Proceeding of the 28th Southern Forest Tree Improvement Conference

Edited by: Steven E. McKeand
            Bailian Li
            Department of Forestry and Environmental Resources
            N.C. State University
            Raleigh, NC  27695-8002

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Foreword

The 28th Southern Forest Tree Improvement Conference was held in Raleigh, NC in cooperation with the Southern Forest Tree Improvement Committee and the Department of Forestry and Environmental Resources in the College of Natural Resources at NC State University. For three days, foresters, tree breeders, scientists, and practitioners met and listened to the excellent presentations that are summarized in these proceedings. We all left Raleigh a bit more informed about what’s happening in southern tree improvement and forest genetics than when we arrived.

Three awards were presented for outstanding contributions to the conference:

The **Tony Squillace Award** is given for the best oral presentation based on content, style and use of visual aids. The winner was Tim Mullin for his talk on “The Impact of Variable Success of Somatic Embryogenesis Among Elite Crosses on Expected Genetic Gain and Diversity of Selected Varieties”

The **Bruce Zobel Award** is given for the best oral presentation by a student. There were two winners this year:

  Josh Sherrill for his talk “Total Inside-Bark Volume Estimation for Loblolly Pine (\textit{Pinus taeda} L.) in Genetic Trials”

  Kevin Potter for his talk “An \textit{Ex Situ} Gene Conservation Plan for Fraser Fir”

Each was awarded $200 in recognition of their achievement.

The **Belle Baruch Foundation Award** includes a $100 award and is given for the best poster. Scott Merkle won for his poster “Light Quality Treatments Improve Pine Somatic Seedling Production Efficiency”

The SFTIC Committee thanks these four individuals as well as all the speakers for their contributions.

The Conference was a success primarily due to the efforts of Dr. Susan Moore and Ms. Becky Townsend with the Forestry Education and Outreach Program (FEOP) at NC State. Our sincere gratitude goes to them. The SFTIC Committee also thanks all the staff, graduate students, and faculty in the Department of Forestry and Environmental Resources that contributed to the success of the conference.

The 28th SFTIC Planning Committee:

Steve McKeand (Conference Chair) and Bailian Li (Local Organization Chair) John Adams, Henry Amerson, Tom Byram, Scott Cameron, Barbara Crane, Mike Cunningham, John Davis, Van Hicks, Gary Hodge, Mike Lee, Early McCall, Scott Merkle, Greg Powell, Dan Robison, Randy Rousseau, Barry Shiver, Chuck Tauer
28th Southern Forest Tree Improvement Conference

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Our Roots: The Start of Tree Improvement in the South

Bruce Zobel

Most of the earlier foresters in the US believed that the characteristics of a tree were primarily determined by the environment in which the tree lived and that parentage was of little concern. For example, one of my professors (F.S. Baker, a famed silviculturist) told me in the late 1930s that to work with genetics of forest trees was useless and we could never change trees in the desired direction by genetic manipulation in our lifetime. Much before this, some was known about the genetics of forest trees but was used in operational forestry mostly in Japan. For example, Miyazaki in 1696 stated, relative to growing Cryptomeria: "For sawing, it is important to select seed of red and handsome trees showing vigorous growth - cuttings must also be collected from young and handsome trees". Since that time, parentage of Cryptomeria has been considered important in commercial production of this species and has been standard in operational forestry there.

Much later, especially studies made in Australia, Sweden, Denmark and South Africa proved that inheritance patterns existed in forest trees and some important characteristics of forest trees were inherited strongly enough so that they might be used as a part of silviculture. The main emphasis on variation in those earlier years related to the importance of seed source. Also, the potentials for the use of vegetative propagation were being assessed. A few excellent and useful results were obtained, some of which are still generally used. However, the opportunity for changing individual trees through control of parentage was mostly ignored, despite the positive information available.

The Application of Forest Genetics:

The general opinion of most foresters was that even though there were some genetic differences with parentage, the use of this information was too difficult and long term to be used operationally. The concept was still strongly held that differences among trees were mainly the result of the differing environments in which the trees developed. Individual tree variation was not well known, and when I started in Texas, persons like C.C. Doak, (a well-known biologist) suggested that I start my genetics program using radiation on loblolly pine. I refused, pointing out that how could we assess results from radiation studies when we did not even know the natural variation patterns among stands or individual trees of the species. Until about the 1950s the subject of genetic control was somewhat known and talked about but few foresters were involved in the actual application of this 'new science'.

Forest Genetics in the Southern Pine Region:

Some early forest genetic studies were done in the early 1900s on southern pines, like those of Phil Wakeley, Keith Dorman and Francois Mergey. Information was obtained on seed source variation. Much of the activities done on the seed sources of the southern pines was primarily

\[1\] Professor Emeritus, NC State University and Senior Associate, Zobel Forestry Associates. 9205 Penny Road. Raleigh N.C., 27606 Email: bjZobel@unity.ncsu.edu
related to the huge program headed up by Phil Wakeley, but assisted both by industry and state forest services. Phil's geographic variation study set the stage for most later genetics work in the southern pines, and yielded excellent results. In addition, the first intensive genetic assessments were related to resin production, which is now only of minor importance. Especially the US Forest Service developed a viable program to improve yields in amount and quality of the resins produced, with most emphasis being on slash pine.

The details about the start of applied genetics on a large scale in the south is unusual and interesting, even though most operational foresters were still antagonistic to the idea of using parentage in silvicultural planning. A major source of interest in the genetics of forest trees was from Syrach Larsen (from Denmark) who wrote a couple of books about the application of genetic principles on forest trees. (Most of his ideas were good, but some were incorrect; however, that did not bother those who read the books because they had little detailed knowledge about genetic principles). The books were well written and translated, and read by some of the leaders in Southern Forestry. In fact, several companies made it mandatory that all their trained foresters should read them.

About that time, Ake Gustafson, a world renowned specialist in radiation in agricultural crops, was asked to give a weeks' seminar on his work while in Houston, Texas. Ake liked forestry and felt that genetics should be used in silviculture, so he asked that he be given one lecture to express his ideas for forestry use. It so happened that the governor, the head of Texas A&M University and the head of the Texas Forest Service were at his presentation, as well as several persons from the press. Gustafson's talk was so well done and interesting that later the administrators got together and decided that Texas should do something about the use of genetics in forestry.

Start of the Texas Forest Tree Improvement Program:

During World War II I was working as a logging engineer harvesting in the redwoods of California after graduation from the University of California. One day, during the worst of the fighting in the islands, I received 'greetings' from the U.S. president inviting me to be drafted into the military services. I asked for the engineers but they said with my background I had to go to the navy. We were then lined up and a major from the Marine Corps walked in and said that 15 of us would have to go into the Marines. He pointed at me and said: "Hey, you" and that was it. Numerous things developed during the war that enabled me to become the only forestry officer in the Marine Corps at Camp Lejeune, NC after obtaining my Lieutenant's commission. During this period the 'bomb' had been dropped on Japan and all of us who were training to invade Japan were suddenly without assignments. While waiting for directions, another forester and I talked the General into developing a forestry plan for the 125,000 acre Marine base (Camp Lejeune NC) which had some fine forests. The next day the other man was transferred to China and I spent the rest of my service time as only forestry officer for the Marine Corps, selling pulpwood, running a sawmill and making a Forest Plan for Camp Lejeune. This enabled me to learn a lot about southern forestry.

After release from the Marines, I was offered a good job managing redwood forests but decided to go back to the University and get my advanced degrees. I was interested in forest genetics,
largely through Jack Duffield, who worked at the Forest Genetic Institute at Placerville, California. There was no such thing as a forest geneticist under which to study at the Univ. of Calif. so I had to be a 'floater' at the university, with an office in Forestry, laboratory in Botany and studies in Genetics.

When I graduated with the PhD degree in 1951, the people in Texas heard that there was a forester trained in genetics so they hired me to head an industry cooperative they were to create for using forest genetics. The objective was to improve growth and form, find drought tolerant trees for east Texas and help obtain disease resistance. We knew practically nothing about individual tree variation in important characteristics in loblolly pine. We studied various agricultural programs and adjusted ours into a simple selection and breeding program that could be applied to forest trees. The first emphasis in Texas was on drought resistance and wood properties. It was successful and widely advertised which resulted in a period when many administrators obtained the idea that if one used genetics in forestry, all problems in forestry could be easily solved. It was particularly hard to get the foresters to support working on genetics even though some higher administrators actually visualized the practicality of a breeding program; their confidence made it easy to get funds from the industry and granting agencies. (For example, we had obtained a 5 year grant from the National Science Foundation to establish a huge genetic study of loblolly pine on the lands of International Paper Company in Bainbridge, Georgia. As that grant was ending, the National Institute of Health asked if it could support the NSF study for 5 more years. Also there was support by individuals; an example was Gunnar Nicholson, president of pulp companies in Georgia and Tennessee. He insisted in giving us annual financial support for studies in the application of genetics, because "it was needed" even though his company in the east had no direct benefits from the more western studies. MY HARDEST JOB AT THIS TIME WAS TO GET PEOPLE TO RECOGNIZE THAT TREE IMPROVEMENT WAS JUST ANOTHER PHASE OF SILVICULTURE AND NOT A CUREALL and SHOULD SO BE USED. When this aspect of forest genetics was being promoted, it was not really believed in, especially by most operational foresters. To make it more acceptable, we entitled it 'tree improvement'.

The Spread and Use of Tree improvement:

Groups in the south became interested in tree improvement, and in a few years Cooperatives were established in Florida (1954) and then at N.C. State University (1956). My coming to head the program in the Carolinas was an interesting 'accident'. Dean Preston from NC State University had pressures from the Eastern Companies to start a genetics cooperative. He asked if our family had ever visited North Carolina, so when I said no, he paid to have our family make a visit there. Meanwhile he had arranged for a meeting of those companies interested in a forest genetics cooperative to discuss their proposal with me. He then offered me the job of setting up a cooperative at NC State University, funded largely by industry. I said no, that I liked what was being done in the Texas Cooperative operating west of the Mississippi River. My boss in Texas heard about our visit and (being an ex-colonel in the army) had me stand at rigid attention while he harangued me about not being loyal to Texas and what opportunities there was there and why did I seek a job in North Carolina. He did not give me a chance to tell him that I had said "no" to North Carolina. His 'attack' made me angry so I went back to my office and called up Dean
Preston and said that I would take his job after all - he said: "Great, we had just decided to offer
double the salary if I would reconsider the no I had given them."

The Texas, Florida and North Carolina cooperatives worked very closely together exchanging
ideas and plant material when suitable.

A Few Suggestions

Later, after official retirement from the NC State cooperative in 1979, I had the opportunity of
starting a number of cooperatives overseas. These were done through our company, Zobel
Forestry Associates, and some general observations about cooperative organization became
clear:

1. Every program, no matter whether it is supported by industry, the government or university
will be asked after 3 to 5 years: "What are we getting for our contribution? What will be the
payoff for our organization?" This is difficult to answer for a forest genetics program because
results take some time to develop. We satisfied our contributors by emphasizing wood and its
uses, its variation and its importance with increased utilization. A major problem was the
utilization of juvenile wood produced when shorter rotations are followed. Our supporters were
quite satisfied with the wood information and the word genetics was hardly mentioned in our
early annual reports. The point is that one has to make sure you can answer this question in a
satisfactory way, which will surely be asked. We observed several really good cooperatives that
were abandoned because they could not satisfy the question to their sponsors.

2. Make sure that you know the species with which you are working. Many decisions must be
made before sound facts are available and one can only make suitable estimates if you know the
species and its reactions. You will initially know very little about the extent and kind of genetic
variation with which you are involved.

3. Make tests simple and understandable. I did a terrible job early in Texas when I wanted to get
information about how to manage seed orchards. Being young and eager, with limited
experience, I designed a 'super study' including all kinds of variables (like species difference,
spacing, kind of grafts, fertilization etc). About ten years later, when I was in North Carolina, I
had a phone call from Hans van Buijtenen who headed the Texas cooperative, (my first graduate
student) as follows: "I hate you". Then he hung up. Later he explained that the study design was
so complex with so many unknown variables that he could get practically no useful provable
data from this huge study. Keep the studies simple as well as the explanations of what has been
found or being done. This is particularly true when using advanced things like biotechnology
because too often the report can become completely non-understandable to the general forester.

4. In forest genetics, the forester usually must take action before the real facts are known. This
can only be done if one knows the species he is working with which enables some assumptions
to be made. Sometimes one is right, as we found for the inheritance of wood density in pines. It
was a required character in our early seed orchards and my boss was horrified that we were
asking the members of the cooperative to spend millions of dollars working with a characteristic
for which we really did not know about the genetic variability. We were lucky with wood
density! However, we were wrong in getting material to root. We knew that branches from older pines would not readily root. But we had the idea that if we grafted an old shoot to a seedling that the ease of rooting seedlings would somehow be passed from the seedlings to the graft. We found that this was not so and the graft continued to respond as if it were still from the old tree. But we did learn the facts that made it easier to get earlier production out of seed orchards through earlier flowering of the grafts because they maintained their physiological age.

Summary:

There is no question about the importance and use of genetics as a silvicultural tool. One who works with more sophisticated systems must always remember that basic plant breeding is necessary if success is to be achieved. Genetics is a good and useful tool for the silviculturist. Of primary importance is vegetative propagation; if foresters do not take advantage of this method of regeneration they will be left behind. I consider vegetative propagation to be a major objective so one can use both the additive and non-additive genetic variation and can develop large numbers of individuals that have special woods, or are resistant to diseases or adverse sites, as has been done so well with some eucalypts in the tropical areas with which I have worked for many years.
Impacts of Tree Improvement on the Forest Products Industry

R. C. Kellison¹

INTRODUCTION

My objective is to discuss the impacts that tree improvement programs have had on the forest products industry in the U.S. South. The period covered is roughly from 1950 to the present, with an occasional reference to the early parts of the 1900s. To assess the impacts, it will be necessary to give a thumb-nail sketch of the changes in the forest industry during that time.

Until about 20 years ago the forest products industry operated without significant competition from abroad. With the exception of Canada, we largely grew our wood and manufactured it into the products that were in demand by society. When the internal economy waned and capacity was high, the excess was exported, but when the reverse occurred we ignored the export market while benefiting from internal consumption. That mode of operation caused foreign buyers to identify us as unreliable suppliers. Those buyers established a lasting relationship with partners on whom they could rely during good times and bad. The relationship of the Nordic countries to Western Europe is the epitome of trust between supplier and buyer (Siry, et al. 2005).

In the early 1980s, the U.S. economy deteriorated badly. Nominal interest rates rose to about 20 percent. That phenomenon, in combination with a high monetary exchange rate, sparked a revolution in the forest products industry. Imports from every corner of the globe came trickling into the U.S., and then they became a flood. Conversely, exports of domestic wood products waned to a slow death.

The ailing forest products industry spawned the interest of arbitragers who visualized a financial gain by buying companies at reduced prices and selling the parts for a sum greater than the whole. In some situations, only a modest amount of cash was offered with the remainder being secured from lending institutions that used the land base and the manufacturing plants as collateral. During this time, many of the forest products companies that were household names ceased to exist. Among those names are American Can, Continental Can, Crown Zellerbach, Owens-Illinois and Scott Paper. Others such as St. Regis and Hammermill escaped the arbitragers only by ‘white knight’ Champion and International Paper coming to the rescue.

In the early 1990s, the economy had righted itself so that the remaining forest products companies prospered to economic highs not seen in the previous 40 years. Northern bleached softwood kraft (NBSK) pulp exceeded $900 per ton, with southern bleached softwood kraft (SBSK) being not far behind. In unison, hardwood bleached kraft pulp, both northern and southern, reached record highs. These good economic times caused a further consolidation within the wood products industry during the late 1990s and the early years of 2000, but this time it was the dominant players in the industry that were swallowing their smaller

¹ President, Institute of Forest Biotechnology, 920 Main Campus Drive, Suite 101, Raleigh, NC 27606. Contact: bob_kellison@forestbiotech.org
counterparts. Among the fallen were Blandon, Union Camp, Consolidated, Champion, Federal Paper Board, Union Camp, and Willamette.

What does all this amalgamation have to do with tree improvement? In short, it has to do with the land base. Starting in the 1930s, when the pulp and paper industry learned that competitive pulp, paper and paperboard could be made from the high-resin southern pines, thanks to Herty Laboratory, Savannah, GA, there was a rush to build new pulp mills. During 1937-38, 18 new pulp mills came on stream. Those mills stretched along the coast from Eastern Virginia into the pine belt of Texas. Almost without exception, those 18 mills and others began compiling a forest land base for timber support. Only a maverick here and there chose to build a mill without the back-up wood resource. Interestingly, those mavericks survived, largely because of landowner assistance programs and because of the camaraderie among wood procurement managers; the wood-poor mills survived because of the wood-rich ones.

The pulp and paper industry went on a binge in the 1960s by diversifying into businesses that were foreign to their core assets, such as rug, furniture, insurance, farming and home construction. With the minor exception of home construction, those investments failed, largely because the buying companies were operating businesses outside of their areas of expertise. Abutting that costly experience was the advent of the chip-'n-saw mills. Nearly every company with significant land holdings invested in that business for the dual purpose of leveraging their timber resource for lumber manufacture as well as a ready supply of chips for the pulp mills. Even though grade-pine sawmills were the core of the land base of some pulp and paper companies, others expanded their operations from the chip-'n-saw operations into high-speed sawmills with production capacities of 80 to 100 million board feet per year, or more. The evolving utilization standards had a significant impact on tree improvement programs because the emphasis shifted to open-pollinated family-block plantings where some families with good growth and tree form were managed for sawtimber rotations, whereas others with fast growth but marginal form were destined for the pulp mill and, later as we shall see, to engineered wood products (EWPs). The open-pollinated family-block plantings have evolved today to control mass pollination (CMP) where the parents are chosen for the attributes specific to the desired end product. The CMPs of today are being eclipsed by clonal forests of tomorrow.

Following the advent of the chip-'n-saw mills by a decade or so were EWPs, chief among which are oriented strand board (OSB) and laminated veneer lumber (LVL). Those allied industries, together with continuous digestors that allowed sawdust to be manufactured into pulp, and power generating plants that used residual bark and refuse wood for fuel allowed the forest industry to liken themselves to the hog industry where everything from the forest was utilized except the squeal.

The forest land base was considered an asset to the financial well being of a company in the early years. It served as collateral for construction of new pulp mills and other assets. During the high-inflation years of the late 1970s and early 1980s, the land base suddenly became a liability in the eyes of the stock analysts. That is one reason why the arbitragers were so successful in buying the whole for less than the parts. Even companies that survived the onslaught began separating their forest lands into wholly owned subsidiaries. Other forest products companies began selling (and continue to sell) their timberlands to new types of forest-land holding
companies such as Timber Management Investment Organizations (TIMOs) and Real Estate Investment Trusts (REITs). The TIMOs are primarily invested in the U.S., but have holdings in New Zealand, Australia, Canada and Brazil. Among the 25 or so organizations that fall into this category, Hancock Timber Resources Group is the standard bearer with about three million acres of timberland. Plum Creek Timber Company is the king among the REIT group, with upwards of eight million acres of timberland. Only a select group of companies continue to hold timberland for wood-resource security. Among those few are International Paper, Weyerhaeuser, MeadWestvaco and Temple-Inland. Even among those giants, non-strategic timberlands are commonly sold to the TIMOs, REITs and others.

**FORMATION OF TREE IMPROVEMENT PROGRAMS**

During and following the years of World War II, the newly coined pulp mills in the South operated on the second-growth forests of colonized agricultural fields that had once supported extensive stands of longleaf pine (Early 2004). Following the demise of the pioneer forest to naval stores and lumbering (Outland 2004) the land was cleared for agriculture. By 1920s much of the land that was abandoned because of poor agronomic soil husbandry and the pervasiveness of the boll weevil had succeeded to native-seeded forests. Even then data compiled by the USDA Forest Service (Alig 1985) and others showed the timber supply to be unsustainable at current usage. In unison, the industry set about to manage their timberlands. The first effort was to plant open lands and, secondly, to site prepare and plant lands from which the timber crop had been harvested. The open-land initiative culminated with the Soil Bank plantings of the late 1950s in which about three million acres of plantations were established with southern pines.

Site preparation of cut-over woodlands varied in quality during the early years. No one knew the quality of site preparation needed and the heavy equipment and other resources were lacking. Heavy equipment that was designed for earth moving was tried with varying results, and herbicides with and without fire received wide-spread attention. With necessity being the mother of invention, the KG-blade, V-blade and root rake came into being. The site preparation equipment also included the rolling drum chopper that was fashioned from a paper-machine drum onto which were welded cutting blades. That vision originated with Wes Sentell, a forester from Arkansas who culminated his career as Woodlands Manager for Tennessee River Pulp and Paper Company, Counce, TN.

It was during this time, also, that forest tree nurseries were established with abandon. Every state forestry service built one or more of the seedling producing factories, and they were aided by nurseries of USDA Forest Service and forest industry. Even though the optimum seedling grade of the southern pines had been quantified by Dr. Phil Wakeley (USDA Forest Service) in the early 1950s the quality of the seedlings produced in the myriad nurseries was highly variable. Thus, the combination of marginal site preparation, poor quality seedlings, and lack of tipmoth control added to the notion that loblolly pine required about four years to become sufficiently established to start meaningful height and diameter growth. It was that notion in addition to the general absence of tipmoth damage to slash pine that caused foresters throughout the loblolly pine region to favor slash pine. Decades passed before the old-line foresters acknowledged that plantation-grown loblolly pine was the superior species in volume production on most soils outside of the deep South.
It was no accident that plantation forestry of the southern pines developed in parallel with tree improvement programs. In fact, Dr. Bruce Zobel was hired as a silviculturist by Texas Forest Service, not as a tree breeder. His foray into forest genetics resulted from a public lecture in Houston, Texas by Dr. Åke Guftasson, a plant breeder from Sweden. The result was the first tree improvement cooperative, with equal participation between public and private agencies. That program, organized in 1951, continues to exist as the Western Gulf Tree Improvement Cooperative. It was joined, in succession, by the University of Florida Cooperative Forest Genetics Research Program and the North Carolina State University-Industry Cooperative Tree Improvement Program. Other tree improvement initiatives were organized by public agencies, viz., USDA Forest Service, Tennessee Valley Authority, and various state forestry services. Many of these programs have waned and others, especially those housed within state forest services, have united with the cooperative programs at Texas A&M, Florida and NC State. The occasional industrial tree improvement program that was not originally aligned with one of the cooperatives has come into the fold, largely because of the wide genetic base maintained by the cooperatives.

**LAND MANAGEMENT**

To sell the tree improvement concept to cooperative members, Bruce Zobel promised a gain in volume production of five percent at the end of a rotation relative to a plantation with unimproved genetic material. Realizing that it would be 10 to 15 years before his promise could be verified and realizing that members of the cooperative would become apprehensive while awaiting the results, the entrepreneurial scientist began studies of wood properties of the southern pines and, to a limited extent, of southern hardwoods. The initial studies showed variability among and within species, and within trees from pith to bark and base to top of crown. Subsequent data eventually showed some of the wood properties, such as specific gravity, to be highly heritable. Thus, the original stop-gap initiative became an integral part of the weighted index for genetic improvement of the southern pines.

As the tree improvement programs progressed, the cooperators learned that the effort included more than just the selection of superior trees and establishment of seed orchards. The next step was progeny testing. The original idea was to evaluate the genetic worth of the selected trees by open-pollinated progeny tests, but flowering in seed orchards at an earlier age than expected, four to six years from establishment, resulted in the decision to forego open-pollination testing in lieu of controlled crossing. A dictum handed down by Dr. Zobel was that the progeny tests were to be established on land that had been site prepared following harvest of the parent stand. In short, tests were not to be established on old fields².

With the rather crude methods of site preparation that were common to the time, performance of the progeny was highly variable. The dictum was then communicated that best performance of genetically improved plant material had to be in concert with the best silvicultural practice, inclusive of site preparation, and that the tests were to be on ‘average’ sites, avoiding the most

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² Because of the high genetic correlation between trees grown in intensively managed research plots relative to those grown in commercial plantations the strategy today is to test the genetic material on the best sites regardless of previous land use.
fertile and least fertile soils. With development of the progeny, comparisons were made with the performance of the genetic material against the trees in commercial plantations. The relatively better performance of the trees in the genetic tests, inclusive of the common check, caused the cooperating members to improve their site preparation methods accordingly. Even though there are many components to site preparation, and no system is optimum for every situation, today’s example might include shearing, piling, disk ing and planting.

In conjunction with the establishment of first-generation seed orchards, it became obvious that plantations established on old fields were superior in tree growth and volume than comparable stands established on cut-over lands. A large part of that difference was attributed to the lack of woody competition, and to residual nutrients from agronomic cropping. Herbicides specific to forestry were developed that were effective in controlling all competing vegetation, and others were specific to the control of competing woody vegetation. Continued evaluation has allowed the herbicides to be prescribed for today’s use by type, amount and timing.

In concert with the control of competing vegetation, plant nutrition was found to be limiting tree growth on most sites. The most notable example was the sites in the Coastal Plain that were phosphorus deficient. Treatment of those sites with phosphate at time of planting was found to be essential for acceptable tree growth. Additional studies across the range of sites common to loblolly pine plantations showed that an application of nitrogen in combination with lesser amounts of phosphorus gave economically attractive returns when applied at time of crown closure (7 to 10 years from planting), and at about 10-year intervals, in combination with thinning, until rotation age is reached (Allen et al. 2005). Some soils have been found to be deficient in potassium, and others in boron and copper. Prescriptions fertilizer applications are now the rule with loblolly pine plantations. More that 150,000 acres receive treatment annually, and more that two million acres of forest plantations, inclusive of repeat treatments, have been treated since the practice was initiated in the 1980s (Allen et al. 2005).

This combination of factors: genetically improved planting stock, optimum site preparation, use of quality seedlings, competition control, optimum plant nutrition and stocking control has increased productivity of loblolly pine plantations from about 3 tons/acre/year in natural stands and 5 tons/acre/year in the original plantations, to as much as 15 tons/acre/year in the most recently established plantations. Tree improvement programs of the southern pines that have progressed through the third generation of breeding, are showing 30 to 40 % volume improvement over the common check. The added productivity is being realized from the best genetic stock benefiting from optimum silvicultural practices.

**SUMMARY and CONCLUSION**

The next step in the improvement of forest trees, in conjunction with continued tree breeding, will involve molecular genetics. Molecular genetics is an extension of tree improvement; the difference is that attention is given to the gene rather than to the genotype. Even then, molecular genetics has multiple uses rather than just genetic engineering. The three components are: (1) vegetative propagation of undifferentiated cells (embryogenesis), (2) gene mapping for marker-aided selection and breeding, and (3) genetic engineering (Yanchuk, 2001).
A segment of forest industry has supported university initiatives in biotechnology research since about 1980, even to the point that some initiated in-house projects. More recently, those separate in-house programs have formed alliances with their competitors, largely because of the cost of the separate programs and the competition encountered in developing intellectual property. The best example of the collaboration among competing companies is ArborGen. That stand-alone company consists of the biotechnology resources of International Paper and MeadWestvaco of the U.S. and Rubicon and Genesis Research of New Zealand. The plant material emanating from ArborGen will accrue first to the sponsoring companies, and subsequently to buyers of the plant material.

Considerable controversy is associated with the release of genetically modified trees, even though some years will elapse before commercial plantations of forest trees are established. To provide a platform for open communication around the benefits and risks of forest biotechnology, a new organization was formed, the Institute of Forest Biotechnology (IFB). IFB is an independent organization that has the objective of working for societal, ecological and economic benefits from appropriate uses of forest biotechnology on a worldwide basis. A major cornerstone of IFB is Heritage Trees®. Heritage trees are defined as those that are threatened, endangered or have intrinsic historic or economic value. American chestnut is the poster child of that initiative. Working with The American Chestnut Foundation (TACF), IFB has convened a group of scientists from Syracuse (ESF), Penn State, NC State, Clemson, Georgia, and USDS Forest Service, to identify the genes in Chinese chestnut that connote resistance to chestnut blight. The results of that research will be used for directing breeding programs (marker-aided selection) and for insertion of the identified genes into American chestnut to give the desired fungal resistance. Programs such as Heritage Trees® are important in that they provide a mechanism for direct social benefit from a new technology. In this vein, regulatory agencies are immune to placing value on a product, whereas society demands a favorable risk/benefit ratio such as provided by the Institute of Forest Biotechnology.

The model envisioned by Bruce Zobel over 50 years ago, for public and private agencies to work together, still lives on. The difference today is that we are equally concerned about a protein produced from a DNA contig as were the silviculturists (geneticists) of yesteryear being concerned about the proper source of seed for plantation establishment. We’ve come a long way, baby!

**LITERATURE CITED**


Tree Improvement and Forest Management in the Evolving Landscape of Forest Land Ownership

Al Lyons¹

Timberland ownership in the United States has evolved over the last twenty-five years as traditional forest products companies have divested timberland to provide capital to improve their manufacturing facilities, to pay down debt, or to provide shareholder returns. Institutional ownership of timberland has accelerated due to these divestitures and passage of the Employee Retirement Income Security Act (ERISA) of 1974. The ERISA laws encouraged institutional investors to diversify away from traditional fixed-income securities and led them first to invest in stocks and commercial real estate. Timberland ownership provided yet another opportunity for diversification improving the risk-efficiency of a mixed-asset portfolio without sacrificing returns. Timberland investments are long-term, in nature, and are attractive to pension funds and institutional investors as they have a long-term investment perspective. Management of these investments is also governed by the ERISA laws which protect the assets of these funds through its fiduciary provisions. The fiduciary must act solely in the interest of the fund with the exclusive purpose of providing benefits.

Hancock Timber Resource Group (HTRG) was established in 1985 and became the first Timber Investment Management Organization (TIMO) dedicated to manage timberland investment portfolios. Since that time, approximately twenty TIMOs have been established and two timberland Real Estate Investment Trusts (REITS) have been formed. While each of these organizations share a common interest in investing in timberland assets, their approach to managing this investment can be quite different. The HTRG philosophy of timberland investment, which includes involvement with cooperatives, research, technology transfer, genetic improvement, and intensive silviculture will be presented to provide the tree improvement community a better understanding of HTRG’s unique approach to timberland investing and our commitment to tree improvement, forest management, and research.

Hancock Forest Management conducts the day-to-day forest management with the goal of improving overall returns, reducing cost, and improving alignment of interests. Intensive forest management is an integral part of the investment strategy and routinely boosts investment performance. HTRG’s business model incorporates applied forest cooperative research, such as tree improvement, to guide sound silvicultural investments, which provide increased investor returns.

¹ Manager of Silviculture and Stewardship, Hancock Forest Management, Harpersville, AL
Issues Facing State Tree Improvement Programs

Russ Pohl1

State tree improvement organizations across the South are confronted by a common dilemma, namely, rapidly decreasing resources in the midst of expanding program demands and opportunities. Legislative appropriations for forestry agencies have been generally declining a number of years. Agencies have responded by looking to other revenue resources, primarily seedling sales, for funding. Yet tree planting in the South remains sluggish. While spectacular gains from clonal forestry in pines are tantalizingly close, poorly funded state agencies drift farther away from capitalizing on the opportunity. Urbanization, restoration, and changing land ownership patterns have shifted tree planting emphasis away from pines and toward hardwood species. This shift suggests a need for improvement efforts in non-pine species, but breeding and testing activities in many species remains non-existent or minimal, at best. With the budgetary constraints typically imposed on state agencies this situation is not likely to change in the very near future.

1 Chief, Reforestation Department, Georgia Forestry Commission, Dry Branch, GA
Improving Forest Productivity Through Biotechnology

Mark Rutter\textsuperscript{1}

Significant gains have been made with loblolly pine through well-developed tree breeding and testing programs. The technology is now available to build on these successes and provide additional gains through clonal selection within families and through genetic transformation.

Over 6,000 clones of loblolly pine have been delivered to customers for clonal testing through ArborGen. A new clonal testing consortium (the ArborGen Testing Service) was established last year with key industrial players. Hundreds of clones were established in tests with the group in 2004 and over 1,000 clones will be tested in 2005. The successful identification of superior clones must be based on well-replicated tests over many sites within recognized breeding zones.

The application of biotechnology to the further improvement of superior loblolly pine clones is becoming a reality. Early field trials are demonstrating the application of biotechnology to the improvement of growth and pine and cottonwood. Significant reductions in lignin and S/G ratios have been demonstrated in Eucalyptus.

\textsuperscript{1} Project Leader, Somatic Embryogenesis Services Group, ArborGen, Summerville, SC
Clonal Forestry: Out of the Lab, Finally

John Pait¹

After 50 years of breeding and orchard production of southern pines, the full realization of genetic improvement is just now arriving in the form of clonal forestry. Several thousand acres of clonal loblolly pine have been planted in the last three years following decades of research at significant costs. Planting loblolly clones will increase by at least an order of magnitude in the next five years. Key drivers for this trend include gains in yield and disease resistance, quality traits, uniformity, speed of deployment per breeding cycle and the development of cost-effective production systems. The dominant business model will be that of a technology provider using a somatic embryogenesis production system funded by private sector investment, infused by cooperative and partnership breeding, and seamlessly delivering elite genetics to forest landowners in the form of planting stock. Clone trials to date indicate substantial gains, high heritabilities, and low levels of G x E. Longer term stand level research for accurate growth and yield prediction and silviculture optimization is still needed. Given the extraordinary gains and higher costs of clonal stock, forest managers have unique opportunities and decisions ahead. The historical value model of the US South in which most of the financial value created by breeding and testing flows to the landowner and almost none flows to the breeder-developer will change. Forest information systems will need to spatially track genotype planting for proper future valuation. Similarly, clonal deployment decisions must take place on a landscape scale, spatially and temporally, to supply the data necessary for forest health and stand development monitoring.

¹ Sr. Vice-President, CellFor Corporation, Atlanta, GA

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Genomic Resources for the Study of Loblolly Pine and Other Conifers

J.F.D. Dean¹

Genomic techniques and technologies are poised to revolutionize many of our approaches to forest biology, as well as forest management. Rapid advancement in the genomic sciences and their infiltration into so many aspects of biology has been facilitated by the rapid release of large datasets to the public via the Internet. Such openness with data release is a relatively new phenomenon, and is predicated on the expectation that important and useful information will be gleaned more efficiently when multiple groups of researchers are allowed to apply a wide variety of bioinformatics tools independently to the analysis of the enormous datasets produced by genomic techniques. One of the challenges facing all biologists is how to keep up with the growing array of databases and bioinformatic tool sets to identify those most useful for addressing particular sets of problems.

Although the development of genomic datasets for conifers has lagged the torrent of information being made available for various model systems, as with other organisms, conifer resources are growing rapidly, and with them come great opportunities to mine the data for new research hypotheses. This presentation will summarize the current content of conifer information available at major public repositories for genomic data, such as GenBank and EMBL. Conifer data housed at smaller online sites, such as Dendrome and Fungen, will also be reviewed. Both structural, as well as functional genomics resources will be covered. A variety of bioinformatic tools available to online users for mining these datasets will also be discussed. As loblolly pine is the conifer species for which the most data is available online, most of the examples to be presented will focus on this species, but examples for other species, particularly the spruces, will also be examined.

¹ Warnell School of Forest Resources, University of Georgia, Athens, GA 30602
The Cellulose Synthase and Cellulose Synthase-Like Gene Superfamily in the *Populus* genome

Laigeng Li, Shiro Suzuki, Ying-Hsuan Sun, and Vincent Chiang

One of the main objectives of growing forest plantation is to provide raw materials for cellulose fiber production. However, it is still unclear how cellulose biosynthesis is genetically controlled during wood formation. In this study we identified all of the possible cellulose synthase (*CesA*) and cellulose synthase-like (*CSL*) genes in the *Populus* genome. There exist multiple *CesA* and *CSL* genes represented by 18 and 26 genomic loci, respectively, in the tree species. The expression of these genes in various tissues was profiled by real-time PCR; and the wood formation-related *CesA* and *CSL* genes were identified. The analysis of the secondary structure of the gene products suggested that CesA and CSL might function in a variety of means to control cellulose properties in wood formation. The application of the genomic information to tree improvement will be discussed.

Acknowledgement: This project is partially funded by Forest Biotechnology Industrial Research Consortium (FORBIRC).
Pitch Canker, caused by the necrotrophic fungus *Fusarium circinatum*, is a fungal disease that has been detected in loblolly pine plantations since 1974 where it causes excessive pitch production and shoot dieback. Fusiform rust, caused by the biotrophic fungus *Cronartium quercuum* f. sp. *fusiforme*, has been a major disease of southeastern conifer plantations since the early 1920's. We are seeking to identify genes that determine quantitative resistance and genetic markers that can be used in resistance breeding. Single nucleotide polymorphisms (SNPs) in candidate genes conferring resistance to these pathogens were identified based on direct DNA sequencing. Candidate genes for disease resistance are categorized as: *Positional Candidates*, referring to candidate genes that are near QTLs for cell wall chemistry like phenylpropanoid pathway genes and cellulose synthase genes, *Expression Candidates*, referring to genes identified by expression analyses (chitinases and myb and WD40 class transcription factors), *Functional Candidates*, referring to genes and regulatory regions whose roles in disease resistance have been identified in other plant systems through sequence homology to loblolly pine ESTs and bioinformatics. A total of 57 candidate loci have been screened first for identification of polymorphic locations that might lead to physiological changes in the disease resistance phenotype, and then selected SNPs have been inquired for their relevance to the variation observed in the phenotypes via association testing of these polymorphisms over an association population of 960 individuals. Progress to date on the successful associations made will be presented; two pathosystems will be compared and contrasted regarding allelic associations of the loci detected to be associated with resistance.
Allelic variation for 13 microsatellite loci was evaluated within the Founder Population of loblolly pine (*Pinus taeda* L.) provided by the three Southern U.S. University/Industry Tree Improvement Cooperatives. Our current sample of the population includes nearly 700 trees selected from natural stands in 290 counties across 13 states. Based on mapping results (data not presented), the 13 microsatellites sampled 10 independent (unlinked) regions of the loblolly pine genome. Average expected and observed heterozygosites among the marker loci ranged from 0.25 to 0.95. Most of the loci were quite informative with heterozygosity rates in the 0.70 to 0.90 range. Analyses of the overall structure of the Founder Population clearly showed the widely known west-east subdivision formed at the Mississippi River. In addition we found evidence for a northwest-southeast subdivision of the population east of the river. The line of this subdivision ran from north Mississippi through central Florida. Two independent analytical approaches revealed this three-way population differentiation for most of the markers. No evidence was found for subdivision west of the Mississippi River, even though samples were included from the Lost Pines area of Texas. Future plans include at least doubling the number of microsatellite loci analyzed to improve the resolution of the population structure analyses and increasing the sample size especially in the more lightly sampled areas of southeast Alabama and southwest Georgia. A detailed understanding of population structure is necessary for developing genetic conservation plans and analyzing association genetic studies aimed at mapping quantitative trait loci (QTL) at the population level.
Identification of a New Retrotransposable Element in Loblolly Pine

M.N. Islam-Faridi\textsuperscript{1}, A.M. Morse\textsuperscript{2}, K.E. Smith\textsuperscript{3}, J.M. Davis\textsuperscript{4}, S. Garcia\textsuperscript{5}, H.V. Amerson\textsuperscript{6}, M.A. Majid\textsuperscript{7}, T.L. Kubisiak\textsuperscript{8}, and C.D. Nelson\textsuperscript{9}\textsuperscript{*}

We initiated a project to locate the genomic position of fusiform rust resistance gene 1 (\textit{Fr1}) in loblolly pine using fluorescent \textit{in situ} hybridization (FISH). Four random amplified polymorphic DNA (RAPD) markers previously found to be tightly linked to \textit{Fr1} were cloned and sequenced, providing a total coverage of about 2 Kb. In order to obtain discernible signal of single-copy sequences using FISH, a minimum of 5 Kb of DNA is required. Therefore, GenomeWalker (Clontech) was used to obtain flanking genome clones and sequences for each of these markers. We were successful in obtaining an additional 2.3 to 3.6 Kb for three of the four markers, totaling 8.7 Kb. Initially, DNA from these three markers was mixed in a single cocktail and used to probe loblolly pine chromosomal spreads. Assuming that each of the markers consisted of single-copy DNA, we expected to observe FISH signals on only a single pair of homologous chromosomes. However, fairly intense FISH signals were observed throughout the entire \textit{Pinus} genome, including loblolly, slash, and longleaf pines. Probing chromosome spreads using DNA clones individually from each marker suggested that two of the three clones contained high-copy DNA. One of the repetitive clones was found to be highly similar to a retrotransposable element in the model angiosperm \textit{Arabidopsis}. Interestingly, this retroelement has not invaded the \textit{Pinus} centromeres or major rDNA sites. More research is underway to study the distribution of this retroelement in various \textit{Pinus} species as well as in closely and distantly related gymnosperms. Work also continues on localization and characterization of the \textit{Fr1} locus in loblolly pine.

\textsuperscript{1}Research Geneticist, \textsuperscript{2}Post-doctoral Scientist, Southern Institute of Forest Genetics, USDA Forest Service, Forest Tree Molecular Cytogenetics Lab, College Station, TX; \textsuperscript{3}Research Scientist, \textsuperscript{4}Associate Professor, School of Forest Resources & Conservation, University of Florida, Gainesville, FL; \textsuperscript{5}Biological Sciences Technician, Southern Institute of Forest Genetics, USDA Forest Service, Gainesville, FL; \textsuperscript{6}Research Technician, \textsuperscript{7}Associate Professor, Department of Forestry & Environmental Sciences, NC State University, Raleigh, NC; \textsuperscript{8}Research Geneticist, \textsuperscript{9}Research Geneticist and Project Leader, Southern Institute of Forest Genetics, USDA Forest Service, Saucier, MS
Molecular Genetics of Cellulose Synthesis in Developing Wood of Loblolly Pine

C. J. Nairn, A. Wood-Jones, W. Lorenz, and J.F. Dean

Cellulose is a major component of wood and wood fiber. The quantity, quality and deposition of cellulose in the secondary cell wall of vascular tissues determine a number of important wood fiber properties and consequently the suitability of wood and wood fiber for various uses. Recent progress in model plant systems has identified a number of genes necessary for cellulose synthesis, however, our knowledge of the molecular and cellular control of cellulose synthesis remains incomplete. Three genes or gene families directly involved in cellulose synthesis have been identified. These include the genes encoding the catalytic subunits of the cellulose synthesis complex, a membrane bound cellulase, and sucrose synthase.

The CesA multi-gene family encodes the catalytic subunits of the cellulose synthesis complex in plant cells. In angiosperms, two different groups, each containing three genes, are necessary for cellulose synthesis in the plant primary and secondary cell wall, respectively. The three secondary cell wall CesA genes are functionally non-redundant paralogs and all three are required for normal cellulose synthesis in vascular tissues as demonstrated by analysis of cellulose deficient mutants in Arabidopsis. Comparative analysis of the CesA gene families from monocot, herbaceous dicot, and woody perennial dicot species indicates that the secondary cell wall CesA genes in angiosperms are orthologous and functionally conserved. A specific member of the γ subfamily of the cellulase (endo-β-1,4-glucanase) multi-gene family, which contains a putative trans-membrane domain, is necessary for normal cellulose synthesis. A gene encoding this enzyme, KORRIGAN, was first identified in cellulose deficient mutants of Arabidopsis. Sucrose synthases are also encoded by a multi-gene family and are believed to supply the substrate, UDP-glucose, to the cellulose synthesis complex.

We have cloned full-length cDNA sequences representing three CesA genes from developing xylem of loblolly pine (Pinus taeda L.). Phylogenetic analysis indicates that these genes are orthologous to the secondary cell wall CesA genes of angiosperms. These three genes are co-expressed in loblolly pine tissues and higher levels of expression are correlated with tissues undergoing secondary cell wall biosynthesis. These data are consistent with conservation of functional roles for orthologous secondary cell wall CesA genes in angiosperms and gymnosperms, suggesting that the gene family and their functional roles evolved prior to the divergence of extant seed plant lineages. We have also isolated full-length cDNA clones for putative orthologs of the membrane bound cellulase and sucrose synthase from developing xylem of loblolly pine. Phylogenetic analysis indicates that these genes are orthologous to those implicated in cellulose synthesis of angiosperms. Preliminary data for expression of these genes are consistent with conserved functions for these components of cellulose biosynthesis in the secondary cell wall of loblolly pine during wood formation. These full-length cDNA clones will facilitate functional analysis and are potentially useful in developing markers for genetic selection strategies and/or cellulose modification through direct gene transfer.

1 Daniel B. Warnell School of Forest Resources, University of Georgia, Athens, GA, USA
Forty Years of Genetic Improvement of Shortleaf Pine in Missouri

D. P. Gwaze¹, R. Melick², C. Studyvin² and M. Coggeshall³

Abstract: Shortleaf pine (Pinus echinata Mill.) is the only native pine species in Missouri, and its restoration is a top priority in the state. Because of the great interest in the species, a genetic conservation and breeding program for the species was initiated in the 1960s by the Mark Twain National Forest, in collaboration with the Missouri Department of Conservation and the Ouachita National Forest. In the 1960s, seed production areas were established by the Missouri Department of Conservation and Mark Twain National Forest. The Missouri Department of Conservation also established provenance tests during the same period. Early results indicated that provenance variation was small, but within provenance variation was large. In the late 1960s, the Mark Twain National Forest selected 66 superior trees from natural stands throughout Missouri. Fifty of the superior trees were grafted into a 1st-generation seed orchard on the Ouachita National Forest in Arkansas. Operational seed collections from the clonal seed orchard were made in 1981, 1983, 1986 and 2003. Currently, all planting and seeding needs in Missouri are met with genetically improved seed from this clonal seed orchard. Open pollinated progeny tests were established in the early 1980s to evaluate orchard parents. A controlled pollinated progeny test was established in 2002 to further evaluate parents in the seed orchard, and to develop a 2nd-generation seedling seed orchard. Progeny test results suggest that genetic variation exists within shortleaf pine, and genetic gain is predicted to be significant. Future challenges and opportunities are discussed.

Key words: Shortleaf pine, selection, testing, breeding, seed production

INTRODUCTION

Shortleaf (Pinus echinata Mill.) is one of the four major southern pines in the United States. It is the only native pine species in Missouri. The range of shortleaf pine has been reduced from an estimated 2.6 million ha to 162000 ha by 1976 in the Missouri Ozarks (Essex and Spencer 1976) due to extensive logging from 1880 to 1920, frequent wildfires and overgrazing (Cunningham and Hauser 1984). Today, sites formerly occupied by shortleaf pine have many hardwoods species, many of which are not as well adapted as shortleaf pine to the dry, nutrient-poor and eroded sites in the Ozark Highlands of Missouri and Arkansas. Currently, these less adapted hardwood species are experiencing problems with oak decline associated with red oak borers and Armillaria root rot. The role of shortleaf pine in maintaining an ecologically stable and productive ecosystem in Missouri is well recognized (Law et al. 2004).

¹ Missouri Department of Conservation, 1110 S. College Avenue, Columbia, MO 65201
² Mark Twain National Forest, 401 Fairgrounds Road, Rolla MO 65401
³ University of Missouri Center for Agroforestry, 10 Research Center Road, New Franklin, MO 65274
In Missouri, shortleaf pine is important for both wildlife habitat and timber products. The cooper (Accipiter cooperi) and the sharp-shinned hawks (Accipiter striatus) nest in shortleaf pine stands in Missouri (Kritz 1989). The red-cockaded woodpeckers (Picoides borealis) prefer mature or over-mature shortleaf pine forests and are endangered because of the overexploitation of mature shortleaf pine trees (Cunningham 1940). Shortleaf pine has excellent stem form yielding high-valued posts and poles. It has dense, strong and easy to work wood valued as sawn timber and pulpwood.

Due to its ecological and economic importance, restoration of shortleaf pine is a priority in Missouri. Consequently, with considerable collaboration over the years, shortleaf pine tree improvement programs were started in the early 1960s by the Mark Twain National Forest and in 1967 by the Missouri Department of Conservation (Stelzer 1981).

The objective of this paper is to give a historical account of the shortleaf pine tree improvement activities in Missouri, provide recent estimates of genetic parameters and genetic gain, and to discuss future options for the genetic improvement of shortleaf pine in Missouri.

**BREEDING OBJECTIVES AND SELECTION CRITERIA**

In the 1960s when the improvement program was started, shortleaf pine was grown primarily for timber production. Breeding objectives were thus related to the requirements of fast-growing trees to provide valuable timber and income. These breeding objectives were focused on an increase in profitability to the landowners and processors through shortening the rotation, increasing volume growth, and by increasing stem quality. A secondary, but important objective was to maintain a broad genetic base. This broad genetic base would serve to buffer against changes in climate, pests and diseases, products or markets. The major selection criteria for shortleaf pine improvement in Missouri were (Stelzer 1980):

- Superior height growth,
- Superior diameter growth,
- Good self-pruning ability,
- Straight bole,
- Small well formed crown
- Resistance to insects, and
- Resistance to diseases.

Today shortleaf pine forests are less important as a source of wood products and more important as a source of ecological services – e.g. biodiversity and mitigation of oak decline. Although selection criteria for ecological purposes may differ from those for timber production, many traits are common to both objectives. For example, one of the most important traits for selection for ecological purposes is broad adaptability. Because growth is a reflection of adaptive traits, such as tolerance to specific environments and tolerance of pests and diseases, selections made for timber production in the past will also be useful for restoring shortleaf pine for ecological purposes.
TREE IMPROVEMENT STEPS

Genetic improvement of any tree species relies on understanding and using variation that occurs within tree populations. Tree improvement increases the value of a tree species through a continuous four-stage process: selection, breeding, testing and seed production. The process is ongoing and recurrent. Thus, genetic gains are accumulated across generations. Trees with an increased frequency of genes contributing towards the traits under selection are selected (plus trees) and comprise what is known as the breeding population. These plus trees are grafted into a seed orchard to produce genetically improved seed. The plus trees are then tested by making controlled crosses among parents and subsequently evaluating the seedlings derived from the breeding program in multiple progeny test plantings. Testing provides the information required for roguing existing seed orchards and also provides a population from which advanced-generation selections are made. Genetic improvement of shortleaf pine in Missouri has followed these same tree improvement steps, namely: 1) selection of plus trees, stands and provenances, 2) establishment of clonal seed orchard, 3) testing of plus trees and 4) mating of best plus trees (Figure 1).

Selection

Provenance selections

Most tree improvement programs in the United States, and indeed in many other countries, started with provenance tests, and the shortleaf pine tree improvement program in Missouri was no exception. Provenance testing aims at defining both the genetic and environmental components of phenotypic variability between trees from different geographic origins. Provenance trials enable a range of seed sources of a single species to be evaluated on a given site. They provide evidence upon which decisions are based for future seed procurement for use in reforestation programs. Large gains in productivity can be made through the selection of the best provenance (or seed source) of a species for a given site and purpose. Thus, provenance testing research normally receives highest priority at the outset of most applied tree improvement programs. In Missouri, the objectives of the provenance tests were to determine if significant and meaningful variation among different provenances exists in shortleaf pine in Missouri, and to determine where and how far these geographic seed sources could be moved without affecting their superiority and adaptability.

In 1967, seed was collected from eight Missouri seed sources (provenances) by the Missouri Department of Conservation. Seedlings were raised at the George O. White State Nursery and eight provenance tests were established from 1968-1970 throughout Missouri’s shortleaf pine range. Details of the provenance tests and the results at juvenile stage have been reported by Brunk (1972, 1977). Two of the tests were established using direct seeding.
Shortleaf pine provenances had excellent survival and growth. Two-year results indicated that there were significant differences among provenances for four of the provenance tests, two of which were established by direct seeding. The early results showed that the Cash provenance (Ozark county) performed consistently well at a number of sites, and that the Hawn provenance (Ste. Genevieve county) did extremely well at the Eminence site. Five-year results showed significant differences in height for four provenance tests, one of which was established by direct seeding. The Cash provenance still maintained its good performance at 3 sites, but had a drastic change in ranks at three sites. It was ranked second at age 2 and its ranking fell to seven at age 5. Measurements at older ages would have provided better data on which to base predictions of growth performance and possible genetic gains to be obtained through the use of specific provenances. Early provenance effects were inconsistent across sites and across ages making it difficult to draw conclusions. Also, many of the provenance tests did not show any significant difference. Therefore, a shortleaf pine tree improvement and orchard establishment program which concentrated on identifying the best individuals regardless of provenance, as done by the Mark Twain National Forest in the 1960s, appeared to be the best option. Small variation among
provenances and large variation within provenance has been reported in other states such as Oklahoma (Tauer and McNew 1985).

In 1952-3 and again in 1956-7, two shortleaf provenance tests (Series 1 and 4) were established at Sinkin Forest Experiment Station by the USDA Forest Service - North Central Research Station. These two tests were part of the Southwide Pine Seed Source Study initiated in 1951 by the Southern Forest Tree Improvement Committee. A total of 52 southwide shortleaf pine seed source tests, comprising 6 series, were established throughout the shortleaf pine natural range. The objective of the committee was to determine the degree to which inherent geographic variation patterns in the four major southern pines is associated with geographic variation in climate and physiography. The information from these tests was to determine if local seed sources are best to use in developing tree improvement strategies, and also to potentially identify non-local seed sources for growing stock purposes. Provenances tested in Missouri came from Arkansas, Louisiana, Mississippi, Missouri, New Jersey, South Carolina, Tennessee and Virginia. Information from all shortleaf pine southwide tests has been used to develop seed source guidelines (Schmidtling 2001). These guidelines indicate that seedlings will survive and grow well if they come from any area having a minimum temperature within 5°F of planting site’s minimum temperature. Seedlings from an area with warmer winters will grow faster than seedlings from local sources; seedlings from an area with cooler winters will grow slower. East-west transfers within a given seed transfer zone are usually successful. The seed transfer guidelines suggest that Missouri should ideally have two seed sources for shortleaf: a southern source consisting primarily of the southernmost tier of counties and a northern source consisting of the species’ remaining range in the state. The validity of these guidelines needs closer scrutiny because planting in Missouri is currently being carried out without regard to these guidelines.

**Individual plus tree selections**

Two selection programs were undertaken: one by the Mark Twain National Forest in the 1960s and the other by the Missouri Department of Conservation in the 1980s. The Mark Twain National Forest goal was to select 70 plus trees (referred to as “superior trees” in the National Forest program) in natural stands throughout Missouri, and a total of 66 plus trees were selected by 1969. These plus trees were well distributed throughout Missouri to ensure that related individuals were not selected. The criteria used to select these plus trees included:

- growth rate and vigor equal to or better than neighboring dominants;
- straightness of stems;
- above average cone production;
- above average ability to self-prune;
- resistance to pests and diseases
- above average branching qualities.

These traits were chosen because of their importance for utilization of shortleaf pine for timber production. Fifty of these plus trees were grafted into a seed orchard on the Ouachita National Forest, near Mt. Ida, Arkansas.

In 1981 the Missouri Department of Conservation also initiated a plus tree selection program for shortleaf pine. The objective was to bring these plus trees together in a clonal seed orchard. The
ultimate goal was to select 100 plus trees, 60 from plantations and 40 from natural stands. By November 1984 twenty-six plus trees had been identified in natural stands and 47 in plantations. The Missouri Department of Conservation program was parallel and not a duplicate program to the Mark Twain National Forest program. This approach was perceived to enhance Mark Twain National Forest’s program by operating a distinct, high level program which could be pooled to develop a new breeding population should either program develop any inbreeding problems. These two programs would be viewed as two sublines (Namkoong et al. 1988) where selections could be brought together in a seed orchard to avoid inbreeding. However, the Missouri Department of Conservation shortleaf pine improvement program was terminated before these selections were used.

Selection of natural stands
Seed production areas were developed in natural stands that had been identified as exhibiting above-average growth, quality and seed production. These were expected to be an interim measure until a clonal seed orchard had been established. In 1967, six seed production areas were established by the Missouri Department of Conservation in collaboration with Mark Twain National Forest. These were located in Franklin, Shannon, Reynolds, Iron (2), and Ste. Genevieve counties.

The total acreage of the Missouri Department of Conservation SPAs was 350 acres (Stelzer 1980). These stands were thinned from below during the early years of establishment, and this thinning served as an initial rouging effort. This thinning was aimed at improving the seed production potential and to improve the genetic quality of the seed collected. No seed was ever collected from these areas, and these have since been abandoned.

In the 1960s, the Mark Twain National Forest established an additional 5 SPAs, which received careful thinning, fertilization and herbicide control of hardwoods. They were never major seed producers and were abandoned in the 1980s after production at the Ouachita seed orchard came on line.

Seed production – development of 1st-generation seed orchard

Fifty of the superior trees selected by the Mark Twain National Forest were grafted into a clonal seed orchard on the Ouachita National Forest in the years 1969 to 1971. The 85 acre clonal seed orchard was located on the Womble Ranger District, Ouachita National Forest, Mount Ida, Arkansas, 35 miles west of Hot Springs. The seed orchard was located in Arkansas, rather than Missouri, because it was thought that establishing the orchard in a more southerly location would increase cone production and reduce age of flowering. It was also less expensive to establish a seed orchard on the Ouachita National Forest, where an active tree improvement program, with necessary personnel, facilities, and equipment already existed. Each of the 50 parents was represented by 160 grafts in the seed orchard planted at 15 x 30 feet. Scion-rootstock compatibility problems prevented grafting success in some plus trees. Grafts were planted in a design to minimize the likelihood of potentially related individuals being planted adjacent to each other. The number of parents was perceived to be enough to conserve the genetic resource
of shortleaf pine in Missouri. Apart from producing seed of high genetic quality, the orchard was also designed to serve as an ex-situ conservation area.

In 2000, the seed orchard was thinned and rogued. Thinning removed trees damaged by the huge ice storm which occurred in December 1999 in Arkansas. Roguing was based on the results from the Boiling Springs progeny test data collected in 1999. Using these results, inferior clones were removed based on height, diameter, straightness and forking. Currently, 33 clones exist in the seed orchard. Seventeen of the original 50 clones were removed from the orchard, and the remaining 33 clones have been thinned by 50%. This roguing has increased the genetic quality of seed without seriously compromising the genetic diversity. Thinning, roguing and natural mortality have reduced the number of trees in the seed orchard to 60% of the original total.

No pesticide or fertilizer has been applied to the seed orchard since 1986. Prior to 1986 Furadan, Guthion, and Pydrin were used to protect the seed orchard from insects, such as Nantucket pine tip moth, cone worms of the Dioryctria species, and the shieldback and leaffooted pine seed bugs. The orchard was fertilized each year with 300 lbs per acre of Ammonium Nitrate (34-0-0) for several years prior to 1986. The ability to control the insects and to fertilize the seed orchard increased production of seed tremendously in the 1980s.

There is a 400 foot wide buffer strip surrounding the exterior of the orchard. This buffer is for pollen exclusion and fire protection, and it is burned each year in the late winter. After the buffer strip is burned it is bush hogged to knock down the brush and hardwood sprouts. It is unknown how effective the buffer strip is in preventing pollen contamination. Pollen contamination could have advantages in increasing the genetic diversity of seed used for restoring shortleaf pine in Missouri. However, pollen contamination may present risks in adaptability when the seed is planted close to the northern edge of the shortleaf pine range in Missouri. The level of pollen contamination needs to be quantified. This information will be critical for ensuring that shortleaf pine seedlings are well adapted to all Missouri growing conditions. In the past the interior of the orchard was bushhugged at least once and preferably twice during the year, and herbicides were used along the rows where the trees were spaced too closely to allow bushhoggging without damaging the trees. Currently, there is little management of the orchard other than management of hardwood regeneration by infrequent bushhoggging.

Excluding small seed collections for open-pollinated progeny tests, seed was first collected from the Mt. Ida orchard in 1981. A total of 215 pounds was collected. A total of 1578 pounds was harvested in 1983. After this second collection there was ample supply of seed in storage and a decision was made to collect only when there was a good seed crop. The third collection was made in 1986 when a massive 2554 pounds were collected. The bumper harvest in 1986 is attributed partly to the use of pesticides and fertilizers. After this large harvest, no cones were picked from the orchard until 2003. Shortleaf pine does not regularly produce large cone crops (Brinkman and Rogers 1967), and few cones were available for picking during that time, partly due to lack of fertilization and protection from cone and seed insects. In 2003, 1500 pounds of seed were collected. This harvest was attributed partly to favorable weather conditions and partly to the thinning and rouging carried out in 2000.
Seedling production and seed requirements

Shortleaf pine is one of the most widely planted tree species in Missouri, with approximately 400,000 seedlings sold to private landowners and public institutions by the George O. White state nursery in 2003 (Figure 2). In 2003 ninety-seven percent of the seedlings were delivered to private land owners, 2.5% to state and 0.5% to Federal government. This represents a sharp drop in shortleaf pine planting by the Mark Twain National Forest, which typically planted 1 to 2 million seedlings annually during the 1980’s.

![Figure 2. Trend in the amount of seedlings sold by the George O. White nursery during the period 1990 to 2003.](image)

All planting and seeding needs in Missouri are currently being met from genetically improved seed collected from the grafted seed orchard in Mt. Ida. The State of Missouri has used 34-80 pounds of seed for planting and 0-109 pounds for seeding per year over the past eight years (Figure 3). Seed requirements for direct seeding fluctuate greatly while seedling production needs are more stable. Thus, average annual seed requirements are approximately 100 lbs for both direct seeding and seedling production. Based on this seed requirement figure, Mark Twain National Forest and Missouri Department of Conservation in combination have about a 30-year supply of shortleaf seed available for future afforestation needs. Storing shortleaf pine for 30 years should not present any problems as shortleaf pine is known to store well up to 35 years (Wakeley and Barnett 1968).
Figure 3. Trend in the amount of seed used for seeding and planting during the period 1996 to 2004.

Testing

Progeny tests were established to:
1) provide a genetic evaluation of the parents, and use this information for thinning the Mt. Ida clonal seed orchard,
2) estimate genetic parameters, and use these genetic parameters to predict genetic gain.
3) convert the progeny test established using controlled crosses to a seed orchard,
4) use the progeny tests for demonstration purposes.

Early 1980s half-sib progeny tests

The first three series of shortleaf pine half-sib progeny tests were established by Mark Twain National Forest. The first major series of open-pollinated progeny tests were established in 1980 at Boiling springs (Houston Ranger District), Blue Buck (Willow Springs Ranger District) and Enough (Potosi Ranger District). Unfortunately all progeny tests died because of severe summer heat and drought. The second series of progeny tests were established in 1982 at Boiling Springs and Enough, and the third series in 1983 at Blue Buck, Boiling Springs and Enough. Unfortunately, both the second and third series progeny tests, except the 1982 progeny test at Boiling Springs, had poor survival. All the families in the three series were derived from the grafted trees in the seed orchard in Mt. Ida. As indicated previously, roguing of the seed orchard in 2000 was based on the 1999 data collected from the 1982 Boiling Springs progeny test.

The Boiling Springs progeny test was assessed at 2, 4, 8, 10 and 17 years of age for height, diameter, volume, and straightness. In order to develop a breeding strategy and to justify the investment in improving shortleaf pine, it is vital that genetic parameters are estimated and genetic gain is quantified. To this end, a comprehensive effort is currently underway to capture and analyze this early data on computer. The 10 and 17 year data were analyzed, and families were found to significantly differ (P < 0.05) in all growth traits, but not for straightness (Gwaze et al. in review). All growth traits were strongly inherited, with age 17 family heritability estimates for height, diameter and volume being 0.46, 0.31 and 0.46, respectively (Table 1).
Heritability estimates decreased with increase in age. Genetic correlations between growth traits were highly positive, suggesting that one can select and breed for one trait without affecting the others.

Percent gains in volume under various selection differentials are shown in Table 2 (Gwaze et al. in review). If the seed orchard is not rogued and all families are allowed to cross pollinate genetic gain in volume production is predicted to be 6.7% and 27.2% for 10-year and 17-year volume, respectively. Roguing the seed orchard by leaving the top 50% of the families results in a 17.8% and 37.6% gain in volume at 10 and 17 years, respectively. Gains in volume were much higher than those for height and diameter. This is expected because volume is a function of diameter and height, and diameter values are squared in volume calculations. Genetic gains in 5-year volume of 10-15% from the 1st generation unrogued shortleaf pine seed orchard have been predicted in Arkansas (Kitchens 1986). Our gain estimates at age 10 were slightly lower than those predicted in Arkansas at age 5, and our gain estimates at age 17 were much higher. Our gain estimates are likely to be biased because they are derived from only one test and there was only one checklot in the test.

Table 1. Overall means and family mean heritability estimates for height, diameter and volume at 10 and 17 years of ages.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Means Age 10</th>
<th>Age 17</th>
<th>Family mean heritability Age 10</th>
<th>Age 17</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height (m)</td>
<td>6.49</td>
<td>11.03</td>
<td>0.67</td>
<td>0.46</td>
</tr>
<tr>
<td>Diameter (cm)</td>
<td>5.17</td>
<td>7.33</td>
<td>0.61</td>
<td>0.31</td>
</tr>
<tr>
<td>Volume(dm³)</td>
<td>4.73</td>
<td>16.13</td>
<td>0.66</td>
<td>0.46</td>
</tr>
</tbody>
</table>

Table 2. Estimated genetic gains (% above checklot) from family selection for height, DBH and volume based on Boiling Springs progeny test.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Age 10 Unrogued</th>
<th>Select Top 50%</th>
<th>Age 17 Unrogued</th>
<th>Select Top 50%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height</td>
<td>5.9</td>
<td>10.1</td>
<td>10.2</td>
<td>12.4</td>
</tr>
<tr>
<td>DBH</td>
<td>1.6</td>
<td>5.1</td>
<td>7.5</td>
<td>10.9</td>
</tr>
<tr>
<td>Volume</td>
<td>6.7</td>
<td>17.8</td>
<td>27.2</td>
<td>37.6</td>
</tr>
</tbody>
</table>

Mid 1980s half-sib progeny tests

In 1986, four half-sib progeny tests were established, two at the George O. White State Nursery at Licking and two additional tests were planted at Sugar Creek, Vienna Ranger District, Shawnee National Forest. All progeny tests were established at close spacing of 1 m x 1 m, and were designed to provide useful data up to age ten and then destroyed. The families in all tests were derived from the grafted trees in the seed orchard in Mt. Ida. These progeny tests were also designed to test differences between bareroot and containerized seedlings. Measurements taken eight years after establishment should provide useful genetic information. The data is being captured on computer and will be analyzed in 2005.
Early 2000s full-sib progeny test
In 1978, a breeding program was started to produce 2-parent controlled crosses from which to make selections for a second generation seed orchard. The breeding program continued until 1985. The plan called for 320 crosses using groups of 6-parent disconnected half diallels with 6 crosses between groups, and a minimum of 600 seeds per cross. Disconnected diallels allow some type of general combining ability and specific combining ability estimates to be obtained while holding down the number of required crosses. Data from this progeny test will also provide estimates of non-additive genetic variance. The controlled crosses were established in a progeny test/seedling seed orchard at George O. White State Nursery, Licking, in 2002.

Development of a 2nd generation seed orchard
The full-sib progeny test established at George O. White State Nursery at Licking in 2002 will be thinned and rogued, and converted to a second-generation seed orchard. This planting will serve a dual function of a progeny test and seed orchard. Early thinning, perhaps at 10 years, to promote flowering and development of wide crowns should be carried out. This early thinning cuts the period that the test will serve as a progeny test to ten years. It is anticipated that an additional 10% gain will be achieved from this second-generation seed orchard. However, the gain could be lower because the seed orchard is not isolated from potential sources of pollen contamination. The pollen contamination is likely to come from the surrounding stands that were established with seeds from natural stands. Locating the 2nd – generation seed orchard at the state nursery will make management and protection of the seed orchard more effective and less expensive. The seedling seed orchard could also serve as a population from which advanced-generation selections are made. Advanced generation selections should be made prior to converting the full-sib progeny test to a seed orchard. Currently, the directions for advanced generation selection and breeding are not clearly defined.

COLLABORATION
Shortleaf pine improvement has been a collaborative effort between Missouri Department of Conservation, Mark Twain National Forest and Ouachita National Forest since the 1960s. This collaborative partnership for shortleaf pine improvement has capitalized on the complementary strengths and interests of the individual institutions. For example, the clonal seed orchard in Mt. Ida was established, maintained and protected by the Ouachita National Forest. The Mark Twain National Forest provided the genetic material and the funding, and the Missouri Department of Conservation extracted, cleaned and stored the seed, and eventually raised most of the seedlings for all reforestation needs in Missouri. The partnership now includes the University of Missouri - Columbia. Continued partnerships between the four institutions will be necessary to make further advances in restoring shortleaf pine and maintaining broad genetic diversity of Missouri’s only native pine species.

IMPACT OF TREE IMPROVEMENT
One of the most notable achievements of the shortleaf pine improvement is that all planting and seeding needs in Missouri are currently being met from genetically improved seed collected from the grafted seed orchard in Mt. Ida.
George O. White State Nursery has observed that seedlings raised from seed collected from the clonal seed orchard in Mt. Ida grow faster than those from wild collections. This was partly attributed to genetics and the size of seed, which was 1/3 larger than from wild collections. The large size is probably due to enhanced growing conditions in the seed orchard - wide spacing and cultural practices such as application of fertilizer and insecticides. The larger size of seedlings raised from seed orchard seed should improve their survival and competitive ability in field plantings.

First-generation selection has been effective for improving growth of shortleaf pine in Missouri. Genetic gain in 17-year volume is predicted to be 27.2% from the unrogued seed orchard. Economic gains could be much higher due to reduced rotation age and improved growth. Further genetic gain is expected in the second generation seed orchard.

Apart from improved growth, use of seed from the clonal seed orchard in Mt. Ida is expected to increase genetic diversity due to the large number of parent trees represented. Most existing shortleaf pine forests have been high-graded, leaving trees of inferior genetic quality and questionable genetic diversity to regenerate the stands. Hence, relying solely on natural regeneration could have a severely negative impact on the future productivity and genetic diversity of shortleaf pine.

One of the most important outcomes of the shortleaf pine improvement program has been the effective collaboration between Mark Twain National Forest, Ouachita National Forest and Missouri Department of Conservation, and more recently with University of Missouri-Columbia, which has resulted in a unique partnership leading to the successful implementation of this applied tree improvement program.

**FUTURE OUTLOOK**

**Seed production**

Genetic improvement of shortleaf pine will continue to be a priority in Missouri because its restoration is receiving renewed attention. The Mt. Ida seed orchard is likely to continue to provide seed for all artificial regeneration needs in Missouri until the 2nd-generation seed orchard at Licking is in production. The second generation seed orchard should produce seed of higher genetic quality than the first generation seed orchard. It is highly recommended that two seedling seed orchards be established, one close to the George O. White State Nursery in Licking and the other on the Mark Twain National Forest using existing surplus seed from controlled crosses. This will ensure that all seed needs are met from seed orchards established in Missouri. In addition, if the current 2nd-generation seed orchard is destroyed by fire or drought, an alternative seed supply of improved seed will be available.

Despite the potential benefits of direct seeding, this regeneration method is not being widely used in Missouri because of limited seed availability. Seed orchard seed in excess of planting requirements should be made available for direct seeding. We recommend that a strategy be developed to make seed available for direct seeding purposes. We also propose that seed be collected from the Mt. Ida seed orchard specifically for direct seeding purposes when the seed...
orchard has a good seed crop. Because shortleaf pine does not produce a good seed crop regularly, we further propose that attempts be made to collect seed specifically for direct seeding from seed production areas (SPAs) established in 1967. Before any seed collection attempts can be made from the SPAs, the SPAs may need thinning and fertilizing to encourage decent cone crops. Given the large acreages with the potential to be restored to shortleaf pine, direct seeding is likely to be a viable method to make a short-term impact on the restoration efforts on both public and private lands in Missouri. Thus, every effort should be made to make seed available to those who want to regenerate shortleaf pine by direct seeding.

**Advanced generation selection**

Although there are no plans for advanced selections in the progeny test established using controlled crosses at the George O. White State Nursery, the test could still serve as a population from which advanced-generation selections are made if the need arises.

**Genetic variation**

The maintenance of high genetic variation within each of our forest trees species is important because genes represent the raw material for present and future trees. Climates surely will change in the future, as they have in the past, and high genetic diversity is the only sure way to insure that future generations of trees accommodate such a change. We support the proposed project that seeks to evaluate the genetic variation and population structure in shortleaf pine using microsatellite genetic markers. Such a project would not only determine genetic variation of shortleaf natural populations in Missouri, but also evaluate the impact of past shortleaf pine forest fragmentation on the species’ genetic diversity. Results of the study will aid in the selection of adaptable seed sources for future regeneration efforts. Quantifying genetic variation in shortleaf pine will be critical for understanding how to manage and maintain diversity in shortleaf pine and its populations. Such knowledge will be incorporated into future shortleaf pine restoration efforts. This study will be consistent with public forest policy of preserving biodiversity, especially at the genetic level.

Future challenges for utilization of genetically improved seed are likely to include, but not be limited to: 1) effective utilization of existing seed stocks, 2) justification for supporting the seed orchard at Mt. Ida under budgetary constraints, 3) contamination of the 2nd-generation seed orchard from adjacent plantings established with wild seed, 4) justification for advanced-generation selections, and 5) increased trends towards natural regeneration.

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Family Composition Changes Over Time in a 17-Year-Old Mixed-Family Loblolly Pine Stand

Joshua P. Adams and Samuel B. Land, Jr.1

Abstract: The family composition of the dominant and co-dominant crown classes (D-C crown class) in a mixed-family deployment of loblolly pine (Pinus taeda L.) was studied. Eight open-pollinated families representing four ideotypes from North Carolina were planted in a random mixture at two sites. Various stand measurements were taken at ages 5, 9, 13, and 17. A dominance percentage was calculated by dividing the number of trees of a given family in the D-C crown class by the total number of trees planted of that family. Family dominance percentages were analyzed with ANOVA at ages 9, 13, and 17. Significant family differences were found at all three ages. Pure-family plots were also present in the same field trial. Since family selection is primarily based on pure-family plot performance, correlations between mixed family dominance percentages and pure-family stand traits were identified.

Keywords: Stand composition, mixture deployment, loblolly pine, selection

Species mixtures have been used to improve growth and quality in forest stands. Oak-sweetgum mixtures can be beneficial to overall stand growth and development (Blackburn et al., 1999), while pure oak stands can stagnate (Meadows and Goelz, 1999). The better growth and quality in a mixed species stand is often attributed to a minimization of intra-specific competition (competition between trees of the same species). In a mixed species stand, inter-specific competition is the dominant form of competition. Use of different species, with differing growth rates, attainable heights, and tolerance levels, minimizes direct competition along the same axis in the stand.

While different species are deployed to achieve this inter-specific form of competition, deployment which exploits the genetic differences in growth rate, attainable height, and shade-tolerance level of a single species could be used to achieve the same competitive effect as a mixed species stand. Such intra-specific tolerance differences have been demonstrated. Hasenauer et al. (1994) found tolerance differences, expressed through mortality associated with stand closure, both between species and within species. Dickmann (1985) classified genotypes into broad categories or ideotypes through identification of differences in tolerance. These ideotypes were defined by tree response to inter-genotypic competition. Three ideotypes (isolation, dominating, and crop) were defined. Classifications of isolation and dominating ideotypes produced maximum individual-tree stem growth at the expense of the stand, while crop ideotype trees maximize growth production of the entire stand.

Loblolly pines are selected from pure-family plots; however, stands are often composed of family mixtures when planted for production. Thus, genotypic response to genotypes of different

1Graduate Student and Professor, respectively, Department of Forestry, Mississippi State University, Starkville, Mississippi, USA
competitive ability is often unknown. Tuskan (1984) found that families do perform differently when grown in family mixtures, inter-genotypic conditions, than in pure-family conditions. Considerations of family-by-family interactions and resulting family composition changes throughout stand development should be made when using mixed-family deployments.

The objectives of this project were to (1) examine stand volume differences that existed between mixed and pure family deployments at age 17, (2) determine if representation of families in the dominant and co-dominant crown classes in the mixed stand changed, and (3) examine the effectiveness of ideotypes, defined by growth and crown characteristics, as a method of classifying families.

METHODS

Plant Material and Experimental Design

Seedlings from eight open-pollinated families of loblolly pine in North Carolina (NC) and one open-pollinated “commercial check” from east-central Mississippi (MS) and west-central Alabama (AL) were provided by Weyerhaeuser Company. The eight families were selected based on 12-year-old progeny tests to represent ideotypes with (1) fast growth with small crown (families NC1 and NC8), (2) fast growth with large crown (families NC4 and NC7), (3) slow growth and small crown (families NC3 and NC6), and (4) slow growth with large crowns (families NC2 and NC5).

Seedlings were planted from April 22 to May 7, 1985 at two sites on the John Starr Memorial Forest (Mississippi State University School Forest) in Winston County, MS. The experimental design was a randomized complete block design with four blocks at each site. The two sites were an old field and a cutover and site-prepared area. Treatments were arranged in split-split plots, with each replication split into three spacings (1.5x1.5, 2.4x2.4, 3.0x3.0-meter). Each spacing was split into a mixed-family deployment and a set of pure-family deployments. The mixed-family deployment did not include the check. Around each pure-family subplot a single or double border row was planted. The interior trees of each pure family subplot covered an area of 0.015 hectares. Survival, dbh, total height, and crown class of all trees were measured at ages 5, 9, 13, and 17 years.

Analysis

Age 17 stand volumes were calculated for families in the mixed-family deployment and the pure-family deployment. Analysis of variance (ANOVA) was used to determine if (1) overall stand volume differences existed between the sum of the eight pure-family-subplots and the mixed-family-plot, and (2) individual families produced different volumes when deployed as a pure family than when deployed in a mixture of families. Duncan’s New Multiple Range Test was used to detect differences between both deployments of a family.

Data from the mixed plots were used to determine if representation of families in the dominant and co-dominant crown classes (D-C crown class) changed with age. All trees classified in the dominant and co-dominant crown classes were summed by family at ages 9, 13, and 17.
“dominance percentage” (DP) was calculated by dividing the number of each family’s D-C crown class trees by the total number of stems initially planted. The DP value was then used as the response variable in ANOVA at each age. The following fixed effects model was assumed:

\[ y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \delta_k + (\alpha\delta)_{ik} + (\beta\delta)_{jk} + (\alpha\beta\delta)_{ijk} \]

where \( y \) was the DP value, \( \alpha \) was the effect of the \( i \)th replication, \( \beta \) was the effect of the \( j \)th spacing, and \( \delta \) was the effect of the \( k \)th family. Variation due to site factors was non-significant and dropped from analysis to simplify the model. Family-rank correlations were determined between the mixed-family DP values and pure-family tree height, BA per acre, and survival. Spearman’s test for independence based on ranks (Spearman 1904) was used to test these correlations.

**RESULTS AND DISCUSSION**

**Volume Differences**

The differences in age 17 stand volume between the pure family deployment (sum of the eight pure family subplots) and the mixed family deployment was not significant (p-value=0.28). The average volumes of the pure family and mixed family deployments were 277.9 cubic meters per hectare and 283.2 cubic meters per hectare respectively. At age 17, deployment of the families in a mixture did not produce a higher or lower overall cubic foot volume yield than the sum of the pure-family subplots. However, the individual family volumes did significantly differ between deployments (p-value<0.0001).

![Figure 1. Deployment-by-family cubic foot volume differences. (NS=mixture and pure deployment volumes were not significantly different, \( \alpha=0.05 \)](image)
Families NC1, NC4, and NC7 all produced significantly greater volumes in a mixed family deployment than in pure-family deployment, while families NC5 and NC6 produced significantly less per acre volume in mixture than pure deployment (Figure 1). This explains why a total volume difference between the two deployments did not exist. As volume increased in the top three families in the mixed family deployment, relative to the pure family volumes, decreases occurred in family NC5 and NC6 that negated those gains. There were also three families (NC2, NC3, and NC8) that showed no significant difference in stand volume between the deployments.

**Dominance Percentage Differences**

Family composition change was investigated to determine the reason for the large significant increases or decreases in volume by five of the families when deployed as a mixture. Trees that are in the D-C crown class will, by definition, be larger in height than neighboring intermediate and suppressed trees and have less mortality; thus, families that have increased their representation in this D-C crown class will make gains in stand volume. Family DP values show that some differences in representation were occurring as early as age nine years (Figure 2). These DP values show that some families have more of the initial trees growing into the D-C crown class (e.g., NC1) and have a greater representation than other families such as NC5. The three families with the highest DP (NC1, NC4, and NC7) were the families that had significantly greater volume production in the mixed deployment than in the pure-family deployment. Similarly, the two families with the lowest DP (NC5 and NC6) were the families that had a significant decrease in volume production in the mixed-family deployment as compared to the pure-family deployment.

![Figure 2. Change in dominance percentage (stems in the D-C crown classes relative to the amount of stems initially planted) for each family.](image-url)
The three spacings also resulted in differences in the number of stems initially planted that grew into the D-C crown classes (Figure 3). As the stand aged the number of D-C crown class trees decreased. The 2.4x2.4-meter and 3.0x3.0-meter spacings initially had nearly identical DP values, but the 2.4x2.4-meter spacing began decreasing more rapidly than the 3.0x3.0-meter spacing after age nine and had a DP value that was 16 percent less by age 17. The 1.5x1.5-meter spacing had far fewer stems in the upper crown classes at each age than the two wider spacings. By age 17 the 1.5x1.5-meter spacing had a DP value 42 percent less than the 3.0x3.0-meter.

Figure 3. Change in the amount of stems in the D-C class relative to the amount of stems initially planted (DP) for each spacing.

ANOVA results showed that both spacing and family independently affected percentages of trees that were in the D-C crown class (Table 1). Significant differences were present at all ages. Interactions between spacing and family were nonexistent at all ages. Duncan’s New Multiple Range Test was used to detect differences among families’ and spacings’ average DP value (Table 2 and 3 respectively).
Table 1. ANOVA results of crown dominance percentage across ages 9, 13, and 17

<table>
<thead>
<tr>
<th>Source</th>
<th>Df</th>
<th>Age 9</th>
<th>MS</th>
<th>F-value</th>
<th>Age 13</th>
<th>MS</th>
<th>F-value</th>
<th>Age17</th>
<th>MS</th>
<th>F-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rep</td>
<td>7</td>
<td>0.009</td>
<td>1.38</td>
<td>0.013</td>
<td>1.88</td>
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<td></td>
</tr>
<tr>
<td>Space</td>
<td>2</td>
<td>0.249</td>
<td>36.40*</td>
<td>0.537</td>
<td>71.24*</td>
<td>1.281</td>
<td>128.15*</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Rep*Space</td>
<td>14</td>
<td>0.014</td>
<td>2.04</td>
<td>0.027</td>
<td>3.57</td>
<td>0.035</td>
<td>3.50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fam</td>
<td>7</td>
<td>0.096</td>
<td>13.56*</td>
<td>0.183</td>
<td>26.15*</td>
<td>0.268</td>
<td>28.25*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rep*Fam</td>
<td>49</td>
<td>0.007</td>
<td>1.04</td>
<td>0.007</td>
<td>0.93</td>
<td>0.009</td>
<td>0.95</td>
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</tr>
<tr>
<td>Space*Fam</td>
<td>14</td>
<td>0.005</td>
<td>0.68</td>
<td>0.009</td>
<td>1.14</td>
<td>0.014</td>
<td>1.37</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>98</td>
<td>0.007</td>
<td>0.008</td>
<td>0.008</td>
<td>0.009</td>
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<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

*-indicates F-value significant at the alpha=0.05

Table 2. Duncan’s test of ranked family DP means

<table>
<thead>
<tr>
<th>Year 9</th>
<th>Year 13</th>
<th>Year17</th>
</tr>
</thead>
<tbody>
<tr>
<td>Family</td>
<td>Mean</td>
<td>Grouping*</td>
</tr>
<tr>
<td>NC1</td>
<td>85.97</td>
<td>A</td>
</tr>
<tr>
<td>NC4</td>
<td>84.82</td>
<td>A</td>
</tr>
<tr>
<td>NC7</td>
<td>82.08</td>
<td>AB</td>
</tr>
<tr>
<td>NC2</td>
<td>77.98</td>
<td>BC</td>
</tr>
<tr>
<td>NC3</td>
<td>76.63</td>
<td>CD</td>
</tr>
<tr>
<td>NC8</td>
<td>73.41</td>
<td>CDE</td>
</tr>
<tr>
<td>NC6</td>
<td>71.84</td>
<td>DE</td>
</tr>
<tr>
<td>NC5</td>
<td>68.29</td>
<td>E</td>
</tr>
</tbody>
</table>

*Means followed by the same letter and case are not significantly different at the 0.05 probability level

Table 3. Duncan’s test of ranked spacing DP means

<table>
<thead>
<tr>
<th>Year 9</th>
<th>Year 13</th>
<th>Year17</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spacing (meters)</td>
<td>Mean</td>
<td>Grouping*</td>
</tr>
<tr>
<td>3.0x3.0</td>
<td>0.82</td>
<td>A</td>
</tr>
<tr>
<td>2.4x2.4</td>
<td>0.80</td>
<td>A</td>
</tr>
<tr>
<td>1.5x1.5</td>
<td>0.71</td>
<td>B</td>
</tr>
</tbody>
</table>

*Means followed by the same letter and case are not significantly different at the 0.05 probability level

Significant family differences in DP were already present at age nine (Table 2), indicating that separation of families occurred before crown closure. As the stand aged, the original separations generally increased between families. The top three families began to exert their dominance over the stand at age nine, and by age 17 had significantly higher representation in the D-C crown class (nearly twice as much as the lowest family NC6). These three families were all members of the fast-growth ideotype, which suggests that a fast-growth/slow-growth ideotype classification may be an adequate method for describing competitive ability. The one exception to the growth-
ideotype trend was NC8, which ranked sixth in mean DP. The crown-size classification showed no relationship with DP. This was unexpected since the “large crown” ideotype might be expected to take more growing space from adjacent “small crown” ideotypes and increase their own representation in the D-C crown class.

The 1.5x1.5-meter spacing had a significantly lower DP value by age nine than the other two spacings (Table 3). Not until age 13 did the 2.4x2.4-meter spacing become significantly less in DP than the 3.0x3.0-meter spacing. As the stand aged, the percentage of trees that were in the D-C crown class decreased. No rank changes were present, but the spread of percentages did increase as the stand aged. This can be attributed to the increased competition following crown closure, which caused differences in dominance to increase.

Variation in DP values among both families and spacings increased as the stand grew older. This can be attributed to increased competition during stand development, causing some families to amplify their representation in the D-C crown class while other families have their representation reduced. The same phenomenon could also be expected by increasing competition using tighter spacings. The 1.5x1.5-meter spacing had a much lower DP value than the other spacings at all ages which would, by amplifying competition in the stand, allow the detection of family differences to occur at earlier ages. Since spacings and families did not interact to affect DP, use of tight spacings could be implemented in progeny tests to cause family DP separation and detection at earlier ages. This would allow families to be selected early in progeny tests for their ability to get into the D-C crown class in a mixed deployment.

**Correlations**

Positive family-rank correlations were found between pure-family-plot heights and mixed-family-plot DP (Table 4). Correlations with height were strong at all ages, reaching their maximum strength at age 13. These positive correlations indicate that families with taller average heights will exert dominance over shorter families in a mixed deployment.

Basal area per acre was significantly correlated with DP at ages nine and above, and the correlation peaked in value at age 13. The correlations were positive and indicate that families which produce more basal area in pure-family plots will have more representation in the D-C crown class in mixed stands. However, these correlations were the weakest of all the pure-family-plot traits, so that basal area’s use for selection purposes will be relatively poor compared to early selection for height or survival.

Survival was significantly correlated with increasing DP. This correlation increased in strength as the stand grew older, peaking at age 17. This trait had the strongest correlations of all the traits studied. Yet, for selection purposes at age nine or 13, correlations were comparable in strength to the height correlations. This correlation must be used with caution. It may give a misleading characterization of a family’s true ability to dominate in the stand. Families with poor survival early in life, which could be attributed to poor genotypic hardiness or merely random mortality, will initially have fewer trees able to grow into the D-C crown class. This gives an early advantage to families with higher juvenile survival that is not attributable to competition.
Families with greater early height growth achieved dominance by the time of crown closure (around age nine) in a mixed-family-stand and established greater representation in the D-C crown class during subsequent years. In a mixed-family deployment, once these families achieved a lead in height they continued that lead through age 17. Doing so allowed these families to exert dominance by receiving needed light while at the same time denying that resource to others. These fast-growth ideotype families (NC1, NC4, and NC7) resembled Dickmann’s (1985) dominating type in this sense. However, they could also fit into his crop type, because these families had the greatest pure-family-plot yields (Table 1). These families will be defined as an “efficiency” ideotype. Families NC2, NC3, and NC8 fit into a “stable” ideotype classification by maintaining a relatively stable percentage of stems in the D-C crown class throughout stand development. These families did not exert dominance, yet they exhibited little loss of stems in the D-C crown class by having neighboring families that were competitors. One type apparent in this study that was not defined by Dickmann is a “submissive” ideotype. Families NC5 and NC6 decreased in representation in the D-C crown class. These two families had the smallest average height at age nine of any family in the study (10.2 feet and 10.4 feet respectively). Once they began to fall behind in height early in stand development, these two submissive families continued to decline in representation in the D-C crown class and would eventually be excluded from the stand.

Table 4. Correlations between pure-family-plot traits and mixed-family-plot DP at age 17.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Age</th>
<th>Spacing (meters)</th>
<th>Average for each Trait and Age</th>
<th>Average for each Age</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1.5x1.5</td>
<td>2.4x2.4</td>
<td>3.0x3.0</td>
</tr>
<tr>
<td>Height</td>
<td>5</td>
<td>+0.24*</td>
<td>+0.31**</td>
<td>+0.30**</td>
</tr>
<tr>
<td>Height</td>
<td>9</td>
<td>+0.59**</td>
<td>+0.49*</td>
<td>+0.48**</td>
</tr>
<tr>
<td>Height</td>
<td>13</td>
<td>+0.68**</td>
<td>+0.63**</td>
<td>+0.48**</td>
</tr>
<tr>
<td>Height</td>
<td>17</td>
<td>+0.62**</td>
<td>+0.60**</td>
<td>+0.48**</td>
</tr>
<tr>
<td>Basal Area</td>
<td>5</td>
<td>+0.14</td>
<td>-0.02</td>
<td>+0.10</td>
</tr>
<tr>
<td>Basal Area</td>
<td>9</td>
<td>+0.25*</td>
<td>+0.25*</td>
<td>+0.24*</td>
</tr>
<tr>
<td>Basal Area</td>
<td>13</td>
<td>+0.34*</td>
<td>+0.52**</td>
<td>+0.30**</td>
</tr>
<tr>
<td>Basal Area</td>
<td>17</td>
<td>+0.21*</td>
<td>+0.44**</td>
<td>+0.28**</td>
</tr>
<tr>
<td>Survival</td>
<td>5</td>
<td>+0.33**</td>
<td>+0.25**</td>
<td>+0.55**</td>
</tr>
<tr>
<td>Survival</td>
<td>9</td>
<td>+0.45**</td>
<td>+0.50**</td>
<td>+0.68**</td>
</tr>
<tr>
<td>Survival</td>
<td>13</td>
<td>+0.75**</td>
<td>+0.57**</td>
<td>+0.79**</td>
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<tr>
<td>Survival</td>
<td>17</td>
<td>+0.86**</td>
<td>+0.66**</td>
<td>+0.85**</td>
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<tr>
<td>Average for each Spacing</td>
<td></td>
<td>+0.40</td>
<td>+0.39</td>
<td>+0.46</td>
</tr>
</tbody>
</table>

**- trait significantly correlated at 0.05

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SUMMARY AND CONCLUSIONS

Differences did not exist in overall cubic foot volume between the mixed-family-plot and the pure-family-plot. However, volume differences did exist between pure-deployments and mixed-deployments for individual families. Three families had large volume increases from pure-plot yield to mixed-plot yield, while two families decreased in volume. A third group of families showed no significant change in volume between deployments.

The percentage of trees planted that reached the dominant and co-dominant crown classes differed by family in mixed-family plantings at ages nine, 13, and 17 years. All of these families were equally represented in the mixture at time of planting (12.5 percent for each of the eight families). The family differences increased with increasing age, and three families had significantly higher DP values by age 17 than the other families. The mixed stand will thereby become less diverse with increasing age. These were the same three families that had increased volume production in the mixed-deployment. Family ranks were constant from age nine to age 17.

Classification of families into ideotypes for fast and slow early height growth was effective for 75 percent of the families in identifying subsequent dominance performance in mixed stands. Ideotype classification for small or large crown types before crown closure was not effective. Selection of families that will dominate in mixed stand can be achieved at an early age in pure-family-plots. Selection based on pure-family height and survival at early ages showed promise. All traits tested increased their correlation with DP as the stand aged.

Many studies have favored height as a selection tool for genetically improved families (McKeand 1988, Foster 1986, and Gwaze et al. 1997). By selecting for height, selection is also being made for families that exert dominance in mixed-family deployment. In light of mixed-species hardwood studies, use of families that all express this increase of dominance with age may not be advantageous for stand level volume production or quality. Based on the hardwood practice of mixing species of different competitive levels, mixtures of dominant families (e.g., NC1, NC4, and NC7) in conjunction with stable families (e.g., NC2, NC3, and NC8) may be optimal.

Acknowledgement
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LITERATURE REVIEW


Heritability and Gain for Early Height Growth and Foliage Retention in Eastern Cottonwood from the Southeast

Jonathan P. Jeffreys and Samuel B. Land, Jr.1

Abstract: Open-pollinated seeds were collected and kept identified by mother tree from 64 natural stands of eastern cottonwood (Populus deltoides Bartr. ex Marsh. var. deltoides) in the southeastern United States. Containerized rooted cuttings from the seedlings were planted in clonal trials at four sites: North Carolina, Florida, Alabama, and Missouri. Heights and late-season defoliation scores were measured at age one through three. Broad-sense heritabilities were 0.28 for third-year height and 0.38 for percent defoliation in October. The genetic covariance, total genetic correlation, and coefficient of genetic prediction were all negative, indicating that height increases as defoliation declines. Gains of 14-percent in height and 19-percent in foliage retention (reduced defoliation) were estimated from direct selection for clone performance over all locations. Ten-percent gains could be accomplished from indirect selection. Examination of ranked clone means indicated that selection should be based on both traits at the same time. Clone-by-test-location interactions were significant for both traits, indicating that increased gains might be accomplished by selection of site-specific clones. The same clones were seldom found in the top five clones at different test locations.

INTRODUCTION

Eastern cottonwood (Populus deltoides Bartr. ex Marsh. var. deltoides) is the fastest growing native commercial forest species in North America (Cooper and van Haverbeke 1990). Rapid growth of the genus has led to the establishment of poplar plantations worldwide. However, it is known that early or premature defoliation can drastically reduce the amount of annual growth and height growth of a tree. Early defoliation can also predispose the tree to disease and other environmental stresses (Land and Jeffreys 2005, Newcombe and others 1994).

Multiple factors can contribute to the defoliation of a tree. Kosola and others (2001) discovered that repeated insect defoliation in a stand of poplars decreased tree growth and increased the rate of top dieback. Ostry and others (1988) reported that defoliation due to Melampsora leaf-rust could reduce growth by 20 to 35 percent. In a study by Chen and others (2001) on Douglas fir, cumulative defoliation over a two-year period was discovered to be negatively correlated with height growth. Objectives of the present study were (1) to analyze early height growth and October leaf defoliation of eastern cottonwood clones from the population in the Southeastern United States, (2) to estimate genetic variation, broad-sense heritability, genetic correlations, and expected gains from direct and indirect selection for three-year height and late-season defoliation in that population, (3) to identify some clones for further testing, and (4) to determine if the same clones can be used at widely dispersed locations in the Southeast.

1 Graduate Student and Professor respectively, Department of Forestry, Mississippi State University, P.O. Box 9681, Mississippi State, MS, USA
MATERIALS AND METHODS

Planting Material

The southeast was divided into six subregions in 1995 by Land and others (2001) for a clonal evaluation of eastern cottonwood from the Southern United States. The three eastern-most subregions, Southeast Atlantic (SA), East Gulf (EG), and East Central (EC), were used for the study reported here. Open-pollinated seeds were collected from mother trees in seventy-two natural stands on various rivers within these three subregions (Figure 1). Seeds collected from 64 of these stands were germinated, and 512 seedling clones were vegetatively multiplied as containerized rooted cuttings for use in four clonal trials (Warwell et al. 1999). These clonal trials were planted in Florida (30° 32.5' N, 84° 35' W), Alabama (32° 02' N, 88° 07' W), North Carolina (35° 58' N, 77° 09' W), and Missouri (32° 02' N, 89° 46' E).

Figure 1. Map of 72 natural Populus deltoides stands from which seeds were collected for production of seedling cuttings to use in the southwide clonal trial. Stands in North Carolina, South Carolina, and eastern Georgia represent the Southeast Atlantic subregion. Stands in western Georgia, southern 80% of Alabama, and eastern Mississippi indicate the East Gulf subregion. Stands in Tennessee, western Kentucky, and the northern 20% of Alabama represent the East Central subregion. Non-filled circles represent two stands.
The rooted cuttings were planted at the four locations between June 1999 and March 2000. The field design at each location was a randomized complete block with three replications. Clones were arranged by origin in subregion split plots. Each clone was planted in a single-tree clone plot. The trees were measured for height and scored for leaf defoliation in October of each of the three years 2000-2002. Height was measured to the nearest tenth of a meter by a vertex laser hypsometer. Leaf defoliation was based on a scoring system. A tree was given a score for the amount of foliage lost. A defoliation score of “5” indicated that 100 percent of the leaves were gone, “4” indicated 80 percent defoliation, “3” indicated 60 percent, “2” was 40 percent, and “1” was 20 percent or less defoliated. These defoliation scores were converted to percent defoliation for analyses. Foliage “retention” was equated with 100 percent minus the defoliation percent.

Multivariate analyses of variance and covariance for height and defoliation over all test locations were performed according to the format in Table 1. PROC GLM and PROC MIXED (SAS 1999) were used to analyze the data. This was a mixed-effects model, with “Subregions” considered a fixed effect. Coefficients for the Expected Mean Squares were taken from the PROC MIXED printout, since there was unbalance in numbers of clones per subregion at each location. Variance components were computed by equating actual mean squares with the expected mean squares. Covariance components were computed in a similar manner from expected cross products that had the same composition of covariance components and coefficients as shown for the variance components and coefficients in Table 1.

Genetic parameters were determined from the estimates of variance and covariance components in the expected mean squares and expected mean cross products. Broad-sense heritability was calculated using the formula:

\[
h^2 = \frac{\sigma^2_{C(S)}}{(\sigma^2_{C(S)} + \sigma^2_{C(S)h} + \sigma^2_{C(S)d})}.
\]

The total genetic correlation was calculated using the formula:

\[
r_G = \frac{\sigma_{C(S)h \times C(S)d}}{\sqrt{\sigma^2_{C(S)h} \times \sigma^2_{C(S)d}}}
\]

where \( h = \) height and \( d = \) defoliation.
Table 1. Composition of expected mean squares (EMS), genetic variance, and phenotypic variance for analyses of height and late-season leaf retention of cottonwood clones in three-year-old trials repeated over four locations in the southeastern United States.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Expected Mean Square&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Location (L)</td>
<td>$\Phi^2_{C(S)R(L)} + k_{14}\Phi^2_{C(S)L} + k_{15}\Phi^2_{SR(L)} + k_{16}\Phi^2_{SL} + k_{17}\Phi^2_{R(L)}$</td>
</tr>
<tr>
<td>Reps in L (R/L)</td>
<td>$\Phi^2_{C(S)R(L)} + k_{12}\Phi^2_{SR(L)} + k_{13}\Phi^2_{R(L)}$</td>
</tr>
<tr>
<td>Subregions (S)</td>
<td>$\Phi^2_{C(S)R(L)} + k_{08}\Phi^2_{C(S)L} + k_{09}\Phi^2_{C(S)} + k_{10}\Phi^2_{SR(L)} + k_{11}\Phi^2_{SL} + Q_S$</td>
</tr>
<tr>
<td>S x L</td>
<td>$\Phi^2_{C(S)R(L)} + k_{05}\Phi^2_{C(S)L} + k_{06}\Phi^2_{SR(L)} + k_{07}\Phi^2_{SL}$</td>
</tr>
<tr>
<td>S x R/L</td>
<td>$\Phi^2_{C(S)R(L)} + k_{04}\Phi^2_{SR(L)}$</td>
</tr>
<tr>
<td>Clones in S (C/S)</td>
<td>$\Phi^2_{C(S)R(L)} + k_{02}\Phi^2_{C(S)L} + k_{03}\Phi^2_{C(S)}$</td>
</tr>
<tr>
<td>C/S x L</td>
<td>$\Phi^2_{C(S)R(L)} + k_{01}\Phi^2_{C(S)L}$</td>
</tr>
<tr>
<td>C/S x R/L</td>
<td>$\Phi^2_{C(S)R(L)}$</td>
</tr>
</tbody>
</table>

Total genetic variance = GV = $\Phi^2_{C(S)}$

Phenotypic variance (Individual-tree basis) = PV = $\Phi^2_{C(S)R(L)} + \Phi^2_{C(S)L} + \Phi^2_{C(S)}$

<sup>a</sup> These are Expected Mean Squares for a mixed-effects model, where the “Subregions” effect is considered a fixed effect (Q_S). There was unbalance in the number of clones per subregion and the number of clones per subregion planted at each location, so the k<sub>xx</sub> coefficients were taken from Type III MANOVA analyses (PROC GLM in SAS). Those coefficients were as follows:

<table>
<thead>
<tr>
<th>$k_{01}$</th>
<th>$k_{02}$</th>
<th>$k_{03}$</th>
<th>$k_{04}$</th>
<th>$k_{05}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.6807</td>
<td>1.5881</td>
<td>4.3243</td>
<td>42.0570</td>
<td>0.9111</td>
</tr>
<tr>
<td>28.9180</td>
<td>86.7540</td>
<td>0.7489</td>
<td>1.8111</td>
<td>25.6110</td>
</tr>
<tr>
<td>76.8330</td>
<td>40.4080</td>
<td>121.2300</td>
<td>8.759</td>
<td>27.5760</td>
</tr>
<tr>
<td>82.7290</td>
<td>82.7290</td>
<td>248.1900</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>$k_{06}$</th>
<th>$k_{07}$</th>
<th>$k_{08}$</th>
<th>$k_{09}$</th>
<th>$k_{10}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>25.6110</td>
<td>121.2300</td>
<td>76.8330</td>
<td>8.759</td>
<td>25.6110</td>
</tr>
<tr>
<td>86.7540</td>
<td>121.2300</td>
<td>40.4080</td>
<td>8.759</td>
<td>25.6110</td>
</tr>
<tr>
<td>40.4080</td>
<td>121.2300</td>
<td>40.4080</td>
<td>8.759</td>
<td>25.6110</td>
</tr>
<tr>
<td>8.759</td>
<td>25.6110</td>
<td>25.6110</td>
<td>25.6110</td>
<td>25.6110</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>$k_{11}$</th>
<th>$k_{12}$</th>
<th>$k_{13}$</th>
<th>$k_{14}$</th>
<th>$k_{15}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>76.8330</td>
<td>40.4080</td>
<td>121.2300</td>
<td>8.759</td>
<td>25.6110</td>
</tr>
<tr>
<td>86.7540</td>
<td>40.4080</td>
<td>121.2300</td>
<td>8.759</td>
<td>25.6110</td>
</tr>
<tr>
<td>40.4080</td>
<td>40.4080</td>
<td>121.2300</td>
<td>8.759</td>
<td>25.6110</td>
</tr>
<tr>
<td>8.759</td>
<td>8.759</td>
<td>8.759</td>
<td>8.759</td>
<td>8.759</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>$k_{16}$</th>
<th>$k_{17}$</th>
<th>$k_{18}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>82.7290</td>
<td>82.7290</td>
<td>248.1900</td>
</tr>
<tr>
<td>82.7290</td>
<td>82.7290</td>
<td>248.1900</td>
</tr>
</tbody>
</table>

k_{17} = 248.1900

51
The coefficient of genetic prediction was calculated using the formula:

\[
CGP = \frac{\sigma_{C(S)hxd}}{\sqrt{PV_h \times PV_d}}
\]

where PV is the phenotypic variance for height or defoliation and \( \Phi_{C(S)hxd} \) is the covariance component. Predicted gain from direct selection \((\Delta G)\) was determined as:

\[
\Delta G = i \times h^2 \times \sqrt{PV}
\]

where \(i\) is the selection intensity (Namkoong and Snyder 1969). The correlated gain from indirect selection \((\Delta CG)\) was determined from the formula:

\[
\Delta CG = i \times CGP \times \sqrt{PV},
\]

where PV is the phenotypic variance of the trait being indirectly improved.

Performance levels were calculated by subtracting the clone’s mean at a given location from the location’s overall mean and then dividing by the standard deviation among clone means at that location. Each clone’s average for its performance levels at the four locations was used in selecting the top-ranked ten clones across all locations for each of the two traits. Actual clone means for each trait, rather than performance levels, were used to pick the top five clones at each location for site-specific selections.

**RESULTS AND DISCUSSION**

Analyses of variance

Analyses of variance indicated significant effects of locations, subregions, and clones within subregions for height and defoliation (Table 2). Location means ranged from 7.3 to 11.0 meters for age-three height and 40.0 to 73.3 percent for average defoliation over three years (Table 3). Interactions between locations and clones within subregions were highly significant for both traits, but the interactions of locations with subregions were not always significant. This indicates that the GxE interaction of clones with locations may require that different clones be selected for different sites. Failure to include this GxE interaction in phenotypic variance of the two traits would cause overestimation of heritabilities, coefficients of genetic prediction, and estimates of genetic gains.
Table 2. Mean squares, mean cross products, F-tests of significance, and estimates of variance and covariance components from multivariate analyses of three-year height and late-season leaf defoliation of cottonwood clones in trials repeated over four locations in the southeastern United States.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>3-Yr Ht. M.S.</th>
<th>Ht.x Defol. M.C.P.</th>
<th>Lf. Defol. M.S.</th>
<th>Var. Comp.</th>
<th>Var Comp or Cov Comp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Locations (L)</td>
<td>679.738**</td>
<td>3711.985</td>
<td>28876.9**</td>
<td></td>
<td>Ht.</td>
</tr>
<tr>
<td>Reps in L (R/L)</td>
<td>10.957**</td>
<td>0.574</td>
<td>157.9 ns</td>
<td></td>
<td>Ht.x Def</td>
</tr>
<tr>
<td>Subregions (S)</td>
<td>50.318*</td>
<td>- 442.620</td>
<td>4099.2*</td>
<td></td>
<td>Defol.</td>
</tr>
<tr>
<td>S x L</td>
<td>6.073*</td>
<td>- 30.582</td>
<td>537.1 ns</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S x R/L</td>
<td>2.709**</td>
<td>- 6.405</td>
<td>276.8**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clones in S (C/S)</td>
<td>3.883**</td>
<td>- 15.266</td>
<td>268.4**</td>
<td>(\Phi_{C(S)}^2) = 0.538</td>
<td></td>
</tr>
<tr>
<td>C/S x L</td>
<td>1.587**</td>
<td>- 3.004</td>
<td>88.0**</td>
<td>(\Phi_{C(S)L}^2) = 0.336</td>
<td></td>
</tr>
<tr>
<td>C/S x R/L</td>
<td>1.023</td>
<td>- 1.468</td>
<td>38.9</td>
<td>(\Phi_{C(S)R(L)}^2) = 1.023</td>
<td></td>
</tr>
</tbody>
</table>

Significance of F-tests are indicated as follows:
- ns = not significant (probability level is greater than 0.05)
- * = significant (probability level between 0.05 and 0.01)
- ** = highly significant (probability level less than or equal to 0.01)

Table 3. Site means for height and defoliation.

<table>
<thead>
<tr>
<th>Site</th>
<th>Mean Height (meters)</th>
<th>Mean % Defoliation in October (average for first three years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Missouri</td>
<td>11.0</td>
<td>73.3</td>
</tr>
<tr>
<td>Florida</td>
<td>10.4</td>
<td>73.3</td>
</tr>
<tr>
<td>Alabama</td>
<td>7.9</td>
<td>40.0</td>
</tr>
<tr>
<td>North Carolina</td>
<td>7.3</td>
<td>73.3</td>
</tr>
</tbody>
</table>

Genetic parameters and expected gains

Estimates of total genetic variance for three-year height and average percent leaf defoliation in October and for the genetic correlation between the two traits are given in Table 4. Phenotypic variances for the two traits are also given. Broad-sense heritabilities are moderate, ranging from 0.28 for height to 0.38 for late-season leaf defoliation. The genetic covariance, total genetic
Table 4. Estimates of genetic parameters and selection responses for height and leaf defoliation of cottonwood clones in trials over four locations in the southeastern United States.

<table>
<thead>
<tr>
<th>Genetic Parameter or Statistic</th>
<th>Trait</th>
<th>3-Yr Ht.</th>
<th>Lf. Defol.</th>
<th>Ht. x Defol.</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Total genetic variance</td>
<td>$\sigma_G^2 = \sigma_{C(S)}^2$</td>
<td>0.538</td>
<td>42.337</td>
<td></td>
</tr>
<tr>
<td>(2) Genetic covariance</td>
<td>$\sigma_G \times \sigma_{C(S)}$</td>
<td>-2.855</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(3) Phenotypic variance</td>
<td>$\sigma_P^2 = \sigma_{C(S)R(L)}^2 + \sigma_{C(S)L}^2 + \sigma_{C(S)}^2$</td>
<td>1.897</td>
<td>110.470</td>
<td></td>
</tr>
<tr>
<td>(4) Broad-sense heritability</td>
<td>$h_b^2 = (1)(3)$</td>
<td>0.284</td>
<td>0.383</td>
<td></td>
</tr>
<tr>
<td>(5) Total genetic correlation</td>
<td>$r_{Gd} = (2) + {\sqrt{(1_{Hr})(3_{Df})}}$</td>
<td>-0.598</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(6) Coef. of genetic prediction</td>
<td>$CGP = (2) + {\sqrt{(3_{Hr})(3_{Df})}}$</td>
<td>-0.199</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(7) Test means (over all locations)</td>
<td></td>
<td>7.43 meters</td>
<td>54.7 %</td>
<td></td>
</tr>
<tr>
<td>(8) Total number of clones tested (all locations)</td>
<td></td>
<td>512</td>
<td>512</td>
<td></td>
</tr>
<tr>
<td>(9) Number of clones selected</td>
<td></td>
<td>5</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>(10) Proportion selected</td>
<td></td>
<td>5/512 = 0.010</td>
<td>5/512 = 0.010</td>
<td></td>
</tr>
<tr>
<td>(11) Selection intensity (i)</td>
<td></td>
<td>2.640</td>
<td>2.640</td>
<td></td>
</tr>
<tr>
<td>(12) Predicted genetic gain (ΔG) from direct selection</td>
<td>$\Delta G = (11)(4)(3)$</td>
<td>1.03 meters</td>
<td>10.6 %</td>
<td></td>
</tr>
<tr>
<td>(13) Predicted correlated gain (ΔCG) from indirect selection</td>
<td>$\Delta CG = (11)(6)(3)$</td>
<td>-0.72 m. ht.</td>
<td>-5.5% defol.</td>
<td></td>
</tr>
<tr>
<td>(14) Percent gain over test mean (direct selection) $%G = [(12)/(7)] \times 100%$</td>
<td></td>
<td>(+) 13.9 %</td>
<td>(+) 19.4 %</td>
<td></td>
</tr>
<tr>
<td>(15) Percent gain over test mean (indirect selection) $%CG = [(13)/(7)] \times 100%$</td>
<td></td>
<td>(-) 9.7 %</td>
<td>(-) 10.1 %</td>
<td></td>
</tr>
</tbody>
</table>

correlation, and coefficient of genetic prediction (CGP) are all negative, indicating that height declines as late-season defoliation increases. Moderate gains of 14 percent in height and 19 percent in leaf retention in October can be achieved from direct selection of one clone in 100 from clonal tests repeated across test locations. Indirect selection of one clone in 100 for less leaf defoliation in October can result in a ten-percent increase in age-three height. Similarly, indirect selection of the tallest clone in 100 at age three can result in a ten percent reduction in late-season defoliation. Note that these direct and indirect gains are estimated for selection of
clones that perform well over all four diverse test locations. Greater gains might be obtained from selection of site-specific clones for each location, since the large GxE interaction of locations with clones within subregions would not be included in the phenotypic variance. Thus, the broad-sense heritabilities and CGP would be larger and result in greater predicted gains for site-specific clones.

**Clones to select for further testing over all test locations**

Performance levels for three-year height and for average three-year October defoliation were used to rank clones for mean performance over all four test locations. The top ten clones by rank for each trait are given in Table 5. The only clone that occurred in the top ten for both traits was 3-1, which came from a mother tree in Hardin County, Tennessee, on the Tennessee River near the Mississippi-Alabama-Tennessee intersection of state lines. For the other clones in the top ten, there were patterns for river systems of origin. Top height-performing clones tended to come from the Tombigbee River and its associated rivers on the west-Alabama side of the EG subregion (four of the top ten) and from the southern half of the SA subregion (Catawba to Oconee Rivers in South Carolina and northeast Georgia)(four of the top ten). Clones with high late-season leaf retention came mainly from the EG subregion (four from the Chattahoochee/Apalachicola system on the border between Alabama and Georgia and three from the Tombigbee system in west Alabama). Only three of the 20 clones in Table 5 came from the EC subregion (includes clone 3-1 mentioned above and two clones from the Mississippi River along the western boundary of Tennessee). None of the 20 clones came from rivers in North Carolina or northeastern South Carolina.

When ranks for the alternative trait were examined, the alternative trait did not always rank very high. However, it appeared that height growth was more correlated with a high score for defoliation, than defoliation was with a high score for height growth. For example, when clones were ranked for height growth, clone 105-5 was ranked second and had a ranking of 105 for foliage retention. While clone 77-4 was ranked second for foliage retention and had a ranking of 18 for height growth. Therefore the negative genetic correlation between defoliation and height is not absolute, and some clones will have a high rank for one trait and a low rank for the other. It is recommended that the clones to select for further testing should be assessed for both traits at the same time. Those that are selected should have a high average for the combined ranks of the two traits. However, for early selection the foliage retention may provide a better measure for indirect selection of future yield than clone height, and it should be given more weight than height growth when combining performance levels. One solution is to use the five clones out of the top 10 for foliage retention that have the highest rank for height growth. The same procedure can be used in selecting the top ten for height growth. These procedures would identify the following 10 clones for further study: 3-1, 80-2, 96-2, 77-4, 80-3, 154C-4, 147-4, 120-6, 100-3, and 92-3.
Table 5. Best performing clones over all locations for mean height and percent defoliation.

<table>
<thead>
<tr>
<th>Top Ranked Clones</th>
<th>Performance Level Rank</th>
<th>Origin</th>
<th>Clone Average (4 loc’s.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Height Foliage Retention&lt;sup&gt;a&lt;/sup&gt;</td>
<td>State</td>
<td>River System</td>
</tr>
<tr>
<td>3-Year Height</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-1</td>
<td>1</td>
<td>6</td>
<td>TN</td>
</tr>
<tr>
<td>105-5</td>
<td>2</td>
<td>105</td>
<td>AL</td>
</tr>
<tr>
<td>92-3</td>
<td>3</td>
<td>80</td>
<td>AL</td>
</tr>
<tr>
<td>141A-5</td>
<td>4</td>
<td>193</td>
<td>SC</td>
</tr>
<tr>
<td>47-1</td>
<td>5</td>
<td>176</td>
<td>MO</td>
</tr>
<tr>
<td>154C-4</td>
<td>6</td>
<td>41</td>
<td>SC</td>
</tr>
<tr>
<td>154B-3</td>
<td>7</td>
<td>216</td>
<td>SC</td>
</tr>
<tr>
<td>147-4</td>
<td>8</td>
<td>37</td>
<td>GA</td>
</tr>
<tr>
<td>120-6</td>
<td>9</td>
<td>95</td>
<td>AL</td>
</tr>
<tr>
<td>100-3</td>
<td>10</td>
<td>57</td>
<td>MS</td>
</tr>
<tr>
<td>3-Year October Foliage Retention</td>
<td>(%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>119-4</td>
<td>31</td>
<td>1</td>
<td>AL</td>
</tr>
<tr>
<td>77-4</td>
<td>18</td>
<td>2</td>
<td>FL</td>
</tr>
<tr>
<td>80-1</td>
<td>36</td>
<td>3</td>
<td>FL</td>
</tr>
<tr>
<td>92-7</td>
<td>48</td>
<td>4</td>
<td>AL</td>
</tr>
<tr>
<td>154A-1</td>
<td>75</td>
<td>5</td>
<td>SC</td>
</tr>
<tr>
<td>3-1</td>
<td>1</td>
<td>6</td>
<td>TN</td>
</tr>
<tr>
<td>80-2</td>
<td>16</td>
<td>7</td>
<td>FL</td>
</tr>
<tr>
<td>96-2</td>
<td>17</td>
<td>8</td>
<td>MS</td>
</tr>
<tr>
<td>80-3</td>
<td>23</td>
<td>9</td>
<td>FL</td>
</tr>
<tr>
<td>68-1</td>
<td>108</td>
<td>10</td>
<td>TN</td>
</tr>
</tbody>
</table>

<sup>a</sup>Foliage retention rank equals inverse of defoliation rank, or 512 minus defoliation rank. Rank “1” for high foliage retention is rank “512” for low defoliation.

**Site-non-specific vs. site-specific clones**

The top five clones at each site for each trait are given in Table 6. There were few similarities among sites. Clone 147-4 was in the top five for height at the Alabama and Florida sites (both of which are in the southern half of the region), and clone 9-5 was in the top five for height at the North Carolina and Missouri locations. The only two clones that were in the top five for both height and foliage retention were 120-4 and 119-1, and this only occurred at the Missouri site. The general lack of repeatability of clones in the top five at different sites confirms the importance of the GxE interactions of locations with clones within subregions.
Table 6. Mean heights and foliage retention of the five best-performing clones for each trait at each site.

<table>
<thead>
<tr>
<th>Location</th>
<th>Clone</th>
<th>Rank</th>
<th>Mean HT (meters)</th>
<th>Clone</th>
<th>Rank</th>
<th>Mean % defoliation</th>
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<tr>
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<td>1</td>
<td>9.1</td>
<td>111-1</td>
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<td></td>
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<td>8.8</td>
<td>45-1</td>
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<td></td>
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<td>8.8</td>
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<td></td>
<td>92-3</td>
<td>4</td>
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<td>92-7</td>
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<td>8.4</td>
<td>77-4</td>
<td>5</td>
<td>23.3</td>
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<td>156A-3</td>
<td>1</td>
<td>13.4</td>
<td>120-4</td>
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<td>147-4</td>
<td>2</td>
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<td>120-6</td>
<td>3</td>
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<td>50B-3</td>
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<td></td>
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<td>7.9</td>
<td>105-1</td>
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<td>40.0</td>
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<tr>
<td></td>
<td>141A-5</td>
<td>4</td>
<td>7.9</td>
<td>109-7</td>
<td>4</td>
<td>40.0</td>
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<td>9-5</td>
<td>5</td>
<td>7.8</td>
<td>111-4</td>
<td>5</td>
<td>40.0</td>
</tr>
</tbody>
</table>

Only eight of the 20 identified site-specific clones for height in Table 6 would have been used if the top ten clones over all locations for height (Table 5) had been selected, and only five of the 20 site-specific clones for foliage retention would have been chosen. This indicates that greater gains may be obtained from selection of site-specific clones (or at least half-region-specific clones for the northern and southern halves of the region) than from selection of non-specific clones for the whole region.

**SUMMARY AND CONCLUSIONS**

Analyses of variance indicated significant effects for locations, subregions, and clones within subregions for height, and late-season defoliation. There was a significant and negative genetic correlation between height and defoliation, indicating that three-year height increases as defoliation declines (i.e., foliage retention increases). Interactions were detected for locations by clones within subregions. This GxE interaction indicates that increased gains may be obtained from selecting different clones for each location. Should this interaction be left out of the phenotypic variance, the heritabilities, coefficients of genetic prediction, and estimates of genetic gains would all be overestimated. Direct selection for traits can result in a 14-percent gain in height and a 19-percent gain in leaf retention. Indirect selection for either height growth or late-
season foliage retention can result in a ten-percent increase for the other trait. However, the negative correlation between height and defoliation was not always reliable for individual clones. It is recommended that a clone should be assessed for both traits before selection is made. It is also recommended that clones be selected for no larger an area than the southern half and northern half of the region, rather than for the whole region. This may allow increased gains by reducing the location-by-clone interaction.

ACKNOWLEDGEMENTS

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LITERATURE CITED


Interacting Genes in the Pine-Fusiform Rust Forest Pathosystem

H.V. Amerson1, T.L. Kubisiak2, S.A. Garcia3, G.C. Kuhlman4, C.D. Nelson2, S.E. McKeand5, T.J. Mullin5, and B. Li5*

Fusiform rust (FR) disease of pines, caused by Cronartium quercuum f.sp. fusiforme (Cqf), is the most destructive disease in pine plantations of the southern U. S. The NCSU fusiform rust program, in conjunction with the USDA-Forest Service in Saucier, MS and Athens, GA, has research underway to elucidate some of the genetic interactions in this pathosystem.

Major genes (R genes) for FR resistance, primarily in loblolly pine and to a lesser degree in slash pine, are being recognized and tagged with genetic markers. These genes, termed Fr genes, are being defined in resistant selections (clones) whose progeny segregate 1:1 for the presence or absence of galls when challenged with specific isolates of Cqf, i.e., the selections are heterozygous with regards to a given R gene. Thus the resistance (R) and the non-resistance (r) alleles of these Fr genes can be followed within specific pedigrees with genetic markers linked to the R genes. Typically 1 or 2 different heterozygous Fr genes have been found in a given selection and to date 8 different Fr genes (Fr1-Fr8) are known among several different resistant loblolly selections. The Fr genes were discovered in first generation, plantation or equivalent Forest Service selections, but some of the Fr alleles are now being followed in advanced generation selections (elite trees) using the previously identified markers.

Some of the loblolly Fr genes are being used to investigate virulence composition in pathogen populations. In that work, progeny from selections segregating for 1 or 2 different Fr genes are challenged with Cqf, either artificially in greenhouse studies (using inocula collected from various field sites) or naturally in field studies. Infection of progeny with a genetic marker defined resistance allele (R1, R2, R3 etc.) denotes virulence in the pathogen population against the specific R-allele of a given Fr gene. The percentage of R-individuals infected, for a given Fr gene, provides a measure for the level of virulence in a particular inoculum. The artificial inoculations have been very informative, showing that the effectiveness of a given R allele may vary greatly from site-to-site, indicative of site-to-site virulence variation. Some R alleles are frequently overcome by virulence in the test inocula at multiple sites while others confer good levels of resistance at many sites.

In concert with genetic marker mapping of Fr genes, and as another approach to analyze virulence composition, an effort is underway to genetic marker map the avirulence gene (pathogenicity locus) AvrI that corresponds to the Fr1 gene in loblolly pine. Once markers tightly linked with AvrI are obtained, we expect to identify the avirulence gene sequence and develop internal markers for allele discrimination. This should allow us to assess AvrI vs. avrI allele frequencies in spore populations of Cqf as a guide for prescribed Fr1 deployment. Upon demonstrating success of this approach, other Avr genes will be similarly investigated. A discussion of the work outlined here will be the focus of our presentation.

1Assoc. Professor, 3Research Analyst, 5Professor, Dept. of Forestry and Environ. Resources, N.C. State Univ., Raleigh, 2Research Geneticist, Southern Inst. of Forest Genetics, USDA Forest Service, Saucier, MS, and 4Retired Research Pathologist, USDA Forest Service, Athens, GA.
Genetic Variation in Wood Quality (MOE) of Coastal Douglas-fir

Randy Johnson¹ and Barbara Gartner²

Douglas-fir (Pseudotsuga menziesii) is of economic importance for forest products industries in the western United States, New Zealand, and parts of Europe. Its primary uses are dimension lumber, piles, plywood and pulp, but it is found in many other solid and composite products as well. In almost all of these capacities, wood density is a good predictor of its economic value and/or its performance because of density’s correlation with strength or pulp yield. Other traits, such as microfibril angle and tracheid length, also affect wood quality, but density has been the trait most examined because of its relative ease of measure and its adequacy as an index for other properties.

Genetic studies in the literature suggest that most of the variation found among trees for wood density is controlled genetically; heritability (h²) estimates for wood density in Douglas-fir range between 0.5 to greater than 0.9. Unfortunately, the reported genetic correlations between wood density and growth rate are strongly negative, ranging from -0.5 to –1.0 (Bastion et al. 1985, King et al. 1988, Vargas-Hernandez and Adams 1991, St. Clair 1994).

Because MOE is more important for utilization than is wood density per se, we decided to examine the genetic variation of MOE in coastal Douglas-fir indirectly with the Director HM-200 ® (Hitman). The Hitman measures the sound velocity through a log, a which is highly correlated with dynamic MOE. Theoretically the relationship is: MOE = green density × velocity squared; see Andrews (2002) for more details.

METHODS AND MATERIALS

Four 20-year-old progeny test sites were chosen from 10 potential sites in the 1st generation Nehalem breeding program (part of the Northwest Tree Improvement Cooperative). The average height of the four sites ranged from 15.4 to 16.5 m and average DBH ranged from 19.1 to 19.4 cm. This series of trials was designed as a reps-in-sets design that tested 10 sets of 40 families (a total of 400 families). Each set was established as three replications with 4 non-contiguous trees per replication (for a total of 12 trees per family per site). We chose a set (set 10) with a higher than average age-11 DBH heritability (0.22 vs. 0.13 for all sets) in order to gain more precise estimates of genetic correlations. The trials were designed so that diagonals could be removed and equal family representation would remain. We measured all trees in set 10 for DBH and selected the diagonals with the higher heritability to fell, thereby improving correlation estimates. One of the 40 families was a full-sib family and was dropped from the analyses, leaving 39 open-pollinated families.

¹ Research Geneticist, USDA Forest Service, Forestry Sciences Lab, 3200 SW Jefferson Way, Corvallis, OR 97331-4401
² Professor, Dept. of Wood Science and Engineering, Richardson Hall, Oregon State University, Corvallis OR 97331-5752
Felled trees were measured for total height. A 4m log was cut from the base of the tree and the sound velocity was obtained with the Hitman. A disk was cut at about 1.5 m and will be subjected to additional measurements. For this report, we are using sound velocity as a proxy for MOE.

Unbiased heritability estimates for height, DBH and sound velocity (“MOE”) were calculated with the formula:

\[
\text{Heritability} = \frac{3 \times \sigma_{\text{family}}^2}{\sigma_{\text{family}}^2 + \sigma_{\text{site-family}}^2 + \sigma_{\text{rep-family(site)}}^2 + \sigma_{\text{error}}^2}
\]

Additionally, cross-site family-means were calculated for age-20 variables (4 available sites), age-11 foliage traits (5 sites) and form traits (10 sites). BLUP estimates were used in place of family-means for height and diameter at age-11 (10 sites) and DBH at age-17 (5 sites). Correlations were calculated for all traits with sound velocity (“MOE”).

**RESULTS AND DISCUSSION**

Heritability estimates are shown in Table 1. Heritability estimates for “MOE” were high at all four sites (0.39 to 0.84) and the unbiased heritability estimate calculated over all four sites was 0.49. In general, “MOE” had larger heritabilities than either of the growth traits (Table 1). The family-by-environmental variation for “MOE” was relatively small; it was only 16% of the family variation.

<table>
<thead>
<tr>
<th>Site</th>
<th>MOE (velocity)</th>
<th>DBH</th>
<th>Height</th>
</tr>
</thead>
<tbody>
<tr>
<td>Over all sites</td>
<td>0.49</td>
<td>0.31</td>
<td>0.22</td>
</tr>
<tr>
<td>Coal Creek</td>
<td>0.58</td>
<td>0.29</td>
<td>0.00</td>
</tr>
<tr>
<td>Sarajarvae</td>
<td>0.84</td>
<td>0.40</td>
<td>0.00</td>
</tr>
<tr>
<td>Slick Rock</td>
<td>0.39</td>
<td>0.31</td>
<td>0.45</td>
</tr>
<tr>
<td>Vesper</td>
<td>0.60</td>
<td>0.69</td>
<td>0.58</td>
</tr>
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</table>

When one considers tree averages, 75% of the variation in MOE in the region (for a given stand age) is found within stands, as opposed to 25% among stands (Johnson et al. 2005). Half of this within-stand variation is controlled by genetics; therefore, over a third of the variation in MOE in the region is impacted by our breeding programs.

The only statistically significant correlations of family means and “MOE” were with DBH, and they were negative (Table 2). This result suggests that selection for improved DBH will result in a decrease in MOE. Similar results are found in the literature for wood density. In bending data from 198 1×1×16 cm sticks (data not shown), the partial correlation coefficient of density and MOE \((r = 0.46)\) was larger than the partial correlation coefficient of microfibril angle (MFA) and MOE \((r = -0.29)\). Therefore, it was not totally unexpected that “MOE” followed the same pattern found for wood density.
Table 2. Family-mean / BLUP correlations with the family mean of sound velocity (“MOE”) and level of statistical significance (italicized and in parenthesis). BLUP estimates are for age-11 height and DBH (10 sites) and age-17 DBH (5 sites).

<table>
<thead>
<tr>
<th></th>
<th>$r_{\text{family mean}}$</th>
<th>Form</th>
<th>$r_{\text{family mean}}$</th>
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<tr>
<td><strong>Height</strong></td>
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<td></td>
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<tr>
<td>age-11</td>
<td>-0.13 (0.40)</td>
<td>Forking</td>
<td>-0.06 (0.73)</td>
</tr>
<tr>
<td>age-20</td>
<td>-0.02 (0.89)</td>
<td>Ramicorns</td>
<td>0.10 (0.53)</td>
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<tr>
<td>age-20*</td>
<td>-0.15 (0.35)</td>
<td>Sinuosity</td>
<td>0.02 (0.91)</td>
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<td><strong>DBH</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>age-11</td>
<td>-0.28 (0.08)</td>
<td>Foliage retention</td>
<td>-0.15 (0.35)</td>
</tr>
<tr>
<td>age-17</td>
<td>-0.33 (0.04)</td>
<td>(SNC)</td>
<td></td>
</tr>
<tr>
<td>age-20</td>
<td>-0.16 (0.31)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* using only the 2 sites with heritability > 0

Acknowledgements: This research was supported by the Sustainable Forestry component of Agenda 2020 (a joint effort of the USDA Forest Service Research & Development and the American Forest and Paper Association), a special grant from USDA to OSU for wood utilization research, the Swiss Needle Cast Cooperative and the Oregon Department of Forestry.

REFERENCES


Genetically Improved Eucalypts for Novel Applications and Sites in Florida

D. L. Rockwood1, G. F. Peter2, M. H. Langholtz3, B. Becker4, A. Clark III5, and J. Bryan6

Abstract: Genetic, silvicultural, and propagation improvements collectively can increase the productivity of *Eucalyptus grandis* (EG) and *E. amplifolia* (EA) grown for mulchwood, energywood, flooring, and other products on agricultural, forest, and non-traditional sites such as reclaimed mined and contaminated lands in Florida and similar areas. *EG* has been grown commercially in southern Florida since the 1960s, initially for pulpwood and now for mulchwood from some 6,000 ha of plantations on flatwoods in the LaBelle-Palmdale area. *EG*’s commercialization was and is facilitated by multi-agency research resulting in some 3,200 *EG* accessions, over 300 maintained clones, 4th-generation clonal and seedling seed orchards, a 5th-generation seedling seed orchard, and a genetic test base exceeding 50 studies involving over 1,000 o-p progenies, 40 control-pollinated progenies, 300 clones, and 30 hybrids. Development of *EA*, suitable for more temperate climates with more frequent and severe freezes, has been less intensive but has followed a similar genetic improvement strategy, with genetic tests in FL, GA, and LA now numbering 20 and including more than 300 accessions, 25 seed orchard o-p progenies, and 50 clones.

Genetic improvement of *EG* and *EA* can increase the commercial feasibility of short rotation woody crops (SRWC) for energywood and phytoremediation. *EG* and *EA* SRWC cost competitiveness will depend on establishment success, yield improvements, harvesting costs, and identifying/using incentives. While SRWC plantations are intended primarily for energy utilization, coproducts and alternative higher-value uses would improve their economic viability and provide an incentive for development. Development of more freeze-tolerance is still of utmost importance for *EG* and *EA*. In addition to significant improvement of freeze-tolerance, increases in productivity, adaptability to various sites, evaluation of wood properties, and determination of propagation options must be addressed. *EG* seed quantity and quality are currently affected by a high level of variability in flowering time and hurricane blowdown in the clonal seed orchard, but genetic gain potential for growth and freeze-resilience may be realized from the 5th-generation orchard. Larger gains are expected from clonal selection and testing. Strong collaboration among public and private partners is necessary.

**Keywords:** *Eucalyptus amplifolia*, *E. grandis*, seed orchards, clones, wood properties, windfirmness, short rotation woody crops, phytoremediation.

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1Professor, 2Associate Professor, 3Graduate Student, and 4Field Program Manager, School of Forest Resources and Conservation, University of Florida, Gainesville, FL 32611-0410
5Forest Products Technologist, Disturbance and Management of Southern Pine Ecosystems Research Work Unit, Southern Research Station, Athens, GA 30602
6Forester, Lykes Bros, Palmdale, FL 33944
INTRODUCTION

EG is grown commercially for mulchwood on ~6,000 ha near Palmdale. This commercialization was made possible by research conducted until 1984 by the US Forest Service in association with several companies (Geary et al. 1983, Meskimen 1983). Since the late 1970s, this research base has been further developed by the University of Florida in collaboration with Lykes Bros. and with support from various agencies to also assess EG as a short rotation woody crop (SRWC) for energywood and phytoremediation in southern and recently central Florida (Meskimen et al. 1987, Rockwood et al. 1989, Rockwood and DeValerio 1986, Rockwood and Geary 1988, Rockwood and Meskimen 1991, Warrag et al. 1990). EG is best suited to southern Florida’s subtropical and tropical flatwoods and muck soils but can also be grown successfully in central Florida (Rockwood 1997). Most available EG seedlots are from GO77, a 4th-generation seedling seed orchard established in 1977 and developed through combined tree selection, progeny testing, and provenance testing. A 5th-generation seedling seed orchard offers genetic gain potential (Rockwood et al. 1989), but larger gains are expected from over 250 clones evaluated for tree size, freeze-resilience, survival, and stem form; some 10 clones were comparable to superior clones previously selected (Meskimen et al. 1987). EG and EA have demonstrated high energywood productivity on reclaimed phosphate mined lands (Segrest et al. 2004).

EA, which coppices prolifically, is an appropriate SRWC for more temperate climates with more frequent and severe freezes and may be grown from central Florida northward to perhaps 50 miles from the Gulf Coast. Development of EA has been less intensive but has followed a genetic improvement strategy similar to EG (Rockwood et al. 1987, Rockwood et al. 1991, Rockwood et al. 1993).

While SRWCs are intended primarily for energy utilization, coproducts and alternative high-value uses would improve their economic viability and provide an incentive for development. Cofiring up to 5% SRWCs is the most cost effective means of creating renewable energy (Segrest et al. 2004). A ready commercial alternative for EG and EA is mulchwood. EG has been used for pallet manufacturing. EG and EA are also very suitable for pulp and paper production, flooring, and very likely reconstituted wood panel products. EA and EG have phytoremediation potential (Pisano and Rockwood 1997, Rockwood et al. 2004).

Genetic, silvicultural, and propagation improvements collectively can increase the productivity of EG and EA for SRWC applications on agricultural, forest, and non-traditional sites such as reclaimed mined and contaminated lands. We report recent results on genetic variability in EG and EA that influence opportunities for these SRWCs in central and southern Florida.

MATERIALS AND METHODS

EG and EA studies established at a Lakeland, Florida, clay settling area (CSA) include a demonstration planting, commercial plantings, and a clone-configuration-fertilizer study (SRWC-90). The demonstration area planted in April 2001 had single and double (0.8m apart) row configurations at 0.9m spacing on top of beds spaced 3.4m apart. After periodic size and survival measurements, approximately half of each block was felled in February 2002. Commercial scale plantings of approximately 8 ha in June 2001, June 2002, and/or October 2002
similarly involved single and double rows on beds, as well as quadruple rows of trees planted 0.8m apart on a macrobed or mound. SRWC-90 involves EG and EA represented by up to six genotypes, two planting configurations (single or double rows per bed), and two fertilizer levels (0 or 100 pounds/acre of ammonium nitrate) in a split-plot design with configuration main plots, species subplots, and genotypes in 6-tree row subsubplots. The initial planting was done in March 2001, and fertilizer treatments were implemented in June 2002. An economic analysis similar to Langholtz et al. (2004) that examined the importance of input costs, harvest prices, progeny, rotation, coppicing, and incorporation of CO₂ mitigation incentives for SRWCs on CSAs in terms of land expectation value (LEV) and annual equivalent (EAE) assumed: a base scenario of 4% interest rate, $1,800 ha⁻¹ site preparation cost, $1,200 ha⁻¹ planting cost, and a carbon price of $5 Mg⁻¹ C.

To assess the significance of clonal variation on wood properties contributing to the use of EG for solid wood products, 53 trees (40 identified, 13 unidentified) in GO77 were felled in March 2004 at 26.6 years of age. Stem disks were taken at 1.3m from 40 identified trees, and boards were cut from the basal logs of 22 unidentified trees. After drying, radial strips were cut from the disks and from eight boards, glued to core holders and sawn to ~1.6 mm thick cross sectional strips. The specific gravity for each radial strip was measured by x-ray densitometry at 0.5 mm intervals with a resolution of 2.5 uM (Clark et al., 2004). In addition, the specific gravities of comparative samples from Lyptus® and baldcypress (Taxodium distichum) were also measured. As there were no clear earlywood and latewood boundaries, the mean specific gravity was determined by simple averaging.

From 1996-2002, GO96, a clonal seed orchard near Palmdale, assembled 49 fast-growing, freeze-resilient clones from several studies. To assess the influence of flowering time variability on seed production in GO96, onset (Early – Days 218-239, Intermediate – Days 240-258, and Late – Days 259-278) and duration (Short - <25 days, Average – 25-50 days, Long - >50 days) of flowering of 29 clones were observed weekly from August through October 2002. Windfirmness of 45 clones in response to three hurricanes in August-September 2004 was assessed in May 2005 by evaluating 1-18 ramets/clone for lean, broken stem, cut stem, blowdown, or mortality (hurricane damage index (HDI) of 1, 2, 3, 4, and 5, respectively), as influenced by DBH and age.

Progeny test SRWC-100 was established on muck soil at Southwest Ranches in July-August 2002 at 2.4 (within paired rows) or 3.7m (between pairs) x 0.9m spacing with 68 open-pollinated (o-p) progenies in 6 reps of 4-tree row plots according to a randomized complete block design (RCBD). Based on 1.3-year-old height, DBH, survival, and tree quality (0=excellent, ..., 4=poor stem straightness/crown form/vigor), SRWC-100 was rogued in March 2004 to create 5th-generation seedling seed orchard GO02. Calculations of basal area/ha (BAH) at 1.3 years (.00007854xDBH² for a live tree, 0 for a dead or missing tree) assumed 3,586 trees/ha. Select trees were subsequently reassessed for height, DBH, and tree quality.

In June 2004, 12.6 ha of operational and research plantings of EG and EA were established in study SRWC-107 near Clewiston, FL, to evaluate cultural and genetic factors important to SRWC energywood production on sandy/muck soil. In the operational single and double (0.8m between paired rows) row plantings (rows or row pairs on 3.1m centers with 0.9m between trees
in rows), equivalent to 1,379, 2,760, or 5,520 trees/ha, 26 EG and two EA progenies were planted in continuous rows of ~306 or 417 trees. Within the operational plantings, cultural and genetic studies were established. The cultural study evaluated three cultures (control, fertilized with 560 kg/ha of 12-10-24 ammonium nitrate, or mulched with 58.3 tons/ha of sugarcane filtercake) in blocks with three replications of two planting configurations (single or double rows) and two EG progenies in 13-tree row plots. The genetic study consisted of 29 EG and six EA progenies planted in June and 37 EG progenies (including three planted in June) planted in July in a RCBD with six replications of 6-tree row plots in a double row configuration. In December 2004, height, tree quality, and survival were assessed in the genetic and cultural trials and in three 10m long plots installed at 75m intervals from north to south in each configuration/progeny combination in the operational planting. In May 2005, the northernmost of the 10m long plots and two rows of the June genetics study were remeasured for height and quality.

RESULTS AND DISCUSSION

EA and EG comparisons before coppicing were similar in the demonstration area and SRWC-90 at Lakeland. In general, 35-month-old EA coppice growth initiated by a Spring 2002 felling of part of the demonstration area was comparable to first rotation growth, but EG coppice growth was much less (Table 1). EA coppice, thus, appears more reliable and vigorous than that of EG.

Table 1. Performance of EA and EG in the demonstration area on a CSA at Lakeland, Florida.

<table>
<thead>
<tr>
<th>Configuration - Trait</th>
<th>EA</th>
<th>EG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single row 44-mo height (m)</td>
<td>13.1</td>
<td>16.7</td>
</tr>
<tr>
<td>Double row 44-mo height (m)</td>
<td>11.9</td>
<td>20.3</td>
</tr>
<tr>
<td>Single row 35-mo coppice ht (m)</td>
<td>6.7</td>
<td>7.7</td>
</tr>
<tr>
<td>Double row 35-mo coppice ht (m)</td>
<td>9.1</td>
<td>7.3</td>
</tr>
</tbody>
</table>

Average EG yields in the commercial plantings at Lakeland are low, as the trees/ha for the single, double, and quadruple configurations are much less than the planted densities (Table 2). The hurricanes of August-September 2004 demonstrated that EG SRWCs on CSAs are at risk of blowdown three to four years after planting or coppicing, as ~85% of harvestable trees were severely damaged; younger EG and EA SRWCs appear much less susceptible to wind damage.

Table 2. Species, culture, establishment date, number (n) of 15m row plots, average tree height (m), DBH(cm), and density (trees/ha) in October 2004 in commercial plantings.

<table>
<thead>
<tr>
<th>Species</th>
<th>Culture</th>
<th>Est. date</th>
<th>n</th>
<th>Ht</th>
<th>DBH</th>
<th>Density</th>
</tr>
</thead>
<tbody>
<tr>
<td>EG</td>
<td>Single</td>
<td>June 2001</td>
<td>15</td>
<td>9.1</td>
<td>5.8</td>
<td>1823</td>
</tr>
<tr>
<td></td>
<td>Double</td>
<td>July 2000</td>
<td>5</td>
<td>12.2</td>
<td>9.0</td>
<td>1583</td>
</tr>
<tr>
<td></td>
<td>Quadruple</td>
<td>June 2002</td>
<td>8</td>
<td>11.3</td>
<td>3.2</td>
<td>2455</td>
</tr>
<tr>
<td>EA</td>
<td>Single</td>
<td>Oct 2001</td>
<td>1</td>
<td>10.1</td>
<td>6.9</td>
<td>3527</td>
</tr>
</tbody>
</table>
In SRWC-90, drought after planting impacted survival. *EG* was first planted in May 2001. Due to high initial mortality, planting was suspended until planting conditions improved with rainfall in July 2001, when *EG* mortality was replaced and *EA* was planted. *EA* and *EG* seedlings typically had survivals exceeding 70%, and *EA* survival usually was over 90%, even at double row configuration of 8,384 trees/ha (Table 3). *EA*, although planted later than and not necessarily as vigorous as *EG*, tended to highest productivity. *EA* and *EG* progenies 5091 and 4200, respectively, were the highest yielding. *EG* progeny 3242, with the highest initial mortality and follow-up planting, was the lowest yielding progeny.

Table 3. *EA* and *EG* mean, low, and high progeny mean height (m), DBH (cm), and survival (%) at 41 months by unfertilized (U, U1, or U2) and fertilized (F) treatments with single (S) and double (D) row configurations on beds in SRWC-90.

<table>
<thead>
<tr>
<th>Row Config</th>
<th>Fert.</th>
<th>Height Mean</th>
<th>Height Low</th>
<th>Height High</th>
<th>DBH Mean</th>
<th>DBH Low</th>
<th>DBH High</th>
<th>Survival Mean</th>
<th>Survival Low</th>
<th>Survival High</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>EG</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>U</td>
<td>8.6</td>
<td>7.5</td>
<td>9.4</td>
<td>6.8</td>
<td>5.3</td>
<td>7.7</td>
<td>85</td>
<td>75</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>8.8</td>
<td>6.9</td>
<td>10.3</td>
<td>6.7</td>
<td>4.5</td>
<td>8.5</td>
<td>82</td>
<td>75</td>
<td>92</td>
</tr>
<tr>
<td>D</td>
<td>U1</td>
<td>7.6</td>
<td>6.9</td>
<td>8.6</td>
<td>4.5</td>
<td>3.9</td>
<td>5.5</td>
<td>83</td>
<td>75</td>
<td>88</td>
</tr>
<tr>
<td></td>
<td>U2</td>
<td>9.5</td>
<td>8.2</td>
<td>10.2</td>
<td>6.3</td>
<td>5.0</td>
<td>7.0</td>
<td>71</td>
<td>50</td>
<td>88</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>11.5</td>
<td>9.1</td>
<td>12.6</td>
<td>8.1</td>
<td>6.0</td>
<td>9.4</td>
<td>59</td>
<td>50</td>
<td>63</td>
</tr>
<tr>
<td><strong>EA</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>U</td>
<td>6.0</td>
<td>4.6</td>
<td>7.8</td>
<td>5.3</td>
<td>3.7</td>
<td>7.3</td>
<td>93</td>
<td>83</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>9.0</td>
<td>7.5</td>
<td>10.0</td>
<td>7.8</td>
<td>6.5</td>
<td>8.7</td>
<td>90</td>
<td>75</td>
<td>96</td>
</tr>
<tr>
<td>D</td>
<td>U1</td>
<td>5.3</td>
<td>4.0</td>
<td>5.9</td>
<td>3.6</td>
<td>2.6</td>
<td>4.1</td>
<td>90</td>
<td>75</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>U2</td>
<td>9.4</td>
<td>7.9</td>
<td>10.6</td>
<td>7.0</td>
<td>5.7</td>
<td>8.1</td>
<td>89</td>
<td>79</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>10.2</td>
<td>8.3</td>
<td>11.2</td>
<td>7.6</td>
<td>6.1</td>
<td>8.8</td>
<td>90</td>
<td>79</td>
<td>100</td>
</tr>
</tbody>
</table>

*EA* and/or *EG* SRWC performance on CSAs is improved with hericiding/disking, bedding, watering/packing seedlings, fertilization with ammonium nitrate, and high planting density. *EA* can be expected to have high survival on well prepared bedded CSAs, respond favorably to fertilization, tolerate high stand densities, and coppice reliably. *EG* has the potential to be the most productive species with thorough site preparation and fertilization and properly timed harvesting. *EG* is best suited for immediate commercialization because of its seed availability, while seed of *EA* is limited. SRWC rotations can be as short as one year, depending on genotypes, initial planting density, culture intensity, harvesting equipment, and local fiber markets.

For the use of Florida grown *EG* for solid wood products, specific gravity and stiffness are two of the most important traits. Clonal variation in wood specific gravity appears significant for Florida grown *EG* (Table 4). Overall, the GO77 samples were less dense than Lyptus®, a *EG* × *E. urophylla* hybrid that serves as a standard for eucalypt lumber, and more dense than cypress. However, the range in density was great among both sets of *EG* samples, and two known and one unknown *EG* clones were as or more dense than Lyptus®.

68
Seed quantity and quality in GO96, the only clonal seed orchard of six EG seed orchards developed in southern Florida, may be affected by high clonal variability in flowering time (Table 5). The earliest clone started flowering on Day 218, the latest on Day 278. The shortest duration for a clone was 10 days, while the longest duration was 86 days. Collectively, some 28% of the clones observed (two early, one intermediate, four late) did not have overlapping flowering times, very likely contributing to low seed quantities and qualities from GO96.

Table 5. Classification of GO96 clones for onset and duration of flowering in 2002 and wind firmness in 2004.

<table>
<thead>
<tr>
<th>Category</th>
<th>Clone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flowering Onset – Duration</td>
<td></td>
</tr>
<tr>
<td>Early – Short; Average;</td>
<td>-; 1506, 2805, 3134; 2798,2814,3187,3756,4950,4952,4954,4958,4959,5123,T3</td>
</tr>
<tr>
<td>Long</td>
<td></td>
</tr>
<tr>
<td>Intermediate – Short;</td>
<td>3043; 2422, 3181, 3323; 4960, 4961, 4962, 4964, 4999</td>
</tr>
<tr>
<td>Average; Long</td>
<td></td>
</tr>
<tr>
<td>Late – Short; Average;</td>
<td>2131, 3914, 4986; 2817; -</td>
</tr>
<tr>
<td>Long</td>
<td></td>
</tr>
<tr>
<td>Windfirmness</td>
<td></td>
</tr>
<tr>
<td>Susceptible (HDI ≥3.0)</td>
<td>577, 1506, 1828, 2773, 2798, 3134, 3914, 4950, 4954, 4962, 4986, 5123, T3</td>
</tr>
<tr>
<td>Average (1.9 &lt; HDI ≤3.0)</td>
<td>2519, 2805, 2807, 2814, 3043, 3187, 3756, 4952, 4958, 4963, 4964, 5126, 5139, T9</td>
</tr>
<tr>
<td>Resistant (HDI ≤1.9)</td>
<td>2131, 2242, 2422, 2817, 3181, 3323, 4959, 4960, 4961, 4999, 5128, 5132, 5137, 5140, 5142, T6</td>
</tr>
</tbody>
</table>

Hurricane damage may reduce GO96 seed production for several years, as 74% of the trees in the orchard sustained some type of damage from the winds that occasionally surpassed 160 km/hour. Tree age and DBH were slightly associated with damage, as only age was correlated with HDI (r=.27), suggesting that the older trees incurred more damage. Some 32% of the clones may be considered susceptible, and 36% appear resistant to damaging winds (Table 5).

The 68 progenies in SRWC-100 varied significantly in DBH, tree quality, and BAH at 1.3 years (Table 6). A wide range in progeny performance evident as early as 4 months, when progeny mean tree heights range from 0.46 to 1.35m, was present a year later as the worst progenies were barely large enough to measure for DBH. After roguing of ~75% of the trees to form GO02, the select trees were more than 2cm larger in average DBH, and the selects from the best progeny were nearly 12m tall. A 5th-generation seedling seed orchard such as GO02 is expected to have good genetic gain potential for growth and freeze-resilience (Rockwood et al. 1989).
Table 6. Summary of *EG* and *EA* progeny means in components of two studies.

<table>
<thead>
<tr>
<th>Trait</th>
<th>No. of Progenies</th>
<th>Progeny Means</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Average</td>
<td>Low</td>
<td>High</td>
<td></td>
</tr>
<tr>
<td><em>EG</em> in SRWC-100</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.3-year DBH (cm)</td>
<td>68</td>
<td>3.74*</td>
<td>0.10</td>
<td>6.50</td>
<td></td>
</tr>
<tr>
<td>1.3-year Tree Quality</td>
<td>68</td>
<td>2.5*</td>
<td>0.0</td>
<td>4.0</td>
<td></td>
</tr>
<tr>
<td>1.3-year Survival (%)</td>
<td>68</td>
<td>79.5</td>
<td>25.0</td>
<td>100.0</td>
<td></td>
</tr>
<tr>
<td>1.3-year BAH (m²/ha)</td>
<td>68</td>
<td>4.06*</td>
<td>0.68</td>
<td>8.93</td>
<td></td>
</tr>
<tr>
<td><em>EG Selects in SRWC-100</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.3-year Height (m)</td>
<td>65</td>
<td>7.1</td>
<td>2.6</td>
<td>11.8</td>
<td></td>
</tr>
<tr>
<td>1.3-year DBH (cm)</td>
<td>65</td>
<td>6.0</td>
<td>1.9</td>
<td>7.9</td>
<td></td>
</tr>
<tr>
<td>1.3-year Tree Quality</td>
<td>65</td>
<td>0.5</td>
<td>0.0</td>
<td>3.0</td>
<td></td>
</tr>
<tr>
<td><em>EG/EA in SRWC-107: Genetics (June and July plantings)</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>June: 0.5-year Height (m)</td>
<td>29/6</td>
<td>1.10*/1.17</td>
<td>0.88/1.12</td>
<td>1.27/1.25</td>
<td></td>
</tr>
<tr>
<td>June: 0.5-year Survival (%)</td>
<td>29/6</td>
<td>65.3/85.6</td>
<td>38.9/66.7</td>
<td>86.1/94.4</td>
<td></td>
</tr>
<tr>
<td>July: 0.4-year Height (m)</td>
<td>37/</td>
<td>0.79*/-</td>
<td>0.56/-</td>
<td>0.93/-</td>
<td></td>
</tr>
<tr>
<td>July: 0.4-year Survival (%)</td>
<td>37/-</td>
<td>88.8/-</td>
<td>61.1/-</td>
<td>100.0/-</td>
<td></td>
</tr>
<tr>
<td><em>EG in SRWC-107: Culture (Control (C), Fertilizer (F), and Mulch (M) amendments)</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C: 0.5-year Height (m)</td>
<td>1</td>
<td>1.12</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>C: 0.5-year Survival (%)</td>
<td>1</td>
<td>82.5</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>F: 0.5-year Height (m)</td>
<td>1</td>
<td>0.86</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>F: 0.5-year Survival (%)</td>
<td>1</td>
<td>61.9</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>M: 0.5-year Height (m)</td>
<td>1</td>
<td>1.1</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>M: 0.5-year Survival (%)</td>
<td>1</td>
<td>88.1</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><em>EG/EA in SRWC-107: Operational (Single (S) and Double (D) rows)</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S: 0.5-year Height (m)</td>
<td>13/2</td>
<td>1.11*/1.23</td>
<td>0.88/1.23</td>
<td>1.40/1.23</td>
<td></td>
</tr>
<tr>
<td>S: 0.5-year Tree Quality</td>
<td>13/2</td>
<td>1.2*/1.7</td>
<td>0.5/1.3</td>
<td>2.7/2.1</td>
<td></td>
</tr>
<tr>
<td>S: 0.5-year Survival (%)</td>
<td>13/2</td>
<td>98.9/100.0</td>
<td>80.5/100.0</td>
<td>100.0/100.0</td>
<td></td>
</tr>
<tr>
<td>D: 0.5-year Height (m)</td>
<td>14/2</td>
<td>1.17*/1.33</td>
<td>0.97/1.24</td>
<td>1.35/1.53</td>
<td></td>
</tr>
<tr>
<td>D: 0.5-year Tree Quality</td>
<td>14/2</td>
<td>1.2*/1.5</td>
<td>0.5/1.4</td>
<td>2.1/1.6</td>
<td></td>
</tr>
<tr>
<td>D: 0.5-year Survival (%)</td>
<td>14/2</td>
<td>95.8*/98.6</td>
<td>75.0/95.5</td>
<td>100.0/100.0</td>
<td></td>
</tr>
</tbody>
</table>

*Significant differences among progenies

While GO02 has good genetic gain potential for growth, larger gains may be expected from clonal selection and testing (Meskimen et al. 1987) involving the some 242 accessions retained in GO02 and over 250 clones that have already been evaluated for tree size, freeze-resilience, survival, and stem form. Some 10 of the 250 clones were comparable to superior clones previously selected (Meskimen et al. 1987). Large numbers of rooted cuttings can be produced from these superior clones, and four clones have been micropropagated commercially. While productivity of cuttings and plantlets may greatly exceed that of seedlings, seedling cost favors the commercial use of improved seedlings (Rockwood and Warrag 1994).

The operational, cultural, and genetic plantings established by Florida Crystals (FCC) illustrate the critical factors for SRWC energywood production (Table 6). In comparison to the 1.3-year-old results from SRWC-100 in which superior trees were over 70% larger in DBH and 100%
greater in basal area/ha (BAH) than the average progeny, the heights of the best 0.5-year-old progenies in SRWC-107 were only 15% greater than average. _EA_ was surprisingly comparable to _EG_ in height and had better survival. The very unusual weather at and following establishment of SRWC-107 also influenced cultural responses, as fertilizer and mulch amendments had unexpectedly similar heights and survivals to the control. Overall, these collective results suggest that FCC, which farms over 70,000 ha in southern Florida and operates the 74 MW Okeelanta Cogeneration Plant by burning bagasse and wood waste, could increase the productivity of operational SRWC plantings of _EG_ for energywood on 800 ha of former sugarcane lands by up to 50% by incorporating appropriate cultural and genetic factors.

Stability of _EA_ and _EG_ progeny performance across these widely different sites in Florida is difficult to assess due to the limited number of progenies common to Studies SRWC-90, -100, and -107 (Table 7) and because of the young age of SRWC-107, although SRWC-107 trees grew ~2m in height from December 2004 to May 2005. Because the CSAs represented by SRWC-90 and mucks represented by SRWC-100 and -107 are much more fertile than the sandy soils on which GO77 and GO96 are located, some GxE interaction is likely.

Table 7. Mean heights (H in m) or BAH (m²/ha) at indicated age in months for _EG_ progeny common to six studies at three sites.

<table>
<thead>
<tr>
<th>Progeny</th>
<th>SRWC-90 H41</th>
<th>SRWC-100 BAH16</th>
<th>SRWC-107 June H06</th>
<th>July H05</th>
<th>Single H06</th>
<th>Double H06</th>
</tr>
</thead>
<tbody>
<tr>
<td>3242</td>
<td>9.1</td>
<td>4.23</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3469</td>
<td>12.6</td>
<td>5.32</td>
<td>0.70</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3931</td>
<td>3.07</td>
<td>0.88</td>
<td>0.87</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4064</td>
<td>12.6</td>
<td>3.21</td>
<td>1.17</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4200</td>
<td>11.9</td>
<td>3.94</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4223</td>
<td>11.3</td>
<td>3.73</td>
<td>1.20</td>
<td>0.95</td>
<td>1.14</td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>11.5</td>
<td>4.06</td>
<td>1.10</td>
<td>0.79</td>
<td>1.11</td>
<td>1.17</td>
</tr>
</tbody>
</table>

The economics for _EG_ grown with sewage effluent (Langholtz et al. 2004) exemplify the merit of using improved SRWC genotypes. Average _EG_ without coppice produced the least return, and _EG_ average with coppice was more profitable. However, faster growing progenies such as _EG_ 3309 increased profitability, especially without coppicing, as LEV was $2967/acre and EAE was $118/acre/year. _EG_ progeny 3309 had significantly higher yields, was most profitable without coppice, and had a LEV of $7,300 ha⁻¹, EAE of $290 ha⁻¹ yr⁻¹, and internal rate of return (IRR) of 29%, with an optimal rotation age of 33 months. Overall returns for the average _EG_ progeny were considerably less, but LEVs still exceeded $2,470 ha⁻¹ (with coppicing). Whether coppicing is preferable to harvesting/replanting depends on relative seedling and coppice yields, seedling establishment costs, and output prices.

For SRWCs produced on CSAs under various scenarios of operational costs, productivity, stumpage price, and incorporation of CO₂ mitigation incentives, LEVs increased with growth rate and biomass stumpage price. LEVs ranged from $-2,789 to $4,616 ha⁻¹ and $-224 to $18,121 ha⁻¹ assuming stumpage prices of $10 and $30 Mg⁻¹. Under these assumptions, marginal increases in LEV per dollar increment in stumpage price range from $264-$293 and $588-$629 for low and high growth scenarios, respectively.
While potential products from SRWCs include mulch, energy, timber, pallets, and fiberboard, the most likely are 1) mulchwood and 2) energywood, a prospective market with much potential for expansion. Successful demonstration of SRWC production and cofiring in Florida could lead to SRWC development in the Gulf Coast region and similar environments. EA has adaptability for the lower Gulf Coast, and improved EG will be suitable for central and southern Florida. SRWCs may also serve as “bridge crops” to restore cogongrass infested CSAs to native forest or productive agricultural lands (Tamang et al. 2004). EA and/or EG–based phytoremediation systems may have SRWC production potential on a wide range of contaminated sites (Rockwood et al. 2004).

While EG and EA growth potential is quite high, SRWC cost competitiveness will depend on establishment success, yield improvements, harvesting costs, and identifying/using incentives. Genetic improvement must continue if EG and EA are to increase in commercial feasibility. Development of more freeze-tolerance is still of utmost importance for EG and EA. In addition to significant improvement of freeze-tolerance, increases in productivity, adaptability to various sites, evaluation of wood properties, and determination of propagation options must be addressed (Rockwood et al. 1993). The EG test base exceeds 50 studies, primarily in southern FL, involving over 1,000 o-p progenies, 40 control-pollinated progenies, 300 clones, and 30 hybrids. EA genetic tests in FL, GA, and LA now number 20 and include more than 300 accessions, 25 o-p progenies, and 50 clones. Strong collaboration among public and private partners is essential for commercializing SRWCs.

CONCLUSION

Genetic improvement of EG and EA can increase the commercial feasibility of SRWCs for mulchwood, energywood, and phytoremediation. EG and EA SRWC cost competitiveness will depend on establishment success, yield improvements, harvesting costs, and identifying/using incentives. While SRWC plantations are intended primarily for energy utilization, coproducts and alternative higher-value uses would improve their economic viability and provide an incentive for development. Development of more freeze-tolerance is still of utmost importance for EG and EA. In addition to significant improvement of freeze-tolerance, increases in productivity, adaptability to various sites, evaluation of wood properties, and determination of propagation options must be addressed. EG seed quantity and quality are currently affected by a high level of variability in flowering time and hurricane blowdown in the clonal seed orchard, but genetic gain potential for growth and freeze-resilience may be realized from the 5th-generation orchard. Larger gains are expected from clonal selection and testing. Strong research collaboration is necessary.

Acknowledgments: We gratefully acknowledge the Common Purpose Institute, Matt Tavtigian, Florida Crystals Corporation, Lykes Bros., and the Florida Institute of Phosphate Research for invaluable assistance in designing, installing, maintaining, measuring, and/or funding these studies.
REFERENCES


Survival and Promotion of Female and Male Strobili from Topgrafting in Third-Cycle Slash Pine (*Pinus elliottii* var. *elliottii*) Breeding Program.

A.M. Medina¹, D.A. Huber², T.L. White³, T.A. Martin³*

In January 2003 the Cooperative Forest Genetics Research Program (CFGRP) at the University of Florida began establishing the slash pine third-cycle breeding population through topgrafting the selected clones. The topgrafting strategy, which has the potential to drastically reduce the breeding cycle, will also eliminate the need for a separate clone bank for breeding (White *et al*., 2003). A study of flowering response to topgrafting was conducted to obtain better understanding and refine the operational use of this technique in slash pine. The objectives of this study were to understand the effect of the genetic material (interstock clones and scion clones) and the interstock crown position on survival and flowering response of topgrafts. Quadrant direction, branch order of the interstock and scion age were assessed as survey data and their effects on survival and flowering response of the topgrafts were also estimated.

The topgrafts were established onto sexually-mature, insect-protected seed orchard trees in nine slash pine seed orchards (first and second generations). Scions were collected from 4 to 6 year-old full-sib block plots for forward selections, and from first generation seed orchards and second generation clone banks for backward selection. The recommended experimental design for each cooperator in a single orchard was one topgraft in each of three crown positions in four different seed orchard interstock clones for a total of 12 grafts per third-cycle selections. The three crown positions were defined as: top (the first two whorls), mid-top (about whorl 4), and mid-crown (usually about whorl 6). Survival, number of female strobili and number of male strobili one year after topgrafting were the response variables of this study. A variation of the modified cleft was the standard grafting method used with the exception of one cooperator that used a regular modified cleft. The statistical analysis was performed in two stages. In the first stage, an analysis of variance (ANOVA), using SAS PROC MIXED (SAS ® Institute 1996), was used to test the effects of the topgraft clone, interstock clone and crown position on the response variables. To test the significance of the variance components for the random predictors a Wald Test was used (Greene, 2000). In the second stage, the effects of quadrant, branch order and topgraft chronological age (adhoc variables) on the topgraft responses were assessed. Given a large number of missing values and inconsistency in the levels of these three new predictor variables among cooperators, the analysis was conducted using a more balanced subset of the full data set for each adhoc variable. The fitted linear model from the first stage analysis (experimental design factors) was the base model to which the three new independent variables (all of them treated as fixed effects) and their interactions were added and fitted in separate analyses using the backward elimination approach as in the first stage.

After one year from grafting a total of 1861 topgrafts (72.6%) from 200 topgraft clones out of 209 were alive. Significant differences in survival were found among cooperators (Table 1) with

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* ¹Graduate Student, ²Research Associate, ³ Director, ⁴Associate Professor, School of Forest Resources and Conservation, University of Florida, Gainesville, FL, USA.
a minimum and maximum mean survival of 46.1% and 86.1%, respectively. Differences in survival among crown positions were also significant (p-value = 0.01) with higher survival rate obtained in the mid-top (75.3%), followed by the top (69.9%) and finally by the mid-crown position (67.2%).

Table 1. First stage analysis of variance, for survival, and female and male strobili production using a full model with all the experimental design factors that were significant at 25% for at least one response variable. P-values are shown for fixed effects and variance components, expressed as percentage of the total phenotypic variance, are shown for random effects.

<table>
<thead>
<tr>
<th>Model effects</th>
<th>Cooperator</th>
<th>Crown</th>
<th>Cooperator*crown</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fixed effects</td>
<td>0.0261</td>
<td>0.01</td>
<td>0.192</td>
</tr>
<tr>
<td>Survival</td>
<td>0.0122</td>
<td>&lt; 0.0001</td>
<td>0.134</td>
</tr>
<tr>
<td>Female flowering</td>
<td>0.0078</td>
<td>0.18</td>
<td>ns</td>
</tr>
<tr>
<td>Male flowering</td>
<td></td>
<td></td>
<td>ns</td>
</tr>
</tbody>
</table>

Random effects

<table>
<thead>
<tr>
<th>Topgraft clone (Topgraft)</th>
<th>5.36%</th>
<th>23.7%</th>
<th>8.6%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interstock</td>
<td>16.28%</td>
<td>12.5%</td>
<td>6.7%</td>
</tr>
<tr>
<td>Ramet</td>
<td>6.00%</td>
<td>ns</td>
<td>6.6%</td>
</tr>
<tr>
<td>Topgraft*interstock</td>
<td>ns</td>
<td>4.1%</td>
<td>3.9%</td>
</tr>
<tr>
<td>Interstock*crown</td>
<td>ns</td>
<td>2.0%</td>
<td>11.7%</td>
</tr>
<tr>
<td>Topgraft*crown</td>
<td>ns</td>
<td>ns</td>
<td>2.9%</td>
</tr>
<tr>
<td>Topgraft<em>ramet</em>crown</td>
<td>11.44%</td>
<td>ns</td>
<td>Ns</td>
</tr>
<tr>
<td>Residual</td>
<td>60.87%</td>
<td>57.7%</td>
<td>59.3%</td>
</tr>
</tbody>
</table>

*a predictor variables in the model are: cooperator, crown (crown position), topgraft (topgraft clone nested within cooperator), interstock (nested within cooperator) and ramet (interstock replication, nested within interstock and cooperator). b effect not significant at the 0.25 level in the fitted model.

Topgrafting was a very effective tool for promoting both female and male strobili. While some topgrafts did not flower, the overall mean and maximum flowering yield per live topgraft was 2.52 and 43 female strobili, and 1.67 and 59 male strobili. After one year from grafting, 84% of the live topgraft clones produced strobili, and almost half of them bore both flower sexes. The expectation in the third-cycle slash pine is to breed 36 out of the 50 selections assigned to each breeding group. The first year results allow us to be optimistic about reaching this expectation in the next few years via topgrafting.

Consistent with topgrafting studies in Pinus taeda and Pinus sylvestris (Gooding et al., 1999; McKeand and Raley, 2000; Almqvist and Ekberg, 2001), the genetic material, scion and interstock clones, had large effects on the flowering response. More than 23 and 8% of the total female and male flowering variation, respectively, were due to differences among scion clones. Given this results it became clear that there is a different potential among slash pine clones to...
produce flowers, especially female strobili, when topgrafted. This fact might compromise the early incorporation of poor topgraft flowering clones into the breeding program throughout topgrafting. Thus, the suitability of topgrafting to shorten the breeding cycle, is also a function of the amount of selected clones that have good flowering response when topgrafted. Clonal differences among interstocks, also large, were less important than the scion clone effect, accounting for 12.5% of the female and 6.7% of the male total variation. In addition, with the large interstock effect on topgraft survival, it results important to identify and select the clones that as interstock promote good topgraft survival and flowering. A low correlation between the flowering capacity of a clone and its suitability as interstock on promoting topgraft flower initiation has been reported in *Pinus taeda* and *Pinus sylvestris* (Schmidtling, 1983; McKeand and Raley, 2000; Almqvist and Ekberg, 2001); and therefore, the practicability of selecting good interstock clones for their flowering performance has been discussed as not promising (McKeand and Raley, 2000; Almqvist and Ekberg, 2001). Given the low topgraft clone by interstock clone interaction on survival and flowering responses in our study, the problem of selecting a good interstock clone can be in part overcome by topgrafting selected genotypes into more interstock clones, this ameliorates the risk of having poor flower initiation and poor survival caused by interstock clone.

Topgraft survival and female strobili promotion showed significant differences among crown positions. The highest survival rate was reached by the mid-top followed by the top crown position. Grafting in the top of the crown was highly superior promoting female strobili followed by mid-top position. Flowering differences among crown position were not significant for male strobili; however, higher overall yields were observed in top and mid-top position compared to mid-crown. When combining survival and strobili production rates in a single index, the top of the crown resulted to be the most efficient promoting female strobili, while mid-top reached the highest efficiency producing male strobili; however, higher proportions of topgrafts allocated in the top and mid-top crown should increase male and female flower production in balanced proportions for breeding practices.

Quadrant showed no significant effect either in topgraft survival or in topgraft strobili promotion; consequently our results do not allow us to make any related recommendation about topgraft quadrant orientation. Branch order was not a relevant source of variation on topgraft response variables with the exception of female strobili production. When testing the effect of the three levels of branch order in a subset of three cooperators, first order branches were significantly superior promoting female strobili, followed by second order branches. Thus, higher proportions of scions grafted on first and second order branches should increase the efficiency for promoting female strobili; however, given that first and second order branches might not be abundant enough for large scale crossing, a lower proportion of scions should also be grafted into third order branches, especially if there is a higher need for male strobili in which the production was not related to the order of the branches. Chronologically older scions (backward selections) produced significantly more female and male strobili when topgrafted. The higher efficiency of older scions promoting both female and male strobili may represent an additional plus in incorporating backward selections in the breeding population; and hence, further studies using a more balanced and larger data is recommended to support these results.
REFERENCES


The Effect of Root Segment Origin, Size, and Orientation in Aspen Rootling Propagation

J. S. Brouard¹, F. Niemi², and L. R. Charleson³

Abstract: A vegetative propagation trial was conducted at two Alberta nurseries using juvenile root segments taken from potted plants of six clones of aspen (Populus tremuloides Michx.). The purpose of this study was to investigate the effect of origin and orientation of root segments on new plant development, and to determine a minimum size of root segment that will still result in acceptable propagation success rates. Five donor plants of each clone were grown at Woodmere Forest Nursery in Fairview, Alberta in 2003. In the fall, stems were removed and the root masses were cold stored over the winter. In the spring of 2004 half of the root masses were shipped to Smoky Lake Forest Nursery. In early June, 2004, root segments were excised and inserted vertically into styroblock cells. Treatment variables were clone, origin (proximal or distal to donor plant stem), root segment length, and orientation (proximal or distal end upwards). Propagation success was assessed at 10 weeks.

There were large differences in propagation success between the two locations; survival was 53% at Woodmere Forest Nursery and only 20% at Smoky Lake Forest Nursery. This difference is believed to have arisen because of heat stress damage in the root donor plants during shipment and setup at Smoky Lake. Due to the resulting large imbalance at Smoky Lake, it was decided to limit further interpretation of the results to the Woodmere location only. Segment length had a large and significant effect on rooting success on both sites. The shortest segments achieved 4% success and the longest 39% at Smoky Lake. The corresponding figures are 5% and 85% at Woodmere. Segment length was by far the most important factor, explaining 42.5% of the total variation at Woodmere. Rooting success appears to be directly proportional to segment length.

The root segments expressed clear polarity, with proximal orientation (root segment planted right way up) showing greater success than distal orientation (root segment planted upside down). There were significant differences in propagation success between clones, although clones explain only 1.3% of the total variation. Root segment origin effects were not significant. Rootling propagation appears to be a viable method for mass clonal propagation of aspen. Root segment length and orientation are critical in ensuring high success rates.

Keywords: Rootling, propagation, aspen, root size, origin and orientation

¹ Isabella Point Forestry Ltd., 331 Roland Road, Saltspring Island, BC, V8K 1V1, Canada. johnbro@saltspring.com
² Daishowa-Marubeni International Ltd., Postal Bag 2200, Peace River, Alberta, T8S 1Y4, Canada. fniemi@prpdmi.com
³ Western Boreal Aspen Corporation, 11420 –142 Street, Edmonton, Alberta, T5M 1V1, Canada. wbac@telusplanet.net
INTRODUCTION

The increased harvest of aspen (*Populus tremuloides* Michaux) in northern Alberta has prompted Canadian forest companies to develop methods for plantation silviculture of this dominant species of the Boreal forest. An efficient, effective and economical propagation method is needed as a pre-requisite for any plantation silviculture.

The Western Boreal Aspen Corporation (WBAC) is a cooperative tree improvement venture involving four Alberta forest companies that harvest aspen for pulp and oriented-strand board (OSB) production. WBAC has been developing a mass vegetative propagation method based on juvenile root segments termed “rootling propagation” (Niemi *et al.* 2003). This method uses small root segments that generate both stem suckers and fine roots to produce an autonomous rootling that can be used as planting stock (Figure 1). Rootling propagation is much easier and cheaper than tissue culture or greenwood cuttings as a means of mass vegetative propagation.

Aspens produce suckers from mature roots and rely on this method for natural regeneration following browse, fire, or other stand disturbance. Sucker stems can be excised from mature roots and induced to form roots under laboratory conditions. Libby (1986) coined the term “steckling” to describe a “plantable rooted cutting”. This term indicates the stem cutting origins of this type of propagule. In a similar vein, Hall *et al.* 1990 coined the term “rootling” to describe plants of root cutting origin. They planted root segments directly into nursery beds to produce bare-root stock. Dreeson and Harrington (1999) developed this method further using potted stock plants grown to provide juvenile root masses for containerized stock production. Dreeson (2001) termed this vegetative propagation method the “root cutting” method.

Given the necessity of growing potted stock plants as root donors, this study was initiated to test the effect of root segment size on propagation success. The origin of the roots and their orientation when planted vertically was also investigated.

MATERIALS AND METHODS

This experiment was replicated at two commercial forest nurseries: Woodmere Forest Nursery at Fairview, and at Smoky Lake Forest Nursery (Coast to Coast Reforestation) at Smoky Lake, Alberta. Six local clones of aspen were used: these are numbered 1176, 1177, 1221, 1223, 1229 and 1230 in WBAC’s clone testing program. Five donor pots were grown at Woodmere in 2003. In the fall, the stems were cut off and the root masses cold stored over the winter. In June 2004, half of the pots were shipped to Smoky Lake. Unfortunately, these were delayed in transit and are believed to have been subjected to excessively hot conditions for a few days. The pots remaining at Woodmere were used directly without transshipment.
At each site, root segments from the six clones were extracted and cut to 13-cm lengths. Each length was cut into two equal halves one proximal to the stem axis and the other distal. Root segments of the following lengths were cut from each half: 3 cm, 2 cm, 1 cm and 0.5 cm (see Figure 2). The root segments were ‘sown’ into styroblock cells (Beaver Plastics, Styroblock 410A – 112 cells / 80 ml/cell. The medium was 2.5:1 peat/vermiculite at Woodmere and peat/perlite at SLFN. Irrigation, lime, gypsum, macronutrients and micronutrients were applied as part of the nurseries standard growing protocol for hardwood seedlings. Segments were ‘sown’ vertically in the styroblock cells according to the pattern in Figure 3.
The experimental design was a split-plot factorial with 2 replicates of 6 clones in whole styroblocks as main plots, and segment length with four levels, and origin and orientation as sub-plot factors each with two levels. Each plot consisted of seven cells arranged systematically.
within the styroblock so that the long segments were on the outside and the short segments in the center of the block.

The trials were established June 2, 2004 and assessed on August 4 after 65 days. The following traits were assessed: number of sprouts per cell, survival (i.e. presence or absence of sucker sprouts) termed ‘rooting success’, height of tallest sprout in dm, and the diameter of the original root segments.

RESULTS

1. Nursery effects

Overall rooting success was 20% at Smoky Lake and 53% at Woodmere (Table 1). This difference is believed to be due to heat stress to the donor pots being delayed in transshipment from Woodmere to Smoky Lake. The Woodmere materials did not suffer from any heat stress.

2. Clone differences

There were significant differences in clone performance as measured by rooting success (Table 1). There were rank order changes across the two sites (Figure 4).

Table 1. Rooting success by clone and nursery

<table>
<thead>
<tr>
<th>Freq</th>
<th>Clone</th>
<th>SLFN</th>
<th>Woodmere</th>
</tr>
</thead>
<tbody>
<tr>
<td>244</td>
<td>1176</td>
<td>24.6</td>
<td>41.5</td>
</tr>
<tr>
<td>244</td>
<td>1177</td>
<td>23.4</td>
<td>50.0</td>
</tr>
<tr>
<td>244</td>
<td>1221</td>
<td>13.8</td>
<td>65.7</td>
</tr>
<tr>
<td>244</td>
<td>1223</td>
<td>17.9</td>
<td>50.9</td>
</tr>
<tr>
<td>244</td>
<td>1229</td>
<td>13.4</td>
<td>55.0</td>
</tr>
<tr>
<td>244</td>
<td>1230</td>
<td>21.2</td>
<td>52.2</td>
</tr>
</tbody>
</table>

3. Segment length

There were distinct size effects. For all clones and all treatments, the longer the root segment, the greater was the propagation success (Table 2 and Figure 5). The optimal segment length is probably around 4 to 5 cm, but these lengths were not tested, since the objective of this experiment was to test how small a segment could be used.

Table 2. The effect of root segment length on propagation success

<table>
<thead>
<tr>
<th>Freq</th>
<th>Length cm</th>
<th>#Sprouts</th>
<th>Success%</th>
</tr>
</thead>
<tbody>
<tr>
<td>336</td>
<td>0.5</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>336</td>
<td>1</td>
<td>0.47</td>
<td>0.43</td>
</tr>
<tr>
<td>336</td>
<td>2</td>
<td>0.96</td>
<td>0.77</td>
</tr>
<tr>
<td>336</td>
<td>3</td>
<td>1.13</td>
<td>0.85</td>
</tr>
</tbody>
</table>
Figure 4. The effect of clone and nursery on propagation success

Figure 5. Propagation success and number of sprouts as a function of segment length
4. Segment origin

Proximal origins had marginally better propagation success than distal ones (proximal = 54% versus distal = 52%). These small differences were not significant (Table 3 & Figure 6).

Table 3. The effect of root segment origin on propagation success

<table>
<thead>
<tr>
<th>Freq</th>
<th>Origin</th>
<th>#Sprouts</th>
<th>Success%</th>
</tr>
</thead>
<tbody>
<tr>
<td>672</td>
<td>Distal</td>
<td>0.6171</td>
<td>0.52</td>
</tr>
<tr>
<td>672</td>
<td>Proximal</td>
<td>0.6952</td>
<td>0.54</td>
</tr>
</tbody>
</table>

Figure 6. Propagation success and number of sprouts as a function of segment origin

5. Segment orientation

Segments planted proximal-end up produced more shoots and had higher success than distal orientations. These differences were larger than those for origin, and were significant (Table 4 & Figure 7).

Table 4. Effect of orientation on propagation success at Woodmere

<table>
<thead>
<tr>
<th>Freq</th>
<th>Orient</th>
<th>#Sprouts</th>
<th>Success%</th>
<th>Height dm</th>
</tr>
</thead>
<tbody>
<tr>
<td>672</td>
<td>Distal</td>
<td>0.60</td>
<td>0.48</td>
<td>1.77</td>
</tr>
<tr>
<td>672</td>
<td>Proximal</td>
<td>0.71</td>
<td>0.57</td>
<td>1.74</td>
</tr>
</tbody>
</table>
The effect of root segment origin

6. Combined effects of origin, orientation and length

The effect of segment length was striking and consistent across all combinations of origin and orientation. (Table 5 & Figure 8). It is clear that orientation is more important than origin. Proximal orientation with distal origin has higher success than distal orientation with proximal origin.

Table 5. Combined effects of origin, orientation and length at Woodmere

<table>
<thead>
<tr>
<th>FREQ</th>
<th>Segment Length-cm</th>
<th>Distal</th>
<th>Distal Proximal</th>
<th>Proximal Distal</th>
<th>Proximal Proximal</th>
</tr>
</thead>
<tbody>
<tr>
<td>84</td>
<td>0.5</td>
<td>0.04</td>
<td>0.06</td>
<td>0.05</td>
<td>0.06</td>
</tr>
<tr>
<td>84</td>
<td>1</td>
<td>0.39</td>
<td>0.48</td>
<td>0.30</td>
<td>0.55</td>
</tr>
<tr>
<td>84</td>
<td>2</td>
<td>0.66</td>
<td>0.82</td>
<td>0.76</td>
<td>0.83</td>
</tr>
<tr>
<td>84</td>
<td>3</td>
<td>0.80</td>
<td>0.87</td>
<td>0.84</td>
<td>0.90</td>
</tr>
</tbody>
</table>

Figure 7. The effects of root segment origin
Figure 8. Combined effects of origin, orientation and length on propagation success at Woodmere

7. Variance components

The variance components for a full split plot model were calculated using SAS PROC VARCOMP (SAS Institute 1985).

The following model was used:

\[ Y_{ijklmn} = \text{Mean} + \text{Rep} + \text{Clone} + \text{Rep*Clone} + L + L*Rep + L*Clone + L*Rep*Clone + \text{Orig} + \text{Orig*Rep} + \text{Orig*Clone} + \text{Origin*Rep*Clone} + \text{Orig*L} + \text{Orig*L*Rep} + \text{Orig*L*Clone} + \text{Orig*L*Rep*Clone} + \text{Orient} + \text{Orient*Rep} + \text{Orient*Clone} + \text{Orient*Rep*Clone} + \text{Orient*L} + \text{Orient*L*Rep} + \text{Orient*L*Clone} + \text{Orient*L*Rep*Clone} + \text{Orient*Orig*L} + \text{Orient*Orig*L*Rep} + \text{Orient*Orig*L*Clone} + \text{Orient*Orig*L*Rep*Clone} + \text{Orig*L} + \text{Orig*L*Rep} + \text{Orig*L*Clone} + \text{Orig*L*Rep*Clone} + e_{ijklmn} \]

where there are i reps, j clones, k lengths, l origins, m orientations, and n trees per plot. All effects and interactions were considered random. Subscripts have been omitted in the model statement for clarity. The percentage contributions to total variance are presented in table 6.
Table 6 Variance components and percentage contribution to total variance Woodmere site

<table>
<thead>
<tr>
<th>Source</th>
<th>Var comp.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rep</td>
<td>0.000000</td>
<td>0</td>
</tr>
<tr>
<td>Clone</td>
<td>0.003909</td>
<td>1.3</td>
</tr>
<tr>
<td>Rep*Clone</td>
<td>0.000400</td>
<td>0.1</td>
</tr>
<tr>
<td>L</td>
<td>0.123000</td>
<td>42.5</td>
</tr>
<tr>
<td>Rep*L</td>
<td>0.001086</td>
<td>0.4</td>
</tr>
<tr>
<td>Clone*L</td>
<td>0.001265</td>
<td>0.4</td>
</tr>
<tr>
<td>Rep<em>Clone</em>L</td>
<td>0.000000</td>
<td>0.0</td>
</tr>
<tr>
<td>orig</td>
<td>0.000497</td>
<td>0.2</td>
</tr>
<tr>
<td>Rep*orig</td>
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<tr>
<td>Clone*orig</td>
<td>0.000780</td>
<td>0.3</td>
</tr>
<tr>
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</tr>
<tr>
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</tr>
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<td>0.0</td>
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<tr>
<td>Clone<em>L</em>orig</td>
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<td>0.0</td>
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<tr>
<td>Rep<em>Clone</em>L*orig</td>
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</tr>
<tr>
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</tr>
<tr>
<td>Rep*orient</td>
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<td>0.2</td>
</tr>
<tr>
<td>Clone*orient</td>
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<td>0.0</td>
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**DISCUSSION**

Segment length was by far the most important factor in explaining rooting success. This was likely due to a combination of factors such as availability of stored carbohydrate reserves, potential sites for new root or sucker primordia, or even availability of residual water reserves to tide over the segment and developing suckers while new fine roots are developing. The optimum length is probably greater than 3 cm but this size was not tested here since we were trying to maximize root segment yield per donor plant.

Origin had little effect on rooting success. The small advantage of proximal over distal origins may best be explained by differences in root diameter close to and away from the stem axis, i.e.
segments of proximal origin are larger than those of distal origin if they are of equal length and from the same root.

The effect of planting orientation might best be explained by hormone gradients in the root segments. On inspecting dug up rootlings, the majority of suckers appear to originate at the proximal end, and the majority of fine roots appear at the distal end regardless of planting orientation.

In many cases, where multiple suckers were observed from a single root segment there was a tendency for one sucker to suppress the others. Usually the first sucker to expose its leaves to the light won this competition and ended up being the dominant or single stem. While horizontal placement of root segments does result in successful rootling production, the authors believe that vertical orientation with proximal side up is the optimal method. Horizontally placed segments are unstable and liable to topple whereas vertically placed segments are better anchored. There may also be more intense competition between suckers originating from horizontal roots with overall lower propagation success. This hypothesis should be tested in some further trials.

The different clones tested here had different rootling success. Such genetic differences in propagation ability have been commonly observed in other species for steckling production as well as tissue culture.

The large nursery effects are believed to have been accentuated by the heat stress that the donor root systems were subjected to in transit. Nonetheless, it can be expected that differences in cultural practices could explain some fairly large differences of response at different nurseries – critical factors could include ambient temperature, moisture availability and light quality and intensity.

With optimum treatment (3-5 cm root segments planted proximal-end up) we can expect 80-90 rooting success. It appears that rootling propagation is a promising method for mass vegetative propagation of aspen.

**LITERATURE CITED**


The Impact of Variable Success of Somatic Embryogenesis Among Elite Crosses on Expected Genetic Gain and Diversity of Selected Varieties

T.J. Mullin¹, M. Lstiburek², J. Pait³, and Y. A. El-Kassaby⁴

From its early beginnings in the 80’s, somatic embryogenesis of conifers has progressed to commercial status in some species (Cyr and Klimaszewska 2002). The current challenge is to produce large numbers of lines for screening and verification of genetic value. Crosses between elite parents selected from advanced breeding programs are used to produce embryogenic cultures in the laboratory, from which somatic seedlings can be propagated. Viable plants recovered from these cultures are planted in varietal tests. The goal is to select and multiply outstanding varietal lines, and to plant these operationally in forest plantations.

Although this technology offers potential for larger genetic gains compared to other deployment schemes, only a fraction of genotypes entering the laboratory culture phase (induction) actually result in viable somatic lines. Individual crosses often vary widely in their ability to generate embryogenic cultures under any given laboratory protocol (MacKay et al. 2001; Pullman and Johnson 2002). The objective of this study was to quantitatively evaluate such differences in embryogenic success, in terms of expected genetic gain in selected varietal mixtures.

METHODS

Stochastic simulation of a recurrent selection program included the generation of elite crosses, followed by testing and selection of superior varietal mixtures (Figure 1).

Figure 1. Schematic description of the recurrent strategy considered in this study.

¹ Research Professor, Department of Forestry and Environmental Resources, N.C. State University, Campus Box 8002, Raleigh, NC 27695-8002, USA
² Lecturer, Faculty of Forestry and Environment, Czech University of Agriculture, Kamýcká 1176, Praha 6 – Suchdol, 165 21, Czech Republic
³ Senior V.P. – Business Development, CellFor Inc., 75 Fifth Street NW, Suite 321, Atlanta, GA 30308, USA
⁴ Professor, Department of Forest Sciences, 2424 Main Mall, University of British Columbia, Vancouver, BC V6T 1Z4, Canada
The recurrent program was initiated by generating 100 founder genotypes “founder population”. Phenotypic observations were due to independent additive genetic effects and environmental deviations. The initial value of narrow-sense heritability was set at 0.3. Single-pair mating was performed among founders (50 controlled crosses). 50 progeny genotypes were generated in each full-sib family “recruitment population” and planted in a test, where each progeny genotype was replicated by ten ramets (10 independent environmental deviations). Following the test evaluation, top two individuals per family were selected to serve as parents in the following generation “breeding population”. This process was repeated for 6 generations. Simulation was done using POPSIM\textsuperscript{TM} software, which is based on infinitesimal model assumptions (Mullin and Park 1995).

In every generation, top 10\% of parents were selected (denoted as “elite parents”) and crossed in a half-diallel mating. Progenies of these crosses entered the embryogenic induction and testing phase of the program. Success in somatic embryogenesis among crosses was considered either constant (CONST) or varied as an exponential distribution of family sizes. In the latter case, success rate was distributed among crosses either at random (EXP-RAND) or as a function of expected family values (EXP-BV). Regardless of the distribution of induction success rate among the elite crosses, the total number of lines field tested was fixed (2,000). The top ten lines were selected and the expected gain of this mixture reported.

RESULTS AND DISCUSSION

Surprisingly, variation in embryogenic propensity (i.e., induction success rates) among elite crosses had a negligible impact on the average genetic gain from the selected varieties (Figure 2).

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2.png}
\caption{Genetic gain in individual generations (1-6) for the three scenarios tested in this study. Bars represent average values of 400 simulation iterations along with confidence intervals (alpha = 0.05).}
\end{figure}

This observation holds across generation due to the relatively small amount of genetic variation among elite families, as opposed to large within-family variation, such that there is a large
potential to select outstanding varieties, even though they may have not have originated from crosses among the very best of the elite parents. The additive variance within families is replenished in every generation and is a function of the initial additive variance (in unselected population) and the inbreeding of parents (which was also considered in this study). The expected gain in selected varietal mixtures

In reality, varietal tests in individual generations will be reevaluated over time as the tests mature. This could lead to revised selections of varieties used in large-scale plantations. At a certain time, new test will become available (from crosses among elite parents in next generation) and the selection will concentrate on these.

It can be expected that as the technology of somatic embryogenesis further develops, this will lead to the normalization of responses among elite lines (Klimaszewska and Cyr 2002). The results of this study indicate that following such normalization and redirecting resources according to expected family values (scenario EXP-BV) would not necessarily lead to an important increase in genetic gains, if the testing effort (overall propagation success) is fixed.

The results from the present study support directing the breeding efforts of cloning programs towards conducting larger number of crosses rather than the common practice of concentrating on the top parents. The use of more crosses increases the chance for capturing superior genotypes through within families’ selection as well as maintaining wider genetic diversity, an important consideration for clonal forestry deployment programs. The observed lack of substantial genetic differences among families when fewer crosses are made reduces the potential gain from these programs. Additionally, the observed minimal impact of family size (i.e., number variety produced/cross) among the implemented three scenarios (CONST, EXP-RAND and EXP-BV) on the captured gain demonstrates that the energy invested on maximizing the induction rate requires further evaluation.

REFERENCES


Genetic Variation in MFA, MOE and Wood Density Among Clones of *Pinus taeda* L.

Fikret Isik\(^1\), Bailian Li\(^2\) and Barry Goldfarb\(^2\)

Breeding and selection for desirable wood properties will be a key factor in determining the global competitiveness of forest industry in the United States. Microfibril angle (MFA), modulus of elasticity (MOE) and wood density are the most important wood characteristic that affect solid wood properties. Forty-five clones from nine full-sib families of loblolly pine were sampled in this study to study genetic variation in these traits. The experimental design was split-plots with seedlings and rooted cuttings of the same full-sib families in whole plots. In rooted cutting plots, 5 to 9 clonal sub-plots were established per family, with each sub-plot consisting of two ramets. The field trials were established in two locations with six complete blocks within each site. Increment cores (12 mm thick) were sampled from breast height of the stems. Wood properties were measured by SilviScan\(^2\) 2, an instrument that combines x-ray densitometry, diffractometry and image analysis to measure a variety of wood properties.

Based on core average values, considerable variation was detected among clones for MFA, MOE and density. Clonal differences explained 26% of the variation in MFA and MOE. Percent of the total variation explained by clones in density (43%) was much higher. Within family clone-mean heritabilities were 0.62 for MFA and MOE. MFA had negative phenotypic and genetic correlations with height suggesting that fast growing loblolly pine clones tend to have acute MFA angles. However, correlations between MFA and diameter at breast height were weak. MFA had also weak correlations with wood density, suggesting that selection for volume or density will not affect MFA in the population studied.

Parents differed significantly for MFA, MOE and density values obtained from the seedlings based materials. Male parents explained greater percentage (9%) of variance for MFA than females (4%). Full-sib families were not different for any of the traits. The results suggested that MFA, MOE and density are repeatable at the family level. Additive genetic effects were the main source of genetic variation. Dominance genetic effects were negligible. MFA had negative but weak phenotypic and genetic correlations with the growth traits. Correlation between MFA and density values was. Selection of parents of loblolly pine for MFA and MOE for deployment in the pine plantations seems promising. Considerable improvement could be realized for fiber properties from traditional selection. The results suggested that emphasis should be given clonal selection for greater improvement of traits.

Radial variations in MFA and MOE and differences among clones and families were examined. MFA decreases from the pith to the bark, whereas MOE increases. The highest MFAs occurred close to the pith for all clones and families. However, large genetic differences (at family and clones levels) were detected in the rate of change over time for MOA and MOE, which should provide opportunities for selection and breeding for these properties in a tree improvement program.

\(^1\) Research Assistant Professor, and  
\(^2\) Professors Department of Forestry and Env. Res., North Carolina State University, Raleigh, NC, USA
Genetic Analysis of Early Field Growth of Loblolly Pine Clones and Seedlings from the Same Full-Sib Families

Brian Baltunis, Dudley Huber, Tim White

The Forest Biology Research Cooperative recently established a series of loblolly pine clonal trials known as CCLONES (Comparing Clonal Lines on Experimental Sites). There are three primary levels of genetic structure in this study (parental, full-sib family, clone) that strengthen the power of CCLONES for examining genetic mechanisms and interactions with cultural treatments and locations. A fourth level of genetic structure can be added by considering the provenance of the parents. This report includes some preliminary results from the genetic analyses of 2nd year growth traits that were recently measured at the CCLONES loblolly pine trials. The specific objectives of this report are 1) to determine heritability estimates for various growth traits for loblolly pine clones and seedlings, 2) to compare the genetic correlations between parents and families when grown as cuttings versus seedlings, and 3) to determine the genotype x environment interaction by looking at the genetic correlations for parents, families, and clones for paired trials.

MATERIALS AND METHODS

The parental population consisted of twenty first-generation and ten second-generation selections from a larger population that is part of the Loblolly Pine Lower Gulf Elite Population. In addition two slow-growing parents were included. These selections represent the Atlantic Coastal Plain (ACP), Florida (FL), and Lower Gulf (LG) provenances of loblolly pine. These thirty-two elite loblolly pine parents were mated in a partial diallel design and created 70 full-sib families from which a total of 2,000 vegetatively propagated clones were generated. Rooted cuttings from approximately 1,000 of these clones from 61 full-sib families and seedlings from the same full-sib families were established at seven field sites across the southeastern United States utilizing a resolvable incomplete block design (Tests A-G).

Each growth variable (2nd year height, height increment, and crown width) was analyzed for cuttings and seedlings simultaneously with a bivariate analysis in ASREML. Narrow-sense heritability ($\hat{h}^2$) was estimated using the corresponding variance components. Type B genetic correlations for general combining ability ($r_{B_{propGCA}}$) and specific combining ability ($r_{B_{propSCA}}$) between cuttings and seedlings were estimated in order to compare parental and family performance between propagule types. In order to quantify the extent of genotype x environment interaction, type B genetic correlations across pairs of trials were estimated for the clonal data.

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1 Graduate student, Co-Director of Cooperative Forest Genetics Research Program, and Director of School of Forest Resources and Conservation, School of Forest Resources and Conservation, University of Florida, Gainesville, FL, USA 32611-0410
RESULTS AND DISCUSSION

There are three provenances represented in this population. Therefore, genetic groups were added to the pedigree file representing the three provenances (ACP, FL, and LG). By doing this we do not assume that all of the parents come from one population, but rather a separate provenance mean was estimated for each of the three genetic groups. The Florida source of loblolly pine ranked highest, followed by Atlantic Coastal Plain and then Lower Gulf provenances for all growth variables measured at all of the tests with one exception. At the northern most test (Test G) in Virginia, the Atlantic Coastal Plain ranked higher than the Florida source for 2nd year height, but the Lower Gulf source still performed the poorest.

Narrow-sense heritability was estimated for all measured variables by site for each propagule type and was always greater for the clones versus seedlings. For example, estimates of $\hat{h}^2$ for 2nd year height of rooted cuttings ranged from 0.14 to 0.26, while for seedlings, $\hat{h}^2$ estimates ranged from ~ 0 to 0.2. Similar trends were observed for the other growth traits measured. Non-additive genetic variance was detected at all of the sites for 2nd year height. Heritability estimates for crown width and height increment of rooted cuttings were more weakly controlled by additive effects than 2nd year height, ranging from about 0.07 to 0.24 for both traits.

At the time of planting, seedlings were generally taller than rooted cuttings, and this trend has continued through year two. However, height increments were very similar between both propagule types. Height increment ranged from 1.1 to 2.1m for cuttings growing in high intensity culture, while for seedlings, height increment ranged from 1.1 to 1.9m. Generally, seedlings also had a wider crown than rooted cuttings.

In order to compare cuttings to seedlings, type B genetic correlations between propagule types were calculated for additive and dominance effects. The genetic correlation between propagule types for additive effects, for example, gives us an indication of whether parental ranks are dependent upon whether their progeny are grown as cuttings or seedlings, while type B genetic correlations for dominance effects measure the correspondence of dominance across propagule types. For all growth traits measured the type B genetic correlation between propagule types for additive effects was strongly high. $r_{B\text{propGCA}}$ ranged from 0.71 to 0.99 for all of the traits. This tells us that the parents ranked the same regardless of propagule type.

The type B genetic correlations between propagule types for dominance effects were more variable. A low $r_{B\text{propGCa}}$ indicates that full-sib families deviated differently around their half-sib family mean depending on whether they were grown as cuttings or seedlings. The most probable reason for inconsistency between dominance effects is a sampling problem. Within a test there were about 32 seedlings per full-sib family, while only 15 clones per full-sib family. Therefore, full-sib means may have been located with more error for cuttings than seedlings. This could also be extended to half-sib families. However, correlations of full-sib family values (GCA parent1 + GCA parent2 + SCA) between propagule types for 2nd year height were moderate to high, ranging from 0.68 to 0.95, indicating that full-sib family performance was independent of propagule type.
The extent of *genotype x environment* interaction was investigated by analyzing data across pairs of trials. The type B correlations were highly variable indicating the presence of GxE on some sites while absent from others. The best type B genetic correlations appear to be between Tests B, C, and D, all of which contained rooted cuttings from the Spring 2002 setting. For example, there were some slight changing of ranks of parents at tests B and D, however, the overall genetic correlation was high \((r_{gxeGCABr} = 0.87)\) indicating little GxE. Not only did full-sib families deviate similarly around their parental means \((r_{gxeSCABr} = 0.82)\) at Tests B and D, but the full-sib families also ranked similarly, indicating little GxE at the full-sib family level. Clonal ranking was also consistent at Tests B and D indicating that a good ranking clone for age two height at Test B also ranked high at Test D, and this was the case at for all paired analyses involving trials originating from the Spring 2002 setting. Extensive *genotype x environment* interaction was observed between some sites. For example, the worst type B genetic correlations were between Test A and Tests B, C, D, E, or F. Rooted cuttings for Test A originated from the Winter 2002 setting, while those from B, C, D, and E originated from the Spring 2002 setting, and rooted cuttings planted in Test F came from the Summer 2002 setting. Perhaps there was a propagation effect that has carried over to the field. A propagation effect of season was observed for rooting as well. The lowest type B genetic correlations for rooting were observed between the winter setting and either the spring or summer settings, while the highest correlations were observed between the two spring settings. Another indication of a propagation (C effect) effect for field growth comes from the genetic correlations involving Test G (cuttings also originating from the Winter 2002 setting). There was a stronger relationship between the clonal rankings at Test A and G, than between the clonal rankings at these sites and any of the other trials.

**CONCLUSION**

All of the growth traits analyzed, 2nd year height, height increment and crown width, were heritable as evidenced by the heritabilities for these traits. However, higher heritability estimates were obtained with clones compared to seedlings for all traits measured. Genetic correlations of additive effects between propagule types indicated that parental rankings were stable regardless of whether their progeny were grown as rooted cuttings or seedlings. There appears to be a propagation effect of the season the cuttings were set that has carried over to field growth. This was evidenced by the type B genetic correlations from paired site analyses.
Accounting for Spatial Variability in Clonal Forestry Trials

S.A. Gezan, T.L. White, and D.A. Huber

Experimental sites in forestry tend to have high environmental variability, which is usually expressed in the form of patches, gradients or both, together with considerable random microsite noise (Costa e Silva et al. 2001). With environmental heterogeneity, the estimates obtained for the analysis of a particular dataset will tend to be highly variable. With an adequate experimental design or by using an appropriate statistical spatial analysis, it is possible to account for part of this heterogeneity and produce considerable improvements in heritabilities and therefore in the precision of genetic value predictions. The goal of this study is to quantify relative efficiencies of using classical and non-classical statistical techniques to account for spatial variability in clonal trials, and also to decide which technique is the most parsimonious and yet performs well across a broad set of environmental conditions.

MATERIALS AND METHODS

The study was based on simulations of single site clonal trials of 2,048 ramets planted in single tree plots on a rectangular grid of 64 rows and 32 columns with 8 ramets for each of the 256 clones. Two factors were considered in simulating the environment over which the different experiments were located: a gradient generated with a polynomial function depending on the x and y coordinates of the grid, and patches that were modeled by incorporating a covariance structure based in a first-order separable autoregressive process or AR1 ⊗ AR1 with nugget (microsite error) (Gilmour et al. 1997). The three surface patterns simulated included: only patches (PATCH), only gradient (GRAD), and a combination of both (ALL). More details of the simulation process can be found in Gezan et al. (2005).

Several sets of spatial analysis techniques were studied and compared with classical approaches. All models and datasets were analyzed using ASREML (Gilmour et al. 2002), and estimated variance components were summarized and empirical correlations (CORR) between the true and predicted clonal values were calculated by surface pattern and statistical model.

The first group of statistical analysis assumed that the errors were independent and identically distributed (ID), and included: classical analysis, simple global trend functions and nearest neighbor (NN) techniques. For the classical analyses the designs considered were: completely randomized (CR), randomized complete block with 8 replicates (RCB), an incomplete block designs with 32 blocks (IB 32), and a row-column design (R-C). The global trend was modeled with 3 different continuous functions of the x and y coordinates: a) Linear, b) Reduced polynomial, and 3) Full polynomial. The NN techniques used linear covariates in the linear model to correct for similar microenvironments which were calculated by averaging residuals of neighbor plots. Here, a number of different variants of Papadakis (PAP) (Atkinson 1969) were implemented.

1 Graduate Student, Professor, Research Associate, respectively, SFRC, IFAS, University of Florida, Gainesville, FL, USA.
The second group of spatial techniques was based in modeling patches by incorporating a separable autoregressive error structure that was fitted with two variants: a) without nugget (AR1⊗AR1), and b) with nugget effect (AR1⊗AR1+\eta). These variants were fitted alone, in combination with design effects (RCB, IB 32 and RC), or with different functions of the global trend (Linear, Reduced and Full polynomial).

RESULTS AND CONCLUSIONS

The use of classical design with independent errors, as is done in many studies using IB 32 and R-C helped to successfully control for trend and patches, but it was not optimal (Figure 1a). The R-C design had average CORR values of 0.89, 0.88 and 0.87 for ALL, GRAD and PATCH surface pattern which were only 0.01 lower than the maximum, and it was the best of the models with independent errors for ALL and PATCH surface patterns, but deficient in GRAD surfaces.

Incorporating an error structure different than independent errors produced an important increase in the average CORR values, with larger improvements in PATCH surfaces (Figure 1a and 1b). Also, differences between classical experimental designs were almost non-existent when the error structure was modeled together with the design effects. As expected, the best results were found in the AR1⊗AR1+\eta error structure with average CORR values as high as 0.90 with the Full-polynomial model, and yielded average variance components close to their parametric values. Finally, failing to model the error structure correctly (as with AR1⊗AR1) produced bias in some variance component parameters (data not shown).

Figure 2. Average correlations between true and predicted treatment effects (CORR) in 3 different surface patterns for classical experimental design analyses and polynomial models fitted for the following error structures: a) independent errors (ID), b) autoregressive without nugget (AR1⊗AR1) and, c) autoregressive with nugget (AR1⊗AR1+\eta).
Promising results were obtained with the Papadakis methods, particularly for surfaces with patches, but also in surfaces with gradients (Figure 2). These methods were almost as good as models that modeling the error structure, particularly those variants that considered more plots and/or covariates. PAP-11 was the best followed by PAP-6. The use of the latter is recommended because of its simplicity (only 4 covariates).

In summary, if simple analyses are preferred, R-C and IB designs should be used. For further improvements, it is recommended to use some form of PAP methods, but several covariates must be tried and tested. Finally, spatial analysis incorporating error structures are promising, and they should be used whenever possible; but the computational requirements and some uncertainty about the correct procedures and model testing limit its practical use.

![Figure 4. Average correlations between true and predicted treatment effects (CORR) in 3 different surface patterns for selected methods: randomized complete block (RCB), row-column (R-C), and Papadakis (PAP).](image)

**REFERENCES**


Clonal Replacement as a Tool for Seed Orchard Managers

C.L. Rosier\textsuperscript{1}, S.E. McKeand\textsuperscript{2}, E.M. Raley\textsuperscript{3}

Topgrafting scion from selective genotypes of loblolly pine (\textit{Pinus taeda} L.) into the crowns of sexually mature seed orchard ramets (interstocks) has been extremely successful in producing both female and male strobili one to two years following grafting. The objective of the current study is to test the feasibility of using topgrafting to replace all or much of the crown of ramets in loblolly pine seed orchards. While there are numerous advantages of clonal replacement to a seed orchard manager, the big question remains - can we afford to do this? In order to develop a detailed economic analysis it is vital to determine the total cost of the operation. In order to do this the following data is needed; 1) scion quality, 2) number of grafts per tree, 3) interstock effects, 4) graft survival, 5) annual flower counts and the number of cones harvested, 6) time required to topwork each tree, and 7) time required for crown management of the grafts to keep the topworked scions dominant.

Clonal grafting survival was significantly different and ranged from 17 to 73\% with a mean of 54\%. Grafting survival also significantly increased as the quality of the scion increased. Flower production was significantly effected by both the topgraft clone and the interstock. Female strobilus production by clone and interstock ranged from 0 to 13.3 and 1 to 21.9 flowers per graft, respectively. Male catkin cluster production was not significant by clone, but was significant by interstock and ranged from 0 to 5 cluster per graft. The biological and economic significance of these results will be discussed.

\textsuperscript{1} Tree Improvement Project Leader, Smurfit-Stone Container Corporation, Fernandina Beach, FL
\textsuperscript{2} Professor, Dept. of Forestry and Environmental Resources, N.C. State University, Raleigh, NC
\textsuperscript{3} Texas Forest Service, Texas A&M University, College Station, TX
Susceptibility of Loblolly x Slash Pine Interspecific F1 Hybrids to Tip Moth Infestation and Fusiform Rust Infection in a South Mississippi Planting

M.T. Highsmith¹, L.H. Lott², and C.D. Nelson³

Tip moth damage and fusiform rust incidence among families of three loblolly pine (Pinus taeda) parent trees from Mississippi, Louisiana, and Texas that were selected for southern pine bark beetle resistance and three slash pines (Pinus elliotti var. elliotti) selected for different levels of fusiform rust resistance, and five of their interspecific F1 hybrids were assessed in a south Mississippi planting. After two years in the field, the rust resistant slash pine interspecific F1 hybrid had the least amount of tip moth damage (caused by Nantucket pine tip moth, Rhyacionia frustrana), and the lowest incidence of fusiform rust disease (caused by Cronartium quercuum f. sp. fusiforme). The highest incidence of fusiform rust occurred on interspecific F1 hybrids between a Texas loblolly pine parent crossed with a rust susceptible slash pine from south Mississippi and one from Georgia. The loblolly pine open-pollinated families had the most height growth compared to their interspecific F1 hybrids.

¹Associate Professor, Department of Natural Science and Mathematics, Shaw University, Raleigh, NC and Department of Forestry & Environmental Resources, NC State University, Raleigh, NC
²Biological Sciences Technician, Southern Institute of Forest Genetics, USDA Forest Service, Saucier, MS
³Research Geneticist and Project Leader, Southern Institute of Forest Genetics, USDA Forest Service, Saucier, MS
Comparing Parameter Estimation Techniques for Diameter Distributions of Loblolly Pine in a GxE Study

B.C. Smith, B.P. Bullock, and S.E. McKeand

Diameter distributions play an important role in stand modeling. Frequency by size class can be estimated from a distribution function with estimated parameters. A number of different distribution functions have been utilized to model diameter distributions, including the Beta, Lognormal, Johnson’s Sb, and Weibull. The Weibull function has been widely used due to its flexibility in modeling reverse-J, skewed, and unimodal shapes and because integration is not required to estimate frequencies. For these reasons the Weibull function was chosen for this study. Two of the main methods which have been used to estimate the parameters of the Weibull function are parameter prediction, in which each parameter is directly predicted from stand-level variables with a regression equation, and parameter recovery, where selected percentiles of the distribution are predicted and used to equate parameters to moments of the distribution. The purpose of this study was to determine the parameter estimation technique most appropriate for young plantation-grown loblolly pine, and to present parameter estimation equations incorporating necessary genotype and environmental information.

MATERIALS AND METHODS

The SETRES-2 study site is located in the Sandhills of NC in Scotland County on very well drained, infertile soil. Established in November 1993, the study is a split-split-plot design with two silvicultural treatments, fertilized and unfertilized, and two provenances (Atlantic Coastal Plain and Lost Pines Texas) with five open-pollinated loblolly pine families from each. The study is divided into nine blocks, with two main treatment plots each containing two provenance sub-plots, with five family sub-sub-plots nested within each provenance plot. 100 trees were planted in each family plot with a rectangular spacing of 1.5 m by 2.1 m, for a total of 18,000 trees. Several measurements, including height and diameter, were taken for every tree at ages 0, 1, 2, 3, 4, 5, 6, 8, and 10. Diameter measurements were taken for the interior 64 trees of each family plot at age 11. Due to the size of the trees relative to the buffer size between treatment plots inducing potential edge effects, only measurements from the interior 64 trees were used for this study.

Theoretical distributions were fitted to the empirical diameter distributions from ages five to 11 using the two parameter Weibull distribution, with the probability density function (p.d.f.) given in Equation (1). The fit method utilized was maximum likelihood estimation (MLE).

\[
f(x) = \frac{c}{b} \left(\frac{x}{b}\right)^{c-1} \exp\left[-\left(\frac{x}{b}\right)^c\right] \quad \text{for } x \geq 0, b > 0, c > 0
\]

where \(b\) = scale parameter

\(c\) = shape parameter

\(x\) = stem diameter

1 Graduate Student, Assistant Professor, and Professor, respectively, Department of Forestry and Environmental Resources, NC State University, Campus Box 8002, Raleigh, NC 27695-8002.
Parameter prediction and recovery models were constructed to estimate the parameters produced by the MLE distribution. Incorporation of various stand level variables as well as family and provenance indicator variables in the models were tested. Regression equations were fit simultaneously using seemingly unrelated regression due to correlated errors.

As Shiver (1988) pointed out, because there is no known underlying distribution function with specific parameters, what are important in modeling diameter distributions are not the individual predicted parameters but rather how well the combinations of predicted parameters reproduce the empirical distributions. Rather than checking the ability of each model to estimate individual parameters, it is desirable to test the fit of a model’s distribution from the estimated parameters. In light of this, the goodness-of-fit of the MLE and the predicted distributions to the empirical distributions were evaluated with an error index proposed by Reynolds et al. (1987). The index is a weighted measure of how well a distribution predicts the number of trees in individual diameter classes. The error index, e.i., is given by:

\[ e.i. = N \sum_{i} w(x_i) \left| \hat{F}(x_i) - F^*(x_i) \right| \]

where \( (x_i) \) is the centerpoint of the \( i \)th d-class

\[ \hat{F}(x_i) = \text{the proportion of distribution predicted in the } i \text{th d-class} \]

\[ F^*(x_i) = \text{the proportion of distribution observed in the } i \text{th d-class} \]

\[ w(x_i) = \text{weight of } x_i \]

\[ N = \text{trees per hectare} \]

When \( w(x_i) \) is set to basal area (m\(^2\)) of diameter \( x_i \), the error index units are basal area per hectare predicted in the incorrect diameter class. If the multiplier \( N \) is dropped from the error index equation, the resulting index yields the percent of BA/ha which was predicted in the incorrect diameter class. For this study, two centimeter diameter classes were used.

**RESULTS AND DISCUSSION**

All of the final models tested performed similarly. The inclusion of a provenance indicator variable was not found to be significant in any of the models. The addition of certain family indicator variables slightly improved the fit of the parameter prediction model, but this would be of limited usefulness as the study families are random typical families. What is interesting to note is that the rank of the mean shape and scale parameters remains fairly stable over time. The percent of the basal area misclassified by the model decreased as age increased for all the models, indicating the accuracy of the fitted distributions are improving over time. The mean e.i. over all treatments, provenances, and ages was lower for the parameter prediction techniques than the parameter recovery techniques.

The recommended parameter prediction model is given in Equation (3), and the recommended parameter recovery model is given in Equation (4). The mean e.i. through age 10 of Equation (3) was 1.291 m\(^2\)/ha, with a minimum of 0.107 and maximum of 4.251 m\(^2\)/ha, compared to a mean e.i. for Equation (4) of 1.389 m\(^2\)/ha, minimum of 0.103 and maximum of 6.137 m\(^2\)/ha. For comparison, the mean e.i. of the MLE distributions was 1.075 m\(^2\)/ha, with a minimum of 0.061 and maximum of 4.446 m\(^2\)/ha. A direct comparison of the methods was not possible through age...
11 due to the lack of dominant height data, but of the parameter recovery models Equation (4) still performed best at age 11.

$$\hat{b} = -0.01393 + 119.02733 \left( \overline{BA} \right)^{0.01393}$$

$$\hat{c} = 7.08377 + 4.90044 \left( \text{Fertilizer} \right) - 7.97094 \left( \text{RS} \right) - 0.34313 \left( \text{Fertilizer} \right) \left( \text{Age} \right)$$

where,

$$\hat{b} = \text{the estimated Weibull scale parameter}$$

$$\hat{c} = \text{the estimated Weibull shape parameter}$$

$$\overline{BA} = \text{mean basal area, m}^2$$

Fertilizer = fertilization indicator variable, 1 if fertilized, else 0

RS = relative spacing, \( \sqrt{\frac{10,000}{\text{trees per ha}}} \)

mean dominant height, m

$$\ln \left( \hat{D}_{25} \right) = 5.89988 + 0.68360 \ln \left( \overline{BA} \right) - 0.20221 \ln \left( \text{Age} \right)$$

$$\ln \left( \hat{D}_{95} \right) = 3.87163 + 0.35889 \ln \left( \overline{BA} \right) + 0.19035 \ln \left( \text{Age} \right)$$

$$\hat{b} = \sqrt{\frac{\left( \overline{D}_{q} \right)}{\Gamma \left( 1 + 2 / \hat{c} \right)}}$$

$$\hat{c} = \frac{2.28823}{\ln \left( \hat{D}_{95} \right) - \ln \left( \hat{D}_{25} \right)}$$

where,

$$\hat{b} = \text{the estimated Weibull scale parameter} ; \quad \hat{c} = \text{the estimated Weibull shape parameter}$$

$$\overline{BA} = \text{mean basal area in m}^2 ; \quad \text{Age} = \text{age of stand, years}$$

$$\overline{D}_{q} = \text{quadratic mean diameter in cm} ; \quad \Gamma \left( \cdot \right) = \text{the gamma function}$$

$$\hat{D}_{i} = \text{the } i^{th} \text{ percentile of the diameter distribution in m, for } i = 25, 95$$

Due to the use of different stand level variables as inputs in the models, Equation (3) is recommended for parameter estimation if dominant height data are available. Otherwise, Equation (4) should be used.

REFERENCES


Preliminary Results for Above- and Below-Ground Bio-Sequestration of a Mature F1 Black Spruce Varying in Site and Family Productivity

John E. Major, Kurt Johnsen2, Marianne Burke3, Debby Barsi1 Lance Kress2 Chris Maier2, John Butnor4 and Moira Campbell1*

Worldwide, efforts to reduce atmospheric CO2 are being explored both by reducing emissions and by sequestering carbon (C). Spruce (Picea) is the major component in many Canadian forest ecosystems and accounts for 1/3 of Canadian inventory. Black spruce (Picea mariana) is the most important softwood species to the forest and pulp and paper industry and accounts for 35% of reforestation in Canada. Below-ground forestry research is difficult, often neglected and sequestration information is severely lacking. Forestry, by tying up C in biomass, in soil and in products, may be an important avenue to increase biologically sequestered C. Although traditional forest genetics research has clearly shown tree genotypes can vary greatly in above-ground volume growth, it is not at all certain that these above-ground growth increases will result in overall increases in C sequestration. This is because trade-offs can exist between above- and below-ground C sinks. Such growth “strategies” can be genetically controlled. Thus, a genetically superior above-ground volume producing genotype may well divert less C below-ground compared to a slower above-ground volume producing genotype. In the early 1990’s a series of studies were conducted to explore genetic variation in drought tolerance of mature black spruce (Picea mariana (Mill.) B.S.P.) trees from a 7 × 7 diallel on 3 sites of varying water holding capacities at the Petawawa Research Forest. A 2 x 2 subset with drought tolerant and intolerant families were examined with the former generating lower osmotic potential, higher turgor, and higher photosynthesis than the drought intolerant families. Thus there are important genetic and site component of varying productivity, which will be analyzed. The trees are now 30-years-old and we are investigating carbon allocation below ground, and above ground. Whole tree harvests were conducted; full tap root systems were displaced using air pressure so that root-to-shoot allocation patterns can be quantified for scaling. Soil cores were also examined for soil carbon and fine root production. The data will be used to estimate carbon fixation of a black spruce plantation and how much carbon is added to a system by planting black spruce. We will be presenting some of the preliminary results from this work.

* 1 Natural Resources Canada, Canadian Forest Service, Atlantic Forestry Centre, Fredericton, NB, Canada
2 USDA Forest Service, Southern Research Station, 3041 Cornwallis Road, RTP, NC, 27709, USA
3 USDA Forest Service, USDA Forest Service, 2730 Savannah Highway, Charleston, SC 29414
4 USDA Forest Service, USDA Forest Service, 705 Spear Street, South Burlington, VT 05403
Expected Genetic Gains and Development Plans for Two Longleaf Pine
Third-Generation Seedling Seed Orchards

C.D. Nelson\textsuperscript{1}, L.H. Lott\textsuperscript{1}, and D.P. Gwaze\textsuperscript{2}

Abstract: Selection and thinning plans were developed for two longleaf pine (\textit{Pinus palustris} Mill.), third-generation seedling seed orchards located in southeastern Mississippi and central Louisiana. The two orchards were part of several long-term experimental field tests designed to investigate genetic variation in height growth and brown spot needle blight (caused by \textit{Scirrha acicola} (Dearn.) Siggers) resistance in a longleaf pine population. Phil Wakeley identified the original population in the 1920s in southeastern Louisiana and E. B. Snyder and H. J. Derr continued to advance the population through selection and breeding for early height growth and brown spot resistance. Our current results suggest that both traits can be improved by another round of selection and deployment through these third-generation seedling seed orchards. Operationally expected genetic gains range from 4.7\% to 9.1\% for height at age 9 years and 3.6\% to 4.3\% for brown spot resistance through age 4 years. These expected gains represent an approximate tripling in early height growth rate and doubling of brown spot resistance compared to the second generation.

Keywords: longleaf pine, grass-stage, brown spot needle blight, disease resistance.

INTRODUCTION

Longleaf pine (\textit{Pinus palustris} Mill.) genetics and breeding research has been ongoing since the 1950s at the Southern Institute of Forest Genetics (USDA Forest Service, Southern Research Station) near Gulfport, Mississippi. As part of this work E. B. Snyder and co-workers (Snyder 1969; Snyder and Derr 1972; Snyder et al. 1977; Snyder and Bey 1978) selected trees in local forests, made crosses among the selections to produce control- and open-pollinated progeny, evaluated the progeny in replicated field trials, and in some instances made second- and third-generation selections for advanced generation breeding and seed orchard development. One such series of experiments produced a second-generation seedling seed orchard (Study 3.10) and field tests designed for the establishment of two third-generation seedling seed orchards (Study 3.45, Part A1). These materials were derived from selections made by Wakeley (1970) in the 1920s in southeastern Louisiana. The current study continues this work with an analysis of the third-generation field tests planted at two locations. Heritabilities and expected genetic gains for the seedling seed orchards are computed and presented for both traits (height growth and brown spot resistance) based on a selection scheme that maintains a high level of genetic diversity while capturing genetic gain using within-family, within-block selection.

\textsuperscript{1} USDA Forest Service, Southern Institute of Forest Genetics, USDA Forest Service, Saucier, MS 39574.
\textsuperscript{2} Missouri Department of Conservation, Columbia, MO 65201.
MATERIALS AND METHODS

Four 9-parent partial diallel tests were established at two field locations— Saucier, MS (Harrison Experimental Forest) and Alexandria, LA (Palustris Experimental Forest, Johnson Tract). The 36 parents were selected from replicated open-pollinated family tests growing at the same two locations (Study 3.10). Combined family and within-family selection was practiced for both brown spot resistance and early height growth. Each parent was crossed with four other parents, resulting in 18 crosses per diallel or 72 crosses for the four diallels. The field design was a reps-in-sets design, where families were replicated (i.e., blocked) within diallels (i.e., sets). Single-tree plots (one tree per family per block) were used in 24 blocks per diallel per location. Spacing was 0.91 m (3 ft) within the rows by 3.0 m (10 ft) between rows. In addition two check lots were included in each block—a bulk local source and an open-pollinated family from a known brown spot resistant selection (Wash 1-77). Trees were evaluated for brown spot infection (% leaf surface symptomatic) in years 1-4 and height in years 2-5, 9, and 18. Additional details can be found in Lott et al. (2001), except that they considered the 9 year-old trees to be 10 years old and the field design to be a randomized complete block with 4 treatments (i.e., diallels) and 24 blocks (i.e., reps) of single-tree plots.

For the purposes of this study we chose to combine the brown spot data over all 4 years providing a brown spot resistance score (BSR1-4) calculated as 100 − (mean brown spot infection over years 1-4). For height growth we chose to analyze the data collected at age 9 years (HT9). This time point provided ample time for the trees to emerge from the grass stage, yet not so much time that above-ground, tree-to-tree competition would affect individual tree growth. Both traits were analyzed with a linear model using SAS Proc GLM (SAS Institute, Inc. 1990). The full model was

\[ Y = L + D + LxD + B(D) + F(D) + Lx(F(D)) + E, \]

where \( Y \) is the observed data for a tree, \( L \) is location (MS or LA), \( D \) is diallel (1-4), \( B(D) \) is block (1-24) within diallel, \( F(D) \) is full-sib family (1-18, as check lots were not included) within diallel, \( Lx(F(D)) \) is location x family within diallel interaction, and \( E \) is residual error. All factors were considered random, with appropriate tests of significance constructed using Satterthwaite’s approximate F-tests (Proc GLM). Variance components (V) and heritabilities (h^2) were calculated as follows, assuming no non-additive variance (Falconer 1981)

\[ V_{F(D)} = (MS_{F(D)} - MS_{LxF(D)}) / t_2 \]
\[ V_{LxF(D)} = (MS_{LxF(D)} - MSE) / t_1 \]
\[ V_E = MSE, \]

where
\[ MS_i = \text{mean square for the } i^{\text{th}} \text{ variance source from the Proc GLM analysis} \]
\[ t_2 = V_{F(D)} \text{ expected mean squares coefficient provided by Proc GLM, essentially} \]
\[ t_1 = V_{LxF(D)} \text{ expected mean squares coefficient provided by Proc GLM, essentially} \]
\[ V_{\text{additive}} = 2 \times V_{F(D)} \]
\[ V_{\text{phenotypic}} = V_{F(D)} + V_{LxF(D)} + V_E \]
\[ h^2 = V_{\text{additive}} / V_{\text{phenotypic}}. \]
A reduced model, specific to the individual locations, was also used to analyze data from each location separately.

For each trait the residuals from the fitted model were studentized (mean=0, variance=1) and then added together to produce a composite variable for evaluating the trees for selection. This variable provides equal weight for selection on brown spot resistance (BSR1-4) and height growth (HT9). Expected gains from selection for each trait were calculated as

\[ G = (\text{mean of selected trees} - \text{location mean}) \times h^2. \]

Expected gains were calculated on individual location and over-location bases using the respective means and heritabilities (Falconer 1981). For each block, we evaluated within-family selection using various selection intensities with and without restrictions based on relatedness or tree-to-tree spacing. Finally, the data provided an opportunity to track the mean performance of this population in the previous (second), current (third), and next (fourth) generation, thus we calculated those means (observed and expected) using height and brown spot resistance at age 3 years (HT3, BSR3).

**RESULTS AND DISCUSSION**

Trees at the Mississippi location (MS) grew taller and suffered less brown spot disease than those at the Louisiana (LA) location. The check lots performed as expected, with the resistant family showing less brown spot infection in year 3 than the susceptible source at both locations (44% vs. 55% infection at MS and 44% vs. 50% at LA). These results also indicate that both field sites provided a good test of brown spot resistance. Full-sib family differences were highly significant (p ≤ 0.001) for both traits (BSR1-4 and HT9) as were Location*Family interactions, suggesting that families should be selected for deployment based on their performance at the individual locations.

Individual-tree heritabilities for both traits by and over locations are shown in Table 1. The heritabilities for HT9 by locations are more than twice as large as the heritability over locations, again indicating the significance of the Location*Family interaction. Over-locations heritability of BSR1-4 was slightly larger than that for HT9 (0.16 vs. 0.12). The BSR1-4 heritabilities were different between sites, 0.37 at MS vs. 0.29 at LA. These results (age 3 growth and brown spot infection levels and heritabilities at the respective locations and the Location*Family interaction for both traits) were very similar to Synder and Derr’s (1972) results with this population at these two test locations in the previous generation.

Expected gains for each trait from selection on the combined trait index (HT9 + BSR1-4) are shown in Table 2. The observed number of families and the maximum number of trees per family selected at each location are given in Table 3. Selection intensities from 1/18 (6%) to 3/18 (17%) on a per-block basis were considered with and without restriction on tree-to-tree spacing and relatedness. The spacing consideration consisted of not taking an otherwise selected tree if it was within the same row and within two positions (i.e., ≤ 1.92 m or 6 ft) of a previously selected tree. The relatedness consideration eliminated an otherwise selected tree if it shared a parent with a previously selected tree. In all cases selections were made and restrictions were applied within the 18-tree blocks (check lots were not considered), keeping the tree(s) with the largest residual value(s).
Table 1. Individual-tree heritabilities for tree height at age 9 years (HT9) and brown spot resistance over years 1 to 4 (BSR1-4) at the Mississippi and Louisiana field locations and both over locations.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Location</th>
<th>Mississippi</th>
<th>Louisiana</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>HT9</td>
<td></td>
<td>0.31</td>
<td>0.29</td>
<td>0.12</td>
</tr>
<tr>
<td>BSR1-4</td>
<td></td>
<td>0.37</td>
<td>0.29</td>
<td>0.16</td>
</tr>
</tbody>
</table>

Note: Heritability estimates assume no non-additive variance.

Table 2. Expected gains in height growth (HT9: %, cm) and brown spot resistance (BSR1-4: %, points) from selection at the Mississippi and Louisiana field locations and over both locations.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Location</th>
<th>Mississippi</th>
<th>Louisiana</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Unrestricted</td>
<td>Restricted</td>
<td>Unrestricted</td>
</tr>
<tr>
<td>HT9</td>
<td></td>
<td>14.3, 64</td>
<td>--,--</td>
<td>29.2, 89</td>
</tr>
<tr>
<td></td>
<td></td>
<td>13.1, 59</td>
<td>12.7, 57</td>
<td>26.4, 81</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12.8, 58</td>
<td>12.2, 55</td>
<td>25.2, 77</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>BSR1-4</th>
<th></th>
<th>Unrestricted</th>
<th>Restricted</th>
<th>Unrestricted</th>
<th>Restricted</th>
<th>Unrestricted</th>
<th>Restricted</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>9.3, 6.8</td>
<td>--,--</td>
<td>9.4, 6.3</td>
<td>--,--</td>
<td>4.8, 3.4</td>
<td>--,--</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8.9, 6.5</td>
<td>8.5, 6.2</td>
<td>8.9, 5.9</td>
<td>8.0, 5.3</td>
<td>4.5, 3.2</td>
<td>4.2, 3.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8.6, 6.3</td>
<td>8.2, 6.0</td>
<td>8.4, 5.6</td>
<td>7.6, 5.1</td>
<td>4.4, 3.1</td>
<td>4.1, 2.9</td>
</tr>
</tbody>
</table>

Notes: ^a Gains for individual locations are based on the assumption that seeds collected at a location are planted on sites that represent that location. ^b Overall gains are based on the assumption that seeds collected at both locations are planted on sites that represent both locations.

Advancing from 2 to 3 selected trees per block had a similar effect on expected gain as going from unrestricted to restricted selection of 2 trees per block. In either case expected gain reductions were not large. A similar trend is noted for number of families selected and maximum number of trees selected per family. Selecting at least 2 trees per block substantially increases the number of families represented in the selected populations (i.e., seedling seed orchards), but has a relatively small effect on expected genetic gain. Given the current development of the stand to age 18 years, we intend to complete the first thinning leaving 2-3 trees per block. Table 4 provides the operational expected gains based on selecting 3 trees per block with the tree-to-tree spacing and relatedness restrictions employed. These gains apply to planting at both sites while collecting seed from one site or the other. As noted above, additional gains can be made by deploying seedlings to sites that are most similar to the test location where the seed is collected.
Table 3. Number of families represented among selected trees at the Mississippi and Louisiana field locations and the maximum number of trees per family selected.

<table>
<thead>
<tr>
<th>Location</th>
<th>Mississippi</th>
<th>Louisiana</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. trees selected per block</td>
<td>Unrestricted</td>
<td>Restricted</td>
<td>Unrestricted</td>
</tr>
<tr>
<td>1</td>
<td>44, 6</td>
<td>--,--</td>
<td>53, 4</td>
</tr>
<tr>
<td>2</td>
<td>58, 8</td>
<td>64, 7</td>
<td>64, 7</td>
</tr>
<tr>
<td>3</td>
<td>66, 9</td>
<td>66, 10</td>
<td>68, 8</td>
</tr>
</tbody>
</table>

Table 4. Operational expected gains in height growth (HT9: %, cm) and brown spot resistance (BSR1-4: %, points) from selection of 3 trees per block, and the number of families and maximum number of trees per family represented in the selected populations.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Location / Selection Scheme</th>
<th>Mississippi / Restricted</th>
<th>Louisiana / Restricted</th>
</tr>
</thead>
<tbody>
<tr>
<td>HT9</td>
<td>4.7, 21.2</td>
<td>9.1, 27.7</td>
<td></td>
</tr>
<tr>
<td>BSR1-4</td>
<td>3.6, 2.6</td>
<td>4.3, 2.9</td>
<td></td>
</tr>
</tbody>
</table>

Data provided by Snyder and Derr (1972) for the second generation of this population allow us to evaluate the effectiveness of selection and to develop a progression of observed and expected mean performances over generations (Table 5). The first-generation parents were for the most part selected in the natural forests of southeastern Louisiana (Lott et al. 2001). Open-pollinated progeny of these selections were field tested at Saucier, MS and Alexandria, LA and they represent the second generation (Study 3.10). Selections made in these tests and mated in the diallel tests described here (Study 3.45 Part A1) produced the third generation that was also evaluated at Saucier and Alexandria. Projecting the fourth generation’s performance (output of the new seedling seed orchards) on representative sites using our expected gain calculations suggests that early height growth and brown spot resistance (both through year 3) will be tripled (HT3 = 40 cm vs. 12 cm) and doubled (BSR3 = 73% vs. 37%), respectively, compared to the observed performance in the second generation.
Table 5. Four generations of improvement in the southeast Louisiana longleaf pine population: Observed generation means (age 3) for generations 2 and 3 at Saucier, Mississippi, and Alexandria, Louisiana, and the expected means at both locations for generation 4.

<table>
<thead>
<tr>
<th>Location</th>
<th>Trait</th>
<th>Generation</th>
<th>Observed 2nd</th>
<th>Observed 3rd</th>
<th>Expected 4th</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mississippi</td>
<td>HT3 (cm)</td>
<td>Observed</td>
<td>16</td>
<td>40</td>
<td>47</td>
</tr>
<tr>
<td></td>
<td>BSR3 (% needles non-symptomatic)</td>
<td>Observed</td>
<td>29</td>
<td>70</td>
<td>76</td>
</tr>
<tr>
<td>Louisiana</td>
<td>HT3 (cm)</td>
<td>Observed</td>
<td>8</td>
<td>26</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>BSR3 (% needles non-symptomatic)</td>
<td>Observed</td>
<td>45</td>
<td>64</td>
<td>70</td>
</tr>
</tbody>
</table>

CONCLUSION

For seedling seed orchard development, we decided to emphasize genetic diversity over gain, and thus used within-family, within-block selection such that no families were purposely culled based on low mean performance. Two to three trees were selected per test block. The first tree had the highest performance (brown spot resistance and height growth) relative to its expected family mean. The second and third trees were the next highest, unrelated to the first tree, and separated by at least two within-row planting positions.

Most (66 of 72) of the full-sib families were represented in the selected population at each location, with an average of about 4 or 5 trees per family. Heritabilities (individual tree basis, across locations) for the two traits were 16% for brown spot resistance (over years 1 to 4) and 12% for height at age 9 years. Operationally, the expected genetic gains for resistance are 3.6% and 4.3% and for height are 4.7% and 9.1% at the Mississippi and Louisiana locations, respectively. The expected levels of brown spot resistance in the next generation exceed 70% (i.e., less than 30% symptomatic leaf area), which is nearly a doubling of resistance over the initial tested generation.

ACKNOWLEDGMENTS

We thank A. G. Kais for his work with Snyder is establishing Study 3.45 and Jim Hamaker for his efforts in producing the plant materials. We also thank J. H. Roberds and M. Stine for useful discussion and comments. Added post-SFTIC, while in review: In honor of those affected by Katrina and Rita we dedicate our efforts and the new seed orchards to restoring the great longleaf pine forests of southern Mississippi and Louisiana.

LITERATURE CITED


Genetic Variation in Young Fraser Fir Progeny Tests

J.L. Emerson¹, J.L. Frampton², and S.E. McKeand³

Fraser fir (Abies fraseri [Pursh] Poir.) is found naturally in only a few stands at high elevations in North Carolina, Virginia, and Tennessee. These forests are associated with important scenic and recreational areas such as the Great Smoky Mountains National Park, Mount Mitchell State Park, the Balsam Mountains, and the Mount Rogers National Recreation Area (Dull et al. 1988). In addition to its recreational and ecological importance, Fraser fir also holds great economic importance as a Christmas tree species. It is grown in plantations as Christmas trees throughout the southern Appalachians as well as other areas of the United States.

Although Fraser fir Christmas tree production is an important industry in the state of North Carolina, there has not been a great deal of previous research on breeding and selection to increase the quality of production. Only one previous progeny test series has been completed with Fraser fir, and it had a limited sampling of seed sources tested at only three sites. A more thorough testing of families from throughout the natural range of Fraser fir was needed. In 1994, a more extensive seed collection was performed, where seeds from over 500 parent trees were collected from the six main populations of Fraser fir (McKeand et al. 1995). Seedlings were grown from the seed collected in 1994 to establish this progeny test series at eight sites in 2000. The objectives of this study were to determine genetic variation among six seed sources of Fraser fir and to estimate genetic parameters for traits important in Christmas tree production. In addition, genetic variation of spring frost damage to the terminal leader and lateral branches in May of 2002, the third year in the field, and how it related to bud flush dates in the nursery as well as parent elevation were studied.

MATERIALS AND METHODS

Seedlings were grown from open-pollinated seed collected during the 1994 range-wide cone collection. Progeny from 188 of these trees were included in this study, and they were from the following six seed sources: Balsam Mountains, Grandfather Mountain, the Great Smoky Mountains, the Black Mountains, Mount Rogers, and Roan Mountain.

In the spring of 2000, seedlings were transplanted from the nursery beds to field trials that were established at eight sites in the mountains of western North Carolina. The families from each seed source were divided equally into two groups, with each group of families being tested at four of the eight sites. A randomized complete block design was used at each site, with 35 replications of single-tree-plots per family. The progeny test sites were located on working Christmas tree plantations and were each managed individually by the landowners according to their standard practices.

¹ Graduate Student, Department of Forestry, N.C. State University, Raleigh, NC, USA
² Associate Professor, Department of Forestry, N.C. State University, Raleigh, NC, USA
³ Professor, Department of Forestry, N.C. State University, Raleigh, NC, USA
The following traits were measured each year for the first four seasons in the field: height, number of leaders, overall tree quality, number of lateral buds, number of whorl buds, number of lateral branches, and number of whorl branches. The quality measurement was an overall subjective rating of the quality of the individual as a Christmas tree, integrating tree density, shape, and overall symmetry. This rating was given on a scale of 1 to 5, with 1 being poor, 3 being average, and 5 being excellent Christmas tree quality. The bud and branch measurements were taken only on a sub-sample, one-third of the families at 4 of the sites. In 2002, the third year in the field, individual trees were rated in the field on the severity of the damage both to the terminal leader and to the lateral branch from a late spring frost. The terminal leader was scored from 0 to 2, with 0 being no damage, and 1 being some damage, where the terminal was elongating when the frost hit but only a portion was killed. A score of 2 was where the entire terminal was killed and a lateral branch became a new leader. The lateral branches were scored from 0 to 3, with 0 being no damage. A score of 1 was for light damage, with approximately 15% or fewer of the lateral branches being damaged. Two was a medium level of lateral branch damage, with approximately 15% to 85% damage, and three was for heavy damage, with over 85% of the lateral branches affected.

Individual-tree within population heritabilities were calculated for all traits in year 4, for height each year in the field, and for frost damage measurements and bud flush dates. Shukla’s (1972) stability variance ($\sigma^2_i$) was calculated for each source to identify those sources showing instability across the eight sites for height growth after four years. Family mean correlations were calculated for all traits in year 4. Family mean correlations were also calculated for the terminal and lateral frost damage, parent elevation, bud flush dates in the nursery for the terminal and lateral buds, and height in the nursery and each year in the field for all sites.

**RESULTS AND DISCUSSION**

Highly significant differences were found among seed sources and families within sources ($p \leq 0.0001$) for height, number of lateral buds, and quality. The seed source mean heights for year four ranged from a high of 114 cm for the Balsam Mountains to a low of 99 cm for Roan Mountain. Large ranges in family means within sources also existed; the family means for year four height within the Balsam Mountains source ranged from 79 cm to 135 cm. The sources showing instability for height across the test sites were the Balsam Mountains and Roan Mountain. Due to the fact that the Balsam Mountains still ranked first for height at seven out of the eight sites, and that Roan Mountain ranked lowest for mean height at five out of the eight sites and was not significantly greater than the lowest source at the other three sites, this instability is probably not of much practical importance. The individual tree within population heritability value for height in year four was the highest of all the traits measured, at 0.44. The heritability values for height varied greatly among the six sources, from 0.15 for the Black Mountains to 0.67 for the Great Smoky Mountains. The high heritability values for height show promise for improving Fraser fir for Christmas tree production via breeding because of the importance of height in determining Christmas tree value. The highly significant family mean correlations seen for height, number of lateral buds, and quality in year four indicate that if selections are made based on height, it will also indirectly select for these other traits.
Significant differences among sources and families within sources were seen for lateral branch frost damage. The heritability for terminal frost damage was only 0.045, but the heritability for lateral frost damage was higher at 0.14. Greater terminal and lateral frost damage were significantly associated with greater height for all years. Parent elevation was negatively associated with progeny height, and higher parent elevation was associated with later lateral bud flush dates in the nursery. Less lateral branch frost damage during the third year in the field was also associated with later terminal and lateral bud flush dates of the same progeny in the nursery. Although taller individual trees appear to be less tolerant of a late spring frost, many of the same families were ranked in the top 25 for height after four years in the field when only those individual trees with no frost damage were included compared to all individuals. Family mean height in years prior to the frost is also highly correlated with family mean height in years after the frost damage occurred. Therefore, it appears that the fast growing families can quickly make up for any loss of height from a late spring frost.

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Using Biotechnology to Help Restore the American Chestnut

S.A. Merkle and G.M. Andrade

Abstract: American chestnut (Castanea dentata) once dominated the Appalachian forests of the Eastern United States, where it was a major timber and nut-producing tree. Beginning in the early 1900s, the tree was devastated by the chestnut blight fungus (Cryphonectria parasitica), which was accidentally introduced from Asia. 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. Our research over the past three years has overcome a number of bottlenecks in somatic embryo production, maturation, germination and conversion. We tested the effects of culture regime (semi-solid versus liquid), cold treatment, activated charcoal and somatic embryo morphology (i.e. cotyledon number) on germination and conversion of the somatic embryos. Cold treatment for 12 weeks was critical for conversion of chestnut somatic embryos to somatic seedlings, raising conversion frequencies for one line to 47%, compared to 7% with no cold treatment. Activated charcoal improved germination and conversion frequency for one line to 77% and 59%, respectively, and kept roots from darkening. For two lines that produced embryos with one, two or three-plus cotyledons, cotyledon number did not affect germination or conversion frequency. We also established embryogenic American chestnut suspension cultures and adapted a fractionation/plating system that allowed us to produce populations of relatively synchronous somatic embryos for multiple lines. Embryos derived from suspension cultures of two lines tested had higher conversion frequencies than those from cultures maintained on semi-solid medium. The improvements in manipulation of American chestnut embryogenic cultures described in this study have allowed over a 100-fold increase in somatic seedling production efficiency.

Keywords: American chestnut, Castanea dentata, somatic embryogenesis, germination, conversion, chestnut blight

American chestnut (Castanea dentata) was once the dominant forest species of the Appalachian Mountains in the eastern United States, with a range that extended from New England to Alabama. At the beginning of the 20th century, American chestnut contributed substantially to many local economies. The highly decay-resistant wood had many uses, including lumber for construction, shingles, furniture and fuel. High in tannin, chestnut was used for telephone poles, mine props, fences and in the leather industry. An annual crop of nuts made chestnut a major source of nutrition for man and wildlife. In the early 1900s, the species was almost eliminated as a forest tree due to the accidental introduction of the fungus Cryphonectria parasitica, which became known as the chestnut blight fungus. The blight is estimated to have killed around 3.5 billion trees (Roane et al. 1986). Since root systems are not infected by the fungus, American chestnut has escaped extinction because of its ability to sprout profusely and repeatedly from
stumps and roots. Today, most American chestnut trees are found as shrubs or small trees in the forest. 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 to develop transgenic trees that would be resistant to chestnut blight fungus.

MATERIALS AND METHODS

Culture initiation and maintenance and somatic embryo production

Immature chestnut burs, collected during summer 2001 and 2002, were provided by The American Chestnut Foundation (TACF) and the American Chestnut Cooperators Foundation (ACCF). Immature, open-pollinated seeds were used as explants. Using previously published protocols (Merkle et al. 1991, Carraway and Merkle 1997), embryogenic cultures were initiated by culturing the immature seeds on Induction/Maintenance Medium (IMM), which was a modified Woody Plant Medium (WPM; Lloyd and McCown 1980) supplemented with 0.5 g/l L-glutamine and 2 mg/l 2,4-D. After 3 months, clusters of repetitive embryos or proembryogenic masses (PEMs) were obtained. For continued culture proliferation clusters of repetitive embryos or PEMs were either transferred to fresh IMM every three weeks. After three to four weeks, 2-4 mm long embryos were selected using a dissecting microscope and transferred to 100 x 15 mm plastic Petri plates of semi-solid Embryo Development Medium (EDM), which was WPM lacking plant growth regulators. Twenty – 25 embryos were cultured per plate in a grid pattern with approximately 0.5 cm spacing. Embryos, which were oriented horizontally on the medium, were allowed to grow for 1 week prior to being subjected to the experimental treatments described below. All cultures were incubated in the dark at approximately 25°C.

Germination and conversion experiments

The cold treatment experiment used three lines. Embryos in this experiment were derived from PEMs that had been cultured on semi-solid IMM. Plates of embryos were divided into 3 groups to be given 0, 6 or 12 weeks of cold treatment. For the 0 weeks cold treatment, embryos from the first set of plates were transferred to GA-7 vessels (Magenta Corp.) containing 80 ml of semi-solid GM, which was the same as EDM, but lacking L-glutamine. Embryos were oriented with their radicle ends inserted into the gelled medium. GA-7s were placed in an incubator under cool white fluorescent light (100 μmol·m⁻²·s⁻¹) with a 16 h photoperiod at 23°C. The remaining plates were wrapped in aluminum foil and placed in a dark refrigerator at 4°C. The second and third sets of plates were removed from the cold after 6 and 12 weeks, respectively, and embryos were transferred to GA7s and placed in the incubator under the same conditions described above. The numbers of germinated (i.e. showing radicle elongation) and converted (producing both roots and shoots) embryos were scored after six weeks in the incubator. The experiment was conducted twice with the same lines and run again with another line six months following the first experiment, in the last case using embryos derived from PEMs collected from size-fractionated suspension cultures.

The AC experiment used 4 lines and embryos were produced using the same protocol described for the first cold treatment experiment. However, in this case, harvested embryos were
transferred to Petri plates containing semi-solid EDM with or without 5 g/l activated charcoal (EM Industries) and allowed to grow for one week prior to storage in the refrigerator for 12 weeks at 4° C. Then, they were transferred to GA-7s containing 80 ml GM with or without 5 g/l AC (depending on whether they were matured on EDM with our without AC) and placed in the same incubator under the same conditions used for the cold treatment experiment. Germination and conversion frequencies were scored after six weeks. The entire experiment was not repeated in time with the same lines, but was repeated with another line three additional times, beginning six months later, with the additional treatment of 1 g/l AC. Embryos in these later replications were derived from size-fractionated suspension cultures.

In separate experiments from those described above, we compared the germination and conversion performances of somatic embryos produced from cultures maintained on gelled medium to those of embryos derived from size-fractionated suspension cultures of the same embryogenic lines. The first experiment used embryos from two lines, while the second experiment, conducted six months later, used embryos from an additional line. In both experiments, embryos were selected from populations derived either from PEMs grown on semi-solid medium or from size-fractionated and plated suspension cultures, and allowed to grow for one week. Then, plates were stored at 4° C for 12 weeks, after which embryos were transferred to GA-7s containing GM with 5 g/l AC and placed in the incubator. Six weeks later, numbers of germinated and converted embryos were scored.

We noticed that most of our lines produced embryos with varying morphology, most notably variation in cotyledon number. Since the embryos with either more or less than two cotyledons appeared otherwise normal, we speculated that these embryos might be capable of producing normal plantlets. Two lines that produced approximately equal proportions of embryos with one, two or three-plus cotyledons were chosen for an experiment to test the effect of this aspect of embryo morphology on germination and conversion. The experiment was conducted twice with one line and once with the other, using embryos that developed from size-fractionated and plated suspension cultures. Embryos were stored at 4° C for 12 weeks, transferred to GA-7s containing GM with 5 g/l AC and placed in the incubator. Six weeks later, numbers of converted embryos were scored.

RESULTS AND DISCUSSION

Cold treatment

Both line and cold treatment affected germination frequency (P < 0.001 and P < 0.007, respectively), although only cold treatment affected conversion frequency (P < 0.001). While some embryos germinated with no cold treatment, none of these completed the conversion process. Six weeks of cold failed to significantly improve germination or conversion frequency over no cold treatment, but 12 weeks of cold improved both, increasing average germination for the two lines from 34% to 51% and average conversion for these two lines from 0% to 12%. When the experiment was repeated with another line with embryos derived from PEMs collected from size-fractionated suspension cultures, cold treatment significantly (P < 0.0014) affected only conversion frequency, and again, only 12 weeks of cold increased it over the no cold treatment, in this case from 7% to 47%. We intend to test if even longer periods of cold treatment will further increase conversion frequency.
Activated charcoal

Since our chestnut cultures must be continuously cultured in medium with 2,4-D to maintain repetitive embryogenesis, we thought that AC might aid normal embryo development and maturation by adsorbing residual 2,4-D from them. In our first experiment with embryos of four culture lines produced on semi-solid medium, chi-square analysis indicated that the addition of 5 g/l AC did not significantly improve either germination frequency or conversion frequency. However, this result may be due to the fact that while AC improved germination and conversion for some clones, others actually had lower germination and conversion on medium with AC. When the experiment was repeated three times with somatic embryos derived from suspension cultures of another line in medium supplemented with 0, 1 or 5 g/l AC, both germination frequency and conversion frequency were improved by the addition of activated charcoal (P < 0.001). Although 1% AC gave the highest germination (77%) and conversion (59%) frequencies, these were not statistically higher than for the 5 g/l AC treatment. The different results with regard to the impact of AC on germination and conversion of embryos from the first experiment with four related lines and the second set of experiments with one line unrelated to the first set may be attributable to genotypic differences. However, it is also possible that embryos from the second set of experiments benefited more from the AC due to the fact that they were derived from suspension cultures, in which the PEMs were bathed continuously in 2,4-D-supplemented medium, rather than sitting on top of gelled medium with 2,4-D.

One qualitative difference we noted in all the AC experiments was that roots of somatic seedlings in the AC medium remained white, while those in medium lacking AC darkened during the 6 weeks the somatic seedlings remained in the GA-7s. Thus, it is possible that one or more of the potential benefits of AC noted by Pan and van Staden (1998), the establishment of a darkened environment or adsorption of undesirable substances, played a role in keeping the roots from browning. In addition, somatic seedlings growing in medium with AC appeared to have a greater tendency to generate branching root systems than those in medium without AC, although this variable was not measured. Given these differences and that our standard embryo production procedure currently uses suspension cultures, we now routinely germinate chestnut somatic embryos on medium with 5 g/l AC.

Culture regime

Embryos derived from suspension cultures converted at a higher frequency than those derived from cultures maintained on semi-solid medium for two of the three tested lines. The first experiment comparing germination and conversion of embryos derived from semi-solid versus liquid medium used two lines. Chi-square analysis of combined data from these two lines indicated that culture regime failed to affect conversion frequency (P<0.09). However, when the two clones were analyzed separately, chi-square results indicated that culture regime improved conversion for somatic embryos of one line, but had no significant effect on embryos from the other line (P<0.08). In the second experiment, which tested a single line, embryos derived from suspension cultures converted at a significantly (P<0.001) higher rate than those derived from material cultured on semi-solid medium. Somatic embryos arising from the size-fractionated suspensions tended to be singularized or in loose clusters compared to the fused clusters of embryos produced on semi-solid medium. Thus, there was probably less damage, particularly to
the radicles, when individual embryos from the liquid suspension were harvested for germination. While the superiority of suspension culture-derived embryos did not hold for every line in these two experiments, we have adopted size-fractionation and plating of suspension cultures as our standard method of embryo production for most of our embryogenic American chestnut lines.

Embryo morphology

Malformed somatic embryos are often reported in somatic embryogenesis studies. No doubt these are often discarded as being unlikely to produce viable plants. We noted high percentages of embryos with single cotyledons or more than two cotyledons in our cultures, but these embryos appeared similar in other respects to those with two cotyledons, with a well-defined shoot apical meristem and radicle. Therefore, we divided populations of embryos from two lines into those with one, two, or three or more cotyledons, put them through our standard germination treatment (12 weeks of cold, culture on EDM with 5 g/l AC) and measured conversion. Overall, the three cotyledon classes did not differ in their conversion frequencies (P<0.06), although embryos from on line with a single cotyledon appeared to have a lower conversion than the other two classes. Based on these results, we feel that we can safely select embryos with two or more cotyledons for somatic seedling production, and in for some clones, even embryos with only a single cotyledon will produce plantlets.

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LITERATURE CITED


Total Inside-Bark Volume Estimation for Loblolly Pine (*Pinus taeda* L.) in Genetic Trials.

J.R. Sherrill, T.J. Mullin, B.P. Bullock, S.E. McKeand, R.C. Purnell, and M.L. Gumpertz

Many tree breeding programs select genotypes based on volume more than any other trait. These programs commonly estimate volume using a combined-variable equation that uses diameter at breast height (D) squared multiplied by total tree height (H) as a regressor on inside-bark volume. The North Carolina State University–Industry Cooperative Tree Improvement Program (TIP) has a long history using the Warner and Goebel (1963) combined-variable equation (Warner-Goebel equation). The Warner-Goebel equation was derived from 74 loblolly pine trees from the upper South Carolina Piedmont with least-squares regression. Combined-variable equations have been effective in fitting prediction equations for total inside-bark volume to small data sets (Spurr 1952). However, differences in taper and bark thickness among individuals or families are not accounted for with a combined-variable equation. The objective of this abstract is to evaluate selection with the Warner-Goebel equation.

**METHODS AND MATERIALS**

A genotype by cultural treatment study at Bainbridge, GA was measured in the 13th growing season. This study was a two by two factorial of weed control and fertilizer treatments in a split-plot design. For the main cultural treatment plots, the control had no herbicide applications until age five and no fertilizer treatments. The herbicide treatment consisted of early woody and herbaceous competition control and all treatments were aerially released at age five. The fertilization treatment was ground applied five times up until age 9.

There were 25 open-pollinated first- and second-generation families arranged as individual-tree subplots. Each complete block was replicated 5 times. Some known relationships existed between mothers and were acknowledged in the estimation of genetic parameters. Approximately 40 individuals from each of 25 families were sampled, 10 from each treatment. Two trees from each family were sampled in most replication/treatment plots.

Sectional data were collected along the stem every 1.2 m to a 7.6-cm top. Inside-bark diameters were used in Smalian’s log volume equation (Avery and Burkhart 2002) to find the inside-bark volume of each stem section. In addition to diameters, total height and height to live crown were measured to calculate crown ratio as a percentage of the total height.

Volumes estimated from the Warner-Goebel equation were compared to those determined by stem analysis of the felled trees. The metric form of the Warner-Goebel (1963) equation was

\[ \hat{V}_{ib} = 0.954560899 + 1.092111257 \left( \frac{D^2H}{10} \right) \]

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1 Graduate student, Research professor, Assistant professor, and Professor, respectively, Department of Forestry and Environmental Resources, NC State University, Campus Box 8002, Raleigh, NC 27695-8002, USA. Manager, Genetics and Tree Improvement, International Paper Co., 1201 W. Lathrop Ave., Savannah, GA 31402, USA. Professor, Department of Statistics, NC State University, Campus Box 8203, Raleigh, NC 27695-8203, USA.
where $\hat{V}_{ib}$ is the estimated total inside-bark volume in dm$^3$ and $D^2H$ is the combined variable in cm$^2$ x m. Selection efficiency was calculated using methods from Falconer and MacKay (1996). The correlated response was

$$CR_y = i h_x h_y r_{xy} \sigma_y$$

where $CR_y$ is the correlated response of indirect selection for trait $y$, $i$ is the selection intensity, $h_x$ is the heritability of trait $x$, $h_y$ is the heritability of trait $y$, $r_{xy}$ is the genetic correlation between traits $x$ and $y$, and $\sigma_y$ is the phenotypic standard deviation of trait $y$.

The response to direct selection on trait $y$ was:

$$R_y = i h_y^2 \sigma_y$$

where $R_y$ is the response from direct selection and $h_y^2$ is the heritability of trait $y$. Selection efficiency was calculated as:

$$S = \frac{CR_y}{R_y}$$

**RESULTS AND DISCUSSION**

The family-mean heritabilities of the measured volume from stem section data and the estimated volume using the Warner-Goebel equation were 0.69 and 0.72, respectively. The standard errors for these two values were 0.10 and 0.09, respectively. The genetic correlation between these two traits was high (0.99 with a standard error of 0.0056). Consequently, the selection efficiency for selection of estimated total inside-bark volume to make gains in measured inside-bark volume was 1.01. Comparison of the total inside-bark volume estimates from the Warner-Goebel equation and from the destructive sample can be seen in Figure 1. The Warner-Goebel equation generally over-estimated the total inside-bark volumes measured in the field. However, this did not affect selection.

![Figure 1. Comparison of prediction lines for estimated volume from the Warner-Goebel equation and measured inside-bark volume.](image-url)
Combined-variable equations are commonly used to estimate volume in many research applications. For TIP trials, individual stems are generally compared using the Warner-Goebel total inside-bark volume prediction equation. Selection for true inside-bark volume by estimating volume with this equation has been an effective practice based on selection efficiency. In fact, family selection for volume estimated by prediction equations was slightly more favorable than by destructively sampling. This high selection efficiency may have resulted from less error variance being associated with estimated volume than with the detailed volume measurement. The measured volume may have had more error variance due to variation in stem taper, bark thickness, environmental variation, and unknown factors. Nonetheless, D and H measurements capture a great deal of the genetic variation in total inside-bark volume. Differences in volume not accounted for by estimating volume with the combined-variable equation are probably small enough for indirect selection to be effective. It is recommended to continue using a combined-variable equation for selection of superior loblolly pine genotypes in the Southeastern US.

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REFERENCES


Selfing Results in Inbreeding Depression of Growth but not of Gas Exchange

Kurt Johnsen¹, John E. Major², and Chris Maier¹, Debby Barsi²

In most tree species, inbreeding greatly reduces seed production, seed viability, survival, and growth. In a previous large-scale quantitative analysis of a black spruce (Picea mariana (Mill.) B.S.P.) diallel experiment, selfing had large deleterious effects on growth but no impact on stable carbon (C) isotope discrimination (an indirect measure of the ratio of net photosynthesis (A) to stomatal conductance (g_w)). It was hypothesized that selfing did not impact carbon gain via leaf level gas exchange but it did impair subsequent utilization of C. Alternatively, both A and g_w may each have been impacted by selfing to the same extent. However, no gas exchange data was ever collected to further test these hypotheses. Here we present photosynthetic gas exchange data collected from three selfed families and three outcrossed families (all the result of controlled pollination) from the same diallel experiment. Photosynthetic responses to intercellular CO₂ concentration (A/C_i curves) were generated on four replicates per family, one block per day, over a four-day period in July. Results indicate no differences between selfed and outcrossed families in maximum carboxylation rate, maximum electron transport, (A) and g_w (both estimated at 370 ppm CO₂ concentration), or the ratio A:g_w. Selfed trees had higher mortality during the experiment thus it is possible that there were potential negative impacts on gas exchange of previously living selfed progeny. However, we clearly show that inbreeding can result in trees that have low productivity despite retaining high levels of leaf level A. Results are consistent with the hypothesis that gas exchange was similar between selfed and outcrossed progeny trees, thus subsequent utilization of C in selfed progeny must have been modified.

Keywords: carbon isotope discrimination, inbreeding, photosynthesis, Picea mariana, stomatal conductance

¹ USDA Forest Service, Southern Research Station, 3041 Cornwallis Road, RTP, NC, 27709, USA
² Natural Resources Canada, Canadian Forest Service, Atlantic Forestry Centre, Fredericton, NB, Canada E3B 5P7
Is the cad-\textit{n1} Allele Associated with Increased Wood Density or Growth in Full-Sib Families of Loblolly Pine?

Q. Yu\textsuperscript{1}, B. Li\textsuperscript{2}, C.D. Nelson\textsuperscript{3}, S.E. McKeand\textsuperscript{4}, and T.J. Mullin\textsuperscript{5*}

A rare mutant allele (cad-\textit{n1}) of the cad gene in loblolly pine (\textit{Pinus taeda} L.) causes a deficiency in the production of cinnamyl alcohol dehydrogenase (CAD). Effects associated with this null allele were examined by comparing wood density and growth of 15-year-old cad-\textit{n1} heterozygotes with their wild-type full-siblings, established in three test series (1, 2 and 3) in two states (South Carolina and Georgia). In each series, cad-\textit{n1} heterozygous selections (A, B and/or C) were crossed with five unrelated wild-type parents, to produce five full-sib families. Series 1 included five crosses each for selections A and B, and series 2 had five crosses with selection C. Five additional crosses for selection A were established in series 3. Each series was established in replicated trials at two different field-test sites, and all tests included a common, unimproved commercial checklot. Test progenies from each cross and at each site were genotyped at the cad locus, and assessed for growth and wood density traits. In all, 839 trees were sampled.

We found evidence of large effects on growth and wood density, associated with the cad-\textit{n1} allele. When all three test series were considered in a combined analysis, cad-\textit{n1} heterozygotes were found to have 5% (\(p = 0.11\)) greater volume than their wild-type full-sibs. However, the phenotypic effects on wood density or growth are not likely due to the cad-\textit{n1} allele alone, and other loci are probably involved. In series 3, selection A cad-\textit{n1} heterozygotes averaged 17% (\(p = 0.07\)) greater volume than their wild type full-sibs, whereas in series 1, they were only 3% greater in volume. This may be due to either different genetic backgrounds between series 1 and 3, or different growing environments. The 17% volume and 3.4% (\(p = 0.04\)) wood density increases in series 3 for cad-\textit{n1} heterozygotes were mainly due to selection A being crossed with two particular second parents. It appears that there is an epistatic effect, in that these two second parents contributed certain genetic components that specifically interacted with cad-\textit{n1} to produce the large positive effect. While substantial gains are possible through deployment of relatives carrying the cad-\textit{n1} allele, these gains are family-specific and must be verified for each cross through field testing.

\textsuperscript{1}Research Associate, \textsuperscript{2}Research Professor, \textsuperscript{3}Research Geneticist and Project Leader, \textsuperscript{4}Professor, \textsuperscript{5}Research Professor, Department of Forestry & Environmental Sciences, North Carolina State University, Raleigh, NC, \textsuperscript{3}Research Geneticist and Project Leader, Southern Institute of Forest Genetics, USDA Forest Service, Saucier, MS

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Cherrybark oak (*Quercus pagoda* Raf.) is an important and highly valued southern hardwood timber species. Previous studies have shown that growth differences do exist among provenances (Greene et al. 1991). Yet this study did not identify any geographic trend in seed source productivity. Substantial variation among families within provenances was also observed by Greene et al. (1991) leading to the recommendation that provenance considerations as well as individual family considerations must be made when making selections.

The objective of this study was to identify growth differences among various seed sources. Determination of potential genetic gains through selection was conducted through estimation of heritability and calculation of genetic gain. Finally, the ability to select for volume at age 15 from juvenile measurements was evaluated through age-age correlations.

**METHODS**

A cherrybark oak provenance-progeny test was established in 1987 on MeadWestvaco property located in Carlisle Co., Kentucky. The test site is on a loess bluff just south of the confluence of the Mississippi and Ohio Rivers. The silt-loam site is composed of the Grenada and Memphis-Loring series. The planting site was previously in agriculture production until 1983, but had been fallow until the spring of 1987. Site preparation included disk ing and sub-soiling prior to planting.

The experimental design was a split block design, eight provenances per block forming the main plots and three to five families per provenance. A total of 37 half-sib families represented the eight provenances, with each family arranged in a five-tree-row sub-plot and planted at a nine-by-nine-foot spacing. Provenances included: Bienville Parish, LA (LA1), Washington Parish, LA (LA2), Okitibbeha Co., MS (MS1), Warren Co., MS (MS2), Washington Co., MS (MS3), Fayette Co., TN (TN1), Lauderdale Co., Haywood Co, Fayette Co., Weakly Co., TN (TN2), and Southampton Co. VA (VA1). Height measurements were taken at ages one, three, five, ten, and fifteen. DBH measurements were taken at ages five, ten, and fifteen. Volume (cubic feet) was calculated using the volume equation: volume = \[0.00007854 \times DBH \text{ (cm)}^2 \times \text{height (m)}\].

Overall differences between provenances and families within provenances were tested using the ANOVA and Duncan’s New Multiple Range Test. Both diameter and height were analyzed at each age of measurement. Estimates of narrow-sense individual tree (\(h^2\)), family (\(h^2_f\)), and within-family heritabilities (\(h^2_w\)) and genotypic and phenotypic correlations were calculated from the estimated variance and covariance components (Becker 1975). The indirect gains estimated by the correlation response (CR) were derived from Falconer and Mackay (1996).

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1 Mississippi State University, Department of Forestry, Starkville, Mississippi, USA.
2 MeadWestvaco, Central Forest Research Center, Wickliffe, Kentucky, USA.
3 Louisiana Tech University, School of Forestry, Ruston, Louisiana, USA.
RESULTS AND DISCUSSION

Early-age survival was generally good in this study for all provenances. Survival percentages remained above 90 percent for all provenances from ages one to ten. At age 10, the Bienville Parish, Louisiana, provenance exhibited the best survival at 97.3 percent, while Okitibbeha Co., Mississippi provenance was the lowest at 92 percent. The high survival rates among all provenances can be attributed to proper site selection, intense grading of the seedlings prior to planting, and the maintenance of the site following planting. Between ages 10 and 15, survival was shown to decrease rather sharply, most certainly related to spacing as the 2.7-by-2.7-meter spacing is creating intense tree-to-tree competition.

Provenance differences did exist for height and diameter at each measurement. Duncan’s new multiple range test showed that differences between the individual provenances were small and in many cases not significant. The two best performing provenances for height and diameter at age 15 were from counties Washington and Warren in Mississippi. While these two superior provenances as well others changed little in rank over time, the provenance from Washington Parish, Louisiana, moved to a higher rank for both height and diameter. Significant differences also occurred among families within provenances for both height and diameter at all ages highlighting the need for family considerations when selecting within a seed source.

Variation among families allows for potential gains to be made through selection within provenances. Family heritabilities ranged from 0.50 to 0.70 for height and 0.55 to 0.70 for diameter. This was much greater than the individual tree heritabilities which ranged from 0.17 to 0.28 for height and 0.20 to 0.36 for diameter. All height heritabilities were high at age one, dropped at age three, and had a modest increase over time until age 15 where there was a slight decrease. Excluding age-one estimates, heritability tended to peak at age ten for all traits and then remain steady or drop by age 15. Overall, dbh heritability was the highest among the three traits. Volume family-heritability ranged from 0.40 to 0.65 which is comparable to height.

Family selection yielded more gains per unit time than combined selection when using height at any age, while selection based on diameter or volume favored a combined selection (Table 1). All traits showed promise at various ages for selection purposes. Combined selection based on dbh, specifically at age-ten, will allow the most gain per unit time to be made. Genetic correlations with volume at age 15 ranged from 0.74 to approximately 1.0. Both age-five dbh and age-ten volume had correlations that were approximately one. However, genetic gains per unit time were highest using selection of DBH at age-ten.
Table 1. Age-age correlated response and genetic gains per unit time with age 15 volume from indirect selection in the 1987 Cherrybark oak provenance test in Carlisle Co., Kentucky.

<table>
<thead>
<tr>
<th>Selected Trait(a)</th>
<th>Correlated Response with Age 15 Volume</th>
<th>Genetic Gain Per Unit of Time(b)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Family Selection</td>
<td>Combined Selection</td>
</tr>
<tr>
<td></td>
<td>(m(^3))</td>
<td>(%)(^d)</td>
</tr>
<tr>
<td>Age 1 Height</td>
<td>0.193</td>
<td>6.0</td>
</tr>
<tr>
<td>Age 3 Height</td>
<td>0.027</td>
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</tr>
<tr>
<td>Age 5 Height</td>
<td>0.027</td>
<td>8.4</td>
</tr>
<tr>
<td>Age 10 Height</td>
<td>0.027</td>
<td>8.4</td>
</tr>
<tr>
<td>Age 5 DBH</td>
<td>0.016</td>
<td>4.9</td>
</tr>
<tr>
<td>Age 10 DBH</td>
<td>0.037</td>
<td>11.6</td>
</tr>
<tr>
<td>Age 5 Volume</td>
<td>0.015</td>
<td>4.7</td>
</tr>
<tr>
<td>Age 10 Volume</td>
<td>0.037</td>
<td>11.6</td>
</tr>
</tbody>
</table>

\(a\) Selection intensity for family selection was 2/37 families (i=2.023) and for combined selection was 5/37 families (i=1.8175) and 10/26 individuals within families (i=0.993). These values were chosen so that a similar number of trees were selected by each method.

\(b\) Unit time is defined as selection age plus 10 years for family selection and 15 years for combined selection.

\(c\) Percent response-correlated response (m\(^3\))/average age 15 volume (0.32 m\(^3\)).

\(d\) Percent gain per unit of time-percent response/unit time

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REFERENCES


Genetic Variation in Basal Area Increment Phenology and its Correlation with Growth Rate in Loblolly and Slash Pine Families and Clones

V. I. Emhart, T.A. Martin, T.L. White, and D.A. Huber

Stand development is the result of complex interactions between genotypes and the environment in which they grow. Research into loblolly and slash pine plantations has shown that diameter growth rates differ between species, and among families within species when planted under common plantation conditions (Jackson 1952, Harkin 1962, Langdon 1963, McCrady and Jokela 1996, Jayawickrama et al. 1998). The time of the year during which the cambium is active varies with climate, species, crown class, seasonal development of leaf area in trees, and different parts of stems and branches (Kozlowski and Pallardy 1997). Throughout the entire life span of a tree, cambial growth is sensitive to available water in the soil (Bouriaud et al. 2005). This study contains the results of 2-years basal area increment phenology study conducted on lands managed by Rayonier located in Bradford County, Florida. The objectives of the study were to assess genetic variation between species, families and clones in basal area growth increment, phenology traits and to estimate genetic parameters.

MATERIAL AND METHODS

The genetic material consisted of half-sib and full-sib families of genetically-improved loblolly and slash pine produced by seedlings and rooted cuttings and planted in 337 m2 family plots in January 1997. The research was concentrated in slash pine families 1, 2, 3, and 10 (S1, S2, S3, and S10 respectively), and loblolly pine family 4 (L4). Understory vegetation competition was controlled chemically and mechanically and fertilization was applied to prevent nutrient deficiency. Phenology was evaluated as growth periodicity increment of the cambial meristem as measured by repeated DBH measurements throughout growing seasons in 2002 and 2003. Diameter increment was assessed once a month in the summer time and every ten to fifteen days during the period of growth initiation and cessation in the spring and fall, respectively. Date of basal area growth initiation, cessation and duration of growth, as well as growth increment, and basal area growth rates were estimated from cumulative basal area growth curves of individual trees. A simple water balance model was computed to estimate soil water reserves at daily time steps, and quantify soil water deficit. The model allowed us to integrate environmental variables as for example, radiation, temperature and rainfall, as well as plot-level variable as transpiration in a common index to determine correlation between basal area growth and soil water availability. Analysis of variance (ANOVA) was used for phenological and growth data separately for each year. PROC GLM in the SAS® System was used to test for significance of random effects (clone), while PROC MIXED was utilized to test the fixed effects (species and families). With so few families, estimates of genetic parameters were restricted to within-family estimates obtained from clonal variation expressed within each of the four slash families and one loblolly pine family. Within family variance and covariance components were obtained using ASREML. Within-family individual tree broad sense heritability and genetic correlations among traits were calculated using standard methods.

1 Graduate Student, Professor, Director, Professor, respectively School of Forest Resources and Conservation, University of Florida, Gainesville, FL 32611
RESULTS AND DISCUSSION

Significant differences at 5% were more apparent among clones within family, than among families or species level in phenological and growth traits both years. In 2002, basal area growth started in second week of March and finished by the end of October (5 and 95% criterion, respectively) for both species. In 2003, basal area growth initiated and finished two weeks earlier than in 2002 for slash pine and loblolly pine families. Annual basal area increment and daily basal area growth rate were larger for all families in 2002 than in year 2003, despite a shorter growing season for some families in 2002. These effects could possibly be due to the differences in amount of rainfall between these two years; year 2002 registered a rainfall of 1405 mm, while 2003 corresponded to 1184 mm (NOAA 2003). In general, loblolly pine tended to accumulate more volume through ages 6 and 7 than slash pine. This was manifested by larger yearly and daily basis basal area increments, but these differences among species were only significant at 5% level for daily basal area growth in 2003. From this study we can conclude that the differences between loblolly and slash pine accumulated slowly over time until ages 6 and 7.

All families showed similar patterns of basal area increment across the growing season in years 2002 and 2003, and shape of the curve were quite similar. In general, basal area increment had a nearly linear trend throughout the growing season, and the differences at the species level and among families within slash pine accumulated across time. Peaks in periodic basal area increment occurred for short (2-3 week) periods in the early spring both years in all families, followed by linear increase in basal area until growth cessation. Significant taxa differences were found in basal area growth rate at certain critical periods, setting a different intercept for the growth line for the better performing taxa (Figure 1).

![Graph](attachment:image.png)

Figure 1: Species mean daily basal area growth increment per time across years 2002 and 2003 for loblolly and slash pine in north central Florida. * Significant differences between species at 5%; + significant differences among slash pine families at 5%.

In 2003, a year with normal rainfall, basal area growth rate was associated with soil water balance (P<0.0001, R²=0.49). In 2002 (a wetter year), there was no correlation between soil water balance and basal area growth rate. Basal area growth rate increases as water soil availability increases, but an excess in water availability in the soil had a negative effect over growth.
At the clone within family level (pooled across families), differences in initiation, cessation and duration of basal area increment in the growing season were more apparent in 2003 than 2002. Traits related to individual tree stem growth such as volume, and yearly and daily basal area increment were different among clones within families in both years. Due to low variation among clones within-family for phenological traits, especially in 2002, within-family individual tree-broad-sense heritabilities were low to moderate, ranging from 0.01 to 0.24. In contrast, within-family heritabilities for stem growth traits were moderate in both years ranging from 0.10 to 0.37. These heritabilities are expected to be smaller than broad sense heritabilities values usually reported, because they are estimated within full-sib families and half the additive genetic variation and one fourth of the non-additive variation occurs among full-sib families (Falconer 1996). In general, phenotypic expression of phenological traits and basal area growth were under weak genetic control.

Both the strength and direction of correlation estimates among phenological traits varied across families and years. At the same time, phenological traits did not differ significantly among clones within family, especially in 2002, so genetic correlations should not be estimated, and just correlations significantly different from 0 were explained. In 2002, genetic correlations between initiation and duration were strong and negative in family L4, S1 and S2 ($r_g=-0.82$ to -0.98), which indicates that genotypes with early growth initiation also had a tendency to grow longer, and the opposite can be true too, that genotypes that initiated later also tended to have a shorter growing season. In 2003, between cessation and duration, the genetic correlation was positive and strong for all families ($r_g=0.89-1$), meaning that clones that stopped growth later also grew longer period of time. With respect to genetic correlations between stem growth variables and phenological variables, there was variation between years in the direction and strength of the correlations. Among the variables we investigated, daily basal area growth rate in both years showed the strongest genetic correlation with basal area increment across all families both years ($r_g=0.96-1$). Correlation of phenology variables with total volume after 2002 and 2003 growing seasons were similar to the patterns of correlation with basal area increment, reflecting consistency between phenology with increment in the year and phenology with cumulative stem growth. These results suggest that in year 2002 and 2003, genotypes that grew faster during the growing season also were the ones with more basal area increment and total volume. Finally, because cambial phenology traits appear to be weakly inherited and to have variation from year to year in the genetic correlations with growth, indirect responses in cambial phenology from selection of bole basal area or volume are expected to be small. None of the basal area growth phenological traits had a significant clone-by-year variance component; in most of traits the clone-by-year variance component was not significantly different from 0. From this analysis we can conclude that for basal area growth phenology and basal area growth rate traits, each trait was genetically controlled by a similar set of genes in year 2002 and 2003. From the selection point of view, high genetic correlation in phenology traits, means that clones within-family are stable across years, genotypes with a large basal area increment one year tend to have a large basal area increment the next year.

Acknowledges: Financial support for this research have been provided by Forest Biology Research Cooperative – UFL and USDA Forest Service (Southern Research Station); study site was provided by Rayonier, Inc.; thanks are also extended to Sean Gallagher, Jason Martin and lab fellows that helped with field measurements.
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On the Origin of Fusiform Rust Resistance in Loblolly Pine

R.C. Schmidtling, C.D. Nelson, and T.L. Kubisiak

Abstract: Studies of geographic variation in loblolly pine 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 disease, while those from elsewhere are more susceptible. The greatest problem with rust infection, on the other hand, is in the center of the distribution, exactly where the frequency of resistant genotypes appears to be lowest. 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 pine is relatively 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. In the northeast, it has been proposed that (2) hybridization with pond pine is the source of resistance to fusiform rust. 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 and cortical monoterpene data were used to evaluate these hypotheses and the results suggest that hybridization is not the primary source of fusiform rust resistance either in the west or northeast.

Keywords: Pinus taeda, Cronartium quercuum f. sp. fusiforme, fusiform rust, disease resistance, evolution, hybridization, Pinus echinata, Pinus serotina.

INTRODUCTION

Fusiform rust (caused by Cronartium quercuum (Berk.) Miyabe ex Shirai f. sp. fusiforme) is the most damaging disease of southern pines in the southeastern United States (Powers and others 1981) and it causes substantial mortality in severe epidemics. In lesser, more common epidemics the disease causes relatively less mortality but moderate to severe degrade in the quality and strength of the main stem and branches. In loblolly pine (Pinus taeda L.), considerable geographic variation in resistance to fusiform rust disease has been found, with resistance decreasing from west to east, except in the northeastern part of the range where trees tend to be nearly as resistant as western seed sources. For the current study we reviewed the available research to evaluate the hypothesis that resistance in western and northeastern sources of loblolly pine originated in related, sympatric species known to be relatively resistant to fusiform rust.

2 USDA Forest Service, Southern Institute of Forest Genetics, USDA Forest Service, Saucier, MS 39574.
DISCUSSION

Geographic Variation

In his pioneering study of geographic variation in loblolly pine, Wakeley (1944) found that a Georgia seed source was much more heavily infected with fusiform rust than western sources from Texas, Arkansas and Louisiana. This study was the first evidence for the now well-known superiority in growth and disease resistance of seed sources from Livingston Parish, Louisiana. The results of the Southwide Southern Pine Seed Source Study (SSPSSS) (Wells and Wakeley 1966) confirmed the relative resistance of the western sources to fusiform rust, and also found a great deal of resistance in a seed source from the extreme northeast of the loblolly pine range (i.e., Maryland). The results of these studies and a study by Grigsby (1973) resulted in large-scale planting of Livingston Parish loblolly pine in areas of high rust hazard in Mississippi, Alabama, Georgia, and Florida (Wells 1985).

Significant seed source-by-planting site interaction in fusiform rust resistance was also observed in the SSPSSS, although the resistant sources from the western part of the range plus the Maryland source are clearly separated from the susceptible sources regardless of the infection level (Figure 1). The eastern and western populations of loblolly pine have been considered to be distinctly different, and the isolating effect of the pine-free Mississippi River basin has been proposed as the mechanism that perpetuates these differences (Wells and Wakeley 1966). This is especially true with regard to resistance to fusiform rust. Sources from west of the river are considered resistant, while those from east of the river are considered susceptible. The one important exception has been Livingston Parish loblolly pine, which is from east of the river but is relatively resistant to fusiform rust.

Studies that have sampled the range of loblolly pine more intensively have shown that the variation in fusiform rust resistance is continuous from west to east. In a range-wide study planted in Arkansas, Grigsby (1973) found no distinct separation between western and eastern sources in rust resistance, rather there was a continuous decrease from west to east, reaching a minimum at the longitude of eastern Georgia, then increasing to moderate levels in the Maryland (MD.) and Delaware (DEL.) sources (Figure 2).

Several regional studies with intensive geographic sampling verified the continuous variation model of Figure 2. Across southern Louisiana, Crow (1958) and Dyer and others (1977) found that resistance to fusiform rust disease decreased from west to east in a continuous manner, with no apparent discontinuity at the Mississippi River. Wells and others (1991) also found that variation in rust resistance across the Mississippi River was continuous, and that there was no distinct separation between western and eastern sources in rust resistance. In a study in Georgia, Sluder (1980) showed a decrease in resistance from west to east across Georgia, reaching a probable low point at the longitude of eastern Georgia. The performance of Livingston Parish is not anomalous when the variation is assumed to be continuous.
Several other studies verify the pattern of fusiform rust resistance of Figure 2. Wells (1966) compared infection of nine Texas seed sources with a source from Livingston Parish, Louisiana and central Georgia. The Texas sources averaged 12% infected, the Livingston Parish source 10% and the Georgia source 32%. Pait and Draper (1983) included sources from east Texas, Maryland and Livingston Parish as well as Florida sources in several plantings in Florida and south Georgia. In all plantings, the Maryland and east Texas sources suffered less infection than the Florida sources. The performance of the Livingston Parish source was comparable to that of the Texas and Maryland sources in all plantings except for one. Other studies further verify the model of Figure 2, for example, Cole (1973) found only minor differences among sources from Georgia and South Carolina, where only small differences might be expected.

The geographic pattern for fusiform rust resistance is difficult to explain, since the lowest concentration of resistant loblolly genotypes occurs exactly where rust infection levels have been the highest, that is, in central Georgia and adjoining Alabama and South Carolina (Squillace 1976). Since fusiform rust infection often causes reduced growth and mortality, natural selection should favor the more resistant genotypes in areas of high infections (Kareiva 1999).
Figure 2. Fusiform rust infection in loblolly pine seed sources from across the natural range, when planted in south Arkansas, plotted against longitude of the seed source (adapted from Grigsby 1973). Also shown is the average infection of SSPSSS seed sources over 10 plantings (adapted from Wells and Wakeley 1966).

One could argue that infection levels are lower in the west and northeast, because that is where the more resistant genotypes are found. That would logically require greater selection for resistance in these areas now or at some time in the past. Warm temperatures and very high humidity at the time of infection of the pine host is a requirement for successful infection (Snow and Froelich 1968). Currently, however, the climate in the central part of the range is more favorable for infection than in the colder northern portions (Arkansas and Maryland) and drier western portions (Texas and Arkansas) of the loblolly pine range.

One popular explanation for the geographic pattern of fusiform rust resistance in loblolly pine involves hybridization with shortleaf pine (Pinus echinata Mill.) in the west and pond pine (Pinus serotina Michx.) in the northeast (Wells and Wakeley 1966).

**Resistance of Western Sources**

The resistance of shortleaf pine to fusiform rust disease is well known. The shortleaf-loblolly hybrid is also quite resistant (Henry and Bercaw 1956) and ample evidence for natural hybridization between loblolly and shortleaf pines exists (Mergen and others 1965). Florence and Hicks (1980) compared putative natural hybrids with loblolly and shortleaf pines and found that
these hybrids contained allozymes from both species, and that they were quite resistant to fusiform rust. However, this provides only circumstantial evidence that resistance to fusiform rust in "typical" loblolly pine comes from introgression with shortleaf pine.

Allozyme analysis provides a useful tool to look at hybridization. An interesting and rare situation exists with regard to allozymes of the isocitrate dehydrogenase (IDH) locus. Shortleaf pine is almost completely monomorphic for one allozyme variant, whereas loblolly pine is almost completely monomorphic for another variant and this variation can be used to detect recent hybrids (Huneycutt and Askew 1989). In a range-wide study of allozymes in loblolly pine, Schmidtling and others (1999) found evidence for loblolly-shortleaf hybrids based on polymorphisms at the IDH locus. The levels of hybridization were low, averaging about 1%, and were highest in the north-central part of the range, rather than in the west (Figure 3).

Figure 3. Map of natural distribution of loblolly pine showing the frequency of the “shortleaf” IDH allele. Numbers in the boxes are the frequencies of the allele at each sampling point (adapted from Schmidtling and others 1999).

Better evidence exists for introgression in the opposite direction, that is, of loblolly pine genes into shortleaf pine (Figure 4). Two range-wide studies of shortleaf pine have shown high
frequencies of the "loblolly" IDH allele in shortleaf pine, especially in the western part of the range (Edwards and Hamrick 1995; Raja and others 1997). The frequency of the loblolly allele was very high in a seed orchard population in southern Arkansas (Schmidtling and Hipkins 2001). Out of 22 clones, four (or 18%) showed evidence of hybridization with loblolly pine using the IDH locus criterion. It is interesting that high frequencies of loblolly IDH alleles occur in populations well north of the current natural range of loblolly pine (Figure 4). This suggests that there may be considerable loblolly pine pollen flow northward in the western part of the natural range.

Figure 4. A map of the natural distribution of shortleaf pine showing the frequency of the “loblolly” IDH allele (adapted from Raja and others 1997 and Edwards and Hamrick 1995).

For resistance to have reached such high levels in western populations of loblolly pine, considerable hybridization would be required, along with selection for the trait. This would presumably result in the inclusion of other traits from shortleaf pine in the western loblolly pine populations. In a morphological study of shortleaf and loblolly pine provenance tests, however,
Wells and others (1977) found that resistant western sources of loblolly pine were no more similar to shortleaf pine than were susceptible eastern sources.

**Resistance of Eastern Sources**

In the northeastern portion of the loblolly pine range, pond pine is commonly associated with loblolly pine, as is shortleaf pine. There is also some evidence for introgression of pond pine genes into loblolly pine (Kang 1967; Saylor and Kang 1973). Some disagreement is found in the literature regarding the relative fusiform rust resistance of pond pine. In one manual, pond pine is listed as "moderately susceptible" compared to loblolly and slash pines (*Pinus elliottii* Engelm. var. *elliottii*), which are listed as "very susceptible" (USDA Forest Service 1972). In artificial inoculation tests, Powers (1972) found that pond pine was very rust resistant, whereas Hedgcock and Siggers (1949) found that it was nearly as susceptible as loblolly pine.

Data on cortical monoterpenes do not show any obvious relationship between pond pine and loblolly pine (Table 1). Pond pine has very high limonene content, 47.9%, whereas loblolly pine averages only 10.5%. Further, loblolly pine from the northeast has much lower limonene content than the range-wide average (Squillace and Wells 1981). If significant introgression of pond pine genes into loblolly pine was occurring in the northeast, one might expect to find a higher limonene content in northeastern loblolly pine sources.

<table>
<thead>
<tr>
<th>Species</th>
<th>Alpha-Pinene</th>
<th>Beta-Pinene</th>
<th>Myrcene</th>
<th>Beta-Phellandrine</th>
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<tr>
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<td>11.1</td>
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<td>11.4</td>
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<tr>
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<tr>
<td>Loblolly pine</td>
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<td>17.0</td>
<td>19.7</td>
<td>20.4</td>
<td>10.5</td>
</tr>
<tr>
<td>Pond pine</td>
<td>10.4</td>
<td>35.7</td>
<td>1.2</td>
<td>1.3</td>
<td>47.9</td>
</tr>
</tbody>
</table>

*a* Unpublished data on file at the USDA Forest Service, Southern Institute of Forest Genetics, Saucier, MS.

Some researchers believe that the form of fusiform rust resistance in the northeastern sources differs from that found in the western sources (Squillace and Wells 1981). An independent origin for the resistance of western sources compared to northeastern sources has been proposed based on evolutionary pressures during the Pleistocene (Wells and others 1991). Some evidence for this has been found in artificial inoculation data (Schmidtling unpublished data).

Geographic variation in fusiform rust infection from artificial inoculation tests of seedlings show a different trend than variation in field studies. It is generally acknowledged that results of artificial inoculation tests do not always conform to the results of field trials in loblolly pine (Anderson and Powers 1985). Schmidtling (unpublished data) found that the northeastern
sources tended to be resistant, as they are in field tests, but that the western sources were not, the opposite of their performance in field tests (Figure 5). This leads us to hypothesize that in field tests, several kinds of resistance, for example, physiological, morphological and phenological are expressed, but in artificial inoculation of post-cotyledon-stage seedlings possibly only one kind is expressed. Eastern sources have a relatively higher frequency of this kind of resistance than do the western sources. Thus, the resistance of the two widely separated populations may have different origins.

Figure 5. Infection of loblolly pine seedlings from across the natural range when inoculated with fusiform rust basidiospores. Data are from artificial inoculation trials of seedlings from seed orchard trees (Schmidtling and others 1999). The dotted line is a linear polynomial equation \( Y=X_1 - X_2^2 \), where \( Y \) = % galled trees, \( X_1 \) = longitude and \( X_2 \) = latitude) fit to the data shown in this figure.

CONCLUSIONS

Recent introgression of genes for fusiform rust resistance into loblolly pine from shortleaf pine in the west or pond pine in the east seems insufficient to account for the relative resistance of these seed sources. Intensive selection pressure due to optimal conditions for disease development at some time in the distant past is a more likely explanation, although hybridization during this time may have provided the genetic variation necessary for the evolution of the resistance observed today.
Schmidtling (in Wells and others 1991) proposed that loblolly pine retreated southward into two populations during the Wisconsin glaciation—Florida and south Texas or Mexico. The climate at that time was much more humid and favorable for fusiform rust infection in the western population (Texas and Mexico) and in the northern part of the eastern population (Florida) (Webb and others 1987). After the subsequent advance northward at the end of the Wisconsin, the populations merged east of the Mississippi River, creating the basis of the present pattern of geographic variation.

The lack of significant recent hybridization as a factor in resistance to fusiform rust disease may make the study of gene-for-gene interaction in host-pathogen analysis more straightforward. The possible west-east difference in resistance mechanisms also should be explored in detailed host-pathogen genetic studies and possibly exploited in breeding for rust resistance. More detailed molecular analysis of the genomes of the involved host species and the actual resistance genes will be required to completely answer this question.

**LITERATURE CITED**


Seedling Resistance to *Phytophthora cinnamomi* in the Genus *Abies*

J. Frampton¹, D.M. Benson² J. Li¹, A.M. Braham¹, E.E. Hudson ¹ and K.M. Potter¹

Cultivation of Fraser fir (*Abies fraseri* [Pursh] Poir.) as Christmas trees is a significant industry in the Southern Appalachians. In western North Carolina alone, 5.5 to 6.0 million trees are harvested yearly with annual revenues exceeding $100 million (McKinley 1996). Regionally, significant mortality occurs in many Christmas tree nurseries and plantations due to root rot disease caused primarily by *Phytophthora cinnamomi* Rands (Benson and Grand 2000). While chemical methods are available for controlling this disease in seedling and transplant beds, chemical control in plantations is stop-gap at best (Sidebottom et al. 1995). Severely infested sites must be abandoned, perhaps permanently, for Fraser fir production.

Since genetic resistance is widely used to combat diseases caused by *Phytophthora* spp. in agriculture and horticulture (Erwin and Ribeiro 1996), earlier research efforts focused on identifying resistant Fraser fir material in greenhouse inoculation trials. These trials have confirmed experiences in highly infested Christmas tree plantations, that Fraser fir is extremely susceptible to *P. cinnamomi*. Overall, mortality of seedlings in one greenhouse inoculation trial was 90% after 122 days but varied significantly among geographic seed sources (Frampton and Benson 2004). In another greenhouse inoculation trial, mortality of 100 Fraser fir open-pollinated families from a single geographic source ranged from 91 to 100% four months following inoculation with 42 families exhibiting 100% mortality (Frampton et al. 2003). Surviving seedlings from both of these studies eventually died and in a follow-up study, all seedlings from 100 open-pollinated families representing each of the six major geographic sources of Fraser fir died within four months of inoculation (Unpublished data, Frampton and Benson 2003).

In the current study, variation of resistance to *P. cinnamomi* was examined in the true firs (*Abies*). Thirty-two species (52 unique taxa) were grown in a greenhouse for two and/or three years from seed. Seedlings were moved to an outdoor lath house, inoculated with rice grains colonized with *P. cinnamomi*, and subsequent mortality assessed biweekly for 16 weeks.

Disease developed rapidly resulting in 87.5% overall mortality after 16 weeks. Final species mortality ranged from 11.3% (*A. firma*) to 100.0% (several species). Hierarchical cluster analysis was used to classify species into resistant (2), intermediate (9), and susceptible (21) groups based on 16 week mortality. All North American *Abies* species were classified as susceptible with the exception of *A. concolor* which was classified as the most susceptible intermediate species. Species classified as resistant and intermediate are native to Eastern Europe and Central Asia plus Japan (*A. firma*). This geographic distribution of resistance may reflect 1) past contact with *P. cinnamomi* during the evolutionary history of the genus or, 2) adaptation to particular environmental factors such as climate or soils that also affect resistance.

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Departments of ¹Forestry and Environmental Resources and ²Plant Pathology, North Carolina State University, Raleigh, N.C. 29765, USA
Although many caveats apply when drawing conclusions from this research, results have helped identify likely sources of *Abies* to be used as resistant rootstock or in hybridization/backcrossing breeding programs in order to improve resistance in susceptible species.

REFERENCES


An Ex Situ Gene Conservation Plan for Fraser Fir

Kevin M. Potter and John Frampton

Abstract: Fraser fir (Abies fraseri (Pursh) Poir.) is an economically and ecologically important conifer species that faces an uncertain future in its natural stands. The exotic balsam woolly adelgid (Adelges piceae Ratz.) has decimated Fraser fir populations during the last 50 years, while changing climate conditions threaten to render Fraser fir’s current habitat on Southern Appalachian mountaintops unsuitable. We have developed an ex situ gene conservation plan to help facilitate the restoration of Fraser fir into its natural stands if such action becomes necessary, and to ensure the continued existence of a genetic resource base for the economically important Christmas tree industry.

This gene conservation plan integrates existing Christmas tree breeding and gene conservation efforts with additional measures to archive Fraser fir genetic resources and to expand the amount of genetic variation included in ex situ conservation. It has four main components: 1) a seed bank representing both major and minor Fraser fir populations, in addition to seeds generated by breeding efforts; 2) a set of conservation plantings, 3) tree breeding elements (provenance and progeny tests, seed orchards, and clone banks); and 4) an archive of genomic DNA. The tree breeding elements already exist, as does a Fraser fir seed bank, which will be expanded.

In its entirety, the gene conservation plan is designed to act as a multiple population breeding system (MPBS) for Fraser fir, with the conservation plantings and the natural stands together serving as the populations upon which long-term tree breeding efforts are based. The MPBS approach involves establishing multiple breeding populations from diverse sources, with the combination of genes from throughout the range of the species ensuring the introduction of varied germplasm into a tree improvement program. Its goal is to generate the genetic variance needed both to adapt to environmental changes and to emphasize economically important quantitative traits in a breeding program.

Keywords: conservation genetics, population genetics, seed bank, tree breeding, Abies fraseri (Pursh) Poir.

Fraser fir (Abies fraseri (Pursh) Poir.) is endemic to a handful of high ridges in the Southern Appalachians of North Carolina, Tennessee, and Virginia. This economically and ecologically important conifer species faces an uncertain future in its natural stands: An exotic insect has decimated its populations over the last 50 years, and changing climate conditions could render its current habitat unsuitable. A gene conservation plan for Fraser fir would help facilitate the
restoration of the species to its natural stands if such action becomes necessary, and will ensure the continued existence of a genetic resource base for Christmas tree breeding.

Figure 1. The range of Fraser fir is limited to high peaks in the Southern Appalachians.

**Justification for a Fraser fir gene conservation plan**

Fraser fir currently exists in six major island-like populations on high ridge systems in the Southern Appalachians: the Great Smoky Mountains in North Carolina and Tennessee; the Black Mountains, the Balsam Mountains, and Grandfather Mountain in North Carolina; Roan Mountain on the Tennessee/North Carolina border; and Mount Rogers in Virginia (Figure 1). Four additional minor populations exist in North Carolina: the Plott Balsams, Cataloochee Balsam, Shining Rock, and Grassy Ridge Bald. The species occurs almost entirely at elevations above 1,300 meters, usually in association with red spruce (*Picea rubens* Sarg.), but Fraser fir becomes the dominant tree species above 1,800 meters (Busing *et al.* 1993; Cain 1935; Whittaker 1956). The populations of Fraser fir are remnants of a boreal forest that extended across much of the Southeast during the peak of the most recent late-Wisconsin glacial period, from 18,000 years to 12,500 years before present (Delcourt and Delcourt 1987; Whitehead 1973; Whitehead 1981).
In addition to its limited distribution, Fraser fir has been severely impacted by human disturbance and the infestation of an exotic insect pest (White 1984). Since the late 19th century, logging and slash fires have dramatically reduced the distribution of Fraser fir and red spruce in the Southern Appalachians (Pyle 1984; Pyle and Schafale 1988; Saunders 1979). More recently, the balsam woolly adelgid (Adelges piceae Ratz.), an aphid-like insect from Europe, has inflicted severe mortality on old-growth Fraser fir forest. First detected on Mount Mitchell in 1957, within two decades the adelgid had spread to all Fraser fir populations (Eager 1984). By the 1980s, the amount of mortality among reproductively mature trees ranged from 44 percent on Roan Mountain to 91 percent in the Great Smoky Mountains (Dull et al. 1988). Additionally, a recent model (Delcourt and Delcourt 1998) predicts the elimination of Southern Appalachian Fraser fir-red spruce forest with a global mean temperature increase of 3° C caused by greenhouse warming. Fraser fir is listed in North Carolina as an imperiled species, as a species of concern federally, and as a species imperiled and vulnerable to extinction globally (Ameroso and Finnegan 2002). The limited distribution of Fraser fir and the threats to its survival in a natural setting are compelling reasons for the systematic conservation of its gene pool (Nicholas et al. 1999).

While more than 90 percent of the extent of Fraser fir stands occur on public lands managed for the continued existence of the species (Dull et al. 1988), this is no guarantee that the species will be able to evolve in response to rapid environmental changes. An *ex situ* conservation plan will help ensure much of the genetic material of these populations is preserved, and could allow for the evolution of genes that might allow natural Fraser fir populations to evolve and survive drastic environmental changes.

**Goals of Fraser fir *ex situ* gene conservation**

Genetic diversity is essential for the long-term survival of species and populations because it provides the raw material for adaptation and evolution, especially when environmental conditions have changed (Eriksson et al. 1993; Rajora and Mosseler 2001). A central objective of genetic resource conservation, therefore, is to maintain genetic integrity and natural levels of genetic diversity, and to enhance genetic diversity in populations and species where it has been eroded (Rajora and Mosseler 2001). The final goal of gene conservation, however, should not be the preservation of only *existing* levels and patterns of variation; it should also allow for the evolution of the species or population (Eriksson et al. 1993). In other words, gene conservation efforts should aim to increase genetic variation by increasing the probability that new alleles will be saved and that genetic variance in quantitative traits will be increased (Namkoong 1997).

*Ex situ* conservation strategies are those that conserve plant genetic resources outside the area of natural occurrence and/or in a controlled manner (Yang and Yeh 1992). These methods can include seed, pollen, and tissue banks; clonal orchards; botanical gardens; and arboreta (McIlwrick et al. 2000). *Ex situ* approaches may also encompass any collection or planting of material not purposefully established or held to regenerate itself naturally, such as provenance and progeny tests (Yanchuk and Lester 1996). *Ex situ* conservation is particularly important for rare and endangered plant species, even when efforts are underway to preserve existing natural populations, because off-site conservation provides insurance against catastrophic events (Holsinger and Gottlieb 1991). After collection from their natural habitat, plants or their seeds
may be propagated elsewhere or stored long-term, with an eventual goal of reintroducing the species into its natural habitat (Brown and Briggs 1991).

*In situ* conservation of forest gene pools, through networks of protected areas, typically represents a more evolutionary dynamic approach than *ex situ* methods, because the target species or population can maintain its full range of evolutionary and ecological functions and processes, and can track the environmental changes to which it must remain adapted (Rajora and Mosseler 2001). Not surprisingly, therefore, forest geneticists have not suggested that *ex situ* collections replace natural populations. Instead, these methods are often implemented to serve as an insurance policy against catastrophes that might eliminate one or more of the few remaining wild populations of a rare or endangered species (Holsinger and Vitt 1997).

Our *ex situ* gene conservation strategy for Fraser fir has two objectives:

1) Preservation of natural population genetic diversity as a source of genetic material for the restoration or augmentation of Fraser fir populations, should this become necessary.

2) Conservation and recombination of genetic resources for the breeding of an economically important tree species.

There should be no conflict between gene conservation for the maintenance of natural ecological processes and for the utilitarian objectives of plant breeding, because the goals of these two strategies are complementary (Rajora and Mosseler 2001; Yanchuk and Lester 1996). Since Fraser fir is widely grown throughout the Southern Appalachians for the $92 million-per-year fresh-cut Christmas tree market (North Carolina Department of Agriculture and Consumer Services 2003), Christmas tree seed orchards and genetic progeny test sites offer a starting place for *ex situ* gene conservation. At the same time, conservation is useful to tree breeding because it focuses on retaining alleles of low frequency that, while of no current value to breeding programs, may become important in the future (Yanchuk 2001). Because tree breeding requires the preservation of a large genetic base from which to select trees with desirable characteristics (Zobel and Talbert 1984), conserving Fraser fir gene diversity should benefit the Christmas tree industry by providing a genetic source for valuable traits such as growth rate and crown form (McKeand et al. 1995).

**Characterization of existing Fraser fir genetic resources**

To design an effective gene conservation program, it is necessary to first identify the present population structure of the species of interest. This includes assessing the genetic and reproductive status of the targeted populations and species (Rajora and Mosseler 2001), the amount of genetic variation within and among populations, the proportion and distribution of rare alleles, and levels of inbreeding (Li et al. 1992). We are using molecular markers, including microsatellites, to achieve these objectives, and are conducting a “gap” analysis to determine which populations are under-sampled in existing Fraser fir tree breeding efforts.

Preliminary results indicate small but significant genetic differences exist among populations, and point toward genetic outlier status for Mount Rogers in Virginia, the most isolated
population. These findings are consistent with a study of Fraser fir isozymes, which found that allele frequencies differed slightly but significantly among five of the species’ populations, most likely as a result of local, restricted mating and genetic drift (Ross 1988). The isozyme research further determined that Mount Rogers had extreme allele frequencies probably associated with a small effective population size at some point in its history, coupled with isolation and with genetic drift. Additionally, a Christmas tree growth trial using open-pollinated seeds from the same five populations found that trees from low-elevation and more southerly sources grew faster than those from high-elevation and more northerly sources (Jett et al. 1993; Li et al. 1988). Heritability analysis revealed moderate to strong genetic control of this trait, and indicated that the degree of genetic control varied within different Fraser fir sources (Arnold et al. 1994).

![Diagram of gene conservation plan for Fraser fir](image)

**Figure 2:** The proposed gene conservation plan for Fraser fir. Boxes with solid borders are existing components, those with dashed lines are new, and the box with dotted lines indicates that the existing seed bank will be expanded. Solid arrows are existing connections and dotted arrows are new.

**PROPOSED EX SITU GENE CONSERVATION PLAN FOR FRASER FIR**

Our proposed *ex situ* gene conservation plan for Fraser fir integrates existing Christmas tree breeding and gene conservation efforts with additional measures to archive Fraser fir genetic resources and to expand the amount of genetic variation included in *ex situ* conservation (Figure 2). The components of this gene conservation plan represent integrated phases of a continuum for plant genetic resource management (Bretting and Duvick 1997). In its entirety, the *ex situ* gene conservation plan is designed to act as a multiple population breeding system (Eriksson et al. 1993; Namkoong 1997; Yanchuk 2001). Such a system should generate the sizeable genetic variance needed to adapt to future changes in environmental conditions and in the economic value of quantitative traits (Eriksson et al. 1993). A multiple population breeding system
(MPBS) emphasizes interpopulation diversity within an array of populations both in the traits targeted for improvement and in environmental adaptabilities. It involves establishing multiple breeding populations from diverse sources, thereby ensuring the introduction of varied germplasm into breeding. It is a dynamic process in which gene conservation is more than gene preservation; it can be coincidental with or supported by long-term breeding, and can eventually create greater genetic variability than what originally existed (Erikkson et al. 1993).

In the gene conservation plan proposed here, a set of conservation plantings could serve – together with natural Fraser fir stands – as the populations upon which the tree breeding efforts are based. Once established, trees in the conservation plantings will be allowed to reproduce, creating new allele combinations that could be of ecological or economic importance. The trees in these populations could serve as a source of seeds for future Christmas tree breeding efforts, while the continued development of interpopulational variation should assure the presence of diversity to meet changing environmental and market conditions.

**Sampling**

If an *ex situ* approach is to succeed in conserving a plant species’ genetic diversity, it must involve a sampling regime that is representative and encompasses the full genetic diversity of the plant’s natural populations (Frankham et al. 2002; Templeton 1991), especially genetic characteristics involved in a plant’s specialization to a habitat (Hueneke 1991). A gene conservation program should attempt to preserve the maximum among-population variation, with the goal of preserving evolutionary potential rather than individual genes (McIlwrick et al. 2000). While it would ideally include at least one example of each alternative allele from each locus (Chapman 1984), this is probably neither practical nor achievable.

There is a strong diminishing return associated with increasing the sampling effort in a population: Increasing the sample size by an order of magnitude will net, on average, only a single additional allele (Holsinger and Gottlieb 1991). Brown and Marshall (1995) determined that a sample of 59 unrelated gametes from a population is sufficient to capture at least one copy of 95 percent of the alleles that occur at a frequency greater than 5 percent. This, they note, can be accomplished in a fully outbreeding species by collecting seeds from 30 randomly chosen individuals in the population, although they suggest a precautionary sample of 50.

The overall objective of our sampling efforts, therefore, is to ensure the collection of viable seeds from at least 50 individual trees in each population. This figure is only a baseline, however, because the collection from each population should be stratified by microhabitat to maximize the chances of sampling unique genotypes (Brown and Briggs 1991; Holsinger and Gottlieb 1991). We propose ensuring that samples from each population are stratified by elevation, because growth trials have demonstrated that Fraser firs from different elevations are genetically different for important growth and morphological traits (Jett et al. 1993; Li et al. 1988).

1) **Fraser fir seed bank**

Seed banks represent a reasonably simple, efficient, and cost-effective approach to the *ex situ* conservation of plant genes (Given 1994; Maunder et al. 2004; McIlwrick et al. 2000). They are
especially suited for the preservation of conifer seeds, which can easily be placed in long-term storage at temperatures between -20 °C and 5 °C (Rajora and Mosseler 2001).

Table 1: Number of seed lots (half-sib families) sampled during the 1994 range-wide Fraser fir cone collection effort, by population, and the number of seed lots with germination rates greater than or equal to 20 percent.

<table>
<thead>
<tr>
<th>Population</th>
<th>Subpopulation</th>
<th>Total seed lots</th>
<th>Seed lots with ≥ 20% germination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Balsam Mountains</td>
<td></td>
<td>73</td>
<td>7</td>
</tr>
<tr>
<td>Black Mountains</td>
<td></td>
<td>124</td>
<td>72</td>
</tr>
<tr>
<td>Cataloochee Balsam</td>
<td></td>
<td>12</td>
<td>7</td>
</tr>
<tr>
<td>Grandfather Mountain</td>
<td>Andrews Bald</td>
<td>90</td>
<td>77</td>
</tr>
<tr>
<td>Great Smoky Mountains</td>
<td>Clingmans Dome/Mount Buckley</td>
<td>51</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>Mount Collins</td>
<td>11</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Mount LeConte</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Mount Sterling</td>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Newfound Gap/Indian Gap</td>
<td>9</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Silers Bald</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Mount Rogers</td>
<td></td>
<td>37</td>
<td>25</td>
</tr>
<tr>
<td>Plott Balsams</td>
<td></td>
<td>23</td>
<td>6</td>
</tr>
<tr>
<td>Roan Mountain</td>
<td>Roan Mountain</td>
<td>66</td>
<td>59</td>
</tr>
<tr>
<td></td>
<td>Grassy Ridge</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Shining Rock</td>
<td></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>521</strong></td>
<td><strong>293</strong></td>
</tr>
</tbody>
</table>

A Fraser fir range-wide cone collection conducted in 1994 will form the foundation of a seed bank for the species (Table 1). That collection harvested 521 open-pollinated seed lots from the major Fraser fir populations during an exceptional cone production year (McKeand et al. 1995). While cones were collected from a range of elevations on 17 mountains across the six major populations, we will undertake additional cone collections to:

a) Resample populations, including the Balsam Mountains and the Plott Balsams, that had many low-germination seed lots;

b) More thoroughly represent populations, such as Cataloochee Balsam and the higher elevations of Mount Rogers, which were not adequately sampled in 1994;

c) Sample minor populations (Shining Rock and the Grassy Ridge Bald outlier near Roan Mountain) and remote subpopulations in the Great Smoky Mountains where collections were not undertaken in 1994;

d) Ensure that the seed bank contains adequate representation of different elevational microhabitats (5,000-5,500 feet, 5,500-6,000 feet, and 6,000-6,500 feet); and

e) Take more samples from populations shown by our molecular marker work to have high allelic richness and/or a disproportionate number of “private” alleles.
Given (1994) recommends including at least 50 seeds from each of 50 to 100 seed lots per population in the seed bank, representing a total of 2,500 to 5,000 seeds per population. We should be able to far exceed this number because an average mature Fraser fir may produce 22,000-30,000 viable seeds in a year (unpublished N.C. Division of Forest Resources data). We will divide each seed lot, making part available for research usage and periodic viability assessment, while setting aside a portion for long-term storage, possibly at the USDA Agricultural Research Service’s National Center for Genetic Resources Preservation. Since each represents a single open-pollinated full-sib family, seed lots are highly useful for Christmas tree provenance and progeny tests. Conversely, seeds produced in seed orchards and conservation banks could be placed in the seed bank for future tree improvement efforts or for other Fraser fir population genetics studies (Figure 2).

One minor drawback in the use of seed banks is that differential seed lot survival in storage, as well as different rates of germination and seedling development among seed lots, could result in artificial selection and the loss of conserved genetic diversity (McIlwrick et al. 2000). This could be addressed by periodic collections of new seed, perhaps every 10-15 years.

2) Components of Fraser fir breeding efforts

The components of tree-breeding research efforts, including provenance and progeny tests, seed orchards, and clone banks, can house a considerable portion of a species’ *ex situ* genetic resource (Lipow et al. 2002). Provenance and progeny tests are also important because they expose diverse genotypes to uniform environments, allowing greater resolution of economically important genetic variation due to minimized local environmental variation (Yanchuk and Lester 1996).

Fraser fir seeds from the 1994 range-wide collection were used to establish the 2000 NC State provenance/progeny test series, which is assessing genetic differences for growth and Christmas tree quality traits among all six major populations (Frampton 1998; McKeand et al. 1995). While most of the trees in this trial will be harvested, cuttings have been taken from select trees for incorporation into a clone bank and Christmas tree seed orchard. These, and earlier established seed orchards and clone banks, represent an invaluable archive of Fraser fir genetic diversity. For example, the N.C. Division of Forest Resources Rattlesnake Knob Clone Bank contains elite and genetic conservation selections from the 1983 Fraser fir geographic variation study. The clone bank encompasses selections from 90 open-pollinated families (10 families from each of nine seed sources with differing elevation classes), from the Balsam Mountains, the Great Smoky Mountains, Mount Mitchell in the Black Mountains population, Roan Mountain, and Mount Rogers.

Components of a tree breeding program could also contribute genetic material to the Fraser fir seed bank and genomic DNA bank (Figure 2).

3) Fraser fir conservation stands

*Ex situ* conservation stands are populations outside a species’ area of natural occurrence that have been propagated from known or unknown stock, or have resulted from the regeneration of
propagated trees. They can have multiple purposes, including conservation, research, recreation, aesthetics, education, and wildlife habitat. Breeding, seedling establishment and vegetative propagation may be uncontrolled or partly controlled (McIlwrick et al. 2000). In these stands, the genetic composition of the target species is allowed to adapt to the prevailing environmental conditions. Because this is a dynamic process, the genetic resources are generally conserved, and stands subjected to unique selection pressures are expected to develop in different directions genetically (Graudal et al. 1995).

We propose establishing a series of Fraser fir conservation stands using plants cultivated from the Fraser fir seed bank. The exact number of conservation stands will rely in part on the logistics of locating appropriate sites, and in part on the measures of population divergence that result from our molecular marker analyses. Each conservation stand could include roughly five trees from 10 to 20 open-pollinated half-sib families (seed lots) from each natural Fraser fir population. The total number of trees in each stand, therefore, might range from 800 to 1,000. Stands this large should contain enough trees to avoid the inbreeding and genetic drift problems to which all ex situ populations are vulnerable (Maunder et al. 2004). To reduce long-term genetic erosion in a wind-pollinated species like Fraser fir, Graudal et al. (1995) recommend including in a conservation stand at least 150, and preferably more than 500, interbreeding individuals from at least 25 randomly chosen half-sib families. They additionally suggest that such stands eventually be expanded to 1,500 or more individuals through regeneration on adjacent areas, since 500 individuals may not be enough to conserve rare alleles (Graudal et al. 1995).

Duplicating each conservation stand will impose extra costs in both time and resources. The replication of conservation stands is necessary, however, to minimize the risk of loss due to unforeseen external events, such as wind throw (Eriksson et al. 1993; Graudal et al. 1995). At the same time, the number of stands should be limited to allow for their proper monitoring and management (Graudal et al. 1995).

These Fraser fir conservation stands, together with the natural stands, represent the breeding units advocated in Eriksson et al.’s (1993) multiple population breeding system (MPBS). This system emphasizes interpopulation diversity within an array of populations, both in the traits targeted for improvement and in environmental adaptabilities. Under this system, in which multiple populations are kept as separate entities, between-population diversity is expected to increase, and ultimately as many alleles will be saved as will be lost if selection for diversity among populations is effective (Namkoong 1997). Diversity among populations can be enhanced by excluding intermating among populations and by selecting for divergent characteristic. This kind of controlled evolution is no different than breeding for economic objectives in which multiple selection regimes are employed for either economic or ecological reasons (Eriksson et al. 1993). At the same time, Holsinger and Vitt (1997) stipulate an important caveat: Even with careful management of conservation stands, inadvertent selection may lead to genetic changes that increase adaptation to the “captive” environment and decrease adaptation to the native environment.

The selection of sites for Fraser fir conservation stands will not be a simple matter. These sites will have to be located above roughly 1,000 meters in elevation on sites free of Phytophthora
root rot and from the potential for infestation by balsam woolly adelgid, although the stands could be configured to allow spraying with insecticide to prevent BWA establishment. To avoid contamination from external sources of pollen, they need to be at least 500 meters away from Fraser fir seed orchards and any other mature Fraser firs (Graudal et al. 1995).

Monitoring and maintenance of the stands will be critical, especially immediately following their establishment (Given 1994). During the first few years, all the conservation stands will require seasonal inspection, including at least one visit annually by an expert who can identify insect or plant pathology problems. Five or six years after establishment, the sites should be monitored annually. Any problems, such as infestation by balsam woolly adelgid or other pests, should be addressed immediately. When the trees reach maturity, after about 20 years, cones can be collected for addition to the seed bank, for Christmas tree breeding research, and for any necessary restoration of degraded natural stands (Figure 2).

4) Fraser fir genomic DNA bank

Archives of genomic DNA extracted from trees can provide an efficient and space-saving method for the indefinite storage and conservation of genetic material (Rajora and Mosseler 2001). This DNA could become helpful in any eventual efforts to genetically transform Fraser fir to confer resistance to important pests and pathogens, such as balsam woolly adelgid or Phytophthora root rot.

We propose extracting DNA from needles collected from Fraser fir natural stands, conservation plantings, and provenance and progeny tests (Figure 2). The DNA will be stored at -80ºC, with half of each sample available for population genetics studies and for genetic engineering research, and half retained for long-term archiving.

CONCLUSION

Eriksson et al. (1993) note that elaborate ex situ conservation methods are practical for only a limited number of commercially important species, as well as for ecological keystone species. We believe that Fraser fir qualifies on both counts. Our proposed ex situ gene conservation plan for Fraser fir aims to incorporate existing tree breeding resources with the expansion of an existing seed bank and the development of conservation stands to ensure that genetic resources are available for the restoration of extirpated or degraded natural stands, and for efforts to breed better trees for the economically important Christmas tree industry. The plan should not only conserve existing genetic diversity in Fraser fir, but should cultivate the development of the genetic variance needed to adapt to changing environmental stresses and market demands.

LITERATURE CITED


Summary of Important Results from Biological Research Conducted by Camcore Over the Last 25 Years

W. S. Dvorak1

We have learned that the ancestor of loblolly pine (Pinus taeda) is shortleaf pine (Pinus echinata) and that the progenitor of slash pine (Pinus elliottii var. elliottii) is most likely an ancient form of Caribbean Pine (Pinus caribaea var. hondurensis). Pinus oocarpa from Mexico and Central America appears to be the evolutionary grandfather of all pine species in the Oocarpae and some species in the Australes subsections. Genetic distances between species in both taxonomic subsections as defined by RAPD markers are often correlated to our ability to make successful wide hybrid crosses. Reproductive cycles of pines vary from 14 to 48 months.

The magnitude of genetic diversity in Mexican pine populations appears to be related to geography. There is little correlation between levels of genetic diversity identified by molecular marker analyses and provenance performance for adaptability and growth, with the exception of studies conducted on P. maximinoi. Ex situ conservation efforts at the species and provenance level have been successful for many of the Camcore species, but pollen contamination between species like P. patula and P. greggii and Eucalyptus grandis and E. urophylla in some exotic environments complicate strategies to maintain gene bases of “pure” species.

The early wood density of species like P. tecunumanii, P. maximinoi, and P. patula var. longipedunculata is higher than that of loblolly pine when trees of both groups are grown as exotics in places like Brazil and South Africa. As a result, these Mexican pines exhibit a more gentle pith-to-bark density gradient than do the US southern pines, and a more stable, uniform wood. Provenance differences in wood density in P. patula and Eucalyptus urophylla appear to be biologically significant. Near infrared (NIR) analysis suggests that regression models developed for a pine species in one country can be used successfully to predict chemical wood properties of selected trees of the same species in another country.

The Mexican pines show great variability in their resistance to Pitch canker (Fusarium circinatum) and Diplodia needle blight (Sphaeropsis sapinea). Several of the Oocarpae pines (P. oocarpa, P. pringlei, P. jaliscana, P. tecunumanii (low elevation) show great tolerance to Pitch canker. Evolutionary history and disease resistance patterns seem to be correlated. Pinus tecunumanii and P. oocarpa also show good resistance to Diplodia needle blight while P. patula and P. greggii are very susceptible. Significant provenance differences in Diplodia susceptibility and strong positive correlations between productivity and disease resistance offers hope for improvement though selection and breeding in some environments.

Conservation approaches used for tropical and subtropical tree species appear applicable to temperate species in the US. Joint efforts are underway by Camcore and the USDA Forest Service to conserve populations of Carolina Hemlock (Thuja caroliniana) threatened by the exotic adelgid (Adelges tsugae) in the highlands of the southeastern US by moving seeds from selected population to southern Latin America. Field conservation banks in Brazil and Chile will serve as gene reservoirs in case local experts cannot control the introduced pest.

1 Professor, Department of Forestry and Environmental Resources, NC State University
Geographic Variation in Shortleaf Pine (*Pinus echinata* Mill.) - Cortical Monoterpenes

R.C. Schmidtling, J.H. Myszewski, and C.E. McDaniel

**Abstract** - Cortical monoterpenes were assayed in bud tissue from 16 Southwide Southern Pine Seed Source Study (SSPSS) sources and from 6 seed orchard sources from across the natural range of the species, to examine geographic variation in shortleaf pine. Spruce pine and pond pine were also sampled. The results show geographic differences in all of the major terpenes. There was no north-south trend in any of the terpenes, but there was clinal variation in alpha pinene from west to east. One source, from New Jersey (SSPSSS) had very low alpha pinene and did not fit the trend, possibly because of hybridization with pitch pine. Some of the western sources had high limonene content, probably as a result of hybridization with loblolly pine, which has high limonene in western populations. Spruce pine had terpene levels similar to shortleaf pine, while pond pine had low alpha pinene and much higher limonene compared to shortleaf pine.


Geographic variation in loblolly pine has been very well documented, but very little has been published on geographic variation in shortleaf pine. Shortleaf pine is not widely planted, because in many situations loblolly pine grows much faster. There is increased interest in shortleaf pine as restoration of native species is becoming more popular. Because shortleaf has the widest north-south distribution of any of the southern pines, it may have the greatest variation among provenances.

Judging from the 25-year analysis of the Southwide Southern Pine Seed Source Study (SSPSSS) (Schmidtling 1995), which may be the only published study on growth of geographic races, variation in shortleaf pine follows a pattern common to many forest tree species. Southern sources are generally less cold-hardy but faster growing than northern sources. This north-south variation appears to be clinal.

In loblolly, populations west of the Mississippi River valley generally are slower growing, survive better and are more resistant to fusiform rust then those east of the Mississippi. It has been hypothesized that the pineless expanse of the Mississippi River Valley serves as a barrier to gene flow among the two populations allowing them to evolve separately. East-west variation in growth of shortleaf pine does not appear to be as extensive as that found in loblolly pine, if it exists at all (Schmidtling 1995). In spite of this, in many tree improvement programs including that of the Southern Region (R-8), the Mississippi river is used as the dividing line to separate western from eastern populations in determining planting zones.

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1 USDA Forest Service, SRS, Southern Institute of Forest Genetics, Saucier, MS, 39574-9344. e-mail: rschmidtling@fs.fed.us
An extensive sampling of xylem monoterpenes of shortleaf and loblolly trees in situ by Coyne and Keith (1972) showed clinal variation in the concentration of alpha pinene from east to west for both species. They also found a tendency for higher limonene contents in the western sources. Terpenes are probably related to insect resistance, qualitatively as well as quantitatively.

Cortical monoterpenes are more useful than xylem monoterpenes for examining population structure because there is generally more genetic variation in the minor constituents and they are affected very little by environment. There is also evidence for a simple inheritance pattern for the cortical monoterpenes (Squillace et al 1980), and they have the additional advantage of being highly variable among populations (Squillace and Wells 1981), at least in loblolly pine. Cortical monoterpenes have not been previously examined in shortleaf pine.

The present study will utilize cortical monoterpenes to examine geographic variation in shortleaf pine, and to explore phylogenetic relationships with other species.

**MATERIALS**

The study primarily utilizes material from a demonstration planting of the SSPSSS shortleaf phase on the Harrison Experimental Forest (HEF) in south Mississippi. All sources used for the 3 different series of the SSPSSS shortleaf phase are included in this planting (Fig. 1). Material from the US Forest Services Southern Region (R-8) tree improvement program located in the HEF clone bank were included, as well as arboretum material (Table 1).

![Figure 1. Map of the southeastern United States showing the natural distribution of shortleaf pine (Pinus echinata Mill.) and the location of the sampled populations. Also shown are the limits of the natural distribution of loblolly pine (Pinus taeda L.).](image-url)
Table 1. Original provenance of the shortleaf pine trees used in the study.

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**METHODS**

The distal 1/8" of 2 buds per sample tree were excised with a razor blade and placed in a vial containing ethel The razor blade was rinsed in ether between each sample tree. Vials were labeled, sealed and stored in a freezer until analysis.

In the laboratory, extracts from the bud tips were assayed for terpenes by gas-liquid chromatography, using a 6.1 m, 4.76 mm, 60/80 mesh chromosorb W column packed with 20% carbowax 20 M.
RESULTS

The results show geographic differences in nearly all the terpenes (Appendix). The major constituents were alpha pinene (averaging 33 percent), and beta phellandrine (averaging 38 percent). The monoterpene composition of spruce pine was similar to shortleaf pine. The composition of pond pine was quite different. Pond pine had very high limonene content (48 percent), and very low beta phellandrine content (Appendix 2). The terpene composition of shortleaf pine cortical oleoresin is comparable to those found by Squillace and Wells (1981) for loblolly pine, except that loblolly pine has higher limonene.

There were east-west trends in the concentration of several constituents. The correlation of alpha pinene content with longitude was $R = 0.85$ (Fig. 2). Alpha pinene content decreased from east to west. The east-west variation appears to be linear, with no discontinuity at the Mississippi River. One source, New Jersey 452 did not fit the pattern of east-west variation. This source has long been suspect as it may be pitch pine or a hybrid.

![Figure 2. Alpha pinene concentration in cortical monoterpenes of buds of geographic races of shortleaf pine plotted by longitude.](image)

Squillace and Wells (1981) intensively examined geographic variation in cortical monoterpenes in loblolly pine. They also found a decrease in alpha pinene from east to west. If their data are plotted similarly to the shortleaf data in Fig. 2, it is apparent that in loblolly pine there is a distinct discontinuity in alpha pinene concentration at the Mississippi River (Fig. 3). Within the eastern population or within the western population, there is no significant east-west trend. This supports the two-population refugium hypothesis for loblolly pine, versus one population for shortleaf pine during the Pleistocene (Schmidtling 2002).
Figure 3. Alpha pinene concentration in cortical monoterpenes of buds of geographic races of loblolly pine plotted by longitude. Data from Squillace and Wells 1981.

Figure 4. Limonene concentration in cortical monoterpenes of buds of geographic races of shortleaf pine plotted by longitude.
A correlation coefficient of $R = 0.39$ between longitude and limonene content indicates a tendency for the western sources to have higher limonene contents, similar to loblolly pine (Squillace and Wells 1981). A plot of limonene content versus longitude, however, indicates that this correlation is due to a few of the western sources having high limonene contents (Fig. 4). This may be due to hybridization with loblolly pine. Western loblolly pine sources also have high limonene contents (Squillace and Wells 1981).

Hybridization of shortleaf and loblolly pines appears to be common in northwestern part of the natural range of shortleaf. Allozyme analysis of some of these same sources has also indicated hybridization with loblolly pine (Raja et al. 1997).

One problem with the hybridization theory is that many of the shortleaf pine sources with probable loblolly parentage are well north of the current loblolly pine distribution (Fig. 1). The Missouri source is perhaps 300 km distant from the nearest loblolly pine. Gene flow as well as long-distance pollen transport is a possibility. Also, the loblolly pine distribution probably extended farther north 5,000 to 7,000 years ago during the Hypsithermal geological period, when the climate was warmer that it is now. Stumps of Scots pine (*Pinus sylvestris* L.) dating from the end of the Hypsithermal have been found well north of the current distribution, indicating that the species expanded northward in the warmer climate, then retreated in the colder present-day climate (Gear and Huntley 1991).

**CONCLUSIONS**

1. There is a moderate east-west trend in alpha pinene content in buds of shortleaf pine.

2. The variation is continuous across the Mississippi River, unlike loblolly pine, where the content varies discontinuously across the river.

3. There is indication of hybridization between shortleaf and loblolly pine in the Limonene content of some shortleaf from the northwestern part of the natural range.

**LITERATURE CITED**


Appendix. Mean values for cortical monoterpenes of geographic sources of shortleaf pine and of spruce pine and pond pine samples. Also included is data on loblolly pine from Squillace and Wells (1981).

<table>
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<th>β-pinene</th>
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<th>myrcene</th>
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Pond pine

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Loblolly pine (Squillace and Wells 1981)

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From Tree Improvement to Species Improvement: Restoration and Conservation Efforts on the Forest Service’s Southern National Forests

Barbara S. Crane

The USDA Forest Service National Forest System (NFS) southern land base in Region 8 (R8) encompasses 13 million acres managed within 17 Administrative Units. These national forests are fragmented across 13 southern states and Puerto Rico.

The national forests were established in the early part of the 1900’s. The original focus was to provide an adequate supply of timber products for the growing population. To meet projected timber needs R8’s Tree Improvement (TI) program was initiated in the early 1960’s. Seed orchards were established in Arkansas, Louisiana, Mississippi, Florida, South Carolina and North Carolina in order to capture the wide geographic variation of seed sources. The orchards would ensure seed needed for reforestation efforts following timber harvesting on the national forests. Some 1900+ superior tree selections were made from six species of pine. Over the next several decades breeding and progeny testing were the main focus of the program. Second generation orchard blocks for the four southern pines were established. In the late 1980’s NFS goals and objectives changed from timber production to ecosystem management and biodiversity. Timber harvesting decreased by 90%, resulting in a drastic reduction in seed needs. The TI program’s objective of genetic improvement for quality timber became obsolete. As a result some orchard components and all progeny testing were terminated. In the early 1990’s R8’s TI program shifted from traditional tree improvement to genetic resource management. Genetic diversity of each species became the priority. The program re-focused on meeting the seed needs for restoration of tree species within endangered ecosystems. More recently, as several pine and hardwood forest ecosystems have been decimated by exotic pests and diseases, genetic conservation has become a major emphasis of the program as well.

Today R8’s Genetic Resource Management Program (GRMP) focuses on perpetuating biological & genetic diversity of tree species on the national forests. Current efforts target restoration and conservation of six conifer and six hardwood tree species. Ecosystem restoration, maintenance and sustainability on National Forest lands are long term commitments. Hence the GRMP continues to manage current species in the orchards, incorporate new species into the orchards and establish seed production areas out on national forests. Ensuring a stable supply of seed is critical for species’ perpetuation. The GRMP promotes species improvement, rather than tree improvement. The GRMP is engaged in several university, research and private partnerships to facilitate conservation and restoration efforts.

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1 Regional Geneticist, USDA Forest Service, Atlanta, GA, USA
A Random Walk Through the History of Breeding for Wood Quality

J. P. Van Buijtenen¹ and T. D. Byram²

The walk begins by accompanying Bruce Zobel on a trip visiting a number of pulp and paper mills in East Texas in search of wood quality and ends up hopelessly lost in a maze of micro-arrays.

In between we will be visiting seed orchards, the Tappi Forest Biology Committee, the clonal forests of the future, the nation’s sanitary landfills, and other interesting landmarks. The choice and order of topics is subject to change without notice.

¹ Professor Emeritus, Department of Forest Science, Texas Agricultural Experiment Station, Texas A&M University, College Station, Texas 77843
² Geneticist, Western Gulf Forest Tree Improvement Program, Texas Forest Service, and Assistant Professor, Department of Forest Science, Texas Agricultural Experiment Station, Texas A&M University, College Station, Texas 77843
The Outlook for Pine Plantation Management in the South

Robert C. Abt

Recent changes in acres and ownership of pine plantations in the South will have potentially significant impacts on future markets and management. The first part of the talk will present an overview of the southern timber supply with specific attention to the changing role of pine plantations and the growing importance of Timber Investment Management Organizations (TIMOs). The increasing share of plantations owned by corporations without processing facilities has implications for silviculture. Since more timber is being transferred through open market purchases, the lack of price premiums for quality will affect a larger proportion of supply. The second part of the talk will explore how current markets deter a focus on quality and give some examples of the types of premiums that may be required if these markets were to emerge in the future.

1 Professor, Department of Forestry and Environmental Resources, NC State University, Raleigh, NC, USA
After 50 Years, Shift Genetic Emphasis Toward the True Tree Value – Pine Sawtimber

H. M. (Mac) Lupold¹

The forest landowner in the South over a rotation receives 75 to 85% of their stumpage income from pine sawtimber. Lumber products derived from today’s sawtimber trees are not the same as 25, 50 or 100 years ago. A lack of clear, dense, heartwood characteristics means few, if any, windows, doors, siding, flooring, boxing, trim boards used in home construction from Southern pine. “Forest Management” has robbed the grade from Southern pine. Coarse grain is the normal grain pattern today due to periodic thinnings to increase diameter growth and reduced age of rotations, plantings produce uniform tree characteristics with increased juvenile wood content which will not meet SPIB #2 strength standards and less than 5% of lumber allows prices twice the species average. The two “true strengths” of Southern pine, treatability and strength, could be in jeopardy if nothing is done now to address forest genetics traits, such as, quicker transition to mature wood, higher percentage summerwood, increase cell wall thickness, determine best microfibral angle, etc. Meanwhile, for the next 25-30 years, how do we manage what we’ve got, and not loose more market share due to poorer lumber properties, and can temperature and chemical modification of the wood provide interim product improvements?

¹ Lupold Consulting, Inc., Camden, SC
Commercialization of Forest Biotechnology: Economic Targets for Enhanced Global Competitiveness of the U. S. Pulp and Paper Industry

G.F. Peter1,2, D.E. White3, N. Sicarelli1, R. De La Torre4, D. Newman4

Abstract: Current economic analyses show that in the U. S., fiber costs represent up to 40% of the total cost for paper manufacturing. In countries located near the equator, these costs are dramatically lower. Commercialization of biological technologies that lead to genetic improvement of tree growth rates, wood and fiber qualities promise to significantly lower raw material costs and to maximize processing efficiencies, minimize environmental impacts, and improve product performances. A multidimensional cash flow model was constructed to estimate the value of changes in growth, wood and fiber properties of loblolly pine on linerboard production. Of the traits modeled increases in fiber tensile strength, specific gravity, and growth were found to be more valuable than reduced lignin content. If trees with a 20% increase in specific gravity captured 20% of the loblolly pine seedling market in the U. S. A., then the overall value of this wood quality improvement just for linerboard production alone was estimated at $300 million per year. These results strongly support the forest industry goal of using biotechnology to improve tree growth and wood quality.

Keywords: Linerboard, profit, specific gravity, microfibril angle, growth rate, Pinus taeda

INTRODUCTION

Commercial interest in forest tree biotechnology comes from the potential to dramatically improve the productivity of plantation grown trees by increasing growth rates and disease resistance as well as by enhancing the efficiency of converting trees into solidwood, pulp and paper products and biomass derived energy and new biobased products (Sedjo, 2004). The commercial success of genetically engineered crop plants that increase yield and require less chemical and energy inputs provides a roadmap to forest products and forest tree biotechnology firm’s application of biotechnological methods to improve select tree species. Like crop biotechnology, forest biotechnology is composed of a suite of technologies. For forest trees these technologies include methods used for clonal or mass vegetative propagation of superior individual tree genotypes, molecular breeding and genetic engineering. These advanced technologies complement traditional breeding programs and share the same primary goal: to create faster growing trees that better resist insects and other pathogens and have wood properties that improve conversion into valuable products (Williams, 2001; Campbell, 2003).

1 School of Forest Resources and Conservation, University of Florida, Gainesville, FL 32611
2 Center for Paper Business and Industry Studies, Georgia Institute of Technology, Atlanta, GA, 30318
3 Institute of Paper Science and Technology, Georgia Institute of Technology, Atlanta, GA, 30318
4 Daniel B. Warnell School of Forest Resources, University of Georgia, Athens, GA
Only a few economic analyses have been published that quantify the potential cost savings for chemical pulp production which could come from increased volume growth and specific gravity, a key wood quality trait. For pines an analysis from the 1970s investigated changes in rotation age and specific gravity of loblolly pine using a linear programming model developed to optimize net profit of a mill that met specific paper quality standards (Van Buijtenen, 1987). The analysis compared 14 different cases for linerboard production and showed that selecting trees with increased volume growth and specific gravity improved mill profitability; however the amount of profit increase was not determined (Van Buijtenen, 1987). More recently Lowe et al. following the method of Borralho (Borrallho, 1993) estimated changes in profitability associated with increases in loblolly pine growth rates and specific gravity for unbleached kraft pulp production (Lowe, 1999). They concluded that deploying seedlings from parents selected for higher specific gravity increased pulp mill profits by about ten fold over parents selected solely for fast growth (Lowe, 1999). This analysis assumed that unbleached kraft pulp was the final product and estimates for the impact of genetic improvements on the profitability of linerboard were not determined.

A research project to identify applications of forest biotechnology that have the greatest potential to enhance the global competitiveness, enterprise effectiveness, and environmental safety of U.S. pulp and paper companies is underway with funding by a Sloan Foundation Center for Paper Business and Industry Studies. The objectives for the first phase of this research program are: (1) cost/benefit comparisons of altering specific softwood fiber traits, (2) biological feasibility of increasing wood growth, improving wood and fiber properties, as well as uniformity of fiber supply through biotechnology, and (3) identification of the potentially most beneficial targets for forest biotechnology research.

**MODELING APPROACH**

The value of changes in growth, wood and fiber properties for linerboard production costs and mill profitability are being estimated with a multidimensional cash flow model, consisting of a forest cost model and a theoretical Greenfield, vintage 1995, integrated Kraft pulp and linerboard mill cost model developed by Jaakko Pöyry Management Consulting (JPC) under contract to the Institute of Paper Science and Technology (IPST) (Figure 1). This mill model has been enhanced by addition of a module to calculate energy recovered when black liquor amount and composition change. To minimize errors due to fluctuations in spot prices for all forest and mill inputs and for the sale price of linerboard, we used real prices obtained from trend price regressions. The linerboard costs and mill profits are projected for year 2020, where the real price of linerboard is expected to drop from current values. Trait modeling predictions were based on empirical pulping and papermaking relationships obtained from the literature and when not available on mass and energy balances. To date all modeling has been conducted with the following basic assumptions: 1) the mill owns the forestland reflected in the lack of transfer pricing for softwood logs, 2) softwood logs are loblolly pine trees grown clonally, 3) all softwood logs for the mill come in as roundwood from company owned land, 4) hardwood (roundwood and chips) and recycled paper are purchased on the open market, and 5) linerboard production is held constant.
To interpret the modeling results it is important to understand the key assumptions and constraints made in developing the forest and mill models as well as the logic that we have followed in estimating the impacts on final costs for the trait changes investigated.

**FOREST COST MODEL ASSUMPTIONS**

1. Plantation comprises solely Loblolly pine
   - Seedling cost is fixed at $0.05
2. Land is located in the lower coastal plain of the Southeastern US
3. Base site index (SI) is 65 (max height in ft. at 25 yrs)
4. Intensive management improves SI from 65 to 78 for the unthinned scenario and 80 for the thinned scenario
   - Shear, rake, pile, bed and hardwood pretreatment
   - Herbicide treatments at planting and year 2 for unthinned scenario and brush control one year after thinning age
   - Fertilizer treatments at years 1, 6, and 10 for unthinned scenario and years 1, 6, and two years after thinning age
5. The land value is fixed and all land is owned by the mill for the sole purpose of supplying wood to the mill. For all rotations the opportunity cost associated with the land is assessed at a real discount rate of 8%.
6. Growth and yield equations for lower coastal plain were obtained from the Plantation Management Research Cooperative at the University of Georgia.
7. Tree diameter classes are estimated with the Weibull distribution function (PDF).
8. Harvesting outputs (efficiencies) are estimated with the Auburn Harvest Analyzer.
9. Transportation is estimated as a function of the distance and a derived hauling rate of $2/mile/load.
10. The forest cost model optimizes the bare land value (BLV) of the plantation.
11. Silvicultural components of the model are constructed in MS-Excel with input variables of site index, trees per acre, cost per seedling, regeneration costs, stumpage prices, discount rate, and land cost. The harvesting and transportation components have multiple input variables.
12. Input variables for cost modeling are per acre land cost, specific gravity, trees per acre, growth (site index), and seedling cost.

**PULP AND PAPER MILL MODEL ASSUMPTIONS**

2. The forest model is based on a yearly mill production of 483,000 ADMT/y unbleached Kraft pulp.
   - Produces 337,000 ADMT/yr (air-dried metric tons per year) of softwood Kraft pulp (70% of virgin fiber needs) at 70 Kappa for top ply and 105 Kappa for bottom ply.
   - Produces 146,000 ADMT/yr of hardwood kraft pulp (30% of virgin fiber needs) at 60 Kappa.
3. Mill also produces 127,000 ADMT/yr of recycled paper pulp.
4. The single paper machine produces about 590,000 MT/y (metric tons per year; 6.5% moisture) of 42 lb linerboard.
   - Top ply is 50% softwood and 50% hardwood
   - Bottom ply is 58% softwood, 10% hardwood and 32% recycled.
5. Black liquor solids and heating values are estimated separately for each pulp.
6. Capital is depreciated over 20 years for all equipment.
7. Inputs prices are based on trend price predictions obtained by linear regression of price data for the last 10-20 years.
8. Model is constructed in MS-Excel as an integrated set of worksheets with a base and proposed case format. All significant input prices and machine variables can be changed manually to determine the effect on production costs and mill profitability.

Table 1 shows a summary of the mill operating parameters, wood and pulp compositions and outputs used in the base case operations for the softwood and hardwood pulps and the top and bottom plys.
<table>
<thead>
<tr>
<th>Scenario</th>
<th>SW Wood Lignin Comp. (%)</th>
<th>SW Wood Density (BD kg/green m³)</th>
<th>SW BS Pulp Kappa</th>
<th>SW Wood Comp.: Cellulose/ Hemi/ Lignin/ Extr (%)</th>
<th>SW BS Pulp Comp.: Cellulose/ Hemi/ Lignin/ Extr (%)</th>
<th>SW BS EA, as Na₂O (% on wood)</th>
<th>Sulfidity (%)</th>
<th>SW BS Pulp Yield (%)</th>
<th>SW BS HHV (BTU/ lb BLS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thin</td>
<td>29</td>
<td>458</td>
<td>105</td>
<td>39 / 23 / 29 / 9</td>
<td>63 / 17 / 16 / 4</td>
<td>10</td>
<td>25</td>
<td>60.3</td>
<td>6705.663</td>
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<tr>
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<td></td>
</tr>
<tr>
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<td></td>
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<tr>
<td>Unthin-LC</td>
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<td></td>
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</tr>
<tr>
<td>Scenario</td>
<td>SW Wood Lignin Comp. (%)</td>
<td>SW Wood Density (BD kg/green m³)</td>
<td>SW TS Pulp Kappa</td>
<td>SW Wood Comp.: Cellulose/ Hemi/ Lignin/ Extr (%)</td>
<td>SW TS Pulp Comp.: Cellulose/ Hemi/ Lignin/ Extr (%)</td>
<td>SW TS EA, as Na₂O (% on wood)</td>
<td>Sulfidity (%)</td>
<td>SW TS Pulp Yield (%)</td>
<td>SW TS HHV (BTU/ lb BLS)</td>
</tr>
<tr>
<td>Thin</td>
<td>29</td>
<td>458</td>
<td>70</td>
<td>39 / 23 / 29 / 9</td>
<td>68 / 17 / 13 / 3</td>
<td>13</td>
<td>25</td>
<td>53.5</td>
<td>6490.115</td>
</tr>
<tr>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Thin-LC</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Unthin-LC</td>
<td></td>
<td>471</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scenario</td>
<td>HW Wood Lignin Comp. (%)</td>
<td>HW Wood Density (BD kg/green m³)</td>
<td>HW Pulp Kappa</td>
<td>HW Wood Comp.: Cellulose/ Hemi/ Lignin/ Extr (%)</td>
<td>HW Pulp Comp.: Cellulose/ Hemi/ Lignin/ Extr (%)</td>
<td>HW EA, as Na₂O (% on wood)</td>
<td>Sulfidity (%)</td>
<td>HW Pulp Yield (%)</td>
<td>HW HHV (BTU/ lb BLS)</td>
</tr>
<tr>
<td>Thin</td>
<td>20</td>
<td>515</td>
<td>60</td>
<td>51 / 27 / 20 / 2</td>
<td>68 / 25 / 7 / 0</td>
<td>12.5</td>
<td>25</td>
<td>58</td>
<td>5592.102</td>
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<tr>
<td>Thin-LC</td>
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<td></td>
</tr>
<tr>
<td>Unthin-LC</td>
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<td>515</td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>
RESULTS

Base Case. Table 2 summarizes the major outputs from the forest and mill models for the base case of growth rate, wood and fiber properties using two forest cost models, for the unthinned model all of the wood goes to the mill as pulpwood and for the thinned model where harvested trees are segregated by stem diameter into three product classes pulpwood, chip-n-saw, and saw timber. In one set of scenarios the opportunity cost of the land is included in the computation of the cost of growing timber (Thin-LC and Unthin-LC). For each forest model the bare land value is calculated the same, the maximal bare land value for all four forest models was 18 years (Table II). In the thinning models, age 10 was the earliest age at which all stems met the minimum pulpwood diameter and stands were thinned to 50% of the trees per acre. Other constraints included were as follows, 20 tons/acre as minimum volume to remove, 65 ft²/acre as minimum residual basal area, and 6.5 in. as minimum Dq (quadratic mean diameter), the minimum age for harvesting solid wood products is 18 years, planting densities less than 400 TPA were not considered sawtimber due to more conic stems, broader crowns, and larger branches. The average specific gravity of the wood coming into the mill is estimated from forest tree biometric data and for the thinned cases is a weighted average of the specific gravities of the 10 and 18-year-old trees. The growth rate is indicated as mean annual increment (MAI) and the land area required for a sustainable supply of all of the softwood needs for the mill is calculated. About one third of the cost of wood comes from tree production while the other two-thirds of the total wood cost come from harvesting and transportation to the mill. Overall the greatest portion of wood is the cost of harvesting the logs. Use of trees for pulpwood, chip-n-saw and saw timber products (thinned) decreases pulp and paper mill profitability by ~20%. The lower mill profit in the thinned scenarios is due to elevated roundwood costs incurred by a higher forest production costs and by increases in the in the haul distance. In this work no attempt was made to estimate the increased profit in the forest that would result from selling a portion of the trees as higher value chip-n-saw and saw timber logs.

Table 2. Summary of Forest Cost Model for the Base Case Data

<table>
<thead>
<tr>
<th>Forest Cost Model</th>
<th>Rot. Age (Yr)</th>
<th>SG</th>
<th>MAI (m³/hect/yr)</th>
<th>Land (Hect)</th>
<th>Wood Cost ($/grn ton)</th>
<th>Liner Cost ($/MT)</th>
<th>Op. Profit (Mil. $)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thin</td>
<td>18</td>
<td>0.46</td>
<td>21.55</td>
<td>235,475</td>
<td>15.30</td>
<td>274</td>
<td>27.95</td>
</tr>
<tr>
<td>Unthin</td>
<td>18</td>
<td>0.47</td>
<td>21.51</td>
<td>85,688</td>
<td>13.27</td>
<td>267</td>
<td>34.23</td>
</tr>
<tr>
<td>Thin–LC</td>
<td>18</td>
<td>0.46</td>
<td>20.63</td>
<td>246,342</td>
<td>16.73</td>
<td>278</td>
<td>25.79</td>
</tr>
<tr>
<td>Unthin-LC</td>
<td>18</td>
<td>0.47</td>
<td>22.03</td>
<td>85,393</td>
<td>14.67</td>
<td>271</td>
<td>31.67</td>
</tr>
</tbody>
</table>

Effect of Reducing Basis Weight on Mill Profits. Reduced linerboard basis weights were analyzed because less damage to the fibers during pulping or decreases in cellulose microfibril angle should increase the tensile strength of fibers and thereby permit basis weight reductions without wet pressing. In this analysis up charges on linerboard sale prices commonly given to high performance linerboard grades were used with our base case wood prices and mill parameters. Figure 2 shows the impact of reduced basis weight on the mill’s profitability can be
quite high. As expected when total annual production is fixed, decreases in basis weight increase mill profitability for both the thinned and unthinned scenarios. The percent increases in mill profit (Figure 2) are greater for thinned forest scenarios because the thinned wood is more expensive, due to increased haul distances (Table 2).

![Figure 2. Effect of Decreased Basis Weight on Profit under Base Case Conditions (Note: Thin and Unthin Labels apply to scenarios both with and without land costs.)](image)

**Effect of Changes in Growth Rate on Mill Profit.** To evaluate a difference in mill profitability as the growth rate of trees increased or decreased by a certain percentage, the site index within the forest cost model was varied by the respective percentage. As the trees’ rate of growth increased, the production, harvest, and transportation costs decreased due to constant, yearly mill consumption. The decreased cost of wood translated into a higher mill profit. The impact of tree growth rate on profitability is illustrated in figure 3. In figure 3 the percent profit change was predicted when growth rate varied from a -20% to +30%. When the site index is increased by 30% (65 to 84.5) timber growth increases ~ 25% and mill profitability is predicted to increase by 5-10%
Effect of Changes in Wood Lignin Content on Mill Profit. The impact on mill profitability of processing wood with reduced lignin contents was modeled by increases in softwood pulp yield. Because no empirical data relates pulp yield with reduced lignin contents, increases in softwood pulp yields were estimated with a mass balance approach by assuming a fixed chemical composition of the base and top ply pulps. Since the yearly mill production of pulp was held constant, the yearly mill wood consumption declined with increases in pulp yield. This decreased wood consumption meant a lower wood cost and reductions in land area required to sustain production. The lower wood cost translated into a higher mill profit. Figure 4 shows the effect of softwood lignin content, at a fixed base case growth rate, specific gravity, and hardwood yield, on mill profitability. In figure 4 the percentage profit change is measured as the lignin content in the wood reduces from 29% to 15%. While there is less than a 1% increase in profit at a lignin content of 25% for all four scenarios, the two thin scenarios show a 7% profit increase at a lignin content of 15%, half of the natural lignin content. While dramatic reductions in wood lignin content lead to large increases in pulp yield, the value to the mill is mitigated by the loss in bioenergy production and the need to purchase more power.

Figure 4. Effect of Change in Wood Lignin Content on Profit
**Effect of Changes in Specific Gravity on Mill Profit.** The impact on mill profitability of processing wood with changes in wood density or specific gravity was modeled by changes in softwood pulp yield. Regression equations relating changes in pulp yield at defined kappa values relative to changes in loblolly pine wood specific gravity were used to predict pulp yields (Kleppe, 1970a). The increases in pulp yield with increases in specific gravity are probably due to less degradation of carbohydrates during pulping in wood with higher specific gravity (Kleppe, 1970b). Since the total yearly production of pulp was held constant, at higher wood densities the yearly mill wood consumption declined. This decrease in wood consumption meant lower pulp mill costs and higher profitability. The impact of specific gravity on profitability is illustrated in figure 5. In figure 5 the percentage profit change is measured as specific gravity varies from 0.30 to 0.80 and can be fairly high. Again the thinned relative to the unthinned cases show a greater impact from changes in specific gravity due to the higher cost of wood that comes from hauling it further in the thinned cases.

![Figure 5. Effect of Change in Specific Gravity on Profit](image)

(Note: Thin and Unthin Labels apply to scenarios both with and without land costs.)

**Effect of all Biotech Changes on Mill Profit.** If we compare the four different changes to the trees, then the greatest increase on mill profits are reduced basis weight followed by increased specific gravity, increased growth rate, and decreased lignin content (Figure 6). The tree improvements that were chosen for Figure 6 are probably achievable through clonal selection or genetic engineering. A 10% reduction in linerboard basis weight, 42 lb. to 38 lb., shows a very good profit increase of forty to fifty percent.
Seedling Costs Reflect the Market Size and Potential of a Return for Investing in Forest Biotechnology. In the above scenarios the seedling cost was fixed at the current market price of $0.05. Table 3 shows what the maximum price for an improved seedling could be. This price was determined by increasing the seedling cost in the forest model to the point where the linerboard cost equals that for the base case and all of the cost savings in the mill due to the wood improvement goes into the seedling. These elevated seedling costs represent the value associated with changes in growth or a wood property and define the upper end of what might be expected in the open market for these improved seedlings. This higher seedling cost can be used to justify the magnitude of investment in forest tree biotechnology research and development. Currently there are over one billion loblolly pine seedlings planted annually in the SE US. If 20% of the seedling market were genetically improved, then the total potential value of the market based just on cost savings for linerboard would be $100-300 million annually depending on the trait. It should be noted that this is a minimal estimate of the market size because it does not include any additional value that would come from reduced production costs of other paper grades or of solidwood products. If these additional cost savings were included, the market size would be expected to be substantially greater.
Table 3. Breakeven Seedling Costs for Growth, Wood Composition, and Specific Gravity within the Thinned Land Cost Scenario

<table>
<thead>
<tr>
<th>Growth Rate</th>
<th>Breakeven Seedling Cost ($/seedling)</th>
<th>Lignin Content</th>
<th>Breakeven Seedling Cost ($/seedling)</th>
<th>Specific Gravity</th>
<th>Breakeven Seedling Cost ($/seedling)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.05</td>
<td>29%</td>
<td>0.05</td>
<td>0.458</td>
<td>0.05</td>
</tr>
<tr>
<td>+10%</td>
<td>0.25</td>
<td>25%</td>
<td>0.09</td>
<td>0.50</td>
<td>0.52</td>
</tr>
<tr>
<td>+20%</td>
<td>0.48</td>
<td>20%</td>
<td>0.29</td>
<td>0.55</td>
<td>1.09</td>
</tr>
<tr>
<td>+30%</td>
<td>0.63</td>
<td>15%</td>
<td>0.47</td>
<td>0.60</td>
<td>1.67</td>
</tr>
</tbody>
</table>

**CONCLUSIONS**

The thinned scenarios have a greater impact on linerboard mill operating profit than the unthinned scenarios due to a higher wood cost. In all four scenarios, a reduction in basis weight provides the greatest profit increase given current biotechnology capabilities. An increase in specific gravity followed by an increase in the growth rate provides the next best value to the mill, respectively. A reduction in wood lignin content provides the least incremental benefit to the mill because of the loss of bioenergy and need to purchase more power.

**Acknowledgements:** We thank B. Borders and B. Shiver of the Plantation Management Research Cooperative at the University of Georgia for providing unpublished growth and yield models used in the forest cost model. We thank members of Arborgen, International Paper, MeadWestvaco, and Weyerhaeuser for their review and suggestions to the models and approaches used for this research. We also thank the Sloan Industry Center for Paper Business and Industry Studies for supporting for this research.

**LITERATURE CITED**


What Do We Do to Improve Wood Properties in a Breeding Program?

Bailian Li, Fikret Isik and Barry Goldfarb

The Cooperative breeding programs in southern US have achieved considerable improvement in growth, stem form, and fusiform rust resistance over two cycles of selection and testing efforts in the last 49 years. Genetic improvement and intensive cultural treatment have improved productivity and reduced rotation ages, but wood quality traits have not been formally incorporated into most breeding programs. As a part of strategy to improve juvenile wood properties in plantation loblolly pine, a research project has been carried out to characterize the genetic variation in wood quality within the elite breeding populations based on the 2nd-generation progeny tests. The purpose is to develop efficient breeding methods for genetic improvement of wood quality traits and for operational deployment of these elite parents for desirable wood quality traits.

The genetic variation among genetically improved materials in wood properties was recently accessed for 179 elite parent trees that have best breeding values for growth, stem straightness, and fusiform rust resistance in the North Carolina State University-Industry Cooperative Tree Improvement Program for loblolly pine. Three best full-sib families were selected for each elite parent. Wood increment cores from five progeny were sampled. A total of 3000 trees were samples from Coastal, Piedmont and Northern breeding populations.

Breeding values for some juvenile wood properties were estimated, including cellulose, lignin content, fiber length and coarseness, and wood density. These are key wood properties that could be improved through selecting and breeding. Based on the phenotypic data, genetic variation and genetic correlations among traits were calculated. Different selection options are evaluated for improving growth and wood properties simultaneously.

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1 Department of Forestry and Environmental Resources, NC State University, Raleigh, NC
Poster Abstracts

&

Extended Abstracts
USDA Forest Service Cooperates with the USDA National Center for Genetic Resources Preservation on a Nationwide ex situ Plant Genetic Resources Conservation Plan

Jill Barbour

Germination Specialist, USDA Forest Service, National Tree Seed Laboratory
Dry Branch, Georgia
jbarbour@fs.fed.us, www.ntsl.fs.fed.us

The USDA Forest Service, National Tree Seed Laboratory and the USDA National Center for Genetic Resources Preservation (NCGRP) have entered into a cooperative agreement to begin conserving plant species through long-term seed storage. Seeds of all forest plant species, located on National Forests and private forest lands of the United States, are to be considered for storage. Seed collections of threatened and endangered plant species can be included with a permit from the US Fish and Wildlife Service. This agreement falls within the ex situ section of the Plant Genetic Resources Conservation Plan developed by the Forest Service.

Seeds are classified as base collections and are stored in a disaster proof cold storage vault at -18° Celsius. Active collections, which may include forest habitat, clone banks, seed orchards, or vegetative material, are maintained by their owners, and the owners have sole responsibility for any plant material distribution.

The National Tree Seed Laboratory is responsible for ensuring the seeds are clean, seed testing, cataloging seedlots, and packaging before the seeds are shipped to the long-term seed storage facility in Ft. Collins, Colorado. Each seedlot receives an accession number and seed information is stored in a database at the National Tree Seed Laboratory and becomes part of the USDA Germplasm Resources Information Network (GRIN), which can be accessed through the internet. Information on the active collections’ location and ownership is kept in order to replenish the accessions when seeds are depleted or lose vigor.

Seed packets with a minimum of 500 seeds and no more than 3,000 seeds per seedlot accession can be stored in the cold storage vault. A retesting schedule is developed for each accession depending on the number of seeds available. Due to the small amount of seed in storage, seed requests need to be filled through the active collections with the owner’s permission. No seed distribution of threatened or endangered species is allowed.

Once procedures are developed for long term seed storage, other vegetative material will be added to the agreement and incorporated in the ex situ plant genetic resources conservation plan.
Genetics of Resistance to *Phytophthora cinnamomi* in Chestnut

Mollie E. Bowles¹ and John Frampton²

¹Graduate Student and ²Associate Professor
Department of Forestry, N.C. State University, Raleigh, NC, USA

*Phytophthora cinnamomi* is the causal agent of ink disease, a deadly root-rot in susceptible chestnut trees. Finding non-lethal methods of selecting Phytophthora-resistant parent trees is emerging as an important issue to The American Chestnut Foundation. Current research involves using molecular genetic markers to verify the type of inheritance of Phytophthora resistance in chestnut and to develop a map of the resistance locus(i). If successful, this work will yield a reliable, non-lethal method of identifying Phytophthora-resistant seedlings; this knowledge is needed to design and implement resistance screening strategies aimed at developing breeding lines of American chestnut (*Castanea dentata*) that are resistant to both blight (caused by *Cryphonectria parasitica*) and Phytophthora.

Preliminary results using controlled inoculations of greenhouse-grown seedlings with *P. cinnamomi* suggest that Chinese chestnut (*Castanea mollisima*) is resistant while American chestnut (*C. dentata*) is largely susceptible. Interspecific F1 hybrids are also resistant indicating that genetic control of resistance is dominant. Resistance segregation patterns in B1 and B1-F2 crosses suggest control by a single locus; however, sample sizes to date have been relatively small. Results have also suggested that the genes for resistance to *P. cinnamomi* and *C. parasitica* are not closely linked. Screening and genetic analysis of two related B1 crosses in the Chinese ‘Mahogany’ line are currently underway (KY 115 x WB 348 and KY 117 x WB 348). Polymorphic AFLP bands that segregate with resistance will be used to map the resistance locus(i).
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, USA. 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. In a research assistance capacity, the RSC has played an important role in newly developed understanding of genetic interactions in the pine-fusiform rust pathosystem and will continue to do so in the foreseeable future. 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 pathogen variation or the effectiveness of fungicides. The RSC remains open to service screening work or research endeavors in an effort to improve forest health.
Genetic linkage maps have been constructed for many species of conifers, however none has been previously published for an *Abies* species. A genetic linkage map of Nordmann fir (*Abies nordmanniana* (Steven) Spach) open-pollinated family 9M was constructed using AFLP and RAPD markers developed from megagametophyte DNA. In all, 556 markers were grouped at LOD 5.0, $\theta = 0.30$ into 19 linkage groups which covered 1977 cM (Kosambi). Framework maps were ordered with interval support $\geq 3.0$ for each linkage group. Accessory markers were attached to the nearest framework marker based on LOD scores and recombination fractions. Significant linkage distortion (approximately 10% of the 556 markers based on a chi-square test at $p \leq 0.05$) from the expected 1:1 Mendelian segregation ratio was recognized. The genome size was estimated to be 2471 cM and this map provided 80% coverage of the genome. Attempts to map a trait locus (based on disease phenotype in an inoculated mapping population) for disease resistance to *Phytophthora cinnamomi* Rands were unsuccessful. This *Abies* linkage map should be important to the Christmas tree industry for marker-assisted selection of useful traits such as pest resistance, branching characteristics, height, growth rate, and post-harvest needle retention.
Towards an Information Concerning Genetic Parameters of European Larch 
(Larix decidua Mill.) on the Base of Progenies from Diallel Crossing 
Evaluation at the age of 31 and 34 Years

J. Frydl¹, J. Sindelar¹, P. Novotny¹

¹Scientific Specialist
Department of Forest Tree Biology and Breeding, 
Forestry and Game Management Research Institute, Jiloviste – Strnady, 
156 04 Praha 5 – Zbraslav, Czech Republic

There are some information concerning evaluation of research plots No. 24 and 25 (key numbers of VULHM research plots registration) located both in Pribyslav Forest Administration and VULHM Jiloviste – Strnady, Gamapole, presented in this report. Complex of 25 progenies of 5 clones from incomplete reciprocal crossing has been evaluated in research plots No. 24 – Pribyslav and No. 25 – Gamapole at the age of 31 and 34 years. Used clones are of Sudeten larch ecotype origin.

Controlled crossing has been realized, within the framework of an extensive breeding project, in spring 1967 in seed orchard at Sternberk, Czech Republic, by Dr. Sindelar (VULHM Jiloviste – Strnady). Research plot has been established in spring 1970 by double grid method in 4 replications. Measurement and evaluation of tested progenies at the age of 31 and 34 years have been oriented to height growth, DBH, volume production and stem form, too. Variability of measured and evaluated characteristics has been examined by analyses of variance (ANOVA) with three variability factors – influence of maternal and paternal clones, replications and respective interactions. There has been calculated heritability both on the base of average effects for individual source of variability, as well as for individual differences. Thus, heritability in both wider and narrow sense (H², h²) has been calculated. It was possible to characterize individual clones used for crossing by their combination ability according to Kraus’ method.

As for results of reciprocal crossings, there have been found statistically important differences, but average magnitudes of these crossings statistically have differed, just a less. E.g., there is progeny from controlled self-pollination represented in research plot No. 25 – Gamapole, too. In this case, depression of evaluated characteristics presupposed theoretically, did not prove as true. It is possible to expect another findings about European larch genetic parameters on the base of evaluation of another research plots established within the framework of extensive breeding projects realized in the Czech Republic both in 1967 – 1968 and in 1983, too.
NAC domain proteins are transcriptional regulators known to control multiple processes in plants including apical meristem maintenance and function. We predict that NAC family members also function in wood development by regulating vascular cambium maintenance, or wood cell division, growth, or differentiation. To test this idea, we are undertaking a functional analysis of NAC068, a poplar NAC domain protein originally identified in wood and vascular cambium EST collections. We are constructing NAC068 over-expressing and RNAi *Populus trichocarpa x deltoides* transgenic plants. These transgenic plants will be examined using microscopy for phenotypic effects in vascular cambium and xylem cell size, shape, and differentiation. We are also constructing NAC068 promoter-GUS fusion *Populus trichocarpa x deltoides* transgenic plants. These plants will be examined for GUS expression in vascular cambium and xylem tissues. Currently, NAC domain proteins have been studied in all meristematic tissues except for the vascular cambium. Therefore, this work will elucidate the role of NAC domain proteins in this important plant tissue.
Genetic Gain and Diversity of Seed Crops under Alternative Management Options in a Clonal Seed Orchard of *Pinus thunbergii*

K.S. Kang¹, D. Lindgren², T.J. Mullin³, W.-Y. Choi¹, S.-U. Han¹ and C.-S. Kim¹

There are various orchard management options to increase genetic gain while conserving genetic diversity, including selective harvesting, genetic thinning, and the combination of both. The practice of selective harvesting improves only the genetic contribution of seed parents, while both seed and pollen parents are improved with genetic thinning. For the production of improved seeds, many factors such as clonal genetic value, selection intensity, fertility variation and pollen contamination should be considered.

The objectives of this study were to evaluate the genetic gain and diversity of seed crops from a *P. thunbergii* clonal seed orchard under different management options, and to determine appropriate selection intensity (i.e., seed collection proportion, thinning rate) in selective harvest and genetic thinning. Additionally, the effects of gene migration from outside the orchard on genetic gain and diversity, and the consequences of alternative management options for seed production are also discussed. This paper reports on different alternatives for seed orchard management to increase genetic gain while maintaining adequate levels of genetic diversity.

**METHODS AND MATERIALS**

*Seed orchard description*

The clonal seed orchard of *P. thunbergii* is located on An-myun island, in the western part of Korea (lat. 36° 3’N, long. 126° 2’E and alt. 35m) and established in 1980. The orchard is planted at 5m x 5m spacing, with approximately equal numbers of grafts. Clonal fertility was estimated from assessments of strobilus production over nine consecutive years.

Additive genetic values for each orchard-parent genotype were obtained from open-pollinated progeny tests (represented by general combining ability, GCA). Parental GCA values for volume growth at age 12 were estimated by the method of best linear unbiased prediction (BLUP), based on height and diameter at breast height measured from field trials.

*Genetic gain and diversity*

Genetic value (G) and diversity (status number, $N_s$) of seed crops were estimated under four management alternatives, as follows:

1) Alternative 1: selective seed harvest from the best 50% of clones, without genetic thinning

\[
G = 0.5 N_r \sigma_s + MC, \quad N_s = \frac{4N_f N_f}{\Psi}\left[1 + (1-2M)(3-2M)N_f\right]
\]

2) Alternative 2: 50% genetic thinning, removing clones with inferior genetic values

\[
G = (1-M) N_r \sigma_s + MC, \quad N_s = \frac{N_f}{(1-M)^2 \Psi}
\]
3) Alternative 3: 75% genetic thinning, a more intensive genetic thinning than Alternative 2
4) Alternative 4: 20% selective harvest after 50% genetic thinning

\[ G = \frac{\left(\hat{i}_{N_{f}, N_{o}} \pm (1-2M)\hat{i}_{N_{f}, N_{o}}\right) \sigma_{A}}{2} + MC \]

\[ N_{f} = \frac{4N_{f}N_{o}}{\Psi[N_{o} + (3-8M + 4M^2)N_{f}]} \]

where \( i \) is selection intensity; \( \sigma_{A} \) is additive genetic variance; \( M \) is gene migration; \( C \) is inferiority of contaminating pollen; \( N \) is the census number; \( N_{f} \) is the number of seed parents and \( N_{m} \) is the number of pollen parents. \( \Psi \) is the sibling coefficient describing fertility variation among clones.

**RESULTS AND DISCUSSION**

Selective seed harvest (Alternative 1), genetic thinning (Alternatives 2 and 3) and the combination of both options (Alternative 4) increased genetic gain over the initial orchard condition (i.e., before thinning). The increase was, however, coupled with a decrease in status number. Genetic gain was highest and diversity (status number) lowest in Alternative 3 under both gene migration scenarios.

In Alternative 1, seeds are collected only from clones with higher genetic values, while the entire orchard remained intact. Selection is only for the seed parents, and not for pollen parents. Under genetic thinning options (Alternatives 2 and 3), seeds are harvested from all of the remaining clones after thinning; thus, as opposed to Alternative 1, both pollen and seed parents are improved by the irreversible removal of clones with lower genetic values. The remaining selected clones contribute to the seed crop as both male and female parents. Selection, therefore, occurs for both parents at the same time and with the same intensity.

In Alternative 4, all clones remaining after the 50% genetic thinning serve as the pollen source, while seeds are harvested from a subset of clones consisting of the top 20% with the highest genetic value. Thus, selection occurs twice; the first is simultaneous selection against inferior clones (pollen and seed parents) at the time of thinning, and the second is the selection against the subset of the remaining clones acting as seed parents only.

![Graph](image)

Figure 1. Relation between genetic value and relative status number \((N_{f})\) for selective harvest.
(left) and genetic thinning (right) with different levels of foreign gene migration ($M$). In these examples, fertility variation ($\Psi$) was set to 2 and contamination inferiority ($C$) equal to $-1$.

Relative gain from orchard management varied with the proportion of selected and/or thinned clones (Fig. 1). The increase in genetic value was not linear relative to the proportion of selected and/or thinned clones in selective harvest and genetic thinning options. Genetic thinning gave greater gain than selective harvest at the same intensity, but this was accompanied by a greater loss of status number.

Strong genetic thinning (e.g., Alternative 3) would remove many clones and subsequently result in a substantial loss of diversity and seed production. However, the combination of genetic thinning and selective harvest would be better than selective harvest alone, because the usual purpose of a seed orchard is to obtain maximum gain with some appropriate level of genetic diversity (Lindgren and El-Kassaby, 1989). Seed production will also recover in a few years as tree crowns develop and occupy the openings created by tree removal (Kang et al., 2003).

**REFERENCES**


Application of Sprinkler System for Control of Cone Insects in a Conal Seed Orchard of Pinus koraiensis

Kab-Yeon Lee¹, Kyung-Jin Cho¹, Sang-Bae Chung² and Jin-Taek Kang¹

The cone insects decreased cone growth and seed production by feeding flesh fruits and by obstructing seed formation. As seed orchards are established as pure forests, there is more damage by insects than in a mixed forest. As Carbofuran (Furadan 3% granule) has been buried around trees to control cone insects, it causes environment problem due to a long stay in the soil. As a replacement for this problem, the need to apply the pesticides, such as Diflubenzuron and Cyfluthrin that cause little pollution and toxicity, was suggested. However, the information on spray periods and spray frequency of the above pesticides is lacking in seed orchards. Therefore the sprinkler system was installed in a Korean pine (Pinus koraiensis) seed orchard, which was managing by the Korea Forest Seed Research Center. The purpose of study was to investigate the effect of sprinkler system and pesticides on the control of cone, and effect on the labor reduction for seed orchard management.

METHODS AND MATERIALS

Korean pine (Pinus koraiensis) is mainly attacked by cone insects such as pine twig moth (Gravitarmata margarotana) and pine shoot borer (Dioryctria abietella). Two kinds of pesticides, Diflubenzuron (25% wettable powder) and Cyfluthrin (2% Emervifiable concentrate) were selected for this study, because they were known to be less harmful compared to Carbofuran, which had been used in seed orchard in Korea. The chemicals were applied for 15 day or 20 day intervals from early June to early August. The sprinkler system was consisted of a water tank, a pesticides tank, a water pump, a pressure pump and the self-generator that supplies the power and the connecting hose and sprinkler nozzle, which carry pesticides to each tree. The spraying nozzles were fixed above the crown as to insecticides dropped onto the cones. Cones from each of the treated groups were collected every month after spraying pesticides to examine cone insect control by sprinkler system.

RESULTS AND DISCUSSION

1. Cone insect control effects by pesticides and spraying intervals

While the damaged cone rate was 67.5% in control group, the cone damage was reduced to 14.8% and 9.1% after spraying Diflubenzuron and Cyfluthrin, respectively. The insect control effects of each pesticide were 86.5% for Cyfluthrin and 78.1% for Diflubenzuron. There were no clear difference between 15 day intervals and 20 day intervals. So it would be more effective to spray 20 days intervals to reduce the costs.

¹ Korea Forest Seed Research Center, Korea Forest Research Institute, 670-4 Suhoeri, Suanbo, Chungju, Chungbuk, 380-940, Republic of Korea
² Department of Forest Science, University of Sanji, 660 Woosandong, Wonju, Kangwon, 220-702, Republic of Korea
2. The control effects by the spraying periods
As shown in Figure 2, the cone insect control effects of pesticide by spraying periods for Korean pine cone insect pests were 93.0% and 94.9% during June and July, however, the control effects reduced a little on September. As the pesticides were applied until early August, the activities of larvae were more increased after that time. So it was suggested that if the effective control would be expected, the application of pesticides should be continued by September.

3. The effective cone insect control management
The present cone insect control in the seed orchard is to bury Carbofuran (3% granule) around individual tree. However due to the lack of labor by the concentration of population to the urban area, it became difficult to manage cone insect by manpower. So the sprinkler system was introduced to the seed orchard.
Figure 3. The comparison of cost for the cone insect control by sprinkler system and manpower

Even though the cost of insect pest control seemed to be higher at the beginning compared to the burying method due to the expense of initial installing system, the gap was reduced gradually as time went by. Five years after installing, the costs for two cone insect control methods became almost the same (Figure 3). In addition, the sprinkler system had advantages to reduce the labor and time because it could spray pesticides large areas in a few minutes. The amounts of pesticides needed for each hectare by the sprinkler system were 200g for Diflubenzuron and 8,000ml for Cyfluthrin, while the burying method for Carbofuran was 501kg (Table 1). The sprinkler system used less pesticide than the burying method, however, its insect control effect was much more increased. Furthermore it was expected that effect on the soil environment would be decreased.

Table 1. The amount of spraying insecticides by treatment each hectare

<table>
<thead>
<tr>
<th>Applying method</th>
<th>Insecticide</th>
<th>Sprayed amount/ha</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sprinkler</td>
<td>Diflubenzuron</td>
<td>3,200 g</td>
</tr>
<tr>
<td></td>
<td>Cyfluthrin</td>
<td>8,000 ml</td>
</tr>
<tr>
<td>Burying in soil</td>
<td>Carbofuran</td>
<td>501 kg</td>
</tr>
</tbody>
</table>
Black willow (*Salix nigra* Marsh.) is the largest and only commercially important willow species in North America. Since this fast-growing tree thrives on floodplains throughout the Eastern United States, it is a potential candidate for phytoremediation of polluted soils. In this study, we initiated adventitious shoot-producing cultures from unexpanded inflorescence explants excised from dormant buds. Explants collected from three source trees growing in Athens, GA were cultured on woody plant medium (WPM) supplemented with three plant growth regulator (PGR) treatments: (1) 0.1 mg/l thidiazuron (TDZ), (2) 0.5 mg/l 6-benzoaminopurine (BAP), or (3) 1 mg/l BAP. Callus induction was observed within three weeks following culture initiation. All three PGR treatments induced adventitious bud formation from all three genotypes. The percentage of explants producing buds ranged from 20% to 92%, depending on genotype and treatment. Although most of the TDZ-treated inflorescences produced buds, these failed to elongate into shoots. Buds on explants treated with BAP elongated into shoots, which were easily rooted in potting mix under high humidity. Shoot regeneration was strongly genotype-dependent, ranging from 7% to 36%, while the number of shoots per explant varied from 1 to 5. The ability of willow inflorescences to produce adventitious shoots may make them suitable targets for *Agrobacterium*-mediated transformation with heavy metal resistance genes for phytoremediation.
Embryogenic cultures of loblolly pine (*Pinus taeda*), slash pine (*Pinus elliottii*), longleaf pine (*Pinus palustris*) and slash pine x longleaf pine hybrids were initiated from immature seeds on an initiation medium containing 3 mg/l 2,4-D and 0.5 mg/l BA. Embryogenic cultures proliferated and somatic embryos developed, matured and germinated following a modified protocol and media originally developed for radiata pine (*Pinus radiata*) somatic seedling production. A discrete, light-sensitive pre-germination stage and a later germination (radicle emergence) stage were identified by the differential response of somatic embryos to light of different wavelengths. Different light quality treatments were applied during the pre-germination and germination steps, using cool white fluorescent bulbs and/or light-emitting diodes (LEDs). In general, red wavelengths provided by LEDs during these steps resulted in higher frequencies of somatic embryo germination and conversion, longer tap roots and more first order lateral roots than the standard cool white fluorescent treatments or treatment with blue wavelengths from LEDs. In addition, exposure to red light allowed germination of somatic embryos of some clones that failed to produce any germinants under fluorescent light. Germination and conversion were further enhanced by sequential application of cool white fluorescent light and red light, resulting in up to 100% germination in one experiment.
A Microsatellite Assessment of Population Architecture and Gene Flow in Fraser Fir

Kevin M. Potter¹, Sedley Josserand³, John Frampton², and C. Dana Nelson⁴

¹Graduate Student, ²Associate Professor, ³Research Technician, ⁴Project Leader,
Department of Forestry and Environmental Resources, North Carolina State University, Raleigh, North Carolina, USA
Southern Institute of Forest Genetics, USDA Forest Service, Saucier, Mississippi, USA

Fraser fir (Abies fraseri (Pursh) Poir.) is a coniferous tree species endemic to a handful of the highest ridge systems in the Southern Appalachians of North Carolina, Tennessee and Virginia. In addition to its ecological importance, Fraser fir is a species with great economic significance in the region, where it is the foundation of North Carolina’s $100 million annual Christmas tree industry.

The six major and three minor disjunct natural populations of Fraser fir have been isolated since the end of the late-Wisconsin glacial period more than 10,000 years ago. Using microsatellite markers designed specifically for this species, we found a relatively small amount of genetic differentiation among most Fraser fir populations (average $F_{ST}$ per locus $\approx 0.04$). This may indicate the presence of fairly extensive gene flow among populations, in the form of pollen wind dispersal over long distances (5-60 km). Alternatively, it may suggest that inadequate time has passed to allow for significant genetic separation among the populations.

Several results appear to point to the presence of pollen-mediated gene flow between populations, the effect of which decreases as the distance between populations increases:

1) Pairwise $F_{ST}$ and genetic distance values showed that populations nearer to each other were generally, but not always, more genetically similar than those more distant.

2) The mean number of alleles per locus was negatively correlated with population isolation.

3) The most isolated population, Mount Rogers in Virginia, was the most genetically differentiated and had the smallest mean number of alleles per microsatellite locus.

These results will be useful in the assembly of a gene conservation plan for Fraser fir, which has experienced severe mortality from the infestation of the balsam woolly adelgid (Adelges piceae Ratz.), an exotic insect from Europe.
Impact of Crop Tree Release on Wood Properties of Pitch x Loblolly Pine Hybrids

M. L. Jackson1, T. R. Fox2

1Research Technician, 2Associate Professor, Department of Forestry, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24060, USA

Wood density and growth was determined for loblolly pine (*Pinus taeda*), pitch pine (*P. rigida*), and F2 pitch x loblolly pine hybrids which underwent crop tree release by crown touching in 1987. The study site was located in Patrick county in the Piedmont of Virginia. Released trees were compared to trees which remained at the original planting density, 6.6 x 6.6 foot spacing. Pitch x loblolly pine hybrids were compared to pitch pine and loblolly pine to determine species differences. Released trees were free to grow on all sides.

Species effects were significant among pitch, loblolly, and pitch x loblolly pine from 1987 to 2003. Diameter and height growth in the hybrids was similar to that in loblolly pine, which were significantly higher than pitch pine. Density of mature wood of hybrids and loblolly pine were greater than pitch pine; 557, 534 and 498 kg/m³ respectively for the three species. Crown touching release significantly increased growth in the pitch x loblolly and loblolly pine. Released hybrids did not differ in mature wood density from loblolly pine, but remained higher than pitch pine. No significant species x release interaction existed.

Within the pitch x loblolly pine hybrids, a few significant family differences were detected. Families 54 x 15A, 57 x 15A, and 78 x 22 were significantly denser than 51 x 23 or KOR P x L, but did not differ from any other families. Family 59 x 7-56 was denser than 51 x 23, but again did not differ from any other family. Among all hybrid families, most individual wood density, and ultimately mechanical properties related to wood density, fell within specified limits for southern pine sawtimber.
Expression Patterns of *Pinus* Defense Genes During Host-Pathogen Interactions

A.M. Morse\(^1\), K.E. Smith\(^2\), D.A. Huber\(^1\), S.F. Covert\(^3\), K.D. Hunt\(^4\), C.D. Nelson\(^5\), and J.M. Davis\(^6\)

\(^1\) Research Faculty, \(^6\) Associate Professor
School of Forest Resources and Conservation, University of Florida, Gainesville, FL
\(^2\) Biological Sciences Technician
Southern Institute of Forest Genetics, USDA Forest Service, Gainesville, FL
\(^3\) Associate Professor, \(^4\) Graduate Student
Daniel B. Warnell School of Forest Resources, University of Georgia, Athens, GA
\(^5\) Research Geneticist and Project Leader
Southern Institute of Forest Genetics, USDA Forest Service, Saucier, MS

*Cronartium quercuum* infects the stems and branches of certain pines and leads to the development of fusiform rust disease. Gall formation is associated with alterations in host metabolism, morphology and development as well as fungal propagation, all of which are likely to be associated with changes in host gene expression. Genetic resistance to fusiform rust exists and controlled inoculation studies have revealed the evolution of specific (gene-for-gene) resistance in pine-*Cronartium* interactions. In slash pine, families can not only be classified as susceptible or resistant to fusiform rust based on gall presence/absence, but also on gall morphology. Small galls on young slash pines often ‘disappear’ as the tree matures, a phenomenon that has been referred to as partial resistance.

To identify candidate genes for conditioning fusiform rust gall morphology and possibly partial resistance, we probed our pine defense-associated array with cDNA derived from RNA isolated from either large or small galls on slash pine. The arrays were statistically analyzed as six pairwise contrasts to distinguish between host tissue and pathogen regulation. A total of 158 genes were identified as significantly regulated in one or more of the contrasts.

Hierarchical cluster analysis comparing transcript abundance for the probes significantly expressed in one or more of the contrasts identified five prominent clades. Two of the clades represent host tissue regulation and contain genes differentially expressed between xylem and phloem. The remaining three clades represent different patterns of pathogen regulation.

A number of genes significantly regulated by fusiform rust had been previously identified as significantly regulated by pitch canker, a disease incited by the necrotrophic fungal pathogen *Fusarium circinatum*. Of particular interest is the fact that a number of defense genes were regulated in opposite directions in the two disease states. Genes encoding chitinase and peroxidase enzymes were suppressed in fusiform rust galls and induced in pitch canker lesions. This result is consistent with the emerging view that biotrophic pathogens, such as *C. quercuum*, actively suppress host defenses in order to complete their lifecycles and cause disease.
Invertases as Genetic Determinants of Sink Strength

P.N. Bocock¹, L.F. Huang¹, K.E. Koch²,³, J.M. Davis²,⁴

¹Graduate Student, Plant Molecular and Cellular Biology Program; ²Professor, Plant Molecular and Cellular Biology Program; ³Professor, Department of Horticultural Sciences; ⁴Professor, School of Forest Resources and Conservation;
University of Florida, Gainesville, FL, USA

Current population growth combined with increasing development and urbanization worldwide is putting strain on the terrestrial ecosystem’s main carbon sink, forests. One way to combat this increasing demand of decreasing forest resources is to manipulate the carbon allocation patterns in trees to direct carbon into the most desirable organs such as stems to meet industrial demands, or roots to help increase the long term carbon storage capacity in the soil. Plants utilize carbon by partitioning the reduced carbon obtained through photosynthesis into different locations within the cell and subsequently allocating it to sink tissues throughout the plant. We are utilizing *Populus* as a model system in which to study invertase and its role in sink strength determination with the aim of applying this knowledge to tree breeding and genetic modifications. Using the newly sequenced poplar genome, we have identified eight acid invertase family members through amino acid sequence similarity searches. Three of these family members encode invertases targeted to the vacuole, while the other five invertases are targeted to the apoplast. With only two exceptions, poplar invertases share the intron/exon structure generally conserved in plants of seven exons separated by six introns. *PtIVR1*, a vacuolar invertase apparently lacks introns and constitutes the first putative intronless invertase found to date. *PtIVR4*, another vacuolar invertase is missing the conserved mini-exon NDPN. Although the absence of this exon is unusual, it is not unprecedented in plant invertases.

As invertase is found in three subcellular locations, we are also taking a transgenic approach in order to elucidate the individual roles of these invertases in sink strength determination. Three groups of transgenic plants have been made expressing Suc2, an invertase from yeast, in the apoplast, vacuole, or cytosol. These transgenics are predicted to have altered sink strengths and/or partitioning phenotypes, and will be used in grafting experiments. These grafts will enable us to mimic the effects of organ specific promoters and thus alter the sink strength of specific organs. We predict these altered sink strengths will lead to altered wood development, storage capacity, and secondary metabolite components.

Though the invertase family has been well characterized in Arabidopsis, it is not possible to determine their respective poplar orthologs based on tissue expression patterns or sequence identity. To address this problem, we are using a microcolinearity approach by identifying invertase gene neighbors on the poplar chromosome with identical gene neighbors on the Arabidopsis chromosome. We are developing a robust statistical procedure for determining the significance of colinearity, which should in turn help us gain insights into the function and evolution of invertase genes.
List of Participants
Bob Abt  
NC State University  
Raleigh, NC 27695

Phone:  
Fax:  
ob_abt@ncsu.edu

John C Adams  
Louisiana Tech University  
PO Box 10138  
Ruston, LA 71272

Phone: 318-257-4985  
Fax: 318-257-5061  
jadams@lans.latech.edu

Joshua P Adams  
Box 9681 College of Natl Resources  
Mississippi State, MS 39762

Phone: 662-325-3635  
Fax:  
jpa18@msstate.edu

Peter Ades  
University of Melbourne  
Forest & Ecosystem Science  
Parkville, VIC 3010  
Australia

Phone: 61-3-83445036  
Fax:  
petera@unimelb.edu.au

Henry Amerson  
NC State University  
2500 Partners II Bldg.  
Raleigh, NC 27695

Phone: 919-513-0012  
Fax: 919-515-7801  
amerson@unity.ncsu.edu

Gisele Andrade  
University of Georgia  
Warnell School of Forest Resources  
Athens, GA 30602

Phone: 706-542-1264  
Fax:  
gisele@uga.edu

Michael J Aspinwall  
NC State University  
3310 Walnut Creek Pkwy Apt L  
Raleigh, NC 27606

Phone: 919-851-9986  
Fax:  
mjaspinw@ncsu.edu

Ryan Atwood  
MeadWestvaco  
PO Box 739  
Ravenel, SC 29470

Phone: 8436-556-8391  
Fax:  
raa4@meadwestvaco.com

Claudio Balocchi  
BioForest SA  
Casilla 70-C  
Concepcion, VIII Reg.  
Chile

Phone: 56-41-390438  
Fax: 56-41-390439  
cbalocchi@arauco.cl

Brian Baltunis  
University of Florida  
PO Box 110410  
Gainesville, FL 32611

Phone: 352-846-0894  
Fax: 352-846-1277  
baltunis@ufl.edu

Jill Barbour  
USDA Forest Service  
2675 Riggins Mill Rd  
Dry Branch, GA 31020

Phone: 478-751-3551  
Fax:  
jbarbour@fs.fed.us

Tori Batista  
NC State University  
1019 Biltmore Hall  
Raleigh, NC 27695

Phone: 919-515-6074  
Fax: 919-515-3169  
tori_batista@ncsu.edu

Andy Benowicz  
CellFor  
PO Box 133  
Brentwood Bay, BC V8M1R3  
Canada

Phone: 250-544-0787x249  
Fax: 250-544-0769  
abenowicz@cellfor.com

Onesphore Bitoki  
Virginia Dept of Forestry  
11301 Pocahontas Trail  
Providence Forge, VA 23140

Phone: 804-966-2201  
Fax: 804-966-9801  
ones.bitoki@dof.virginia.gov

Tom Blush  
MeadWestvaco Forest Research  
PO BOX 1950  
Summerville, SC 29484  
USA

Phone: 843-851-4768  
Fax: 843-875-7185  
thomas.blush@meadwestvaco.com

Philip N Bocock  
1226 NW 33RD AVE  
Gainesville, FL 32609

Phone: 352-846-0881  
Fax:  
pnbocock@ufl.edu

Mollie E Bowles  
NC State University  
209 Lafayette Rd  
Raleigh, NC 27604

Phone: 919-624-2367  
Fax:  
mebowle2@ncsu.edu

Jean Brouard  
Isabella Point Forestry Ltd  
331 Roland Rd  
Salt Spring Island, BC V8K1V1  
Canada

Phone: 250-653-2335  
Fax:  
johnbro@saltspring.com

Richard L. Bryant  
International Paper Company  
719 Southlands Rd.  
Bainbridge, GA 39819

Phone: 229-246-3642  
Fax: 229-243-0766  
richard.bryant@ipaper.com

Tom Byram  
WGFTIP  
Bldg 1042 Agronomy Rd TAMU 2585  
College Station, TX 77843-2585

Phone: 979-845-2523  
Fax: 979-845-3272  
tbyram@tfs.tamu.edu
Keith Byrd  
MeadWestvaco  
PO Box 1950  
Summerville, SC 29483  
Phone: 843-851-4605  
Fax: 843-875-7185

Michael Cheveldave  
Syngenta  
3054 Cornwallis Rd  
Research Triangle Park, NC 27709  
Phone: 919-541-8531  
Fax: 919-541-8585  
mcheveldave@shaw.ca

Vincent Chiang  
NC State University  
Campus Box 7247  
Raleigh, NC 27695  
Phone: 919-513-0098  
Fax: 919-513-7801  
vchiang@ncsu.edu

Wan-Yong Choi  
Korea Forest Research Institute  
44-3 Omokcheon, Kwonsun  
Suwon, Kyonggi 441-350  
Republic of Korea  
Phone: 82-31-290-1120  
Fax: 82-31-292-8468  
wychoi@foa.go.kr

Tommy Conwell  
Gulf States Paper Corp  
PO Box 48999  
Tuscaloosa, AL 35404  
Phone: 205-562-5497  
Fax: 205-562-5496  
tconwell@gulf-states.com

Jeffery Dean  
University of Georgia  
Warnell School of Forest Resources  
Athens, GA 30602  
Phone: 706-542-1710  
Fax: 706-542-8356  
jeffdean@uga.edu

Jeff Donahue  
PO Box 345  
Hagan, GA 30429  
Phone: 912-739-4613x11  
Fax: 912-739-1861  
jeffrey.donahue@ipaper.com

Phil Dougherty  
MeadWestvaco  
PO BOX 1950  
Summerville, SC 29483  
Phone: 843-851-4750  
Fax: 843-875-7185  
pmd6@meadwestvaco.com

Bill S Dvorak  
CAMCORE, NC State University  
1110 Grinnells Lab, Box 7626  
Raleigh, NC 27695  
Phone: 919-515-6424  
Fax: 919-515-6430  
bill_dvorak@ncsu.edu

Jennifer L Emerson  
NC State University  
Campus Box 8002  
Raleigh, NC 27695  
Phone: 919-388-4312  
Fax: jlemersn@ncsu.edu

Veronica I Emhart  
University of Florida  
134 Newins-Ziegler Hall  
Gainesville, FL 32611  
Phone: 352-846-0903  
Fax: vemhart@ufl.edu

Elhan S Ersoz  
UC-Davis  
Dept of Plant Sciences, 1 Shield Av  
Davis, CA 95616  
Phone: 530-304-2933  
Fax: eersoz@ucdavis.edu

Phil Dougherty  
MeadWestvaco  
PO BOX 1950  
Summerville, SC 29483  
Phone: 843-851-4750  
Fax: 843-875-7185  
pmd6@meadwestvaco.com

Barbara Crane  
USDA Forest Service  
1720 Peachtree Rd NW  
Atlanta, GA 30309  
Phone: 404-347-4039  
Fax: 404-347-4154  
barbaracrane@fs.fed.us

Jennifer L Emerson  
NC State University  
Campus Box 8002  
Raleigh, NC 27695  
Phone: 919-388-4312  
Fax: jlemersn@ncsu.edu

Carlyle Franklin  
NC State University  
Campus Box 8006  
Raleigh, NC 27695-8006  
Phone: 919-515-3852  
Fax: 919-515-7559  
carlyle_franklin@ncsu.edu
<table>
<thead>
<tr>
<th>Name</th>
<th>Company/Institution</th>
<th>Address</th>
<th>Phone</th>
<th>Email</th>
<th>Fax</th>
</tr>
</thead>
<tbody>
<tr>
<td>Randy Johnson</td>
<td>USDA Forest Service</td>
<td>3200 SW Jefferson Way, Corvallis, OR 97331</td>
<td>541-750-7290</td>
<td><a href="mailto:randyjohnson@fs.fed.us">randyjohnson@fs.fed.us</a></td>
<td></td>
</tr>
<tr>
<td>Don Kaczmarek</td>
<td>ArborGen</td>
<td>PO Box 840001, Summerville, SC 29484</td>
<td>843-832-5073</td>
<td><a href="mailto:djkacz@arbogen.com">djkacz@arbogen.com</a></td>
<td></td>
</tr>
<tr>
<td>Kyu-Suk Kang</td>
<td>Korea Forest Research Institute</td>
<td>44-3 Omokcheon, Kwonsun, Suwon, Kyonggi 441-350, Republic of Korea</td>
<td>82-31-290-1120</td>
<td><a href="mailto:kangks@foa.go.kr">kangks@foa.go.kr</a></td>
<td></td>
</tr>
<tr>
<td>Gogce C Kayihan</td>
<td>Wasatch State Forestry Board</td>
<td>4339 SW 21st Ln, Gainesville, FL 32607</td>
<td>352-371-3313</td>
<td><a href="mailto:gogce@ufl.edu">gogce@ufl.edu</a></td>
<td></td>
</tr>
<tr>
<td>Bob Kellison</td>
<td>Institute of Forest Biotechnology</td>
<td>920 Main Campus Dr Ste 101, Raleigh, NC 27606</td>
<td>919-424-4464</td>
<td><a href="mailto:bob_kellison@forestbiotech.org">bob_kellison@forestbiotech.org</a></td>
<td></td>
</tr>
<tr>
<td>John S King</td>
<td>Michigan Technological University</td>
<td>1400 Townsend Dr, Houghton, MI 49931</td>
<td>906-482-6303x13</td>
<td><a href="mailto:jsking@mtu.edu">jsking@mtu.edu</a></td>
<td></td>
</tr>
<tr>
<td>Harry Labhart</td>
<td>Gulf States Paper Corp</td>
<td>PO Box 48999, Tuscaloosa, AL 35404</td>
<td>205-373-8515</td>
<td><a href="mailto:hlabhart@gulf-states.com">hlabhart@gulf-states.com</a></td>
<td></td>
</tr>
<tr>
<td>Julietta Labhart</td>
<td>Gulf States Paper Corp</td>
<td>202 Coyote Ln, Gordo, AL 35466</td>
<td>205-364-0260</td>
<td></td>
<td>205-373-3443</td>
</tr>
<tr>
<td>Clem Lambeth</td>
<td>Weyerhaeuser Company</td>
<td>810 Whittington Ave, Hot Springs, AR 71901</td>
<td>501-624-8510</td>
<td><a href="mailto:clem.lambeth@weyerhaeuser.com">clem.lambeth@weyerhaeuser.com</a></td>
<td></td>
</tr>
<tr>
<td>Sam B Land</td>
<td>Mississippi State University</td>
<td>531 Chuck Wagon Dr, Brandon, MS 39042</td>
<td>601-591-5307</td>
<td><a href="mailto:sam_land@bellsouth.net">sam_land@bellsouth.net</a></td>
<td></td>
</tr>
<tr>
<td>Gregory N Leach</td>
<td>International Paper Company</td>
<td>4025 Hwy 178, Jay, FL 32565</td>
<td>850-675-0929x121</td>
<td><a href="mailto:greg.leach@ipaper.com">greg.leach@ipaper.com</a></td>
<td></td>
</tr>
<tr>
<td>Kab-Yeon Lee</td>
<td>Korea Forest Research Institute</td>
<td>670-4 Soohoi, Suanbo, Choongiu, Choongbuk 383-940, Republic of Korea</td>
<td>82-43-8503-3001</td>
<td><a href="mailto:leeky99@foa.go.kr">leeky99@foa.go.kr</a></td>
<td></td>
</tr>
<tr>
<td>Baolian Li</td>
<td>NC State University</td>
<td>Box 8002, Raleigh, NC 27695</td>
<td>919-515-6845</td>
<td><a href="mailto:baolian_li@ncsu.edu">baolian_li@ncsu.edu</a></td>
<td></td>
</tr>
<tr>
<td>Laigeng Li</td>
<td>NC State University</td>
<td>840 Main Campus Dr, Raleigh, NC 27606</td>
<td>919-513-0075</td>
<td><a href="mailto:laigeng_li@ncsu.edu">laigeng_li@ncsu.edu</a></td>
<td></td>
</tr>
<tr>
<td>Larry H Lott</td>
<td>USDA Forest Service</td>
<td>23332 Hwy 67, Saucier, MS 39574</td>
<td>228-832-2747</td>
<td><a href="mailto:lholt@fs.fed.us">lholt@fs.fed.us</a></td>
<td></td>
</tr>
<tr>
<td>Victor Sierra Lucero</td>
<td>Forestal Mininco SA</td>
<td>AVDA Alemania #751, Los Angeles, Chile</td>
<td>56-43-405350</td>
<td><a href="mailto:victor.sierra@forestatl.cmmpc.cl">victor.sierra@forestatl.cmmpc.cl</a></td>
<td></td>
</tr>
<tr>
<td>Mac Lupold</td>
<td>Lupold Consulting Inc</td>
<td>224 Chestnut Ferry Rd, Camden, SC 29020</td>
<td>803-713-0422</td>
<td><a href="mailto:mac.c@charter.net">mac.c@charter.net</a></td>
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</tr>
<tr>
<td>Al Lyons</td>
<td>Hancock Forest Management</td>
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<tr>
<td>John Major</td>
<td>Canadian Forest Service</td>
<td>1350 Regent St, Fredericton, NB E3B 5P7, Canada</td>
<td>506-452-3262</td>
<td><a href="mailto:jmajoar@nrccan.gc.ca">jmajoar@nrccan.gc.ca</a></td>
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</tr>
<tr>
<td>Early McCall</td>
<td>Rayonier</td>
<td>PO Box 819, Yulee, FL 32041</td>
<td>904-225-5393</td>
<td><a href="mailto:early.mcall@rayonier.com">early.mcall@rayonier.com</a></td>
<td></td>
</tr>
<tr>
<td>Maria McDougall</td>
<td>NC State University</td>
<td>118G Wolf Village Bldg F, Raleigh, NC 27695</td>
<td>256-653-7505</td>
<td><a href="mailto:mmcdougall35762@yahoo.com">mmcdougall35762@yahoo.com</a></td>
<td></td>
</tr>
<tr>
<td>Name</td>
<td>Affiliation</td>
<td>Address</td>
<td>Phone</td>
<td>Email</td>
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<tr>
<td>Steven E McKeand</td>
<td>NC State University</td>
<td>Box 8002, Raleigh, NC 27695-8002</td>
<td>919-515-6073</td>
<td><a href="mailto:steve_mckeand@ncsu.edu">steve_mckeand@ncsu.edu</a></td>
<td></td>
</tr>
<tr>
<td>Alex Medina</td>
<td>University of Florida</td>
<td>PO Box 110410, Gainesville, FL 32601</td>
<td>352-262-3578</td>
<td><a href="mailto:alexfitz@ufl.edu">alexfitz@ufl.edu</a></td>
<td></td>
</tr>
<tr>
<td>Scott Merkle</td>
<td>University of Georgia</td>
<td>School of Forest Resources, Athens, GA 30602</td>
<td>706-542-6112</td>
<td><a href="mailto:merkle@forestry.uga.edu">merkle@forestry.uga.edu</a></td>
<td></td>
</tr>
<tr>
<td>Larry G Miller</td>
<td>WGFTIP</td>
<td>Bldg 1042 Agronomy Rd, TAMU 2585</td>
<td>979-845-2523</td>
<td><a href="mailto:lmiller@tfs.tamu.edu">lmiller@tfs.tamu.edu</a></td>
<td></td>
</tr>
<tr>
<td>Larry K Miller</td>
<td></td>
<td>230 Sarah Rd, Kennewick, WA 99338</td>
<td>509-627-4103</td>
<td><a href="mailto:tregyn1@yahoo.com">tregyn1@yahoo.com</a></td>
<td></td>
</tr>
<tr>
<td>Susan E Moore</td>
<td>NC State University</td>
<td>Campus Box 8003, Raleigh, NC 27695</td>
<td>919-515-3184</td>
<td><a href="mailto:susan_moore@ncsu.edu">susan_moore@ncsu.edu</a></td>
<td></td>
</tr>
<tr>
<td>Daniel Morrow</td>
<td>International Paper</td>
<td>4025 Hwy 178, Jay, FL 32565</td>
<td>850-675-0929 x122</td>
<td><a href="mailto:dan.morrow@ipaper.com">dan.morrow@ipaper.com</a></td>
<td></td>
</tr>
<tr>
<td>Alison Morse</td>
<td>University of Florida</td>
<td>118 Newins-Zeigher Hall, POB 110410, Gainesville, FL 32611</td>
<td>352-846-0883</td>
<td><a href="mailto:ammorse@ufl.edu">ammorse@ufl.edu</a></td>
<td></td>
</tr>
<tr>
<td>Nicholas Muir</td>
<td>Temple-Inland</td>
<td>229 N Bowie St, Jasper, TX 75951</td>
<td>409-383-2552</td>
<td><a href="mailto:nicholasmuir@templeinland.com">nicholasmuir@templeinland.com</a></td>
<td></td>
</tr>
<tr>
<td>Tim Mullin</td>
<td>NC State University</td>
<td>Campus Box 8002, Raleigh, NC 27695</td>
<td>919-515-3644</td>
<td><a href="mailto:tim_mullin@ncsu.edu">tim_mullin@ncsu.edu</a></td>
<td></td>
</tr>
<tr>
<td>Campbell J Nairn</td>
<td>University of Georgia</td>
<td>Forest Res. 3-211, DW Brooks Dr, Athens, GA 30602</td>
<td>706-542-1885</td>
<td><a href="mailto:jnairn@forestry.uga.edu">jnairn@forestry.uga.edu</a></td>
<td></td>
</tr>
<tr>
<td>Dana Nelson</td>
<td>USDA Forest Service</td>
<td>23332 Hwy 67, Saucier, MS 39574</td>
<td>228-832-2747 x209</td>
<td><a href="mailto:dananelson@fs.fed.us">dananelson@fs.fed.us</a></td>
<td></td>
</tr>
<tr>
<td>Ronald Overton</td>
<td>USDA Forest Service</td>
<td>Purdue Univ., Pender Hall, West Lafayette, IN 47907</td>
<td>765-496-6417</td>
<td><a href="mailto:roverton@fs.fed.us">roverton@fs.fed.us</a></td>
<td></td>
</tr>
<tr>
<td>John Pait</td>
<td>CellFor</td>
<td>75 5th St NW Ste 321, Atlanta, GA 30308</td>
<td>404-526-6176</td>
<td><a href="mailto:jpait@cellfor.com">jpait@cellfor.com</a></td>
<td></td>
</tr>
<tr>
<td>Liliana Parisi</td>
<td>University of Florida</td>
<td>PO Box 110410, Gainesville, FL 32611</td>
<td>352-846-0894</td>
<td><a href="mailto:lparisi@ufl.edu">lparisi@ufl.edu</a></td>
<td></td>
</tr>
<tr>
<td>Gary F Peter</td>
<td>University of Florida</td>
<td>PO Box 110410, Gainesville, FL 32611-0410</td>
<td>352-846-0896</td>
<td><a href="mailto:gfpeter@ufl.edu">gfpeter@ufl.edu</a></td>
<td></td>
</tr>
<tr>
<td>Russell Pohl</td>
<td>Georgia Forestry Commision</td>
<td>5645 Riggins Mill Rd, Dry Branch, GA 31020</td>
<td>478-751-3520</td>
<td><a href="mailto:gpohl@gfc.state.ga.us">gpohl@gfc.state.ga.us</a></td>
<td></td>
</tr>
<tr>
<td>Kevin M Potter</td>
<td>NC State University</td>
<td>Campus Box 8002, Raleigh, NC 27695</td>
<td>919-515-6074</td>
<td><a href="mailto:kpotter@unity.ncsu.edu">kpotter@unity.ncsu.edu</a></td>
<td></td>
</tr>
<tr>
<td>Greg Powell</td>
<td>University of Florida</td>
<td>PO Box 110410, Gainesville, FL 32611</td>
<td>352-846-0895</td>
<td><a href="mailto:glpowell@ufl.edu">glpowell@ufl.edu</a></td>
<td></td>
</tr>
<tr>
<td>Robert C Purnell</td>
<td>International Paper</td>
<td>719 Southlands Rd, Bainbridge, GA 39819</td>
<td>229-246-3642</td>
<td><a href="mailto:robert.purnell@ipaper.com">robert.purnell@ipaper.com</a></td>
<td></td>
</tr>
<tr>
<td>Fred Raley</td>
<td>WGFTIP</td>
<td>Bldg 1042 Agronomy Rd TAMU 2585</td>
<td>979-845-2523</td>
<td><a href="mailto:fraley@tfs.tamu.edu">fraley@tfs.tamu.edu</a></td>
<td></td>
</tr>
</tbody>
</table>
Ross Whetten
NC State University
Campus Box 8002
Raleigh, NC 27695-8002
Phone: 919-515-7578
Fax: 919-515-8149
ross_whetten@ncsu.edu

Carol Young
USDA Forest Service
1579 Brevard Rd
Asheville, NC 28806
Phone: 828-667-5089 x102
Fax: 828-665-2187
cyoung@fs.fed.us

Qibin Yu
NC State University
Box 8002
Raleigh, NC 27695-8002
Phone: 919-515-5029
Fax:
qibin_yu@ncsu.edu

Barbara Zobel
Raleigh, NC
Phone:
Fax:

Bruce J Zobel
Professor Emeritus, NC State University
PO BOX 37398
Raleigh, NC 27627
Phone:
Fax:
bjzobel@unity.ncsu.edu