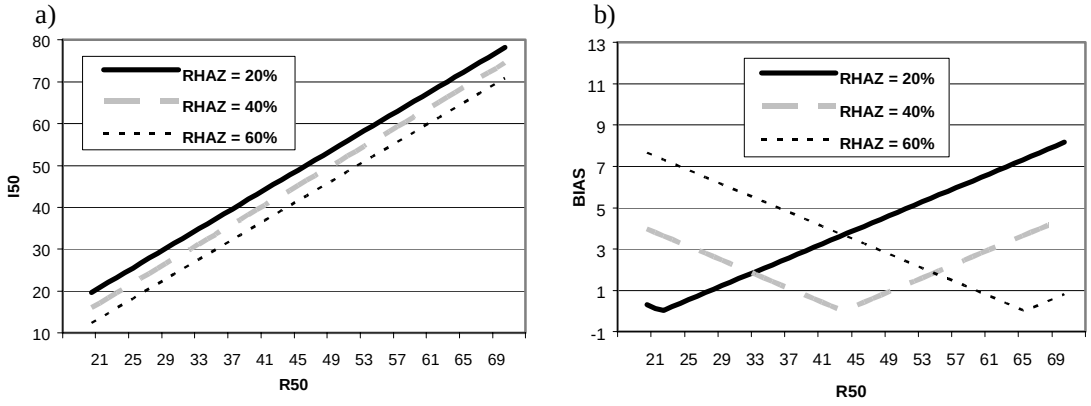

Figure 1. Relationship between realized (I50) and predicted (R50) percentage rust incidence at three levels of rust hazard (RHAZ) representing approximately one standard deviation below the mean (20%), the mean (40%), and one standard deviation above the mean (60%) rust hazard for the 43 trials: a) Regression of I50s on R50s according to I50=1.169*R50-0.1839*RHAZ (adj-R^2=0.361), b) Bias of the predictions, measured as the absolute difference between R50 and I50.

Additionally, to validate the general predicting quality of R50s, the relationship between R50 and I50 was assessed through the simple linear regression model $I50 = \beta_0 + \beta_1 * R50$. The analysis showed a moderately positive correlation between R50 and I50 ($r=0.563$). This model's low adj-R^2=0.317 indicated that the precision for predicting I50 values was low and influenced by factors other than R50s; however, the highest adj-R^2 obtained from the multiple regression analysis was 0.386, indicating that other unknown factors may be involved in rust resistance and/or that a higher experimental precision must be achieved by using more and better replicated trials. Although the I50 equation (I50=-13.18+1.29*R50) had intercept and slope significantly different from the expected values for accuracy ($\beta_0=0$ and $\beta_1=1$, respectively), the predictions were reasonably unbiased (Figure 2). I50 was slightly over predicted for R50s smaller than 45%, meaning R50 predicts that a seedlot will get slightly more rust than actually observed, and slightly under predicted for seedlots with R50s greater than 45%. In other words, at R50 <45%, R50s predict seedlots to be slightly more susceptible than observed and vice versa above 45%. However, the bias was never more than a few percent.

In general, most studies comparing predicted and realized rust incidence in slash pine have been done across different levels of rust hazard, and most of them have demonstrated that average I50 values are very well predicted by R50s, with differences always smaller than 4% (Sohn and Goddard 1979, Hodge *et al.* 1993, Lopez-Upton *et al.* 2000, Vergara *et al.* in review, Vergara *et al.* in preparation). These previous studies agree with the general results found here. Even though the 1995 R50s (White *et al.* 1996) seem reasonably accurate, adding weight to the hypothesis of inflated variances in the 1988 R50s (Dhakal 1995), further research must inspect the behavior of rust resistance at different levels of rust hazard to confirm or to correct the hypothesis of proportional resistance.

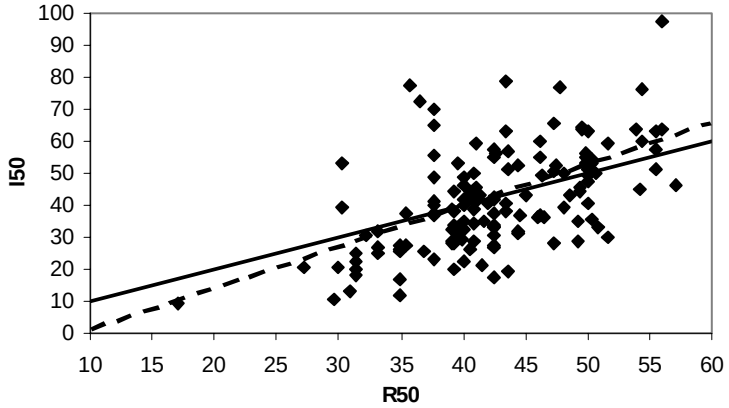

Figure 2. Comparison of observed (I50) versus predicted (R50) percentage rust incidence. The solid line represents the ideal relation between realized and predicted values with 100% accuracy. The dashed line is the actual regression of I50s on R50s according to I50=-13.18+1.29*R50 (adj-R^2=0.317).

Validation of BVVs

When G_TREEVOLs were regressed against BVV and other regressors including RHAZ, SI, R50, and all two, three, and four-way interactions, the final model including only significant terms had 12 effects including R50 and some of its interactions with a low adj-R^2=0.221. Because this model did not have a reasonable biological interpretation, a new full model was analyzed deleting R50 and all its interactions. In this new analysis, the most parsimonious model was:

G_TREEVOL=5.744*BVV + 0.0645*SI - 0.23202*BVV*SI
with an adj-R^2=0.214.

This adj-R^2 was similar to the final model obtained using all the variables, showing either that the terms that were dropped, while statistically significant, contributed very little toward explaining the variability of G_TREEVOL or that BVV and R50 were colinear in explaining the variation of G_TREEVOL (i.e., high covariance between BVV and R50).

Using the latter model, BVV predicted G_TREEVOL very well at some SIs, but not well in others (Figure 3a). To determine the slope of each regression line, the model was solved for SI=18, 21 and 24 m (approximately –1, 0 and +1 standard deviations from the mean SI of 43 trials), resulting in the following partial models;

When SI is 18 m, G_TREEVOL=1.16+1.57*BVV,
When SI is 21 m, G_TREEVOL=1.35+0.87*BVV, and
When SI is 24 m, G_TREEVOL=1.55+0.17*BVV.

The best predictions were at SI=21m, with a slope close to 1 (0.87). At low SIs (i.e., SI=19m), BVV underestimated G_TREEVOL, and at high SIs (i.e., SI=24m) BVV strongly overestimated G_TREEVOL (Figure 3b). Consistently low G_TREEVOLs for good sites, regardless of seedlot BVV, might be related to having selected trees in low SI stands in the 1950s through 1960s, as the selections may have been adapted to restricted nutritional conditions and high competition. When growing in near optimal conditions, with fertilizer, mechanical soil preparation and/or herbicide, the genetic advantages of the selected trees may not be displayed.

Another possible explanation is the scale effect of measuring gains in percentage. If there is a constant gain in TREEVOL across site indices, the percentage gain would be smaller when site indices are larger and larger in poor site indices, as is observed in Figure 3a. To test this idea, DIF_TREEVOL (TREEVOL *I*-*U* difference in m^3) was regressed against same former variables, after replacing BVV with DEV (predicted deviation in volume between *I* and *U* in m^3). In the final model, DIF_TREEVOL=0.5076*DEV (adj-R^2=0.048), SI was not significant in explaining absolute gains and therefore confirmed some scale effect in measuring gains as a percentage.

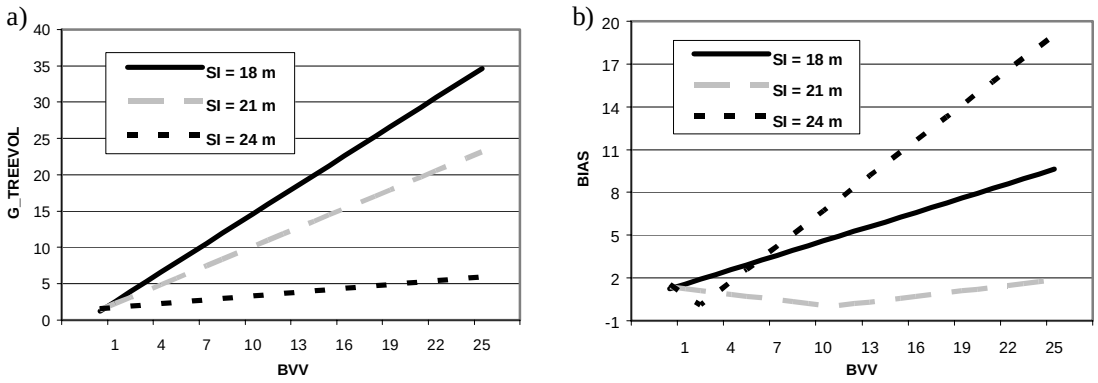

Figure 3. Relationship between realized gain in individual tree volume (G_TREEVOL) and predicted volume breeding values (BVV) at three levels of site index (SI) representing approximately one standard deviation below the mean (18m), the mean (21m), and one standard deviation above the mean (24m): a) Regression of G_TREEVOL on BVV according to G_TREEVOL=5.744*BVV + 0.0645*SI - 0.23202*BVV*SI (adj-R^2=0.214), b) Bias of the predictions, measured as the absolute difference between G_TREEVOL and BVV.

Since all regressions of realized gains in individual tree volume had low adj-R^2 values, two factors that could confound both the assessment of individual tree volume gains and the prediction of BVV through BLP should be considered: 1) since rust mortality favors individual tree volume by providing more space to the living trees, BVVs from row-plot data could be impacted by a seedlot's rust resistance and observed realized gains in block plots could also be impacted, and 2) the measurements of G_TREEVOL from the 10 to 18 years used in the analysis could confound realized gains if age affects performance.

The full model regression of realized gains in stand yield (G_MAI) on BVV and RHAZ, SI, R50, and all two, three, and four-way interactions produced an extremely low adj-R^2=0.07. As for G_TREEVOL, the DIF_MAI (MAI *I-U* difference in m^3 ha^{-1} $year^{-1}$) was regressed against the same regressors (except replacing BVV by DEV), and the adj-R^2 was again very low. Thus, these results provide little insights about the relationships among the studied variables.

Most of the variation found in G_MAI in this study is likely due to experimental noise. Realized gains in MAI were not significantly influenced by any of their most probable predictors such as BVV, R50, RHAZ, or their interactions. Mortality not due to rust infection, such as from poor microsites, plantation effects, lightning, and other causes probably greatly influenced the realized gains in stand yield. With the database analyzed here, it was not possible to validate or determine if the BVVs are accurately predicting stand yield.

Variation in microsites can greatly affect within-replication uniformity in field trials. To compare the volume performance of seedlots, progeny tests should have a replication size smaller than 0.1 ha (Matheson 1989). These realized gain trials usually exceeded this limit, and replication numbers were low. Both problems clearly contributed to the

excessive noise found in this study. We suggest trials with medium-size plots (i.e., 36 to 49 total trees, 16 to 25 measurement trees), no more than six entries per replication, and at least 10 replications to overcome these problems. However, a simulation study should be done before to estimate a sufficient design to maximize the amount of information obtained and statistical precision.

CONCLUSIONS

Validation of predicted rust resistance breeding values by comparing expected to realized rust incidence (R50 to I50) in 43 trials suggests that the 1995 R50s are reasonably accurate, although they are not very precise. Also, there is evidence that higher rust incidence is predicted by R50 in low rust hazard sites than in high rust hazard sites, which suggests that the hypothesis of proportional resistance should be revised. Most of the known variation in rust incidence can be assigned to the R50s; however, the influence of rust hazard levels should not be ignored when making new predictions.

Validation of BVVs was difficult using either individual tree volume or stand yield realized gains in large rectangular plots. BVVs were better correlated with realized gains in individual tree volume than with realized gains in stand yield. Realized gains in individual tree volume were predicted imprecisely by BVVs, with very different accuracies depending on the level of site index, being most accurate for seedlots tested in trials with average site indices (approximately 21 m) but less accurately for trails with low or high site indices.

The large amount of noise associated with stand yield made it impossible to validate volume predictions using realized gains in stand yield. This experimental error was likely due to mortality from rust infection and other sources, along with the inherent imprecision of large rectangular plots. This last issue could be improved by optimizing trial design.

ACKNOWLEDGMENTS

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Field Performance of Shortleaf Pine Half-Sib Families Though 10 Years in the Ouachita Mountains of Arkansas

John C. Brissette[1] and James P. Barnett[2]

Abstract:-- Shortleaf pine (*Pinus echinata* Mill.) seeds collected from six half-sib families were grown as both bareroot and container stock and outplanted on two sites in the Ouachita Mountains of Arkansas. Survival and growth were measured at years 1, 3, 5, and 10 after planting. Stock-type and family interacted to affect height at year 1 on one site. There were no other interactions through 10 years. Container stock performed consistently better than bareroot at each interval measured. There were differences among half-sib families for diameter and height on both sites for some measurement periods. Family ranks for total height early in the experiment correlated with 10 year performance on both sites.

Keywords: Artificial regeneration, container, bareroot, half-sib family, *Pinus echinata*.

INTRODUCTION

In the Ouachita and Ozark Mountains of Arkansas and Oklahoma, shortleaf pine (*Pinus echinata* Mill.) is usually planted on south- and west-facing slopes where soil moisture is often limited. One advantage cited for container-grown southern pine seedlings compared to bareroot planting stock is better performance on harsh, drought-prone sites (Barnett and Brissette 1986). Superior survival and growth of container seedlings on dry sites has been shown for longleaf pine (*P. palustris* Mill.) (Amidon et al. 1982, Goodwin 1980), and for loblolly pine (*P. taeda* L.) (Goodwin 1980, South and Barnett 1986).

Shortleaf pine has not been compared in as many stock-type trials as the other southern pines. Ruehle et al. (1981) compared container and bareroot shortleaf pine in an ectomycorrhizae study on two sites in the Ouachita Mountains. The container seedlings survived better and grew taller and to a larger diameter than the bareroot stock on the site with the best soil moisture characteristics. However, on the site with the drier moisture regime, the container seedlings were poorer than the bareroot seedlings in both survival and growth. The authors concluded that the container seedlings, which were considerably smaller, required more intensive site preparation to perform as well as the larger bareroot stock. However, when we matched seedlings with less disparity in shoot size, survival and growth were greater for container seedlings on two sites in the Ouachita Mountains for up to 10 years after planting (Barnett and Brissette in press). We attributed the initial superior field performance of the container seedlings to larger root systems and overall better shoot-to-root balance.

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The use of half-sib family seed collections, rather than seed orchard mixes, for reforestation has been increasing across the southern United States for several years. One advantage of maintaining family identity is that variation in rates of seed germination and seedling growth in the nursery can be reduced. Thus, nursery culture can be tailored to individual families, or groups of families, to grow seedlings of higher quality than possible when a bulked seed collection is sown. Similar advantages in growth may be achieved when outplanting is by family as well. While the practice of producing and planting seedlings by family has become commonplace for loblolly pine, it is used little for the other southern pines.

This experiment was designed to test whether stock-type and half-sib family interact to affect field performance of shortleaf pine seedlings planted on typical reforestation sites in the Ouachita Mountains. We recently reported results of this study through 10 years with an emphasis on the stock-type comparison (Barnett and Brissette in press). This paper focuses on half-sib family performance.

MATERIALS AND METHODS

Seeds were collected at the USDA Forest Service Ouachita and Ozark seed orchard near Mount Ida, Arkansas. The seed orchard has three blocks, each representing a geographic subregion. One block contains selections from the eastern half of the Ouachita National Forest, another from the western portion of that forest, and the third from the Ozark National Forest. Trees in the orchard are numbered such that the 100s are east Ouachita, 200s west Ouachita and 300s Ozark. Seeds for this study were from two families in each block, selected based on survival in a number of progeny tests (Brissette and Barnett 1989).

The staff of the Weyerhaeuser Company Magnolia Forest Regeneration Center in southwest Arkansas grew the bareroot seedlings. Seeds were sown April 16, 1986, with a Weyerhaeuser-designed precision seeder that accommodated sowing individual lots of seeds in each row along a nursery bed. Seeds from an orchard mix were sown in the two outside rows with the families sown on the interior of the nursery bed. Families were assigned randomly to the rows and re-randomized for seven replications along the bed. Nursery cultural practices, such as fertilization and root pruning, were applied based on the best judgment of the nursery manager. Top pruning was not done. Seedlings were hand lifted February 2, 1987, and kept in cold storage (approximately 3 ºC) until they were planted.

Container seedlings were grown at the USDA Forest Service laboratory in Pineville, Louisiana. The containers were hand seeded April 23, 1986, into Ray Leach "Stubby" cells filled with a 1:1 peat-vermiculite medium. The volume of each cell was approximately 115 cm^3. One tray held 98 cells at a density of about 500 per m^2. Each tray was sown with a single family, with five replications of each family. Following germination in a greenhouse the trays were moved to raised benches outside in full sunlight where the seedlings were grown and hardened off.

The two planting sites were typical of sites artificially regenerated on the national forests in Arkansas at that time. One site was on the Magazine Ranger District of the Ozark National Forest and the other on the Winona Ranger District of the Ouachita National Forest. Both sites are physiographically in the Ouachita Mountains, in areas gently rolling to rolling with shallow, rocky soils. They were clearcut and site prepared as part of a commercial operation. Both sites were ripped following the contours during site preparation. Container seedlings were planted December 9 and 10, 1986 on the Magazine and Winona sites, respectively. Bareroot stock was planted February 11, 1987 at both sites.

A split-plot, randomized block experimental design with six blocks was used at each site. At both sites, slope position was the blocking variable. Stock type was in the whole plots with 5 degrees of freedom for error (dfe). Family and stock type by family interaction were in the subplots with 50 dfe. Because the families followed in this study were purposely, not randomly, chosen from among those available in the seed orchard, family was considered a fixed effect. Experimental units were 25 tree row plots. Analysis of variance (ANOVA) was used to test for the stock type x family interaction, and for stock type and family main effects for a number of response variables. When the family main effect was significant ($p=0.05$), family means were separated by the Waller-Duncan Bayes Least Significant Difference (BLSD) procedure (Petersen 1985). Data analysis was separate for each site.

The experiment was evaluated at 1, 3, 5, and 10 years after planting and a range of response variables were measured as the trees grew. Survival was measured at each inventory. Ground line diameter was measured at year 1. Diameter breast height (dbh) was measured at years 5 and 10. Total height was measured at years 1, 3, and 5. Quadratic mean diameter (QMD) and plot basal area (BA) were determined at year 10. In addition, families within each stock type x block combination were ranked for each response variable and correspondence between early performance (years 1 and 3) and 10-year performance was tested using Spearman's Coefficient of Rank Correlation (Steele and Torrie 1980).

RESULTS AND DISCUSSION

When outplanted, the bareroot seedlings were larger, both in root collar diameter and shoot length, than the container seedlings but the container stock had greater mean root volume and a more favorable shoot-to-root ratio than the bareroot seedlings (Brissette and Barnett 1989). Overall survival exceeded 94 percent at both sites the first year after planting and remained at 90 percent or higher through year 10 (Barnett and Brissette in press). Neither the stock type x family interaction nor the main effect of family influenced survival at any measurement period. However, the stock type main effect was significant on the Winona Ranger District planting site at each measurement. Container-grown seedlings consistently survived better than bareroot seedlings on that site.

With respect to the measures of growth, diameter and height, there was a stock type x family interaction in total height one year after planting at the Winona site. The family

tallest when grown in containers was the second shortest from bareroot (Brissette and Barnett 1989). There were no other interactions between stock type and family through 10 years.

The main effect of half-sib family was significant for diameter and/or height at each site at some measurement periods. On the Magazine site, families differed in diameter at years 1 and 5, while there were no differences in diameter among families at the Winona site (Tables 1, 2). At year 1, the diameter of Family 322, the largest at the Magazine site, was 12 percent greater than Family 219, the smallest, and 5 percent more than the overall mean.

Site	Response variable	Year	Stock Type x Family	Stock Type	Family
Magazine	Survival	1	0.5	0.1	0.3
		3	0.3	0.2	0.6
		5	0.1	0.3	0.5
		10	0.1	0.3	0.7
	Diameter	1	0.1	0.002	0.02
		5	0.9	0.03	0.02
		10	0.9	0.4	1.0
	Height	1	0.3	0.0002	0.2
		3	0.9	0.008	0.0001
		5	0.7	0.008	0.0001
	Basal Area	5	0.3	0.02	0.054
		10	0.3	0.2	0.7
Winona	Survival	1	1.0	0.005	0.9
		3	0.9	0.03	0.9
		5	0.9	0.02	0.9
		10	0.6	0.048	0.9
	Diameter	1	0.7	0.0006	0.2
		5	0.9	0.004	0.1
		10	0.9	0.0001	0.2
	Height	1	0.048		
		3	0.7	0.004	0.03
		5	0.9	0.003	0.054
	Basal Area	5	0.8	0.008	0.2
		10	1.0	0.0001	0.4

Table 1. Significance levels (probability of greater F) from analyses of variance for survival and growth of shortleaf pine half-sib families grown as bareroot and container stock and planted on two sites in the Ouachita Mountains.

After 5 years, the difference between largest (202) and smallest (342) families at Magazine was 15 percent, while diameter of the largest family was still 5 percent greater than the overall mean. Container seedlings had larger diameters at years 1 and 5 at Magazine and years 1, 5, and 10 at Winona (Table 1, Barnett and Brissette in press).

There were differences among families in total height at both sites (Tables 1,2). At the Magazine site, family affected height at both 3 and 5 years after planting. The difference between the tallest and shortest families was 14 percent both times. At year 3, Family 202 was 7 percent taller than the mean, while at year 5 the tallest (still 202) was 6 percent taller than the mean. At the Winona site, differences among families were only significant at year 3. Family 115, the tallest, was 12 percent taller than Family 322, the shortest, and 5 percent taller than the overall mean. Through the 10 years of the study, container seedlings were consistently taller than bareroot seedlings at both sites (Table 1, Barnett and Brissette in press).

Family	Planting Site				
	Magazine				Winona
	Ground line diameter, yr 1 ---(mm)---	DBH, yr 5 ---(cm)---	Total height, yr 3 ---(cm)---	Total height, yr 5 ---(m)---	Total height, yr 3 ---(cm)---
103	5.4 bc[1/]	3.6 abc	162 bc	3.2 a	113 abc
115	5.7 ab	3.8 ab	170 ab	3.2 a	120 a
202	5.3 bc	3.9 a	173 a	3.3 a	118 ab
219	5.2 c	3.7 abc	159 cd	3.2 a	114 abc
322	5.8 a	3.5 bc	152 d	2.9 b	107 c
342	5.4 bc	3.4 c	152 d	3.0 b	109 bc
Mean	5.5	3.7	161	3.1	114

Table 2. Significant ($p < 0.05$) differences among half-sib families on two planting sites in the Ouachita Mountains. [1/] Within a column, values followed by the same letter are not significantly different.

Plot basal area integrates both survival and growth performance. Basal area was determined at years 5 and 10, and family had no effect on BA at either site (Table 1). Container seedlings had greater BA than bareroot stock at both sites at age 5 and at Winona at age 10 (Table 1, Barnett and Brissette in press).

Spearman rank correlation coefficients were significant at both sites. At the Magazine site, family rank for year 1 total height correlated with family rank for year 10 BA (Table 3). At Winona, family ranks between year 3 total height and both 10 QMD and BA at year 10 were highly correlated (Table 3).

Site	Early Performance	Age 10 performance	
		QMD[1/]	Plot Basal Area
Magazine	Ground line dia, yr 1	-0.025 (0.9)	0.002 (1.0)
	Total height, yr 1	0.271 (0.1)	0.425 (0.01)
	Total height, yr 3	0.028 (0.9)	0.314 (0.06)
Winona	Ground line dia, yr 1	0.164 (0.3)	0.166 (0.3)
	Total height, yr 1	0.253 (0.1)	0.295 (0.08)
	Total height, yr 3	0.662 (<0.0001)	0.619 (<0.0001)

Table 3. Spearman rank correlation coefficients (r) and probabilities (in parentheses) for half-sib family performance at years 1 and 3 with performance at year 10 on two sites in the Ouachita Mountains. [1/] QMD = quadratic mean diameter of the surviving trees.

CONCLUSIONS

If quality seedlings are planted, either bareroot or container-grown shortleaf pine will survive well on prepared sites in the Ouachita Mountains. Interactions between half-sib family and stock type might impact initial field performance on some sites. However, it is unlikely that such interactions will persist. Consequently, over the long term, family performance is consistent regardless of how seedlings are produced. Container stock generally performs better than bareroot, especially on poorer sites where both survival and growth are less.

Among half-sib families, the largest in diameter or tallest in height have a 12-15 percent advantage over the poorest performers and a 5-6 percent edge over the mean. Spearman rank correlation coefficients indicate that once established there is some stability in relative performance among families.

These results suggest that there are enough differences among half-sib families of shortleaf pine that maintaining family integrity during seedling production will improve overall quality of planting stock. In addition, some improvement in growth can be achieved by selecting the best performing families. On the other hand, planting diverse families will not result in a marked reduction in overall field performance. All appropriate management alternatives should be considered when planning to artificially regenerate sites in the Ouachita Mountains with shortleaf pine.

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Slash and Loblolly Pine Productivity on Reclaimed Titanium Mined Lands in Northeast Florida

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Abstract:--Titanium mining often occurs on forestlands that previously supported productive pine plantations. The productivity of reclaimed mined lands is uncertain, based on observations that tree height on reclaimed lands is less, perhaps due to compaction. This paper summarizes early results from field studies initiated in December 1999 on unmined and reclaimed mined lands near Green Cove Springs, Florida, using two slash (*Pinus elliottii var. elliottii)* and loblolly (*Pinus taeda*) pine progenies, three fertilizers (granulite, diammonium phosphate, and a 16-4-8 blend at 36lbs N/acre (40.3 kg N/ha)) each, one herbicide (glyphosate), one dry humate addition, one mycorrhizal inoculation, subsoiling, and various combinations thereof to determine silvicultural treatments that optimize pine productivity on reclaimed mined lands. Mined sites had significantly higher survival but shorter trees than the unmined lands. A combination of treatments, including pines genetically superior for growth and disease resistance, may afford the opportunity for sustaining pine productivity on titanium mined lands.

Keywords: *Pinus elliottii var. elliottii*, *Pinus taeda*, fertilization, subsoiling, pest incidence, humate

INTRODUCTION

Titanium mining is a major industry in Florida, but its impact on site productivity is difficult to assess (Poulin and Sinding, 1993). Current extraction techniques include dredge and satellite mining that removes existing vegetation and pushes approximately 25 cm of topsoil into berms. The dredge or other heavy equipment removes the underlying 1.5 to 4.5 m of soil. The material is macerated into a homogenized slurry mix containing humic matter, soil residuals, humates, water, and the titanium ore and other select minerals. This process generates tailings that are used to partially backfill the excavation site and a black turbid wastewater created when the organically rich spodic (Bh) horizon is masticated prior to the separation process. The water is pumped into treatment ponds where the suspended solids often form a very stable colloidal suspension. Carefully monitored additions of H_2SO_4 or $FeCl_2$ lower the pH of these waters to 4.5 whereupon the humates precipitate allowing the purified water to be returned to the mining operation or discharged into steams if it conforms to state standards. The humate material is allowed to dry before the previously removed topsoil is redeposited over the dried pond prior to its reclamation with trees or other suitable vegetation.

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Mined soils are problematic for companies reclaiming mined lands. These soils are typically homogenized by the mining and retain virtually none of the soil structure that benefits vegetation through favorable bulk density, soil strength, and porosity. Competition for water and nutrients is especially critical on reclaimed sites due to the upheaval of the spodic horizon and the degradation of the stored topsoil. For reclamation practices to be effective, water and nutrients must be available in the soil, and roots must be able to penetrate the soil.

Current reclamation policy includes the redeposition of topsoil to the mined area. Unfortunately the bermed topsoil is often degraded due to the effects of long-term storage. Additionally, soil compaction by heavy equipment has detrimental effects on the soil (Zabowski et al., 1996). Topsoil amendments can effectively enhance the productivity of these sites.

Silvicultural practices can assuage some of these limitations. Controlled drainage reduces the nitrogen and phosphorus export as well as the total suspended solids lost from a watershed (Amatya et al., 1998). Conserving the total organic carbon concentration in the soil preserves electrical conductivity levels. Bedding improves survival by increasing root zone aeration, reducing compaction, and prolonging water availability. Trees grown on infertile soils respond prominently to fertilization and to increased nutrient allocation afforded by weed control. The enhanced growth potential and disease resistance of genetically superior stock may augment the productivity of reclaimed lands.

Mathey (2001) evaluated the productivity of slash pine as old as 16 years on reclaimed titanium mined lands. Tree heights were significantly higher on unmined sites, although there was a high degree of variability. While nutrients were not significantly different between unmined and reclaimed sites, pH was slightly higher on reclaimed sites (5.1 vs. 4.6). Furthermore, compaction was greater on the reclaimed sites and was suspected as the primary reason for reduced height. Subsoiling is positively related to the growth of pines in the southeastern United States (Slay et al., 1987). The cost of subsoiling reclaimed mined sites would be minimal since mining companies are typically replete with the necessary equipment.

The purpose of the field studies reported here was to determine the silvicultural and genetic treatments, or combination of treatments, that optimize the productivity of pine planted on reclaimed mined lands in northeast Florida.

MATERIALS AND METHODS

Approximately 13km south of Green Cove Springs, Florida, unmined and reclaimed sites in close proximity were paired to minimize topographic variations. Unmined sites were not disturbed by any mining activity or affected by additional humate. All reclaimed sites had undergone reclamation at roughly the same time interval prior to planting and did not have any wetland inclusions.

Studies 1 and 2 were planted on reclaimed satellite mined and unmined lands on poorly drained, low nutrient spodosols (Hurricane and Leon series, CRIFF group D) endemic to

the flatwoods of northeast Florida. Study 1 involved three treatments (C, G2, and D2 in Table 1) replicated three times on paired unmined and mined sites according to a randomized complete block design, with six more treatments in one replication. In Study 2, four of the same nine treatments were replicated three times in a randomized complete block design on an unmined site and twice on a paired mined site with adjacent non-subsoiled and subsoiled blocks, five of the nine treatments were only in one replication, and three humate treatments were also included in one replication in the subsoiled block. Study 1 was planted in mid-December 1999 on beds prepared up to three months earlier, Study 2 in mid-January 2001 on beds that had settled for 4 to 6 months. Loblolly progeny 1-1313 was planted after the slash pine progeny 164-58 (selected for fast growth and resistance to fusiform rust). Each gross treatment plot had 6 beds at 11 feet (3.4m) by 11 trees at 5 feet (1.5m), for a planting density of 792 trees/acre (1,955 trees/ha). Three fertilizers were evaluated: granulite, a 5-3-0 organic derived from anaerobically digested sewage sludge, diammonium phospate (DAP, 18-46-0), and a 16-4-8 blend with balanced micronutrients (Mg, Mn, Cu, and S). The fertilizers were manually broadcast over the beds eight weeks after planting in amounts calculated to provide 36lbs/acre of nitrogen (40.3kg/ha). Weed control using 4% glyphosate (Roundup) was implemented in May 2000 and 2001. The second year fertilizer treatments for Study 1 were broadcast in March 2002. A 0.35% aluminum humate amendment was also broadcast. The mycorrhizae treatment was three ectomycorrhiza (*Pisolithus tinctorius*) tablets per planting hole prior to closure.

Treatments **CHP** and **CHHP** (Table 1) were established in dredge mined areas to determine any significant difference with satellite mining. Dredge mining techniques had exclusively been performed until recently when satellite mining became more efficacious. The **CHP** site is identical to the control plots installed over the satellite-mined areas. The **CHHP** site had a preponderance of humate material close to the surface of the topsoil.

Tree height and survival were measured annually in June. Pitch canker, fusiform rust, and insect incidences were also noted. In July 2001 and 2002, several additional measurements were performed. Topsoil depth (horizons A and E) above the spodic horizon or reclaimed humate layer was measured at 15 random locations on each unmined and reclaimed site with a trenching shovel and meter stick. Soil strength was measured with a soil penetrometer at 10 random samples from a spot located between beds in each location. Groundcover was assessed as vegetation type and percentage cover at 10 one square meter plots randomly located between beds for each location and year. Height, survival, and soil data including topsoil depth, soil strength, and percent vegetative cover were subjected to ANOVA using GLM and Duncan's tests in SAS to determine significant treatment differences. Pearson's correlations were also calculated.

Treatment	Study 1		Study 2		
	Unmined\ No	Mined\No	Unmined\No	Mined\No	Mined\Sub
C	3	3	3	2	2
G0R	1, *1*	1, *1*	1, *1*	1, *1*	1, *1*
G0H					1
G2	3	3	3	2	2
D0R	1	1	1	1	1
D0H					1
D2	3	3	3	2	2
B0R	1	1	1	1	1
B0H					1
B2	1	1	1	1	1
H0		1		1	1
M0	1	2	1	1	1
CHP				1	
CHHP				1	

Table 1. Number of replications (66 tree gross plot, 36 tree net) of silvicultural treatments (C= bedding only, **G** = bedding + granulite, **D** = bedding + DAP, **B** = bedding + blend with micronutrients, **M** = bedding + mycorrhizae, **H** = bedding + humate, **CHP** = bedding + humate pond, **CHHP** = bedding + humate pond with extra humate; **R** = herbicide; **0** = broadcast at planting, **2** = rebroadcast in year 2) applied to slash (loblolly in italics) pine in the unmined and mined components of Studies 1 and 2 with (Sub) and without (No) subsoiling.

RESULTS AND DISCUSSION

Tree height was greater on unmined sites than on reclaimed locations in both studies (Table 2). In Study 1, land types, treatments, and land type*treatment interaction were significant (Type and Treatment $p<.0001$ at $\alpha=.05$, Type*Treatment $p<.0010$ at $\alpha=.05$). In Study 2, no differences were detected between subsoiled and non-subsoiled sites, but significant effects of land types, treatments, subsoiling*treatment, type*treatment, and type*treatment*subsoiling were observed ($p<.0001$ at $\alpha=.05$).

Conversely, survival was higher on mined sites (Table 2). In Study 1, spring drought stress and probably J-rooted seedlings reduced survival at the unmined site. Much mortality occurred before May 2000, and entire rows within treatment plots were dead by the end of the first year. There were significant effects due to land types, treatments, and land type*treatment interaction ($p<.0001$ at $\alpha=.05$).

Survival in Study 2, reflecting the influence of another spring drought following planting, was more varied than in Study 1, but still higher in the reclaimed sites (Table 2). Not surprisingly, the subsoiled site had the best survival by alleviating the detrimental effects of soil compaction on early establishment. Future survival trends should be more revealing. Roots elongate through the soil by growing through larger pore spaces or

enlarging pores smaller than their own diameter. Land types, subsoiling, treatments, subsoiling*treatment, type*treatment, and land type*treatment*subsoiling were significant ($p<.0001$ at $\alpha=.05$).

Treatment	Study 1 – 3 years				Study 2 – 2 years					
	Unmined\No		Mined\No		Unmined\No		Mined\No		Mined\Sub	
	H	S	H	S	H	S	H	S	H	S
C	3.71	48.1	2.47	82.4	1.15	80.5	1.15	80.5	1.77	93.1
G0R	3.05 *3.93*	30.6 *86.1*	2.76 *2.34*	80.6 *97.2*	1.75 *1.85*	66.7 *80.6*	1.04 *1.46*	75.0 *97.2*	.88 *.99*	91.7 *94.4*
G0H									1.24	97.2
G2	4.03	66.7	3.15	92.6	2.25	72.2	1.18	81.9	1.04	93.1
D0R	3.76	30.6	2.41	91.7	2.19	61.1	1.20	66.6	1.64	91.7
D0H									1.60	83.3
D2	3.81	61.1	2.81	79.6	2.14	50.0	1.36	54.2	1.36	76.4
B0R	3.90	8.3	2.69	91.7	2.47	22.2	1.45	55.6	1.53	80.8
B0H									1.24	86.1
B2	3.41	58.3	3.00	83.3	1.80	36.1	1.23	50.7	1.36	54.2
H0			2.72	91.7			.96	88.9	1.10	97.2
M0	3.40	16.7	2.69	87.0	1.48	63.9	1.38	81.9	1.62	75.0
CHP							NA			
CHHP							NA			
Mean	3.79 A	50.6B	2.76B	86.4 A	1.93A	60.9B	1.26B	73.3A	1.36B	83.6A

Table 2. Height (H in m) and survival (S in %) by silvicultural treatment and species (loblolly in italics) in Studies 1 and 2.

Pest incidences were relatively unimportant in both studies. Virtually no pitch canker was noted, fusiform rust was evident only in Study 1, and insect incidence, primarily tip moth, tended to be higher on mined sites. Fusiform rust in Study 1 at three years exceeded 10% in only the **C**, **G2**, and **D2** treatments on the unmined site. Insect damage was notably higher on the non-subsoiled mined site, with average incidence still less than 20%, but surpassing 60% in the **G0R** treatment at three years.

All treatments except **C** and **M0** included fertilizer. The generally poor soils associated with pine flatwoods negatively influence the allocation of biomass and the aboveground net primary production (ANPP) (Gower et al. 1994). The redistribution or addition of available nutrients on the unmined sites through weed control and/or fertilization should increase the ANPP and LAI due to the increase in biomass or a shift in the present biomass from belowground to aboveground. The diminished growth seen on the reclaimed site is likely caused by an externality, such as compaction, not present on the unmined site.

Where nutrient resources are likely to become limiting to growth, fertilizers and vegetative control are often complementary (Goncalves, 1997). The availability of such nutrients is critical to the productivity of pine trees in north central Florida (Polglase et al., 1992). The initial effectiveness of fertilizer treatments can be reduced by the presence of weeds and hardwoods, and weed control measures may be required to ensure successful pine seedling establishment. Future measures may not be necessary as the crown coverage begins to shade out and suppress competition (Goncalves, 1997). Fertilization prior to canopy closure is most likely to increase future volume because trees in the early stages of development depend on the soils where nutrients are limiting (Miller, 1981).

Penetrometer measurements confirmed suspicions about the reclaimed sites, as soil strength increased with soil depth (Figure 1). Soil strengths also differed significantly between unmined and reclaimed sites in Studies 1 and 2 ($p<.0001$ at $\alpha=.05$). Root elongation decreases as soil strength increases. Compaction usually increases both bulk density and soil strength. Root growth in moist soils is generally limited by bulk densities ranging from 1.45kg/cm^3 in clays to 1.85kg/cm^3 in loamy sands (Brady and Weil, 1999). The effects of tractors and other heavy equipment are especially damaging if the soil is wet when trafficked. Subsoiling can reduce the soil strength and density as related to resistance to penetration, but the effects typically last less than 18 months on sandy soils.

Vegetative cover was significantly less on reclaimed sites in both studies ($p<.0001$ at $\alpha=.05$). Presumably, the storage of the topsoil in berms decreased viability of autochthonous plant life. Plant species on the unmined sites included saw palmetto, gallberry, blackberry (*Rubus spp.*), grasses (various *Poaceae* genera), and various berries (*Vaccinium spp.*). Patchy outcroppings of grasses, etc., only appeared on the reclaimed sites after the wind had dispersed seeds from neighboring unmined tracts This effect made the application of herbicide less problematic, if not unnecessary. Furthermore, correlations between the percentage of cover and soil strength (Table 3) support assertions regarding survival of seedlings on the reclaimed sites and compaction.

The depth of the topsoil layer in Study 2 varied from 24 to 49cm on the unmined to 25 to 49cm on the reclaimed sites (Figure 2). Study 1 had slightly more variation with the unmined site ranging from 20 to 45cm, but the reclaimed depths were from 13 to 49cm. The topsoil harbors most of the biological activity. For reclaimed sites to equal unmined sites in productivity, at least 30cm of topsoil should be redistributed over the area after mining.

The pedigrees of the slash and loblolly progenies used in these studies provide some opportunity to speculate about how genetic variability in these species may influence productivity. Slash progeny 164-58 has breeding values of 23 and 25 for volume and fusiform rust resistance, respectively. As these breeding values are exceeded by only some 20 other open-pollinated progenies that may be publicly available for northeast Florida, use of less superior planting stock for replanting mined lands could result in less growth and more fusiform rust (Vergara et al., 2003) by slash pine than what has thus far

been observed in these two studies. Loblolly pine progeny 1-1313 has a Piedmont origin, however, and may consequently underestimate loblolly productivity, especially when intensive silviculture is practiced. Open-pollinated progenies of Gulf Coast or Florida origin have the potential to increase loblolly productivity by up to 15 and 30%, respectively, in northeast Florida (D. Huber, personal communication).

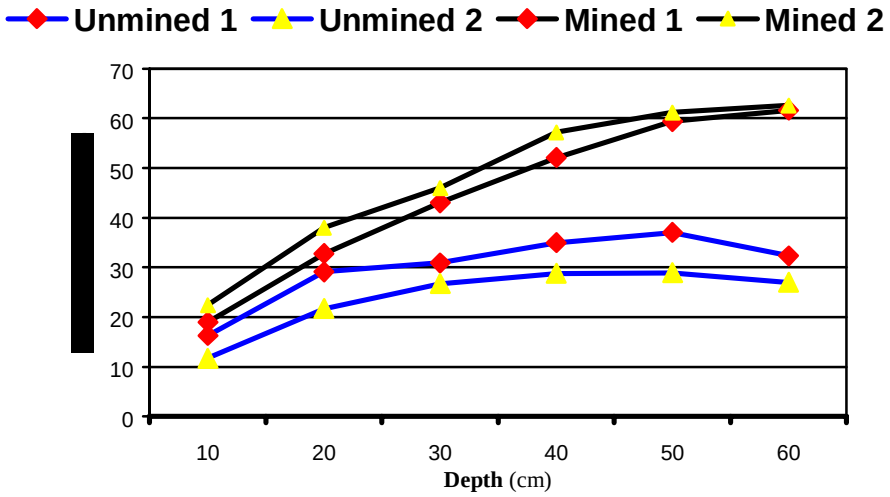

Figure 1. Soil strength with depth for Studies 1 and 2 unmined and reclaimed sites.

Study	Soil Depth (cm.)					
	10	20	30	40	50	60
1	-.44*	-.44*	-.64*	-.67*	-.67*	-.65*
2	-.12	-.18	-.72*	-.62*	-.64*	-.52*

Table 3. Pearson correlations between percent cover and soil strength at six soil depths in Studies 1 and 2. (*significant at α=.05)

CONCLUSION

Current recommendations derived from Studies 1 and 2 are preliminary. Reclaimed sites had higher survival but shorter trees. Subsoiling increased survival and should be considered for reclaimed lands. Nutrient deficiencies common to the flatwoods may be overcome through proper site and species selection, appropriate fertilization, and/or reallocating existing nutrients through the application of herbicides. Clearly, compaction limits the productivity of reclaimed sites, although there is no way currently to reclaim these areas without the use of heavy machinery. Reclaimed sites have the potential for productive forestry through the application of these methods over time.

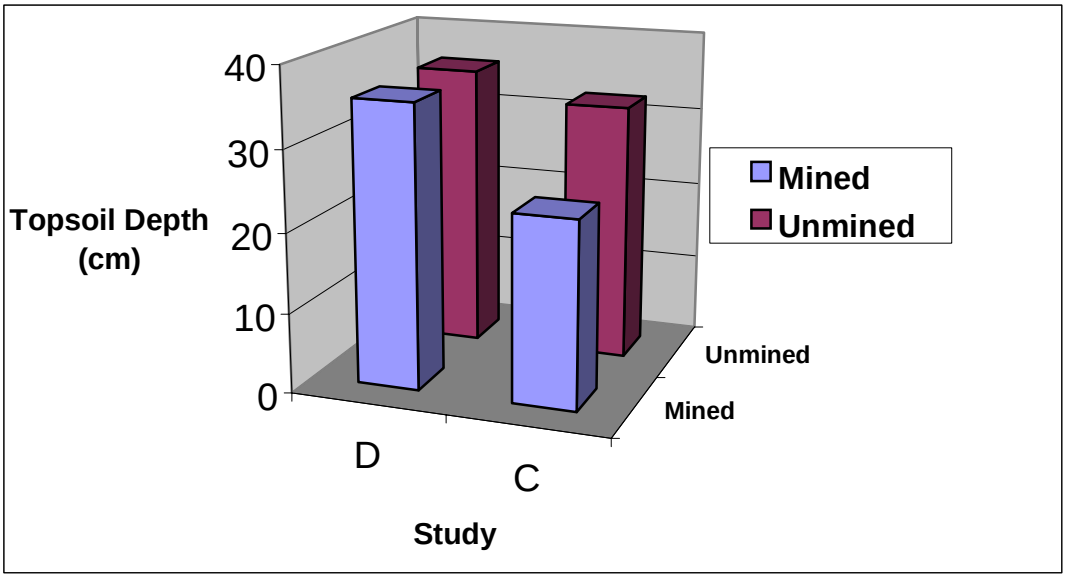

Figure 2. Topsoil depths for Studies 1 and 2 unmined and reclaimed sites.

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We acknowledge general assistance and support from Iluka Resources Inc., assistance and materials by Rayonier Forest Research Center, significant participations by Ted Goodman of Iluka and Tom Fox of Rayonier, reviews of slash and loblolly pine breeding values by Early McCall and Dudley Huber, and assistance with study establishment from Mark Torok and Richard Cardellino.

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Performance Effects of Outside Pollen on Seed Orchards In and Out of the Genetic Source Area

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Abstract

Contamination by outside pollen sources is recognized as a serious problem that reduces the genetic gain obtained from open-pollinated seed orchards. A mitigating tactic is to locate an orchard outside the source area into a region with higher performance, e.g. Virginia source clones established in the more southerly, and high performing, Atlantic Coastal source region. Previous studies have emphasized estimation of the degree of contamination. This study sought to quantify the impact on realized gain from two Virginia source orchards, one located in the Virginia source area, and another located in Georgia. Field performance of progeny from controlled crosses using an orchard pollen mix were compared to open-pollinated progeny. Contamination by outside pollen resulted in a significant increase in volume growth for the Georgia progeny, but no significant effect on the Virginia orchard progeny. Outside pollen resulted in decreased performance in straightness ratings for both orchards, with the negative effect more pronounced with the Georgia orchard progeny.

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Transgenic Loblolly Pine Trees from Diverse Elite Families

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Abstract

Loblolly pine (*Pinus taeda* L.) has been the focus of genetic improvement for nearly 100 years because of the value of this species to the forestry industry. The application of gene transfer technology to loblolly pine improvement has been limited by the regeneration of transgenic tissue into plants. We have developed gene transfer systems that allow the regeneration of trees after the transformation of embryogenic cultures from a large number of genetically diverse families.

Genetic transformation was achieved by biolistic and *Agrobacterium*-mediated techniques. Biolistic transformation efficiency was increased by identifying the optimal target using secondary somatic embryogenesis and by determining the long-term effects of tissue culture manipulations. Improvements to selection and the tissue culture system facilitated the production of stable transformants from 72% of the cell lines attempted from 15 elite families, with an escape rate of less than 1%. Molecular analysis of transgenic trees produced from biolistic transformation found that 36% of the trees had three inserts or less. Transgenic trees produced by biolistics have exhibited normal morphology for up to five growing seasons, to date.

An *Agrobacterium*-mediated transformation system was developed for loblolly pine using tissue culture and selection procedures of the biolistic system. *Agrobacterium tumefaciens* has been used to produce transgenic trees of clones from elite loblolly families, as well as clones of *P. radiata* and *P. taeda* x *rigida*. Genomic blot analysis of *Agrobacterium*-transformed somatic embryos is ongoing. Field tests with *Agrobacterium*-transformed loblolly and the hybrid loblolly have been established each year since 2001. The efficiency of the *Agrobacterium* transformation system has made it possible for ArborGen to scale-up for high-throughput gene testing in a conifer. Transgenic trees have been produced with genes for lignin modification, accelerated growth, and flowering control.

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Genetic Analysis of a Disease Resistance Gene from Loblolly Pine

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Abstract: Rapid advances in molecular genetics provide great opportunities for studies of host defense mechanisms. Examination of plant responses to disease at the cellular and molecular level permits both discovery of changes in gene expression in the tissues attacked by pathogens, and identification of genetic components involved in the interaction between host and pathogens. Expression of specific proteins, which is one type of defense mechanism, may offer the host a weapon to protect it from invasion of pathogenic attack. Recently, we have isolated a novel antimicrobial protein gene (PtAMP) from loblolly pine during our gene screening effort. Studies of molecular characterization show that the PtAMP gene shares limited similarity with previously reported antimicrobial proteins in the amino acid sequences, but it contains some common features, e.g. DNA sequences and protein structure, with those antimicrobial proteins. The function of this novel antimicrobial gene has been analyzed at the *in vitro* and *in vivo* levels. Antimicrobial assay data showed that the purified PtAMP protein has strong inhibitory activities against a variety of pathogenic bacteria and fungi. Furthermore, the gene for the PtAMP was transferred into the tobacco genome via *Agrobacterium*-mediated transformation. Ectopic expression of the PtAMP protein in transgenic tobacco plants confers resistance to bacterial and fungal phytopathogens. Our data suggest that the PtAMP gene has the potential through genetic manipulation to protect plants from a wide range of plant pathogens. Analysis of its function provides further understanding of plant defense mechanisms in loblolly pine.

Keywords: Loblolly pine, disease resistance, antimicrobial protein, transgenic plant, forest biotechnology

INTRODUCTION

Due to continuous exposure to microbial pathogens, animals and plants have evolved various highly effective mechanisms to fend off microbial invaders. A widespread defense strategy in many living organisms involves the production of small peptides (i.e., proteins) that exert antimicrobial activity (Broekaert et al. 1997). Well-known examples of antimicrobial peptides (AMP) are the cecropins that accumulate in the hemolymph of many invertebrates in response to injury or infection and the magainins that occur in the

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skin and in the gastrointestinal system of amphibians to protect them from microbial invasion. Another class of antimicrobial proteins is formed by the cysteine-rich peptides, which in contrast to cecropins and magainins, have a complex disulfide bond-stabilized three dimensional folding pattern. Defensins, occurring in mammalian, insect and plant systems, are a major group among cysteine-rich antimicrobial peptides (Broekaert et al. 1995). In general, those defensins share some common features in their structural and functional properties, suggesting that this class of AMP peptides acts on molecules and/or cell structures common to a wide range of microbial organisms.

Cysteine-rich antimicrobial peptides play an important role in host defense against infectious agents. This type of protein exhibits basic and small molecules, and often possesses a broad spectrum of inhibitory activity against several major taxonomic groups of microbes. As products of single genes, antimicrobial peptides can be synthesized in a swift and flexible way. Also, because of their small size they can be produced by the host with a minimal input of energy and resources. More importantly, the simple genetic bases make it amenable for genetic manipulation and easier to develop antibiotic-like biotechnological products.

Unlike animals, plants do not have an innate immunity system. However, the antimicrobial proteins in plants form the defense system similar to the innate immunity in animals. It is believed that antimicrobial proteins are deployed by plants and play an important role in certain stages of plant development when a plant or plant tissue is under vulnerable conditions. In general, plant seeds are especially rich in antimicrobial proteins, and the level of antimicrobial proteins is several fold higher compared to the tissues such as leaves and flowers from a developing plant (Wang et al. 2001) as they could contribute to improved seed production and seed germination. Plant response to pathogen infection is known to result from the activation of a specific cellular program on the part of the plant and is frequently associated with a certain degree of cellular damage (Huang et al 1998). This finding was also supported by an early observation, inhibition of the fungus (*Cronartium fusiforme*) by loblolly pine (Jacobi, 1982). Resistance of plant callus to infection has been attributed to antimicrobial metabolites exuded by the host tissues (Campbell et al. 1965). In a similar manner, tissue culture is an active process, triggered in response to environmental and phytohormonal clues, that is also accompanied by a certain level of cell injury. We have observed that in addition to pathogen infection, the expression of various defense-related genes has been found to be activated during in vitro tissue culture (Huang et al 1998).

As part of an ongoing study of genetic mechanisms of host resistance in loblolly pine, we have begun a characterization of antimicrobial properties present in the tissues of loblolly pine. Several plant proteins with antibacterial or antifungal activity have been reported, but to date none has been reported from a gymnosperm. Using molecular biological approaches, we have recently isolated a loblolly pine gene encoding an antimicrobal protein (PtAMP). Based on the protein sequence, this AMP represents a novel type of plant antimicrobial peptide; however, it contains AMP conserved domains and multiple disulfide bridges and can therefore be considered as the counterpart of cysteine-rich AMPs from animals. Thus, the PtAMP may be a potent inhibitor of the growth of a wide

range of microbial pathogens. In order to determine the antimicrobial feature of the protein, this gene has been cloned and expressed in *E. coli* and in transgenic plants. In this report, we demonstrate that the PtAMP gene from loblolly pine expressed a novel antimicrobial protein, which has strong inhibition against a broad group of pathogenic microorganisms including bacteria and fungi. The PtAMP gene was able to contribute an enhanced disease resistance while expressing in the target plants. We believe that the PtAMP can be used to engineer plants to confer effective and durable resistance in plant against microbial pathogens.

MATERIALS AND METHODS

Plant materials and tissue culture

Loblolly pine (*Pinus taeda* L.) seeds were aseptically germinated *in vitro*. Then the shoot apices were excised from these germinating seedlings and placed on a modified LePoivre medium (LP) for callus induction using a modification of the method developed by Aitken-Christie et al. (1988). Thus, both the callus derived from these young seedlings and seedling tissues (i.e. control) from the same seed source were used in parallel for the studies of gene expression.

Gene identification and cloning

Total RNA was isolated from both apical meristematic cultures and normal seedling tissues (i.e., controls) according to Huang and Tauer (1996). A cDNA library representing all expressed genes in the callus cultures of loblolly pine was constructed in a λ-ZAP vector (Stratagene, La Jolla, CA) using the poly(A) RNAs prepared from the total RNA. In order to identify the special group of genes expressed in the treated tissues, analysis of RNA (i.e. transcripts) differential display was performed using the differential display kit (Genhunter Corp., Nashville, TN). Similarities and differences in gene expression were uncovered by comparing the transcripts in the callus cultures to those from controls. Thus, distinguishing DNA fragments of co-migrating cDNA bands were separated through DNA sequencing gel electrophoresis and differential display (DD) fragments were excised from the differential display gels. Finally the resulted DD cDNA fragments were confirmed by reverse northern blotting analysis.

Several selected distinguishing cDNA fragments were used as the probes to screen the cDNA library of loblolly pine callus to obtain a full-length cDNA of the gene of interest. To recover the cDNA inserts, the pBluescript phagemids were excised with ExAssist helper phage and the host strain *E. coli* XL-1 Blue (Stratagene, La Jolla, CA). The nucleotide sequences were determined using thermal cycle sequencer with dye terminators and the ABI automatic sequencer (Applied Biosciences, Foster City, CA). Sequence analysis was performed using the MacVector program (Oxford Molecular Group). Similarity analysis to known sequences and to expressed sequences tag (EST) in GenBank was assessed by the BLAST program of the National Center for Biotechnology Information (NCBI) (Bethesda, MD).

Bioassay for antimicrobial activities

For the antimicrobial assay, the PtAMP protein was expressed in vitro and purified with affinity chromatography. The microplate-reading method was used to determine antimicrobial activities of the recombinant PtAMP protein against a selected group of bacteria and fungi as described by Jin (2001). The inhibitory effect was assessed by calculation of the 50% inhibition concentration (CI_{50}) of the PtAMP protein to each of the bacterial and fungal strains.

Functional studies of the gene in transgenic plants

For the construction of a plant transformation vector, the cDNA coding for the PtAMP protein was cloned into the plasmid pBI121 (Clontech), and the gene was placed under the control of the CaMV35S promoter. This gene fusion construct was introduced into the strain LBA4404 of *Agrobacterium tumefaciens*, which was then used for transformation of tobacco leaf explants according to the leaf-disc transformation method (Horsch et al. 1985). Transgenic tobacco plants were recovered in vitro and confirmed to contain the PtAMP gene using Southern blot analysis or PCR DNA amplification with the PtAMP specific primers. T_1 plants of transgenic tobacco were subjected to bioassay for plant protection. Non-transformed tobacco plants served as controls. All plants were inoculated with one of the two virulent strains of *Pseudomonas syringae* pv *tabaci* AT81 and AT2004 by infiltration under aseptic conditions. After inoculation, plants were kept at the appropriate humidity. Disease symptoms of those plants were evaluated at 4 and 12 days after infection.

RESULTS AND DISCUSSION

The cDNA library from loblolly pine callus was screened with the selected differential display cDNA fragments and several interesting cDNA clones were obtained. Sequence analysis indicated that one of the identified cDNA clones encodes a cysteine-rich protein, although its nucleotide sequences did not show a high degree of homology with any known genes in the Genbank database. Cysteine rich in this protein is an important feature as many other antimicrobial peptides isolated to date contain numerous cysteine residuals, which contribute a high stability to the proteins by forming disulfide bridges. Thus, this cDNA clone, named PtAMP (as isolated from loblolly pine, *Pinus taeda*) was chosen for further characterization.

First, we cloned the PtAMP gene into the expression vector pET30C+ in order to heterologously express the AMP protein *in vitro*. In general, any recombinant proteins can be effectively expressed in the model system, *E. coli* cell, which also facilitates subsequent purification of the target protein. However, expression of this antimicrobial protein in *E. coli* cells was a challenging task due to the nature of toxicity of the AMP protein to the microbial host. After testing a variety of expression systems, an *in vitro* expression system based on a combination of bacterium and bacterial phage was identified, which allowed us to isolate an adequate amount of the AMP protein. We also

identified the critical point in the growth phase of the host cells, which permitted the host cells to synthesize an optimal amount of the AMP protein before the host cells were inhibited by the toxic protein. Subsequently, the expression product was purified using affinity chromatography.

Purified PtAMP protein was tested *in vitro* for inhibitory activity against a variety of microorganisms. Preliminary results, utilizing a highly pathogenic strain of *Pseudomonas (Ralstonia) solanacearum* (a vascular pathogen that causes severe wilting and eventually death) indicated that the purified PtAMP protein had very strong inhibitory effect to bacterial growth. As shown in Fig. 1, the viability of the bacterial cells was 55.1% when *P. solanacearum* was grown in a medium supplemented with 3 ug/ml of purified PtAMP protein. Antimicrobial assay was also carried out with six fungal phytopathogens, five bacterial phytopathogens, and baker's yeast. Very strong inhibition (IC50 <10 ug/ml) was observed against six microorganisms and strong to moderate inhibitions against all other microorganisms tested (Table 1). However, the PtAMP protein was not toxic to plant cells (data not shown) when we conducted toxicity assays with both loblolly pine and tobacco cells *in vitro*. The addition of 100 ug/ml of purified PtAMP protein to plant cell suspensions showed no effect on plant cultures, which was evidenced by no decline in cell viability and proliferation rate.

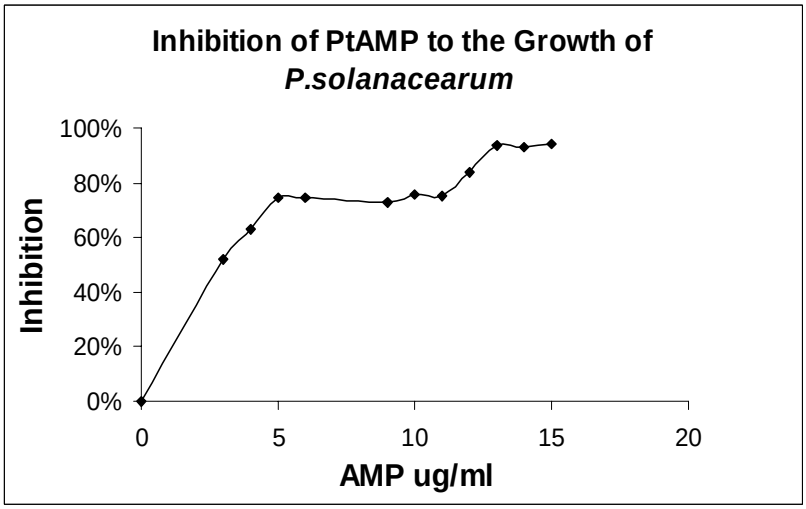

Figure 1. Bactericidal effect of the PtAMP protein on Pseudomonas solanacearum. The inhibition dynamics of various concentrations of the PtAMP on the pathogen grown *in vitro*.

In order to test *in vivo* expression of the antimicrobial gene in target plants, two expression vectors were constructed, containing the PtAMP gene and other regulatory elements such as enhancer sequences. The gene fusion was used to transform the

Bacteria or fungi	IC_{50} (ug/ml)*
Clavibacter michiganesis	<2
Pseudomonas syringae	<3
Pseudomonas solanacearum	<3
Fusarium oxysporum	<2
Collectotrichum obiculare	21
Saccharomyces cerevisiae	30
Thielaviopois basicola	75

Table 1. The PtAMP protein was tested for antimicrobial activity with a range of bacterial and fungal pathogens. Microbial growth inhibition was measured at the protein level (microgram per milliliter) when 50% of inhibition (IC_{50}) was observed.

leaf-disks of tobacco via the *Agrobacterium*-mediated transformation system. The transformants were selected by their resistance to the selection agent (kanamycin) and transgenic tobacco plants were regenerated from shootlets by transferring to a root-induction medium containing kanamycin (10 ug/ml). Thus, fertile transgenic plants were obtained, which appeared normal in phenotype but were confirmed to possess intact copies of the PtAMP gene by standard Southern blot analysis (data not shown). In the first assay, we tested in vitro antimicrobial activity against virulent strains of *Pseudomonas syringae* pv *tabaci* which causes wild-fire disease on tobacco plants. PtAMP protein was partially purified from the PtAMP-expressing plants and the extract from the transgenic plants was used in the in vitro antimicrobial assay. The expressed PtAMP protein from transgenic plants clearly inhibited bacterial growth (Fig. 2), whereas extracts from the control plant (non-transformed) showed no inhibition to the growth of bacteria (data not shown). This result also suggests that the recombinant PtAMP protein from transgenic plants was folded correctly and had its full function. To determine the effect of disease resistance, the plant expressing PtAMP and non-transgenic control plants were grown under aseptic conditions. Following this, the plants were inoculated by infiltration with two highly pathogenic strains of *Pseudomonas syringae* pv *tabaci* AT81 and AT2004. Seven days after infection, disease symptoms such as chlorsis and necrosis appeared at the inoculation sites of control plants but not on transgenic plants (data not shown). Fourteen days after infection, the control plants showed severe disease symptoms and eventually died with severe leaf wilting and stem rot after 21 days (data not shown). In contrast, little necrotic lesion was observed on leaves of PtAMP-

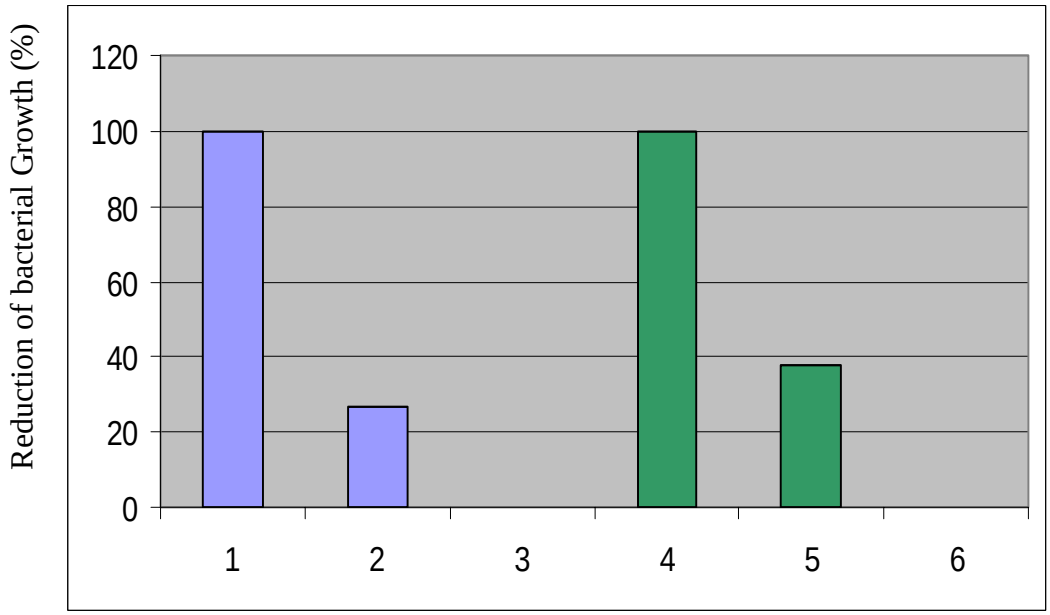

Figure 2. Inhibitory activity of the plant extract from the transgenic tobacco plant expressing the PtAMP. Plant extract was added into bacterial growth medium and bacterial growth rates were determined after 15 hours of incubation. Samples 1 and 2 represent the relative growth of *Pseudomonas syringae* pv *tabaci* PTBR2004, and samples 4 and 5 for the growth rate of *Pseudomonas syringae* pv *tabaci* PT81. Samples 2 and 5 were grown in the medium containing the plant extract from the transgenic plant, and samples 1 and 4 were grown in the medium containing the plant extract from non-transformed plant.

transgenic plants challenged by the same pathogenic organisms (data not shown). Taken together, the results clearly suggest that overexpression of the PtAMP gene in the host plants confers an enhanced host resistance to *Pseudomonas syringae* pv *tabaci* AT81 and AT2004, two major pathogens of tobacco.

CONCLUSIONS

In summary, the PtAMP gene appears to be a novel antimicrobial gene from loblolly pine and encodes a small protein with the molecular size of 11.7 kDa. Purified PtAMP protein shows strong antimicrobial activities against both fungal and bacterial phytopathogens but no toxicity to plant cells. Analysis by Southern blot with loblolly pine genomic DNA indicated that the PtAMP gene belongs to a single or low copy gene family. When its expression pattern was investigated by Northern blot analysis, expression of the PtAMP gene had strong correlations with the status of seed damage and attack of phytopathogens. Thus, the results from this study suggest that the PtAMP protein has great value for engineering disease-resistance in crop plants.

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Light Quality Effects on Germination and Conversion of Southern Pine Somatic Embryos

S. A. Merkle[1], B. L. Upchurch[2], P. M. Montello[1], X. Xia[1], and D. R. Smith[3]

While induction of somatic embryogenesis for the major southern pines and production of somatic seedlings from these cultures has been reported, major bottlenecks remain that prevent the adaptation of somatic embryogenesis for mass cloning of these trees. These bottlenecks include culture initiation, embryogenic culture capture, continued culture proliferation, somatic embryo development, maturation, germination and conversion to somatic seedlings. Each of these bottlenecks must be overcome for somatic seedlings to be delivered for planting in large numbers, at an acceptable cost and, perhaps most importantly, on a predetermined timetable. One of the most serious bottlenecks standing in the way of industry-wide adoption of this technology is the production of vigorous somatic seedlings from somatic embryos produced by the cultures (i.e. the maturation, germination and conversion steps).

The objective of the experiments described here was to determine the effects of light quality on maturation, germination and conversion of loblolly pine (*Pinus taeda*), slash pine (*P. elliottii*) and longleaf pine (*Pinus palustris*) somatic embryos and on early growth of the resulting somatic seedlings. Evidence of a synergistic effect between light quality and high CO_2 on in vitro growth was reported in a patent by Tisserat et al. (2000), although this work did not involve pine somatic seedlings.

In a first, preliminary experiment, six slash pine lines were generated following a protocol based on that of Smith (1996). Briefly, cultures, which were maintained by serial transfer on semisolid EDM6 medium (Smith 1996), were proliferated in liquid EDM6 medium for three weeks, then cultured on EMM2 medium (Smith 1996) in the dark until they reached the early cotyledonary stage. Then, embryos were cultured for 6 weeks on Pre-germination Medium (Smith 1996), followed by 6 weeks on Germination Medium (Smith 1996), under different light treatments provided by either standard cool white fluorescent bulbs at full-strength or with 1 or 2 layers of shade cloth, or by LEDs, using a Percival E30-LED Plant Growth Chamber. LEDs supplied pure red (670 nm) wavelengths. In this experiment, somatic embryos cultured under cool white fluorescent light with no shade cloth began germination (i.e. radicle elongation) earlier than other treatments, but those cultured under red light ultimately had the highest germination frequency after 100 days (57% versus 48% for cool white). In addition, embryos germinated under red light developed green hypocotyls and cotyledons, while those germinated under cool white fluorescent light had red hypocotyls and cotyledons.

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For the second experiment, somatic embryos from 5 loblolly pine lines and 1 slash pine line, produced using the same protocol described above, were cultured under cool white fluorescent light (90 $\mu mol \cdot m^{-2} \cdot sec^{-1}$) and red light at two intensities (24 $\mu mol \cdot m^{-2} \cdot sec^{-1}$ and 59 $\mu mol \cdot m^{-2} \cdot sec^{-1}$). Red light at both intensities gave higher overall germination frequencies (63-65%) compared to the cool white fluorescent light (25%). Furthermore, both red light treatments allowed germination of somatic embryos from two clones that failed to germinate under cool white fluorescent light. Germinants under both red light treatments had longer tap roots than those germinated under cool white fluorescent light and those germinated under the higher intensity red light had significantly more first order lateral roots.

In the final experiment, mature loblolly, slash pine and longleaf pine somatic embryos, produced as described above, underwent 3 weeks of treatment on Pre-germination Medium under either red or blue (470 nm) light. Embryos were then transferred to germination medium, with half of the embryos coming from the red light environment maintained under red light, while the rest were transferred to blue light. Embryos initially incubated on Pre-germination Medium under blue light were divided between blue and red light treatments when transferred to Germination Medium. A continuous cool white fluorescent light treatment was also included as a control. After 6 weeks, the embryos cultured under red light throughout had the highest germination and conversion percentages (76% and 48%, respectively), and those cultured under blue light throughout the lowest (25% and 15%, respectively). Reciprocal transfers (red to blue, blue to red) gave intermediate results, although embryos cultured on Pre-germination Medium under blue light, followed by culture on Germination medium under red light had similar germination and conversion rates to those cultured continuously under red light. Cool white light was inferior to red light for germination of somatic embryos, but allowed a higher frequency of epicotyl elongation. However, very large standard errors for the light quality treatment effects, probably due to the inclusion of 3 species in the experiment, made conclusions regarding statistical significance of the treatments difficult to interpret.

Preliminary experiments combining elevated light intensity (up to 330 $\mu mol \cdot m^{-2} \cdot sec^{-1}$) with elevated CO_2 (up to 1300 ppm) indicated that raising these two variables may produce more vigorous somatic seedlings once they are transferred to potting mix, compared to the standard treatments (100 $\mu mol \cdot m^{-2} \cdot sec^{-1}$ and ambient CO_2, 300 ppm). Our future plans call for combining light quality and elevated CO_2 during in vitro culture to test for their effects on somatic embryo germination and conversion.

ACKNOWLEDGEMENTS

The work reported here was funded by the Georgia Traditional Industries Program in Pulp and Paper (TIP3). We wish to thank Steve Wann (International Paper Co.) for advice on design of experiments, personnel at the Georgia Forestry Commission's Flint River Nursery for supplying material for initiating slash and loblolly pine cultures and Dana Nelson and Larry Lott (USDA Forest Service) for supplying material for initiating longleaf pine cultures.

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Loblolly Pine Karyotype Using FISH and DAPI Positive Banding

M. N. Islam-Faridi [1,2], C. Dana Nelson [2], T. L. Kubisiak [2], M. V. Gullirmo [1], V. H. McNamara [1], S. Ramakrishnan [1], H. J. Price [1] and D. M. Stelly [1]

INTRODUCTION

The pines *(Pinus, 2n = 2x =* 24) include many commercially important timber species. *Pinus* spp. have 12 pairs of chromosomes, with 10 or 11 pairs of long metacentric chromosomes and one pair of short sub-metacentric chromosomes (Sax and Sax 1933). The pines have been studied extensively with conventional cytological techniques (Mergen 1958; Borzan and Papes 1978; MacPherson and Filion 1981; Schweizer 1980; Hizume et al. 1990). Molecular cytology, *in situ* hybridization (ISH), coupled with conventional cytological techniques can provide more accurate information about genomes (Heslop-Harrison 1991; Leitch and Heslop-Harrison 1992; Leitch et al. 1992). Well-spread metaphase chromosomes that are free of cell walls and cytoplasmic debris are a prerequisite for ISH. Since the chromosomes of pine are extremely large, well-spread metaphases are very difficult to obtain (Doudrick et al. 1995; Jacobs et al. 2000; Schmidt et al. 2000). We report a modified somatic chromosome preparation technique that was used to improve chromosome spreading and morphology in loblolly *(Pinus taeda* L.) and slash *(P. elliottii* Englm.) pines. Fluorescent ISH (FISH) was then used to locate 18S-28S ribosomal, 5S ribosomal, and telomeric DNA sites in these plant species, and to facilitate the development of karyotypes for each.

MATERIALS AND METHODS

Plant Material: Seeds from an open-pollinated loblolly pine clone (LSG-62, kindly provided by Dr. Tom Byram, Texas Forest Service) were treated with 1% hydrogen peroxide (H 2 O 2) to break dormancy and then germinated on moist filter paper in petri dishes at 24C in the dark. Likewise, seeds from an open-pollinated slash pine clone (LA-11) were germinated, except with treating to break dormancy.

Slide Preparation: Healthy roots, 0.06 cm to 1.5 cm long, were excised and pretreated in 0.15% colchicine (Sigma, P-9754) for 7 h at room temperature in the dark and then fixed in 3:1 ethanol (95%) – acetic acid. The roots were treated enzymatically as described by Jewell and Islam-Faridi (1994). The digested root tip was macerated on a cleaned slide in 3:1 ethanol-acetic acid and then squashed under a cover glass in 45% acetic acid. The slides were stored at –80C.

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Probe DNA and Nick Translation: Whole plasmid with 18S-28S (and 5S rDNA) insert was labeled by nick translation using biotin-14-dATP (BioNick Labeling System, Invitrogen Inc.) and digoxigenin-11-dUTP (Dig-Nick Translation Mix, Roche). The *Arabidopsis* type telomere repeat ((TTTAGGG)n , about 300bp, kindly provided by Dr. Tom McKnight ,Texas A&M University) insert was labeled by BioNick labeling system (Invitrogen, Inc.).

In situ Hybridization: A standard *in situ* hybridization technique was followed as published by Islam-Faridi and Mujeeb-Kazi (1995) and Islam-Faridi et al. (2002). The probe hybridization sites were detected with FITC conjugated avidin-dcs (for biotin labeled probe) and/or rhodamine conjugated sheep anti-dig (Roche) followed by rhodamine conjugated anti-sheep (Jackson Immuno Research Lab, Inc.) (for dig labeled probe). The preparations were counterstained with DAPI and mounted with Vectashield (Vector Laboratories Inc., CA).

Microscopy: Digital images were recorded from an Olympus AX-70 Epifluorescence microscope with suitable monochrome filter sets (Chroma Technology, VT), using a 1.3 MP Sensys (Roper Scientific) camera and the MacProbe v4.2.3 digital image system (Applied Imaging, Santa Clara, CA). Images were processed with MacProbe v4.2.3 and Adobe Photoshop 6.0.

Karyotype Analysis: Each chromosome arm was measured three times from its extremity to the centromere after zooming 400% to minimize the error using Optimas v6. The length measurements yielded the following values: a) total chromosome length, b) centromeric indices, c) relative length, d) position of FISH signals and e) luminescence of FISH signals. Six well spread cells were chosen for karyotype analysis from each line. In each cell, chromosomes were numbered arbitrarily from 1 to 24, and paired on the basis of 18S-28S rDNA, 5S rDNA, telomere repeat FISH signals and DAPI positive bands.

RESULTS AND DISCUSSION

Our modified chromosome preparation yielded well-spread metaphase chromosomes in loblolly and slash pines (Fig. 1). A relatively high mitotic index was observed for both pine species. A single root yielded as many as 731 mitotic divisions, mostly metaphases. Chromosome morphology was sharp and clear after *in situ* hybridization, as evidenced in the following photomicrographs. Strong DAPI positive bands occurred in various patterns near or around the centromeres of 23 chromosomes (11 pairs + one) in loblolly pine and 22 chromosomes (11 pairs) in slash pine. Distinct DAPI positive bands appeared at both sides of the primary constrictions (i.e., centromeres) in some chromosomes. In both species numerous light or weaker DAPI bands appeared interstitially in either or both arms of the chromosomes.

Light to strong telomere repeat FISH (TR-FISH) signals appeared at or near the centromeric sites in nine or possibly 10 pairs of chromosomes in both species (Figs. 1a and c). TR-FISH signals also appeared interstitially and pairs of snake-eyed signals

appeared near the end of most of the chromosomes. TR-FISH signals are found to be associated with DAPI positive bands but not all DAPI positive bands are associated with TR-FISH signals. DAPI positive bands located at the end of chromosomes are found to be associated with TR-FISH signals. This is the first report of this phenomenon.

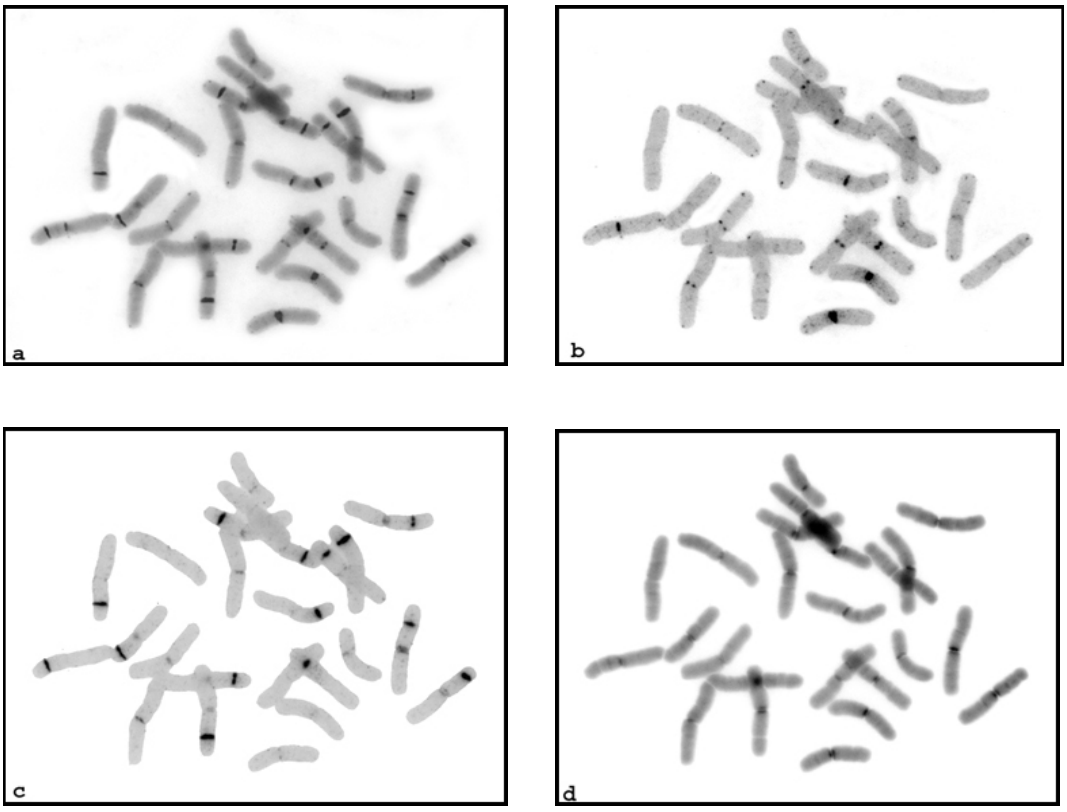

Figure 1. Fluorescence *in situ* hybridization of 18S-28 rDNA and telomere repeat probes to a mitotic metaphase spread of **(a)** loblolly pine (LSG-62). Individual signals from 18S-28S rDNA and telomere repeat are represented in Figures 1b and c, respectively; **(b)** FISH of 18S-28S rDNA sites (image from Cy3 filter followed by inversion); **(c)** FISH of telomere repeat sites (image from FITC filter followed by inversion) and **(d)** gray scale followed by inverted image of DAPI stained chromosome.

Seven intercalary 18S-28S rDNA sites have been identified both in loblolly and slash pines (Figs. 1a and b, 2a). Eight or possibly nine distinct 18S-28 rDNA sites appeared near or around the centromeric regions in slash pine and as many as nine sites were observed in loblolly pine.

None of these 18S-28 rDNA sites were associated with the DAPI positive bands or proximal TR-FISH sites. To date, this is the largest number of 18S-28S rDNA sites ever reported for a pine species. We hypothesize that the higher number of 18S-28S rDNA sites in this study as compared to previous reports (Doudrick et al. 1995; Jacobs et al. 2000; Hizume et al. 2002; Siljak-Yakovlev et al. 2002) is due to the improved method of

chromosome preparation. One major 5S rDNA site was observed on a long metacentric chromosome and two minor sites were observed on two other different metacentric chromosomes in both loblolly and slash pines.

We have clearly categorized 10 large pairs of metacentric chromosomes, one smaller pair (11 th pair) of sub-metacentric chromosomes, and the smallest pair (12 th pair) of sub-metacentric chromosome in both species. This is in contrast to previous reports of 11 pairs of long metacentric chromosomes and one pair of short sub-metacentric chromosomes.

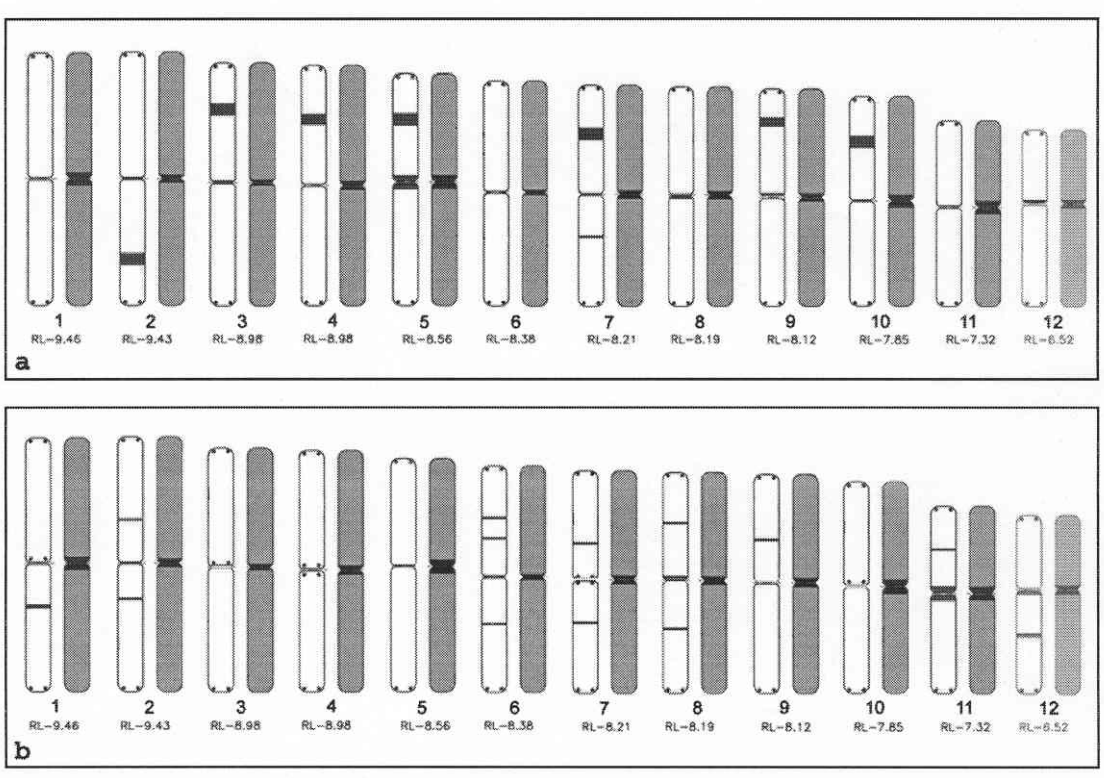

Figure 2. Ideogram of FISH based karyotype of loblolly pine (LSG-62). **(a)** The banded (Cy3 fluorochrome) signal corresponds to 18S-28S rDNA sites **(b)** shaded (FITC fluorochrome) signal corresponds to telomere repeat sites. Shaded ideogram represents DAPI positive bands (dark).

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Fourth-Year Results from a Clonal Test of Loblolly Pine

Barry Goldfarb, Fikret Isik, Anthony LeBude, Steve McKeand and Bailian Li[1]

Abstract

In November and December 1998, two experimental plantings were established using rooted cuttings from 450 clones of eight unrelated full-sib families of loblolly pine (*Pinus taeda* L.). Clones from four of the families (282 clones) were planted in South Carolina and clones from the other four families in Florida (168 clones). Both tests were laid out as randomized complete blocks with nine blocks and one ramet/clone/block. Height, survival and rust resistance were measured annually and diameter was measured at age four. Best Linear Unbiased Prediction of clone genetic values were estimated for height and volume at age four. Estimated genetic gains from various clone selection strategies, the effect of increasing or decreasing the number of ramets for testing on genetic gain, height age-age and trait-trait genetic correlations were estimated.

Estimated genetic gain was highly sensitive to the intensity of clonal selection. Selecting the single best clone from each test resulted in an estimated gain of 13% (SC) and 14% (FL) in height at age four over the test average (all the clones). The single best clone from each family at each site (four in total) resulted in an estimated gain of 10% (SC) and 10% (FL). However, if six clones were selected from each family (twenty-four in total), gain was reduced to 8% (SC) and 6% (FL) in height. The genetic correlations between height at age one and height at age four were low (0.60 at SC, 0.58 at FL), but increased to 0.96 (SC) and 0.97 (FL) between heights at ages three and four. Simulations using test parameters showed that estimated gain increased with the number of ramets tested up until ten ramets per clone, but did not increase appreciably with ramet numbers above ten. Moreover, approximately 90% of the gain could be obtained using only six ramets. These data, additional details and the implications of these results will be discussed.

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Volume Gains of Rooted Loblolly Pine Clones at Age 10 in Florida and Alabama

Early McCall[1] and Fikret Isik[2]

Abstract:--An estimate of the growth potential of clonal lines produced from elite crosses compared to trees grown from seed is needed to justify clonal forestry programs. A series of age 10 loblolly pine (*Pinus taeda* L.) rooted cutting tests planted in Nassau County, Florida and Monroe County, Alabama are some of the oldest clonally replicated studies in existence. A 3 X 3 factorial produced 9 cross families from which 4-6 clones were produced as rooted cuttings from seedling hedges. Clones and seedlings were planted in a randomized complete block design with split-plots for seedlings and rooted cuttings. Clone genetic values were estimated by Best Linear Unbiased Prediction method and genetic gains were estimated for various clone selection scenarios. Average volume gain over the family mean estimate by choosing the best clones from each family was 12.6%. The top clone of the 45 tested clones yielded 39.8% more volume than the grand mean. The top five and the top 10 clones had 30% and 23% more volume gain than the grand mean, respectively.

INTRODUCTION

Clonal forestry is becoming reality with recent successes in rooted cutting and somatic embryogenesis (SE) research. The economic returns of clonal forestry depend heavily upon identification and replication of individuals within a crossed family exhibiting superior growth and tree quality improvements. With SE technology, an unending supply of elite genetically clonal lines can be produced from one seed. The ability to cryogenically store lines to be reproduced later is the primary advantage over clonal production using clonal hedges and rooted cuttings. As might be expected, SE technology is expensive and it is important to determine how much more growth can be expected from choosing among the best clonal lines with the added benefit of starting with seed from crosses of the best available parents.

Before the development of SE technology, clonal forestry was investigated using rooted cuttings (Foster et al., 1987). To generate a clonal line using rooted cuttings, one seedling from a cross was hedged to produce cuttings for several other hedges. After production of a number of genetically identical hedges, cuttings were rooted for clonal testing. Hedged trees may be biologically limited to about 3-4 years of useful life before maturity causes a decline in rooting success although the exact number of years of useful

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life for southern pines is yet to be determined[1].

One of the oldest series of well-designed rooted cutting/seedling tests (Frampton, 1989) has completed the tenth growing season. Results after six growing seasons were published (Frampton, et al. 2000; Isik, et al. 2003). Clones selected from older rooted cutting tests cannot be rejuvenated and reproduced for operational planting, however rooted cutting clonal tests can provide an estimate of the growth gain from clonal forestry.

METHODS

A 3x3 factorial mating design was used to produce nine full-sib families of loblolly pine (*Pinus taeda* L.). Seedlings of full-sib families were used to establish clonal hedges. One year after hedge establishment, up to six clonal lines of cuttings were rooted from each of the nine crossed families. Seedlings from the same full-sib families and rooted clonal lines were established in a split-plot field design. There were a total of 45 clones tested in addition to the nine families established from seed. The two field studies were established on a bedded flatwoods site in Nassau County, Florida and on an old-field Upper Coastal Plain site in Monroe County, Alabama. Clones and seedling were randomly allocated to main plots and replicated 6 times at each site.

Both sites were measured for height and diameter at age ten and were analyzed for volume. Clone genetic values were predicted by the Best Linear Unbiased Prediction method and fitting a linear mixed model. Given the large differences in growth between the two sites, volume per tree was transformed for combined analysis by dividing the individual tree volumes by the standard deviation of each site. Variance components were estimated by SAS Mixed procedure and using REML method.

RESULTS

There were no significant differences in volume between from rooted cuttings vs. seedlings for either the Florida or the Alabama tests at age 10 although trees at the Alabama location grew about twice as fast as those at the Florida location (Figure 1).

This indicated that the rooted cuttings were growing as well as trees originating from seed. Cloning a family for genetic testing did not show slower or faster growth, but may yield greater within family genetic gains compared to the seedling based testing and selection (Isik et al. 2002).

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[1] Personal communication with Dr. Barry Goldfarb. NC State University College of Natural Resources

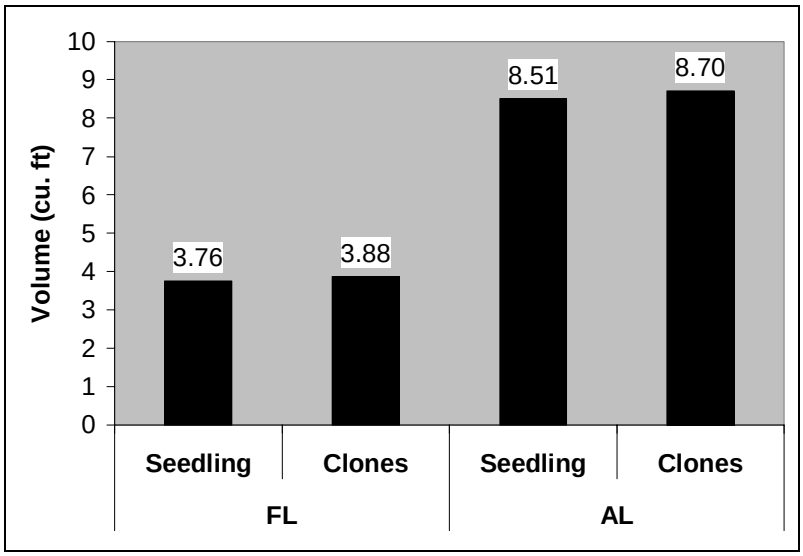

Figure 1. Volume from seedlings and clones of the same families at two sites at age 10.

Genetic factors including female and male general combining ability (GCA) effects, specific combining ability (SCA) and clonal differences explained about 33% of the total phenotypic variance (Figure 1). Genetic differences among females explained about 2.6% of the total variation in volume. Differences among males accounted for 6.5%, whereas SCA of females and males explained 7.4%. However, genetic differences among clones explained a greater percentage (12.7%) of the total phenotypic variation than GCA and SCA effects.

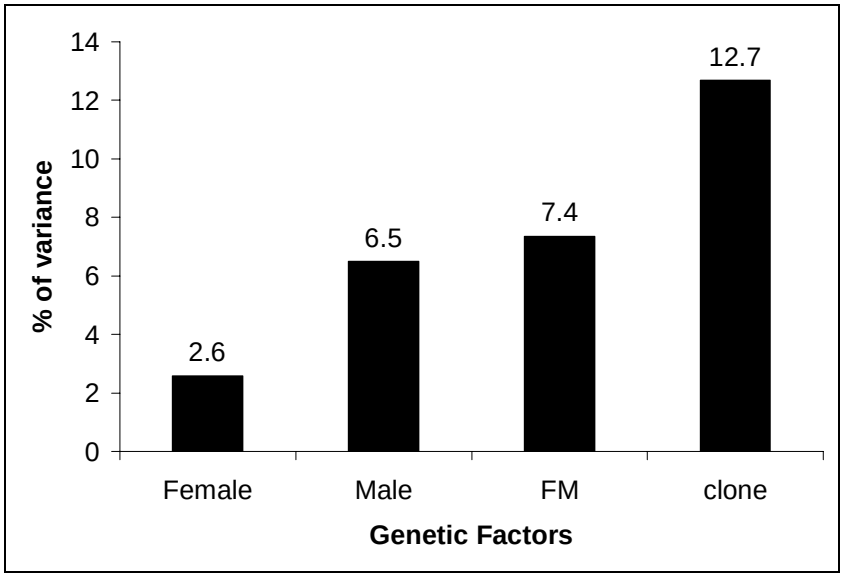

Figure 2. Percentages of the total phenotypic variance for volume explained by the genetic effects at age 10

Studies at NCSU using six-year results of these tests have shown the advantages of cloned family testing for within-family selection compared to the within-family selection from seedling testing option (Isik, et al. 2002). Therefore in this study we compared the best clone genetic values to its family breeding values instead of the seedlings (Figure 3). The best clone within a particular full-sib family had 3.9% (family 64) to 24.9% greater genetic gain (family 21) than their family breeding values. The average of the best clones provided 12.3% additional volume gain compared to their corresponding family mean. This would be the gain expected from using the clone in a vegetative propagation program. For use in an orchard program, selection of the same clones for their breeding values yielded 2.9% to 16.5% genetic gains over their family breeding values.

The low gains for family 64 are mostly because the means of clones within that family are tightly grouped with low variation between them. High gains are most likely to be realized with a clonal program by choosing very good families such as family 65 and family 35 with a high degree of variation among clones and then choosing the top performing clones to propagate.

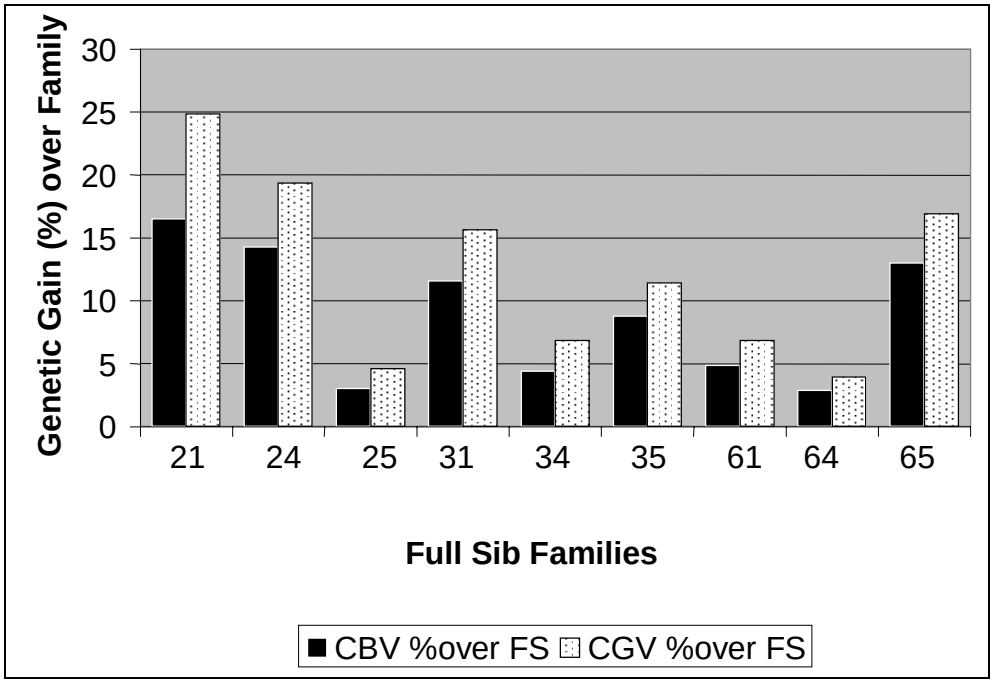

Figure 3. Genetic gains of the best clone breeding values (black bars) and clone genetic values over the respective family breeding values for volume at age 10.

The choice of the best clone per family was the same in the Florida and Alabama tests in six of the nine crossed families. In the three cases where the best clone was not the same, the top performing clones in one test was very close to the top in the other test. This is notable considering the distance between the two test locations and the obvious difference in site quality. The fact that clones ranked the same at both locations demonstrates the precision of clonal replication, which can sample several different microsites with the exact same genotype (Isik et al. 2002).

The volume increase of the best clone for each full-sib family can be important to estimate the gains achievable within a family. However many of the best clones may come from just a few of the best families. The best clone across both tests came from full-sib family 65 and had 39.8% more volume compared to the mean of all 45 clones (Figure 4). Each clone was represented by an average of 20 trees between the Florida and the Alabama tests. The top five clones, collectively represented by 106 trees, had 30 % more volume than the average. All five of these clones came from either full-sib family 65 or family 35. All of the top 10 clones came from either family 65 or family 35 except for one. Even choosing the top 10 clones yielded over 23.3% more volume gain than the test average.

Although no rust incidence was taken at age ten, Frampton, et al. (2000) reported significantly different rust incidence between seedlings vs. cuttings at age six for both sites. Rust incidence for the Nassau site at 22.3% for the seedlings and 15.6 for the cuttings. At the Alabama site, rust incidence was considerably higher at 51% for seedlings and 46% for cuttings. Clones had lower rust incidence than the seedling of the same families.

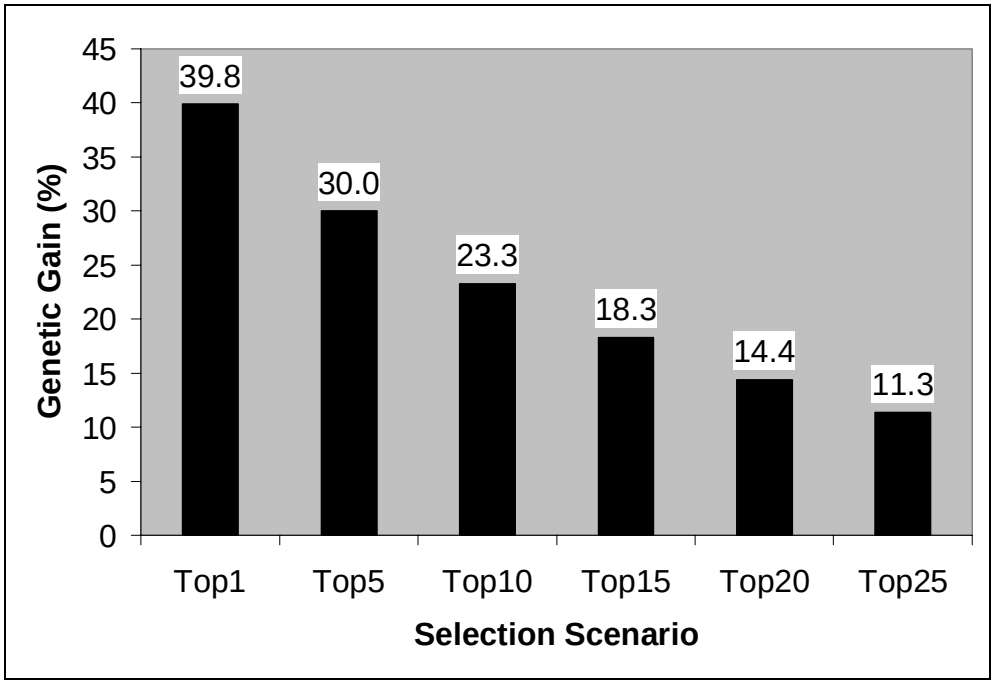

Figure 4. Percentage volume gain expected over the grand mean (averages of all 45 clones) by choosing the top clones out of 45 clones regardless of families. Each clone genetic value prediction was based about 20 ramets tested at two sites.

SUMMARY

The average volume gain from choosing the best nine clones (the top clone of each family) was 12.3% compared the mean of all of the rooted cutting families. Choosing the top five clones out of the 45 clones tested no matter what cross they came from yielded 30% more volume at age 10. All of these came from two crosses, however it may be less risky to accept less gain and choose good clones from some of the other full-sib families for operational deployment.

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Improving the Rooting Ability of Stem Cuttings from Virginia Pine and Fraser Fir Christmas Trees by Stumping

Christopher Rosier[1] and John Frampton[2]

Keywords: vegetative propagation, maturation, crown position, indole-3-butyric acid, 1-naphthaleneacetic acid, cloning, Christmas trees, *Pinus virginiana*, *Abies fraseri*

The Christmas tree industry in North Carolina is largely based in the mountainous western part of the state where Fraser fir (*Abies fraseri* [Pursh] Poir.) is grown, which represents over 96% of all the state's Christmas tree production. Production of Christmas trees is a valuable industry in the western part the state because it provides a source of income to a largely rural region in which there are few other economic opportunities (Frampton, 2002). Fraser fir is a desirable Christmas tree due to several characteristics including dark blue-green foliage, excellent post-harvest needle retention, pleasing aroma, and natural Christmas tree shape. However, Fraser fir has two significant problems as a Christmas tree species including extreme susceptibility to the introduced insect, the balsam wooly adelgid (*Adelges piceas* Ratz.), and to an introduced root rot fungus, *Phytophthora cinnamomi* Rands.

The most common Christmas tree species in the piedmont and coastal regions of North Carolina is Virginia pine (*Pinus virginiana* Mill.). Virginia pine has several characteristics that make it a deserving Christmas tree species including its rapid growth (3 to 5 years to harvest), short needles, good branch structure for holding ornaments, pleasant pine scent, and dark green color. However, Virginia pine also has several significant problems as a Christmas tree species. Chief among these problems are poor stem form, non-uniformity, and extreme susceptibility to damage by the Nantucket pine tip moth (*Rhyacionia frustrana* (Comstock)). In fact, due to the cumulative effect of these and other problems, growers typically only market about 50% or less of Virginia pines planted.

Asexual propagation by stem cuttings could help meet future demand for elite Fraser fir and Virginia pine Christmas trees. Due to the high market value of Christmas trees (relative to forest trees), genetic improvement for desirable Christmas tree characteristics can be justified. Once selected, desirable genotypes could be propagated for both archival purposes as well as commercial use (Zobel and Talbert, 1984). However, one of the major limitations in the effective use of vegetative propagation is the developmental process of maturation (Zobel and Talbert, 1984). Maturation has been shown to increase the time for root initiation to occur, decrease rooting ability, and decrease the growth rate of cuttings following rooting (Zobel and Talbert, 1984). This is a common problem in progeny testing where trees are allowed to grow over an extended period of time, but by the time their genetic potential is determined, the material is too mature to root.

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Hedging, or stumping, is an important stock plant management technique that maintains juvenility, allows for increased shoot production, allows for easier cutting collection, and reduces sexual reproduction (Hartmann et al., 2002). Previous research with Fraser fir (Wise et al., 1985), loblolly pine (*Pinus taeda* L.) (e.g., Cooney, 1999), radiata pine (*Pinus radiata*. Don) (e.g. Bolstad and Libby, 1982; Fielding, 1954; Libby et al., 1972), Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] (Black, 1972) and Norway spruce (*Picea abies* L.) (Bentzer, 1993) has demonstrated that continuous hedging of a stock plant provides a way to increase cutting production and maintain juvenility.

Two experiments were designed to investigate the possibility of increasing production and rooting of vertically oriented (orthotropic) shoots from Fraser fir and Virginia pine. *Experiment 1*, was designed to test the effects of age, stumping, and auxin treatments on shoot production and subsequent adventitious root formation. *Experiment 2*, was established to describe quantitatively the effects of stumping treatments and crown position on the rooting ability of stem cuttings from both species. Fraser fir Christmas trees were hedged to 1 whorl (trees in the field 3 and 5 years) or 1, 3, and 5 whorls (trees in the field 7 years). Virginia pine stock plants were stumped to ¼ original height, ½ original height and ¾ original height. Intact (nonstumped) control trees were also identified for comparisons.

Fraser fir

Rooting percentages increased as the age of the stock plant decreased and the severity of the stumping treatment increased. In the 3-year-old stock plants, no significant differences occurred when auxin (4 mM IBA) was applied to cuttings collecting within the same stumping treatment. However, when collected from the 5- and 7-year-old stock plants, cuttings treated with auxin (4 mM IBA) always rooted more frequently than the non-auxin treated cuttings collected from the same stumping treatment. The highest rooting percentage occurred when 3-year-old stock plants were stumped to the first whorl regardless of whether they had been treated with (51%) or without auxin (50%).

Primary root production generally increased as the severity of the stumping treatment increased and age of the stock plant decreased. Root production was always greater in auxin treated cuttings, compared to nonauxin treated cuttings. The greatest production of primary roots (8.1) occurred when 3-year-old stock plants were stumped to the first whorl.

The effect of height consistently explained more of the variation in every age class and stumping height than horizontal position, with the exception of the 7-year-old nonstumped controls; however, in this treatment, height did explain a significant portion of the variation. In general, rooting percentages increased as the age of the stock plant decreased and the severity of the stumping treatment increased. In the nonstumped controls of the 3- and 7-year old trees, rooting percentage increased as the distance from the base of the stem decreased. This phenomenon is less obvious in the stock plants

stumped to the first whorl, most likely due to the limited distance from the base of the stock plant to where the stumping treatment was applied.

Virginia pine

Stumping height, auxin type and concentration significantly affected rooting percentage. In general, rooting percentages increased as the severity of the stumping treatment increased. When IBA was applied, the highest rooting percentage (73%) occurred when stock plants had been stumped to ½ original height and treated with 2 mM. When NAA was applied to the base of the cuttings, the highest predicted rooting percentage (76%) using the nonlinear regression analysis occurred when trees where stumped to ¼ original height and treated with 4 mM.

Primary root production, was significantly affected by auxin type and concentration, and increased with increases in auxin concentration, regardless of the type of auxin applied. When IBA was applied, the predicted number of primary roots produced increased with increases in auxin concentration to peak at 3.1 for cuttings treated with 12 mM. When NAA was applied, the number of primary roots produced dramatically increased with increases in auxin concentration to peak at 5.5 when 12 mM was applied.

When compared to the position traits, primary needle length explained more of the variation at every stumping height. Percent rooting and total root length were significantly affected by primary needle length and continued to increase as the length of the primary needle increased. For the crown position variables in stumped stock plants, the effect of height consistently explained more of the variation in rooting than horizontal position for the stumped stock plants. In the nonstumped stock plants, distance from the main stem was the most influential position variable.

In conclusion, the current findings suggest that asexual propagation by stem cuttings could provide some assistance in meeting future demand for elite Fraser fir and Virginia pine Christmas trees. This management technique may prove to be most useful in bulking up clonal material following progeny test selections or field selections for trees that exhibit a desirable characteristic such as pest and disease resistance. Research is underway to assess field growth of cuttings following rooting.

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USDA Forest Service Forest Health Protection Resistance Screening Center

Carol H. Young[1]

Abstract

The Resistance Screening Center (RSC) is operated by the Forest Health Protection unit of the USDA Forest Service, Southern Region, State and Private Forestry. The Center is located at the Bent Creek Experimental Forest near Asheville, NC. The Center evaluates seedlings for resistance to disease, primarily fusiform rust (caused by *Cronartium quercuum* F. sp. *fusiforme*) and pitch canker (caused by *Fusarium circinatum*) as a service to tree improvement specialists, seed orchard managers, scientists, government agencies, research institutions, universities, and private industry. Testing enables clients to obtain information on the relative resistance of their materials in much less time than is possible in field progeny tests. The RSC has the flexibility to modify current screening procedures to accommodate specialized requests. This allows researchers to use the RSC as an additional experimental tool. By using information from the Resistance Screening Center tests, trees producing resistant progeny can be identified or questions may be answered concerning such things as the nature of variation in the rust fungus or the effectiveness of fungicides.

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High Efficiency Transformation of Loblolly Pine (*Pinus taeda* L.) Using Green Fluorescent Protein as a Vital Screenable Marker

Wei Tang and Ronald J. Newton[1]

Poster Abstract

An engineered green fluorescent protein (m-gfp5-ER) gene under the control of the 35S Cauliflower Mosaic Virus promoter was used to develop a facile and rapid loblolly pine (*Pinus taeda* L.) transformation system via *Agrobacterium tumefaciens*-mediated transformation of mature zygotic embryos. Green fluorescent protein has been introduced into three different loblolly pine families that are considered recalcitrant to transformation. The m-gfp5-ER gene produced bright-green fluorescence easily detectable and screenable in loblolly pine tissue 3-30 days after explants were co-cultivated with *Agrobacterium*. A high-level of GFP expression was detected in transgenic cells, tissues, and plants, and was localized in specific cells derived from cotyledons, hypocotyls, and radicles of mature zygotic embryos. Furthermore, in vitro and in vivo monitoring of GFP expression permitted a rapid and easy discrimination of transgenic shoots, and drastically reduced the quantity of tissue to be handled and the time required for the recovery of transformed plants. Integration of the m-gfp5-ER was confirmed by polymerase chain reaction (PCR), by Southern and northern blot analysis, and by junction DNA sequence analysis. Molecular analysis of *Agrobacterium* T-DNA loci in transgenic loblolly pine demonstrated that most of transgenic plants were derived from single transformation events. GFP-expressing shoots were also observed in loblolly pine explants co-cultivated with *Agrobacterium* but cultured in a medium without the selective agent kanamycin. This provides the opportunity to regenerate transgenic plants without using selectable-marker antibiotic-resistance genes, which will enhance the commercialization of transgenic plants.

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Antibiotics Effects on the Elimination of *Agrobacterium tumefaciens* from Loblolly Pine (*Pinus taeda* L.) Zygotic Embryo Explants and on Transgenic Plant Regeneration

Wei Tang and Ronald J. Newton[1]

Poster Abstract

Three antibiotics were evaluated for their effects on the elimination of *Agrobacterium tumefaciens* during the genetic transformation of loblolly pine (*Pinus taeda* L.) using mature zygotic embryos as targets. *Agrobacterium tumefaciens* strains, EHA105, GV3101, and LBA 4404, all harboring the plasmid pCAMBIA1301, which carries the selectable marker gene, hygromycin phosphotransferase (hpt) controlled by the cauliflower mosaic virus 35S promoter and terminator, and the uidA reporter gene (GUS) driven by the cauliflower mosaic virus 35S promoter and the terminator of nopaline synthase gene, were used in this study. Exposure to 350 mg/l carbenicillin, claforan, and timentin respectively for up to 6 weeks did not eliminate the Agrobacteria, while antibiotics at 500 mg/l eradicated them from the co-cultivated zygotic embryos. All three antibiotics increased callus growth and shoot regeneration at 350 mg/l and 500 mg/l each, but reduced callus growth and shoot regeneration at 650 mg/l when compared with controls. Putative transgenic calli were selected for continued proliferation and differentiation on 4.5 mg/l hygromycin-containing medium. Transformed calli and transgenic plants produced on a selection medium containing 4.5mg/l hygromycin were confirmed by GUS histochemical assays, by polymerase chain reaction (PCR), and by Southern blot analysis. These results are useful for future studies on optimizing genetic transformation procedures in loblolly pine.

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Geographic Information Systems(GIS) and Virtual Reality Models (VRM) to Improve the Analysis of Genetic and Silvicultural Trials

Rafael Rubilar, Steve McKeand and H. Lee Allen[1]

Poster Abstract

GIS systems have become core tools for mapping needs in forestry. During the last decade software platforms have expanded the basic capabilities of data storage and retrieval in map formats. Complex overlay procedures, terrain analysis, 3D modeling, spatial and geostatistical tools, and remote sensing integration have increased the power of space related information. In addition, new VRM have emerged as improved tools for visualization, simulation and teaching.

Research field trials in forestry are usually established to minimize spatial environmental variation, however exploring this assumption "ex ante" or "ex post" has been always tedious and uncertain and has lacked the power of visualization and analysis. Powerful spatial statistical analyses and interpolation analyses may be integrated to visualize site variability, remove environmental trends or integrate those to conventional statistical analyses. We investigated the "ex post" analysis of a research trial using ARCMAP/GIS and ARCScene VRM tools (ESRI, Inc) in order to explore their utility for trial analysis. Detailed sampling activities investigating specific physiological or ecological process may take full advantage of GIS and VRM tools capabilities to understand site variability and locate highly representative sampling points. That information may be used for modeling based on the same spatial information.

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Microprojectile-Mediated Genetic Transformation of Embryogenic Sweetgum Cultures

M. J. Walsh and S. A. Merkle[1]

Poster Abstract

With its rapid biomass accumulation, sweetgum (*Liquidambar styraciflua*) may be particularly suitable for phytoremediation purposes, especially if it can be engineered with genes that enhance tolerance and/or accumulation of heavy metals. Sweetgum has been transformed using *Agrobacterium tumefaciens*-mediated transformation of shoot explants or nodule cultures, followed by adventitious shoot regeneration, and by microprojectile bombardment of nodule cultures. We tested microprojectile bombardment of embryogenic cultures as an alternative means to produce transgenic sweetgum trees, with the long-term goal of engineering the species with genes conferring heavy metal resistance. Embryogenic cultures were obtained by culturing immature seeds on an induction/maintenance medium (IMM) with 2,4-D and BA. Cultures proliferated as proembryogenic masses (PEMs) with monthly transfer to fresh medium and formed suspensions following transfer to liquid IMM. Bombardment parameters were optimized using transient GUS expression. Different osmotic conditioning and selection agents and concentrations were tested to determine suitable levels of these agents for sweetgum PEMS. PEMs representing 4 different genotypes were size-fractionated and bombarded with pBI426, which contains a translational fusion between the GUS and the NPTII coding region, under the control of a double 35S promoter. Following selection on proliferation medium supplemented with 50 mg/l of kanamycin, 8 kanamycin-resistant sweetgum lines were recovered, all from one of the bombarded genotypes. While all lines remaining kanamycin-resistant were GUS- and PCR-positive, and Southern analysis indicated stable integration of the transgenes, neither the transclones nor the untransformed control line from which they were derived were capable of producing somatic embryos, having apparently lost this potential over time. Sweetgum transclones cryostored for several months maintained GUS expression.

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Rooting Cuttings of Northern Red Oak (*Quercus rubra* L.)

Matthew H. Gocke, Barry Goldfarb and Daniel J. Robison[1]

Poster Abstract

Several techniques have been used experimentally to vegetatively propagate northern red oak (*Quercus rubra* L.), including: 1) rooting juvenile softwood cuttings in intermittent mist, 2) rooting shoots originating from mature buds grafted onto juvenile root stocks, and 3) *in vitro* shoot proliferation of juvenile or mature shoots followed by *in vitro* rooting. Of these techniques, rooting juvenile softwood cuttings has provided the most consistent results for northern red oak (NRO).

Juvenility (or at least the associated ability to form adventitious roots) disappears rapidly among progressive flushes of growth in NRO seedlings. Decreased rooting has been reported for NRO shoots obtained from progressive flushes of growth produced within a growing season, as well as shoots representing flushes obtained from successive seasons of growth. However, as with many other tree species, the process of maturation in NRO can be slowed by pruning to encourage juvenile shoot production. Optimizing the number of juvenile cuttings produced from each stock plant is necessary for efficient rooted cutting production systems. In addition, rooting conditions must be determined for the shoots produced under these pruning regimes.

Two NRO rooted cutting studies are currently being conducted at NCSU. The objective of the first study is to evaluate the effects of stock plant pruning location, diameter, and age on new shoot production. Treatments include pruning first-year seedlings, as well as one-, two-, and three-year-old seedlings to the base of the first, second, third, or fourth flush of growth produced during the first growing season. The objective of the second study is to evaluate the ability of the shoots produced in the first study to form adventitious roots. Treatments include three rooting hormone levels (0.5%, 1%, and 1.5% IBA) and a control (45% EtOH). Preliminary results from both studies will be presented.

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Progress with American Chestnut Somatic Embryogenesis

G. M. Andrade and S. A. Merkle[1]

Poster Abstract

American chestnut (*Castanea dentata*), once the dominant forest species of the Appalachian Mountains in the eastern United States, was devastated during the early twentieth century by the introduction of the chestnut blight fungus. As part of an effort to restore the species to the forest, we have been working with embryogenic cultures of the species, aiming to establish a reliable somatic embryogenesis system for mass clonal propagation, as well as for genetic transformation with potential anti-fungal genes. While initiation of embryogenic cultures from immature ovules of American chestnut has become routine, bottlenecks still remain for embryo maturation, germination and conversion. Effects of cold stratification, gellan gum concentration and activated charcoal on somatic seedling production were investigated. Studies of other variables, such as the effects of light quality on germination and conversion, are underway. Using five genotypes, clusters of proembryogenic masses maintained on WPM with 2 mg/l 2,4-D were transferred to basal WPM for somatic embryo development. Individual cotyledonary-stage embryos (2-4 mm) were cultured for 10 days on basal medium prior to storage at 4^{o} C for 12 weeks in the dark. These embryos germinated at an average frequency of 23% following transfer to WPM basal medium in GA7 vessels in the light. Embryos that did not receive cold treatment or were stored for only 6 weeks failed to germinate. Embryos on WPM with 5 g/l activated charcoal (AC) germinated at the same frequencies as those cultured without AC, but AC prevented darkening of taproots. Embryos cultured on 3 or 6 g/l Phytagel germinated at higher frequencies following 12 weeks cold storage than did those cultured on 10 g/l Phytagel. To date, over 30 somatic seedlings representing 3 genotypes have been transferred successfully to greenhouse conditions.

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Evaluation of the Inheritance of Tip Moth Susceptibility Using Pine Hybrids Planted in Southeast Mississippi

M. T. Highsmith[1,2], L. H. Lott[3], D. P. Gwaze[4], D. Lominy[1] and C. D. Nelson[3]

Poster Abstract

Nineteen families of susceptible and resistant pine parent species and their interspecific hybrid progenies were quantitatively assessed for Nantucket pine tip moth (*Rhyacionia frustrana* Comstock) damage in a study in southeast Mississippi. The seeds for this study were leftover from previously performed experiments and the collection did not provide for a balanced set of families. However, it did include seven slash pine families, two shortleaf families, two loblolly families, three three-way hybrid families, one testcross hybrid family, and two F1 hybrid families of susceptible and resistant parents. The loblolly (L1 and L2) and shortleaf (Sf1 and Sf2) pine parents were susceptible, while the slash (S1 to S7) parents were resistant. The hybrids were produced from controlled pollinations that included eight additional parent trees along with the S1 slash pine parent and both of the shortleaf (Sf1 and Sf2) parents. The eight additional parent species included two F1 hybrid parents; one of which was a slash x longleaf, combining two resistant parent species and the other was a longleaf x shortleaf, combining a resistant and a susceptible parent species. The F1 hybrids in this study were the progeny of the S1 slash parent and a loblolly parent. The three-way hybrids were the progeny of the slash x longleaf F1 hybrid parent and the Sf1 and Sf2 plus one additional shortleaf parent, while the testcross hybrid was produced from the longleaf x shortleaf F1 hybrid parent and a slash pine parent.

The F1 hybrid families and the three-way hybrid families were susceptible to tip moth attack like their susceptible loblolly and shortleaf parents, but tip moth damage on all of these families was significantly higher than that on the slash pine parent and testcross hybrid families. The phenotypes of the pure species and hybrid families supports a dominant mode of inheritance for susceptibility to tip moth in three different ways: (1) the phenotype of the F1 hybrids expressed the phenotype of the susceptible parents, (2) the phenotype of the three-way hybrids expressed the phenotype of the susceptible shortleaf parents when those parents were crossed with an interspecific F1 hybrid of two resistant parents, and (3) the testcross hybrid expressed a level of susceptibility that was intermediate to that of the susceptible and resistant parents when the longleaf x shortleaf F1 hybrid parent was backcrossed to a resistant slash pine parent.

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Evaluation of an Open-Nucleus Model for Forest Tree Breeding

M. Lstibůrek and T. J. Mullin[1]

Poster Abstract

Open-nucleus breeding was evaluated by stochastic simulation. Methodology was developed for unrestricted migration rate between two tiers (main and elite). Genetic progress in the breeding population was iteratively maximized for a wide range of restrictions on diversity varying from strong family selection to balanced within-family selection. The model assumed genetic parameters typical for growth traits in conifers and accounted for inbreeding depression. Comparison was made with a single population without hierarchical structure assuming constant testing effort. A seed orchard was established in each breeding cycle as a selected subset of the breeding population. The extra gain achieved by assigning better mates into the elite population was counteracted by increased group coancestry (relatedness) among seed orchard selections. The size of elite tier was found to have little importance in this study. When more effort was concentrated into elite crosses, potential for inbreeding in the seed orchard crop increased.

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Note: Also presented as a contributed paper.

Characterization of a Bacterial Contaminant in Loblolly Pine Tissue Culture

M. J. Cook[1], J. J. Clark[1], J. W. Anderson[2], A. D. Junkins[3], C. K. Karr[4] and M. R. Becwar[1]

Poster Abstract

Bacterial contamination of immature seed explants can decrease the efficiency in initiating and establishing pine embryogenic cultures. A type of contaminant commonly observed surrounding the megagametophyte explant of immature loblolly pine (*Pinus taeda* L.) appears as a white to creamy halo on the surface or within the culture medium. This contaminant appears within 24-48 hours after culturing the immature seed. The severity of the contamination varies by year and family. The pattern of contamination among seeds pooled from several cones did not suggest operator technique as the source of contamination. Tests with seeds separated by cone during sterilization and culture suggest that the source of the contamination was from seeds of specific cones. To better understand the causal bacterium and provide insight to help prevent or decrease the occurrence of contamination, several isolates taken from different contaminated explants were identified. This identification was done by PCR amplification of a 500bp sequence of a 16S ribosomal RNA gene. The closest match in terms of sequence similarity was *Erwinia amylovora*. *Erwinia* species are the causal agents of several economically important diseases. For example, fire blight (*E. amylovora*) in the *Roseaceae* family that affects apple and pear trees, and soft rot (*E. carotovora*) in several crops including potato. Our results suggest that *Erwinia* may also infect immature loblolly pine seeds and is a probable causal agent of this type of bacterial contamination in pine tissue culture. We have been able to significantly reduce the negative impact of this contamination and increase culture establishment efficiency by separating seeds by cone for sterilization and culture.

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A Functional Genomics Pipeline in Loblolly Pine and Eastern Cottonwood

Bob Kodrzycki[1], Paul Sanders[2], Murray Grigor[2], Samantha Roberts[1], Kim Winkeler[1], Kirk Foutz[1], Heather Holley[1] and Carl Huetteman[3]

Poster Abstract

Advances in transformation technology allow functional genomics techniques that are commonly applied to *Arabidopsis thaliana* to be considered for tree species. ArborGen is applying the principles of high throughput functional genomics originally developed for Arabidopsis to both hardwood and conifer trees as a means to screening genes that affect wood quality traits and productivity. The ability to demonstrate gene function in commercially important tree species is an essential technology for developing improved tree products based on gene transfer. Starting with a large database of ESTs isolated from *Pinus radiata* and *Eucalyptus grandis*, a systematic approach to uncovering gene function is in progress. This integrated functional screen consists of bioinformatics characterization, cell-based assays, and *Arabidopsis thaliana* screens to identify candidates for high throughput functional testing in two commercially important species: *Populus deltoides* (Eastern cottonwood) and *Pinus taeda* (Loblolly pine). Efficient gene transfer methods are being used to introduce large numbers of genes into these tree species and methods for early detection of transgene function are being developed based on phenotypic and chemical composition screens. These methods will enable ArborGen to functionally test several hundred genes per year in commercial tree species. These functional screens in trees are being used to identify candidate genes that are expected to affect key commercial traits in plantation forestry. This integrated system for characterizing tree genes including bioinformatics, cell-based assays, Arabidopsis screens, and high throughput Pine and Populus screening systems will be described.

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Field Selection of American Sweetgum Transformed for Herbicide Resistance

C. A. Huetteman, Y. Zhao, K. C. Gause, H. D. Wilde and B. T. Parks[1]

Poster Abstract

One American sweetgum clone from an open-pollinated parent was transformed with *Agrobacterium tumefaciens* containing the gene for acetolactate synthase (ALS), and regenerated through organogenesis. Seventy independently transformed lines were selected in vitro in the presence of an ALS-targeting herbicide. Containerized ramets of the 70 lines were established at an irrigated fiber farm in South Carolina, in May, 2002. Over 1,000 trees were planted in a completely randomized design with up to 15 ramets per transline. Establishment survival in June was near 100 percent. Two months after planting, the actively growing trees received one over-the-top application of a tank mix of two ALS-targeting herbicides to evaluate resistance in each transformed line. Thirty days following application, over 85 percent of the planted ramets from three lines displayed no damage symptoms. Sixteen additional lines had a mean damage rating less than "slight". Twelve of these nineteen lines had first-year heights that were not significantly different from the non-sprayed control line. One transformed line was significantly taller than the control clone (103 cm vs 91 cm mean height, respectively). At the end of the year, only 11 of 1,027 individual trees (1 ramet from each of 9 lines and 2 ramets of another line) died due to herbicide damage. Although the test was terminated after only one growing season, at least four lines were sufficiently resistant to be considered for further plantation development.

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Loblolly Pine Karyotype Using FISH and DAPI Positive Banding

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Poster Abstract

A loblolly pine (*Pinus taeda* L.) karyotype has been developed based on fluorescent *insitu* hybridization (FISH) using cyto-molecular landmarks including plant telomere repeat, 18S-28S rDNA and 5S rDNA probes and DAPI positive bands. Somatic chromosome spreads of loblolly pine root tips were prepared using a modified enzymatic digestion technique. We observed ten pairs of long metacentric, one pair of long sub-metacentric and one pair of short sub-metacentric chromosomes. All the chromosomes showed characteristic DAPI positive bands (A-T rich regions) near and/or around the centromeres. At least one DAPI positive band was also observed in intercalary positions on all chromosome arms. Plant telomere FISH signals were observed towards the end of each chromosomal arm as expected. In addition, most of the chromosomes showed telomeric sites near and/or around the centromeres except for one or possibly two chromosomes. A total of seventeen 18S-28S rDNA sites were identified per haploid genome. Eight of these were located near and/or around the centromeres and seven were at intercalary positions. One major 5S rDNA site was observed in an intercalary region of a metacentric chromosome that lacked 18S-28S rDNA sites. One or possibly two minor 5S rDNA sites were observed near the ends of two different chromosomes. We are also developing a slash pine karyotype for direct comparison with loblolly as well as a comparison with a previously published slash karyotype (Doudrick et al. 1995, Journal of Heredity 86:289-296). Finally, we will provide an update on our progress toward using BAC clones as FISH probes on pine chromosomes.

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Note: Also presented as a contributed paper.

Molecular Pathology of Pitch Canker Disease

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Poster Abstract

The inciting agent of pitch canker disease in Pinus species is the necrotrophic fungal pathogen *Fusarium circinatum* (teleomorph *Gibberella circinata*). Pitch canker disease has been identified in the southeastern United States, as well as in California, Mexico, Japan and South Africa. The disease is episodic in nature and can reach epidemic proportions with potentially devastating consequences for both managed and natural forests. Symptoms of pitch canker disease include discolored lesions (cankers) on stems and branches that generate profuse amounts of resin (pitch). Infected shoots eventually desiccate due to reduced water transport caused by pathogen development in vascular tissues.

As with most diseases of pine, pitch canker disease is not well understood with respect to mechanisms of fungal pathogenicity, disease development, and disease resistance. To gain insight into the processes associated with disease development, we used a method called differential display of messenger RNA. This method was coupled with gene expression array analysis to identify genes from the pine host and from the fungal pathogen that are regulated differently during the disease state compared to the free-living states of host and pathogen. The functions of these genes appear to be associated with plant defense and desiccation, however many genes of unknown function were also identified.

The next goal of these studies is to identify genes that are associated with resistance, and to compare and contrast the gene expression programs that are associated with resistance vs. susceptibility. The large-scale screening of loblolly pine clones for resistance to pitch canker disease, which was carried out as part of the ADEPT project (Allele Discovery of Economically-important Pine Traits), is a major step toward this goal. Analysis of the screening study data revealed that resistance was heritable on the clone mean basis. A mixed linear model was used to predict genotypes that are highly resistant or highly susceptible to pitch canker disease. Ultimately it is hoped that association genetics approaches - such as those utilized in the ADEPT project - will identify host genes and alleles that condition pitch canker disease resistance. The genes identified by differential display serve as candidate genes for evaluating this approach.

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