Proceedings

29th Southern Forest Tree Improvement Conference

Tree Improvement in North America: Past, Present, and Future

Joint Meeting

Western Forest Genetics Association
&
Southern Forest Tree Improvement Committee

Galveston, TX
June 19-22, 2007

Proceedings of the 29th Southern Forest Tree Improvement Conference

Tree Improvement in North America: Past, Present, and Future, WFGA/SFTIC Joint Meeting, Galveston, TX. June 19-22, 2007

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Foreword

The first ever, joint meeting of the Southern Forest Tree Improvement Conference and the Western Forest Genetics Association was held June 19-22 in Galveston, TX. This meeting, the 29th SFTIC and the 51st gathering of the WFGA, focused on our shared history, compared and contrasted present strategies and attempted to chart our collective futures. Invited speakers for the opening session included James B. Hull who addressed the changing face of forestry in Texas and the nation, Steven McKeand and Glenn Howe who spoke on the history of tree improvement in the southeast and the Pacific Northwest, respectively and Bradley St. Clair who outlined novel uses of tree improvement data to address climate change issues. During the final plenary session, John Helms addressed the rapidly changing social environment in which foresters must function, Robert Kellison and Adam Costanza each spoke about the adoption of forest biotechnology, and Clark Binkley addressed the valuation and impact of forestry research. During the intervening two days of concurrent sessions, participants were privileged to hear 52 contributed papers and view 11 posters on topics that included selection, progeny testing, marker aided breeding, genetic diversity and gene conservation, physiology, and climate change as well as a variety of other topics.

Two changes were made to accommodate the different meeting styles of the two groups. Publication of papers and extended abstracts was optional. Articles submitted by their authors for publication at the time of the meeting are included in the first part of this proceeding. A complete set of pre-meeting abstracts are included as a separate section. The second change was that the two groups combined awards for outstanding contributions to the conference. All participants were eligible regardless of their affiliation. The awards presented included the following:

The **Tony Squillace Award** given by the SFTIC for the overall best oral presentation based on content, style and use of visual aids was awarded to **Alex Mangini** for the presentation entitled "A south-wide rate test of esfenvalerate (Asana® XL) for cone and seed insect control in southern pine seed orchards."

The **Bruce Zobel Award** given by the SFTIC for an outstanding oral presentation by a student was presented to **Shiqin Zu** for the presentation entitled "Genetic diversity and hybridization in natural stands of shortleaf pine (*Pinus echinata* Mill.) and loblolly pine (*Pinus taeda* L.)".

The **Critchfield Award** presented by the WFGA for an outstanding oral presentation by a student was made to **Daniel Chmura** for the presentation "Integration of crown morphology and leaf-level physiology as a tool for explaining differences in aboveground productivity among elite families of loblolly and slash pine."

The **Belle Baruch Foundation Award,** normally made by SFTIC for the best student poster was presented jointly this year by the WFGA and SFTIC. The award was made to **Lisa Worthen** for the poster entitled "Male genotype influences seed set and seed size in controlled crosses of American chestnut (*Castanea dentate* [Marsh] Borhk)."

We would like to thank Penny Sowell, who made all of the physical arrangements and coordinated the registration procedure, breaks, and the banquet. Larry Miller and Fred Raley also aided during registration and organized the presentations with the audio visual staff at the hotel.

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Photo: David Reid

The 118 conference participants represented 11 countries and 19 states within the US.

Table of Contents

Presentations

Poster Abstracts

Pre-Meeting Abstracts

Invited Papers

Contributed Papers

Presentations

Southern Pine Tree Improvement – A Living Success Story

Steven E. McKeand[1], Bruce J. Zobel[2], Thomas D. Byram[3], and Dudley A. Huber[4]

The U.S. South can boast of the productivity, quality, and value gains realized from plantation forestry that silviculturists and tree breeders have developed over the past 50+ years. From the beginning of tree improvement programs in the region, the focus has been on selecting, breeding, testing, and planting trees that provide landowners with the greatest return on their investments (e.g. Zobel 2005). The agrarian culture, available land, favorable political and social attitudes towards production forestry, productive soils, and a moderate climate all favor the growth of plantation forestry in the South. The trend in recent years has been for increasing intensity of forest management of these acres (Allen et al. 2005). With global demand for timber products increasing at the same time as the area of the world's forests is decreasing, increased productivity of southern plantations has local, regional, national, and global implications. These plantations help provide timber to meet increasing demands while simultaneously reducing the environmental footprint of industrial forestry by growing more wood on less area.

Foresters in the southern United States are responsible for over 75% of the nation's tree planting, and over 95% of these seedlings are genetically improved loblolly and slash pines (McKeand et al. 2003). Deployment practices such as planting only the best open-pollinated (OP) families to the best sites are resulting in dramatic increases in productivity. Increased resistance to fusiform rust disease, especially in slash pine, has also had major impacts on plantation yields (Vergara et al. 2004).

In the early 2000's, 59% of the loblolly and 43% of the slash were annually deployed as OP families by companies and small landowners (McKeand et al. 2003). Over the last 10 years, seed orchard managers have had great success in developing methods to mass produce full-sib families for operational planting. The gains from improved quality and yield are very impressive when both the female and male parents are selected (e.g. Bramlett 1997, Bridgwater et al. 1998, Jansson and Li 2004). In 2007, the companies that have been mass producing full-sib seedlings for operational planting were surveyed to determine how many Mass Control Pollinated (MCP or CMP) seedlings have been produced. Since 2000, Over 94 million full-sib family seedlings have been planted in the South (Figure 1). Propagation of selected clones has also become a reality via somatic embryogenesis (SE), and the gains to be realized from planting these outstanding genotypes are tremendous (e.g. Pait 2005). To date, almost 10 million seedlings of somatic embryogenic clones have been planted, and the numbers are increasing annually.

Over the last 50+ years, forest products companies, government agencies, landowners, and most recently institutional investment firms such as Timber Investment Management Organizations (TIMOs) and Real Estate Investment Trusts (REITs) have invested millions of dollars in tree breeding and seed orchard management. The value to landowners and the returns on these

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investments have been tremendous. We recently surveyed members in the three southern tree improvement cooperatives to determine the members' annual investment in breeding and seed production. Approximately $14 million is spent annually to breed and produce improved loblolly and slash pine seeds. Annual seedling production is about 1 billion seedlings or about 1.8 million acres at 566 trees per acre average for an annual investment of less than $8 per acre for genetically improved loblolly and slash pine seedlings. Some simple economic analyses using publicly available growth and yield models show that planting improved seedlings on site index 70 land ($SI_{25years}$ = 70') on a 30 year rotation with two thinnings and a mix of products will yield a PV of $15 per acre per percent improvement in growth. Assuming a conservative 30% value gain over unimproved, this is an astounding PV to the landowner of $450 per acre for planting better seedlings that can be obtained at very modest marginal costs.

There are many ways to evaluate the investments in southern tree improvement (e.g. Porterfield et al. 1975, van Buijtenen 1984, Talbert et al. 1985, McKeand et al. 2006). To date, every analysis we have seen shows that the returns on investment in breeding and planting genetically improved loblolly and slash pines are very high. Even with the changes in land ownership and the loss of the integrated forest products companies (Byram et al. 2005), we are optimistic that tree improvement and intensive silviculture will continue to be mainstays of forest management in the South. The challenges to the large tree improvement cooperatives are numerous, but support is still strong, and gains continue to be made.

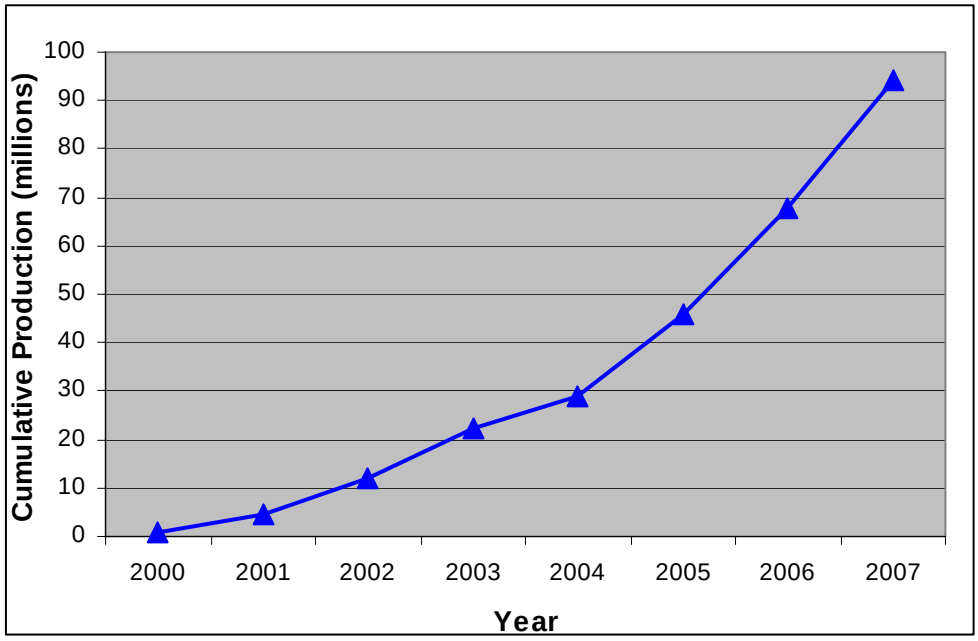

Figure 1. Operational planting of full-sib families of loblolly pine has become a reality. Since 2000, over 94 million full-sib family seedlings have been planted by landowners in the southern United States.

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LITERATURE CITED

Allen, H.L., T.R. Fox, and R.G. Campbell. 2005. What is ahead for intensive pine plantation silviculture in the South? South. J. Appl. For. 29:62-69.

Bramlett, D.L. 1997. Genetic gain from mass controlled pollination and topworking. J. For. 95(3): 15-19.

Bridgwater, F.E., D.L. Bramlett, T.D. Byram, and W.J. Lowe. 1998. Controlled mass pollination in loblolly pine to increase genetic gains. The Forestry Chronicle 74(2): 185-189.

Byram, T. D., T.J. Mullin, T. L. White, and J.P. van Buijtenen. 2005. Tree Improvement: Alternative visions for the next decade. Southern Journal of Applied Forestry 29(2): 88-95.

Jansson, G. and B. Li. 2004. Genetic gains of full-sib families from disconnected diallels in loblolly pine. Silvae Genetica 53(2):60-64.

McKeand, S.E., R.C. Abt, H.L. Allen, B. Li, and G.P. Catts. 2006. What are the best loblolly pine genotypes worth to landowners? J. For. 104:352-358.

McKeand, S.E., R.P. Crook, and H.L. Allen. 1997. Genotypic stability effects on predicted family responses to silvicultural treatments in loblolly pine. South. J.Appl. For. 21:84-89.

McKeand, S., T. Mullin, T. Byram, T. White. 2003. Deployment of genetically improved loblolly and slash pine in the South. J. For. 101(3): 32-37.

Pait, J. 2005. Clonal forestry: out of the lab, finally. P. 16, In: Proc. 28th Southern Forest Tree Impr. Conf., Raleigh, NC. 203p.

Porterfield, R.L., B.J. Zobel, and F.T. Ledig. 1975. Evaluating the efficiency of tree improvement programs. Silv. Gen. 24:33-44.

Talbert, J.T., R.J. Weir, and R.D. Arnold. 1985. Costs and benefits of a mature first-generation loblolly pine tree improvement program. J. For. 83:162-166.

van Buijtenen, J.P. 1984. Genetic improvement of forest trees through selection and breeding. P. 457-488 in Wenger, K.F. (ed.) Forestry Handbook, 2nd Edition. John Wiley & Sons, NY.

Vergara, R., T.L. White, D.A. Huber, B.D. Shiver, and D.L. Rockwood. 2004. Estimated realized gains for first-generation slash pine (*Pinus elliottii* var. *elliottii*) tree improvement in the southeastern United States. Can. J. For. Res. 34:2587-2600.

Zobel, B.J. 2005. Our roots: the start of tree improvement in the South. P. 1-5, In: Proc. 28th Southern Forest Tree Impr. Conf., Raleigh, NC. 203p.

Forest Biotechnology: Its Place in the World

Robert Kellison[1]

Abstract: Forest biotechnology is on the cusp of scientific breakthrough. Great advances have been made in two of the three components of the science: asexual propagation and association genetics. The third component, genetic engineering, is in various stages of development. Genome sequencing, a segment of association genetics, has been done for only one forest tree, *Populus tricocarpa*, but a concerted effort is being made to gain the resources for sequencing loblolly pine (*Pinus taeda*). Sequencing of that species, with a genome about seven times larger than that of the human genome, was once thought to be a prodigious task, but with advances in technology the prognosis is that the job can be completed in two to three years at a cost of $25 to $30 million. However, sequencing is only a start. The association and function of the genes, the bread and butter of the effort, can only be determined by meticulous laboratory and field testing.

Progress has been made in engineering the traits for tree growth, wood properties, cold, drought and herbicide tolerance, and insect resistance. However, the only genetically engineered tree species that has been released for commercial use is Bt-resistant European black poplar in China. There is opposition to commercial application of trees, engineered specifically for fast growth and increased yields, by those whose stance is that the value accrues only to 'big companies'. It will remain for traits that have broad societal benefits, such as conservation of threatened and endangered species and biofuels, for acceptance to be gained. Even then some countries will benefit before others, not because of the science, which is universal, but because of organized resistance. Regardless, forest biotechnology continues to progress and, combined with conventional tree breeding programs, will be hugely beneficial in housing and feeding the world's population in centuries to come.

INTRODUCTION

The objective of this paper is to give a thumbnail sketch of forest biotechnology at its present state of development and tie it to its background and to the future. As is becoming evermore obvious, forest biotechnology will become commonplace in our lifetime. The voice of present-day adversaries of the science will soothed because the results of technology will produce benefits on which society is dependent while real and perceived adverse effects to ecological systems and the environment will be neutralized. The catalyst for those accomplishments will be the cellulosic products from trees that will range from transportation fuels to foodstuff. As with all advancing technology, the developed economies will be the first to reap the benefits of the science, but only a short time will elapse before the developing economies of the world will also enjoy the benefits.

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Tree Improvement

Planting of forest trees has a historic basis in every society where native forests once covered the terrain: Middle East, Western Europe, northern Africa, Asia inclusive of Japan and India. It wasn't until the last vestiges of the 19th Century that the practice became highly successful on a broad scale. Until that time, the emphasis was on planting native species, but with the source or provenance of the seeds being generally ignored. Beginning about 1900 a major effort was devoted to planting trees of exotic origin in places far removed from their indigenous populations. Examples include the movement of sitka spruce (*Picea sitkensis*) and Douglas-fir (*Pseudotsuga menziesii*) from the Pacific Northwest (U.S.) to Western Europe, radiata pine (*P. radiata)* from California to Australia, Chile and New Zealand, yellow pines from the southern U.S. and Mexico to southern Africa, and the shuffling of poplars (*Populus* spp.) to and from all parts of the world. In more recent times, consistent with attention being given to species and provenances, there has been a mass movement of the North American southern pines (*P. ellottii, P. taeda)* being established in extensive plantations in southern South America and southern China. To that list, the eucalypts (*Eucalyptus* spp.) and on a more limited basis, acacias (*Acacia* spp.) have been dispersed to nearly every country within the Tropics and Warm Temperate zones of the world (Burdon and Libby 2007).

Along with the mass movement, tree improvement programs, inclusive of selection of the species and provenances most fit for the area being planted, were initiated. Refinement of tree improvement programs with selection and interbreeding of plus-tree phenotypes had its origin in Scandinavia during the World War II years. It was patterned on the principle of agronomic-crop breeding. Beginning in the 1950s that effort was copied in nearly every region of the world where plantation forestry was a priority, and that included indigenous as well as exotic forestry. A microcosm of the indigenous-forest effort exists right here in the southern United States where successful tree improvement programs of loblolly and slash pines, and to a limited extent of other conifers, are housed at the universities of Texas A& M, Florida, and North Carolina State. In only the third generation of breeding, huge genetic gains have been achieved in adaptability, volume production, tree form, pest resistance and wood properties (McKeand *et al.* 2006).

Forest Biotechnology

A limitation of rapid genetic gain in tree improvement programs is the long time to sexual maturity and the comparable time for genetic testing of the selections and their offspring. Enter forest biotechnology, which is an extension of tree improvement with the work being done at the gene rather than the tree level. In this treatment of the subject, biotechnology has three components: asexual propagation, genomics and genetic engineering (Yanchuk 2001).

Vegegative propagation. Vegetative propagation, a form of asexual propagation, has been with us for thousands of years, as evidenced by the inhabitants of Mesopotamia ripraping the banks of the Tigris and Euphrates Rivers and their tributaries with clonal poplars and willows to help control flooding and soil salinization. Those genera are relatively easy to vegetatively propagate, but not so with many others species of both angiosperms and gymnosperms. Some of the species of commercial importance, such as loblolly pine, can be vegetatively propagated in their juvenile state, but once they progress toward the equivalence of puberty in humans they become progressively harder to clone, and at some point most genotypes become recalcitrant to vegetative propagation.

In case of uncertainty about the value of vegetative propagation, clonal forestry offers significant genetic gain in uniformity that translates into added volume gains, ease in plantation management and manufacturing efficiency. In addition, the technology is absolutely essential if genetic engineering is to be accomplished in forest trees. As opposed to agronomic crops, which can be inbred and then outcrossed to distribute a single genetic modification into its progeny, forest trees are generally resistant to inbreeding and, as a result, are powerless to set seeds with the intact genetic modification. The only procedure for genetic engineering to be successful in forest trees is for each genotype to be modified at the embryonic stage.

To overcome the recalcitrance of selected genotypes, somatic embryogenesis offers a viable alternative and, in the process, it adds the benefit of 'having your cake and eating it too'. That benefit is the ability to store a portion of the manufactured embryos in cryopreservation while another portion is used to test the genetic worth of the clone of which the plantets are a part. Somatic embryogenesis is accomplished by selecting plant material in the blastocyst stage of the embryo, *i.e.,* undifferentiated tissue. By coddling the tissue through various laboratory procedures, mature embryos can be produced *en masse,* all of the same genotype. The naked embryos, *i.e.,* without a seed coat can be coaxed to germinate and develop into plantlets suited for plantation establishment (Pait 2004).

Genomics. Genomics is the study of the arrangement of genes on chromosomes. The exercise in genomics that most people can associate with is the huge effort expended on sequencing the human genome. Despite the millions of dollars and multitude of years spent on the project the job is only partially complete. Knowing the location of a gene on a specific chromosome has little value until the function of that gene is known. The correlation between the genes and their functions and interactions are being slowly developed, but it will take years before the task is complete.

Other organisms with smaller and less complicated genomes have been or are being sequenced, most notably *Arabidopsis thaliana*, a species of the mustard family. Good correlations exist between the genotypes of the least and the most advanced plants, and also between the least and most advanced animals. To that end, *A. thaliana* has served as a good model for rice (*Oryza sativa*), wheat (*Triticum* spp.), corn (*Zea mays*), soybean (*Glycine max*) and other food, forage, and fiber crops, inclusive of trees. To date the only forest tree species to have its genome sequenced is black cottonwood (*Populus tricocarpa*). Efforts are now in progress to have the genome of a conifer sequenced. The species selected for that endeavor is loblolly pine because it has the most advanced quantitative breeding base of any conifer in the world. The advancement results from the ongoing tree improvement programs of the three major cooperative programs in the South, each of which has been in progress in excess of 50 years. The genotyping of a tree of this species will serve as the template for all other pines and, in fact, all other conifers because of genome similarity of the gymnosperms.

The number of genes in loblolly pine is about seven times larger than that of the human genome. With the time and money expended for the much smaller human genome, why take on the colossal task for a conifer tree? The answer, in addition to the available genetic base from tree breeding and the evolutionary aspects of a long-lived organism, is that the technology for gene

sequencing has developed so rapidly within recent years that the job will require only a fraction of the time and resources that it would have consumed several years ago. The initial estimate was that the five-year effort would cost $130 million for partial sequencing and related research. Now the estimation is that complete sequencing can be done for $25 million in half the projected time. One day we will know the gene or genes responsible for fusiform rust (*Cronartium fusiforme*), for example, and will be able to silence those genes by over expression or under expression or by inserting a gene from an unrelated plant, such as *A. thaliana,* into loblolly pine to give resistance to the disease.

Even today genomics is having a positive effect on plant breeding through at least two technologies: marker aided selection (MAS) and quantitative trait loci (QTL). Through quantitative genetics, MAS has application for a number of traits, including vegetative propagation. Even though the gene or genes for rooting, for example, are unidentified the association can be made by the presence of a marker gene in a genotype that roots well. In like vein, QTLs operate on the principle that the location of a gene or genes on various chromosomes might account for a percentage of the gain to be achieved by their presence. The scenario might be that the identified genes account for only 46% (or some other number) of the variation of the trait, but that assurance is money in the bank when dealing with recombinant genetics.

Genetic engineering. Genetic engineering is the only component of forest biotechnology that has generated opposition to the science. A major reason for the concern is the visualized grotesqueness of the offspring that might be generated from the insertion of a gene from a mouse into a tree, for example. In reality, it will be a gene from a soil bacterium such as *Bacillus thuringiensis* that connotes insect resistance or *Agrobacterium tumefaciens* that gives resistance to glyphosate, the active ingredient in Roundup Ready® that will be the transferred gene. Both of the bacterial genes for insect resistance and glyphosate tolerance have been successfully inserted in trees for experimental purposes. Gene insertions or modifications have also been made for lignin modification (Chiang 2003), tree growth, and cold and drought.

The only genetically engineered tree species that has been released for commercial use is *Populus nigra* in China, with the inserted gene being *B. thuringiensis.* Initial reports from 2003 revealed that about 400 acres of such trees had been planted, but the information since then of additional area established or success of the original plantations has been muted (Wang 2004)

Of The Future

I predict that, in the U. S., genetically engineered trees will not find a use for increased growth in the foreseeable future. Increased tree growth and yield will primarily come from tree improvement programs, inclusive of asexual propagation. To that end, tree improvement and forest biotechnology fit together like hand and glove. Genetic engineering is nothing without asexual reproduction and asexual reproduction is nothing without breeding programs to produce ever-improved genetic recombinants.

I further predict that the first benefits from genetic engineering to commercial forestry in our society will be for insect resistance and tolerance to glyphosate. The research has been largely completed for those traits with the use of *B. thuringiensis* and *A. tumefaciens.* Beyond that, value-added products such as pharmaceuticals, carbon sequestration, bioremediation, pulp and

paper manufacture, and most importantly conservation of threatened and endangered tree species and bioenergy will gain priority over traits specific to growth and yield. The concentration on species conservation and bioenergy will conclude this paper.

Tree conservation. A number of tree species in the U.S. are threatened by extinction from attacks by foreign pests, either insects or diseases. The tree species that has attracted most attention as threatened or endangered is American chestnut (*Castanea dentata).* Occupying a range throughout the Appalachian Mountains, the eastern portions of the Central and Lake States and into southern Ontario with a tree count in the overstory crown class of approximately three billion, the species was killed to ground level during a 40-year period beginning about 1900. The pest was a fungus (*Cryphonectria parasitica*) of Oriental origin that was accidentally introduced to New York in the late 1800s on ornamental stock of Chinese chestnut (*C. mollisima*) (Sisco 2004). Attempts to find trees resistant to the fungus and to introduce hypovirulent strains of the fungus have largely met with failure even though some research on those subjects is still in progress.

The program that is showing progress for restoration of the species was initiated in 1989 by The American Chestnut Foundation (TACF) with the goal of producing a tree with 15/16 American chestnut and 1/16 Chinese chestnut through a backcross breeding program (Sisco 2004). The program is nearing its completion through the fourth backcross.

To supplement the backcross breeding program, research has been initiated to identify the two or three genes in Chinese chestnut that connote resistance to the disease. Funded by the National Science Foundation, the anticipated identification of the causal genes will allow the results to be used in a conventional tree improvement program or it will allow insertion of the genes for resistance from Chinese chestnut into American chestnut. Utilizing forest biotechnology to restore the icon of forest tree species to its natural range will be of significant positive social and environmental value.

Bioenergy. Unsettled conditions in the Middle East, Nigeria and Venezuela, from whence the U. S. obtains most of its imported crude oil, have driven the price upwards to $60/barrel. At that price, alternative fuels such as ethanol begin to look very promising for transportation needs.

Corn has been the sole feedstock for commercial ethanol manufacture in the U. S. largely because of favorable legislation that imposes $0.51 per gallon tariff on imported ethanol and a federal subsidy of $0.54 per gallon. The federal subsidy is complemented by an added subsidy in a number of states in the Midwest where corn is the major agricultural crop. The economics has driven the price of corn from $2.00/bushel in March 2006 to futures of $4.38/bushel in March 2007. With the incentives and crude oil at $55 to $60 per barrel ethanol producers could pay from $3.65 to $4.54 per bushel of corn and still realize a 12% return on investment. (Runge and Senauer 2007).

The escalation in corn prices has adversely affects the price of ethanol, but it also causes a ripple effect in the economy. Specifically impacted are the cattle, hog and poultry producers, and the effect is beginning to play out in food prices. In addition to the economy being adversely affected, the realists are questioning the environmental impacts of a corn crop that is estimated to

cover 90.5 million acres in 2007, the highest acreage since World War II. Lands with low soil fertility, poor internal drainage, high erosion potential, and forest conversion are being brought into the program. The majority of those lands will require added nutrients, a product of fossil fuels, to make them economically productive. In addition, monocultural farming with corn planted year-on-year requires additional nutrients and greater application of pesticides to control competition from weeds, insects and diseases than occurs with rotational cropping.

The limitations of corn for ethanol have resulted in the search for alternative crops. Switchgrass (*Panicum vergatum)* has received considerable attention as a cellulosic resource that could help solve the energy dilemma, as have trees and other plants. The limitation to those crops is the time and cost of extracting the sugars from the cellulose of plant fibers and then the fermentation of the sugars to ethanol. To date, a half dozen or so enzymes were needed for the sugar extraction even before the fermentation process. As plant biotechnology intensifies, however, more efficient enzymes are being discovered or designed. Organizations such as Novozymes and Genencor are devoting their whole energy to the identification and manufacture of such enzymes. It will be only a matter of time until the extraction and fermentation process is greatly expedited.

Switchgrass and plant residues such as corn stover and wheat straw are championed by some enthusiasts as the cellulosic materials for ethanol production. Acknowledging the benefits from those materials, they have the serious limitations of collection and storage on an annual basis. The calculation of one ethanol plant manager was for one tractor-trailer load of switchgrass every six minutes to equate to the capacity of the plant for corn ethanol production (Runge and Senauer 2007). With such limitations, the subject turns to trees. A major advantage of trees is that they store on the stump. No need for huge storage facilities of the material that is essential for annual crops.

Research is in progress to increase the cellulose content of trees with a commensurate reduction of lignin. Positive results have been achieved with aspen (*Populus tremuloides)* (Chiang 2003) and research is in progress to do the same with a conifer, such as loblolly pine. The latter species with its wide adaptability to diverse sites and a wide geographic base, which ranges from Maryland and Delaware south to Florida and west to Texas makes it a suitable candidate for bioenergy plantations. The vision is that those plantations will surround a decommissioned pulp mill, many of which dot the southern landscape, that will be retrofitted for ethanol production. Estimates are that the retrofitting of such a pulp mill could be done at about 20% of the cost of a new ethanol plant (Kelley 2006). After all, the facilities are in place for everything but the cellulosic conversion: wood supply, chipping operation, power plant, digesters, refiners, transportation, etc.

Even with the advances being made in converting cellulose to fuel the benefits need not stop there. The host of products that can be made from fossil fuels can also be made from plant material. And, on top of that, the sugars from plants, especially from genetically engineered trees with their elevated cellulosic content, are candidates for the manufacture of foodstuff. One day we will be competing with termites for the carbohydrates sequestered in trees.

Summary

Biotechnology is the coming science of the 21st century. The involved scientists have only nipped the tip of the iceberg in ferreting out the cause and effect of genes that inhabit every living thing on earth. Forest biotechnology will parallel the advances made in the farming of flora and fauna on which civilization depends, but it will do so only in conjunction with related sciences such as conventional tree breeding.

The emphasis in the short run will be concentrated on disciplines that benefit society as a whole. In this treatise, I've addressed conservation of threatened and endangered species and bioenergy as the two disciplines that will most rapidly get public support. Engineered trees for faster growth and greater yields per unit area of time will, in the short run, continue to get negative publicity because of the perception that the benefits will accrue to 'big companies'. Following acceptance of specialty crops for the good of the whole will set the stage for acceptance of value-added products such as trees engineered for fast growth, tolerance to adverse sites, and exotic plantations. The application of forest technology will first accrue to the owners of large industrial tracts of land, then to the REITs and TIMOs, and lastly to the non-industrial private landowners.

Because of the internationalization of biotechnology one country will not benefit to the exclusion of another one so far as the science is concerned. The difference in application will come in acceptance. The adversaries of the science will delay acceptance in some countries while it is readily implemented elsewhere. Worldwide acceptance will come only when there is worldwide need.

LITERATURE CITED

Burdon, Rowland D. and William J. Libby. 2006. Genetically Modified Forests: From Stone Age to Modern Biotechnology. Forest History Society Issues Series. Durham, NC. 79 pp.

Kelley, Steve. 2006. Forest Biorefineries: Reality, Hype or Something in Between? Paper Age (March/April): 46-48.

Lu, Shanfa, Ying-Hsuan Sun, Rui Schi, Catherine Clark, Laigeng Li and Vincent L. Chiang. 2005. Novel and mechanical stress-responsive microRNAs in *Populus trichocarpa* that are absent from Arabidopsis. The Plant Cell 17:2186-2203.

McKeand, Steven E., Robert C. Abt, H. Lee Allen, Bailian Li, and Glenn P. Catts. 2006. What are the best loblolly pine genotypes worth to landowners. JF 104(7):352-358.

Pait, J. A. 2004. Propagation and cloning. Forest Biotechnology in Latin America. In: Proceedings, Workshop Biotechnologia Forestal, Univrsidad de Concepción, Concepctión, Chile. pp 41-48.

Runge, C. Ford and Benjamin Senauer. 2007. How Biofuels Could Starve the Poor. Foreign Affairs. Council on Foreign Relations. Washington, DC. 5 pp.

Sisco, P. H. 2004. Breeding blight-resistant American chestnut trees. J. Am. Chestnut Foundation XVIII(1):12-16.

Wang, Huoran. 2004. The state of genetically modified forest trees in China. In: Preliminary Review of Biotechnology in Forestry, Including Genetic Modification. Forest Genetic Resources Working paper 59. Forestry Depart., FAO, Rome. 11 pp.

Yanchuk, A. D. 2001. The role and implications of biotechnological tools in forestry. Unasylva 204(52):53-61.

Increasing the Efficiency of Breeding Without Breeding Through Phenotypic Pre-selection in Open Pollinated Progenies

Yousry A. El-Kassaby[1] and Dag Lindgren[2]

Abstract: Unlike classical methods used by forest tree breeders that rely on pre-determined mating designs to construct pedigreed materials for testing and selection, the concept of Breeding Without Breeding (BWB: El-Kassaby et al., 2007) was introduced to allow the assemblage of full-sib (FS) and half-sib (HS) families from seed orchards' wind-pollinated offspring without conducting any crosses. The method relies on using highly informative molecular markers (e.g., SSRs) and pedigree reconstruction methods to unravel the genetic relationship among individual's offspring. Fingerprinted large wind-pollinated families are required to allow the assemblage of FS and HS families with reasonable size for field testing. To maximize the method's efficiency while minimizing methodological efforts, we propose the inclusion of phenotypic pre-selection from existing open-pollinated family tests to substantially reduce the number of fingerprinted individuals. The proposed application (merging mass selection with BWB) capitalizes on the efficiency of mass-selection in identifying groups of superior individuals and the use of pedigree reconstruction to delineate the paternal parents of the phenotypically selected individuals, hence complete pedigree tracking. Methods for expanding the BWB utility through either slight modification of the production populations' structure or the introduction of desired genotypes through pollen management techniques are presented. The most breath-taking possibility offered by BWB is offering opportunities to abandon not only clonal archives and crosses but also field testing. If both maternal and paternal pedigrees could be reconstructed in commercial plantations originating from a seed orchard, the same type of mass selection could be performed for the orchard's clones and long term breeding could be practiced in commercial plantations rather than of investing efforts and resources on specialized progeny test trials.

Keywords: Breeding Without Breeding, pre-selection, mass selection, pedigree reconstruction, seed orchards

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CONCEPTUAL APPROACH

The concept of Breeding Without Breeding (BWB: El-Kassaby et al., 2007) is extended to include phenotypic pre-selection from open-pollinated family tests to maximize the method's efficiency while minimizing methodological efforts. The proposed application (merging mass selection with BWB) capitalizes on the efficiency of mass-selection in identifying groups of superior individuals (Lindgren and Wei 1994, 2007) and the use of pedigree reconstruction to delineate the paternal parents of the phenotypically selected individuals, hence complete pedigree tracking.

The BWB scenario presented by El-Kassaby et al. (2007) starts with the selection of the top 15 performing individuals form a 40-parent seed orchard to from a nucleus population. The nucleus population consisted of a half-diallel (105 full-sib families, each with 100 individual) that was created after pedigree reconstruction and the top 40 individuals (forward selection) from the progeny test were selected to establish the new orchard using group-merit selection (Figure 1)

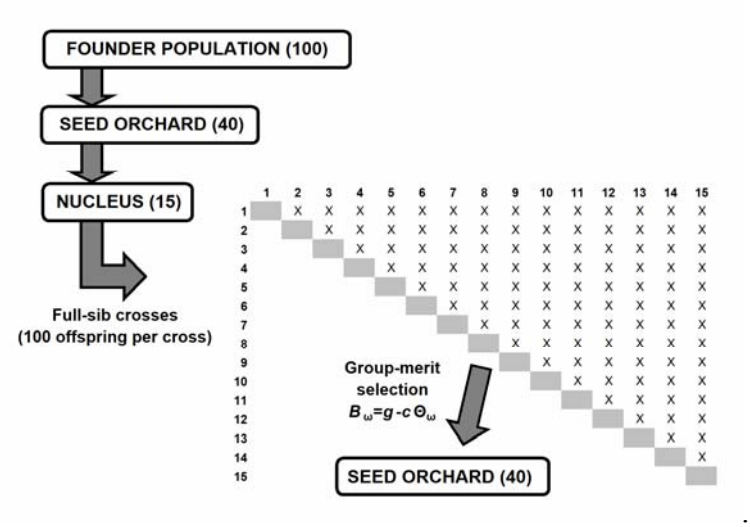

Figure 1. Schematic description of the breeding strategy implemented in El-Kassaby et al. (2007). The founder population is equivalent to a phenotypically selected breeding population in which the top 40 parents form the original seed orchard population and the partial diallel represents the FS and HS produced through pedigree reconstruction and the selected best individuals within these families form the parents for the new production population (seed orchard).

The offspring available for selection from the top 15 families represents the remaining individuals after the elimination of those resulted from contamination (assumed at 40%), sired by the other 25-orchard parents (assumed at 70% of the within orchard pollination) and selfing (assumed at 1%). If selection of the best individuals, for forward selection, is done after pedigree reconstruction (i.e., mating among the top 15 parents), then a large number of individuals are required for fingerprinting to permit capturing and identification the top 15 parents' offspring; however, the application of phenotypic truncation selection, prior to

fingerprinting, will drastically reduce the fingerprinting and restricting laboratory efforts only to those selected individuals by orders of magnitude. The genotype-dependent laboratory costs will be almost negligible. The estimated gain – effective number relation for the 40 selected individuals could be adjusted at different levels by the parameters of the group merit selection algorithm to a desired level.

Lindgren and Wei (1993; 2007) compared different selection methods and highlighted the efficiency and ease of mass-selection (phenotypic selection). The bases for their approach was truncation selection on an index where each individual within each HS and/or FS family generates its own index value that is based on a weight of this individual's family value and its phenotypic value. For the particular selection scheme phenotypic selection, the family value has zero weight and all weight is on the individual phenotype. By varying this weight over all possible positive values, a graph could be constructed relating gain and status number (diversity measure) given the number of selected individuals. That graph (example in Figure 2) shows the upper bound for the gain that can be achieved at a specific status number (diversity) and selection intensity. Alternative selection methods such as between-family selection, within-family selection, and combined index selection are also illustrated. Figure 2 demonstrates the efficiency of phenotypic selection in capturing both high gain and high effective number.

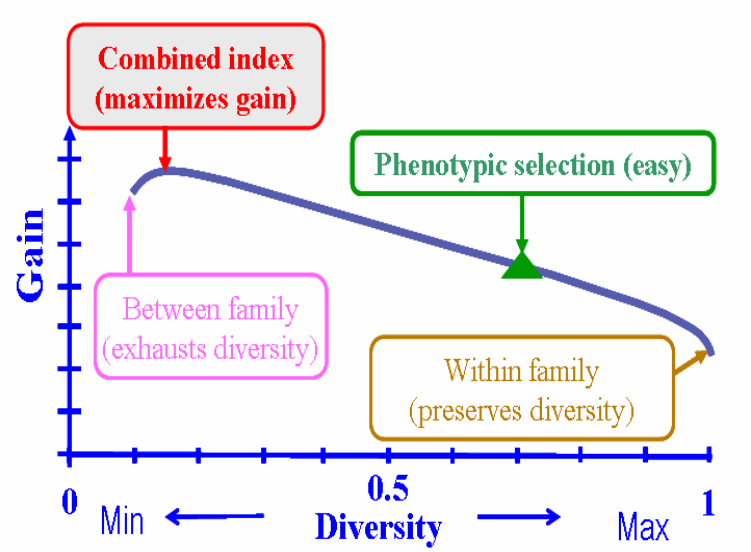

Figure 2. Relation between gain and diversity (relative status number) following selection in a population with a simple family structure. The graph is generated by an optimizing selection procedure where the candidate population was composed of an infinite number of large unrelated full-sib families of equal size. Heritability was set at 0.25 and 10 percent of the individuals were selected (Modified from Lindgren and Wei 1994).

At this stage, no assumption were made regarding knowing the pedigrees or the breeding values of the selected individuals, thus simply why should we consider BWB and invest unwarranted expenses on fingerprinting and pedigree reconstruction where the desired number of top ranking phenotypes to obtain the desired gain and diversity could be selected? The reasons for advocating the use of a scheme involving phenotypic pre-selection followed by pedigree

reconstruction and group merit selection is the methods ability in offering better results. These considerations include: 1) phenotypic selection is only approximately optimal in real world applications (Lindgren and Wei 2007), the model by Lindgren and Wei (1994) contained some simplifications, so some of the results are correct only for special cases, 2) phenotypic selection is efficient, but it does not control the point where maximum gain and effective number occur in the way it can be made by pedigreed material, 3) it provides an opportunity to place more emphases on the best segment of the breeding population (Lindgren 2005), 4) the proposed model (Lindgren and Wei 2007) is a one generation model that does not consider the occurrence of inbreeding in future production populations, thus knowing pedigree of the selected material offers advantages for better control of inbreeding and co-ancestry structure in advanced generations, and 5) provides control to the commonly considered unsophisticated and uncontrolled mass-selection making it more acceptable.

The utility of the BWB concept could be expanded to a host of other applications. For instance, since breeding program deals with larger genetic resource base than seed orchards, we propose two options for expanding the seed orchards' genetic base. One that involves the inclusion of few extra ramets representing the additional parents required for completing the breeding population into seed orchards. The impact of these extra ramets on the orchard's pollen pool and genetic gain will be very limited due to their low frequency and their infusion could be viewed as a tool to expand the diversity (Lindgren and El-Kassaby 1989). The other, involves the introduction of the desired clones into the orchard's pool through their pollen by means of supplemental mass pollination (El-Kassaby et al. 1993). Thus the proposed design allows long term breeding without clonal archives or controlled crosses. Additionally, some of the offspring sired by pollen contamination may be worthy of inclusion in the breeding stock, and thus offers a mechanism for enriching the breeding stock with fresh material. The most breath-taking possibility offered by BWB is to abandon not only clonal archives and crosses but also field testing. The pre-selection is efficient and does not require knowledge of pedigree, not even maternal. If both maternal and paternal pedigrees were reconstructed in commercial plantations originating from a seed orchard, the same type of group merit selection could be performed for the orchard's clones as well for practicing long term breeding in commercial plantations instead of investing a lot of effort and resources on specialized progeny test trials.

In conclusion, we recommend the application of mass-selection in identifying superior individuals before applying BWB for controlling the pedigree and group merit selection. This scheme is ideal for untested seed orchards where open-pollinated progeny trials exist. The substantial reduction of the number of pre-select individuals requiring for fingerprinted allows for greater opportunities to broaden the selection for greater flexibility.

LITERATURE CITED

El-Kassaby, Y.A., S. Barnes, C. Cook, and D.A. MacLeod. 1993. Supplemental-mass-pollination success rate in a mature Douglas-fir seed orchard. Can. J. For. Res. 23:1096-1099.

El-Kassaby, YA, M Lstibůrek, C Liewlaksaneeyanawin, GT Slavov and GT Howe. 2007. Breeding without breeding: approach, example, and proof of concept. *In*: Proc. IUFRO, Low Input Breeding and Genetic Conservation of Forest Tree Species (Isik, F., ed.). Antalya, Turkey, 9-13 October 2006. pp. 43-54. http://www.akdeniz.edu.tr/english/iufro/2007.pdf

Lindgren, D. 2005. Unbalances in tree breeding. *In*: Proc. Nordic Forest Tree Breeders and Forest Geneticists, Status, Monitoring and Targets for Breeding Programs (Fedorkov, A., ed.). Syktyvkar, Russia, 13-15 September 2005. ISBN 5-89606-249-4. pp. 45-56. http://www.nordicgenecar.org/documents/proceedings.pdf

Lindgren, D. and Y.A. El-Kassaby. 1989. Genetic consequences of combining selective cone harvesting and genetic thinning in clonal seed orchards. Silvae Genet. 38:65-70.

Lindgren, D. and R.P. Wei. 1994. Gain versus effective number. *In*: Proc. Nordic Group for Tree Breeding (Lee, S., ed.). Edinburgh, 6-10 October 1993. pp. 64-177.

Lindgren, D. and R.P. Wei. 2007. Low-input tree breeding strategies. *In*: Proc. IUFRO, Low Input Breeding and Genetic Conservation of Forest Tree Species (Isik, F., ed.). Antalya, Turkey, 9-13 October 2006. pp. 124-138. http://www.akdeniz.edu.tr/english/iufro/2007.pdf

Development of Reference Karyotypes for Longleaf and Shortleaf Pines using Fluorescence *in situ* Hybridization†

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Abstract: The Southern Institute of Forest Genetics is developing reference karyotypes for each of the major southern U. S. pine species— loblolly (*Pinus taeda*), slash (*P. elliottii* var. *elliottii*), shortleaf (*P. echinata*) and longleaf (*P. palustris*)— using 18S-28S rDNA, 5S rDNA, and *Arabidopsis*-type telomere repeat (ATR) sequence probes and AT-rich banding. Reference karyotypes for loblolly and slash pines have been completed. Preliminary results for the rDNA genes show that both shortleaf and longleaf pines contain seven major intercalary 18S-28S rDNA sites. Shortleaf pine showed as many as three major and six medium-to-minor centromeric 18S-28S rDNA sites, while longleaf pine showed one major and six medium-to-minor centromeric 18S-28S rDNA sites. Both species showed one major and one minor site for 5S rDNA. There are as many as five ATR sites with different degrees of signal intensities located near the centromeres of each chromosome in both longleaf and shortleaf pine. In addition, strong AT-rich bands were found to flank the centromeres of most chromosomes in both species. Complete karyotypes for shortleaf and longleaf pines are being developed for comparison to each other and to the existing loblolly and slash pine karyotypes.

Keywords: cytogenetics, molecular cytology, ribosomal DNA, telomere repeat sequence

INTRODUCTION

The genus *Pinus* ($2n = 2x = 24$) includes many economically and ecologically important species worldwide. According to Sax and Sax (1933) all pines have eleven pairs of long metacentric chromosomes and one pair of short sub-metacentric chromosomes. The conventional cytology technique known as C-banding has been used extensively to characterize the pines, but found to be ineffective in discriminating the chromosomes except for the one pair of short sub-metacentric chromosomes (Mergen 1958; Borzan and Papes 1978; MacPherson and Filion 1981; Hizume and others 1990). Molecular cytology, *in situ* hybridization (ISH) coupled with conventional cytology, can provide information that greatly facilitates the individual identification of chromosomes (Heslop-Harrison 1991; Leitch and Heslop-Harrison 1992; Leitch and others 1992; Hizume and others 2002; Doudrick and others 1995). A reference karyotype

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(i.e., chromosome-specific description of a genome) is a pre-requisite for advanced genetic and genomic studies and such karyotypes for loblolly and slash pines have been completed (Islam-Faridi et al. 2007 and Doudrick et al. 1995, respectively). Here we report the preliminary FISH results for developing reference karyotypes for both longleaf pine and shortleaf pine using 18S-28S rDNA, 5S rDNA, and *Arabidopsis*-type telomere repeat (ATR) sequence probes and AT-rich banding.

MATERIALS AND METHODS

Plant Sample and Slide Preparation

Prior to germination, seeds from two shortleaf pine parental clones (WO-5 and WO-12) were treated with 1% H_2O_2 followed by cold, moist stratification (6 wk), while seeds from two open-pollinated longleaf pine parental clones (EM17 and EM24) were treated with 1% H_2O_2 only. Germinated seedlings were transferred to potting mix and allowed to grow in a greenhouse prior to root sampling for cytological analysis. Actively growing root tips, about 1.5 cm long, were excised and pretreated in 0.15% colchicine (Sigma, USA) for 7 hr at room temperature in the dark. Root tips were then fixed in 2:1:1 95% v/v ethanol, glacial acetic acid, and double distilled water. The fixed root tips were treated with 3% w/v cellulase RS and 1% w/v pectolyase Y23 as described by Jewell and Islam-Faridi (1994). The digested root tips were macerated on a 95% v/v ethanol-cleaned glass slide followed by light squashing under a clean cover glass as described by Islam-Faridi and Mujeeb-Kazi (1995).

Nick Translation and *In situ* Hybridization

Whole plasmids of 18S-28S rDNA and 5S rDNA were labeled with biotin-16-dUTP (Biotin-Nick Translation Mix, Roche, Germany) following instructions provided by the manufacturer. An ATR DNA sequence $(TTTAGGG)_n$ of about 300 base pairs (kindly provided by Dr. T. McKnight, Texas A&M University) was labeled with digoxigenin-11-dUTP (Dig-Nick-Translation Mix, Roche, Germany). Labeled DNA was dot-blotted to verify incorporation of labeled nucleotides. A standard hybridization technique was used as described elsewhere (Hanson et al. 1996; Islam-Faridi et al. 2002). The probe hybridization sites were detected with Cy3-cojugated streptavidin (Jackson Immuno Research Laboratories, USA; for biotin labeled probe), and fluorescein conjugated anti-digoxigenin (Roche, Germany; for dig labeled probe) – followed by fluorescein conjugated anti-sheep (Roche, Germany). The preparation was counter stained with DAPI (4 ug/ml) and mounted using Vectashield (Vector Laboratories, USA).

Microscopy

Digital images were recorded from an AxioImager Z-1 Epi-fluorescence microscope with suitable filter sets (Chroma Technology, USA), using a COHU High Performance CCD Camera and the Metafer v4 MetaSystems Finder digital image system (MetaSystem Inc., USA). Images were processed with Ikaros and ISIS v5.1 and then further processed with Adobe Photoshop CS v8 (Adobe Systems, USA). The Metafer software allows scanning of the whole slide in less than

5 min and records the coordinates of each cell, while the "photo gallery" aspect of Metafer ensures the re-capture of the same cell in repeated experiments.

RESULTS AND DISCUSSIONS

We have modified a technique for preparing pine somatic chromosome spreads that consistently provides a high number of metaphase cells in root tip samples of the southern pine species. As many as 731 mitotic divisions, mostly in metaphase, have been observed from a single root tip preparation. In addition, chromosome morphology generally appears to be sharp and clear after *in situ* hybridization. This feature is critical for obtaining accurate measurements of total chromosome lengths, centromere indices, and distances from centromere to FISH signal positions. Strong DAPI positive (AT-rich) bands occurred in various patterns near or around the centromeres of 22 chromosomes each of longleaf pine and shortleaf pine. Numerous light or weaker DAPI bands appeared interstitially (i.e., area between a centromere and telomere) in some chromosomes of both species.

FISH experiments were carried out in two phases. First, we used 18S-28S and 5S rDNA clones (both labeled with biotin) on longleaf pine and shortleaf pine chromosome spreads. The hybridization sites were detected with Cy3 streptavidin. The 5S rDNA site was identified in an earlier experiment, so there would no confusion of its location with the 18S-28S rDNA sites (Figs. 1 and 2). FISH images were captured as described above, then the slides were incubated in 0.5X SSC for 2 hr to remove the probes and then re-probed with ATR. In our earlier experiments we observed that the first probe hybridization sites could not be eliminated completely. To obtain a better image we re-used the rDNA probes in the second FISH experiment along with the ATR probe (Figs. 1 and 2).

One major and one minor 5S rDNA sites were detected in both longleaf pine and shortleaf pine. Similar results were reported in loblolly pine (Islam-Faridi et al. 2007). Seven major interstitial 18S-28S rDNA sites were observed, similar to other pine species (e.g., loblolly pine, Jacobs et al. 2000 and Islam-Faridi et al. 2007, and slash pine, Doudrick et al. 1995). One major and six medium-to-minor 18S-28S rDNA sites were identified at or around the centromeres in longleaf pine, and three major and six medium-to-minor 18S-28S rDNA sites were identified in shortleaf pine. One of the interstitial sites of shortleaf pine is heterologous, i.e., one homologue showed a major 18S-28S rDNA FISH signal and the other showed a medium-to-minor signal. These results indicate that the medium-to-minor 18S site contains fewer repeat units of 18S-28S rDNA sequence than the other homologue. A similar result was reported in slash pine (Doudrick et al. 1995), suggesting that at least for this cytological feature shortleaf pine appears more closely related to slash pine than to loblolly pine or longleaf pine. However other genetic evidence suggests that shortleaf pine is more closely related to loblolly pine or longleaf pine than to the other southern pines (Dorman 1976; Wagner et al. 1991; Krupkin et al. 1996; Dvorak et al. 2000).

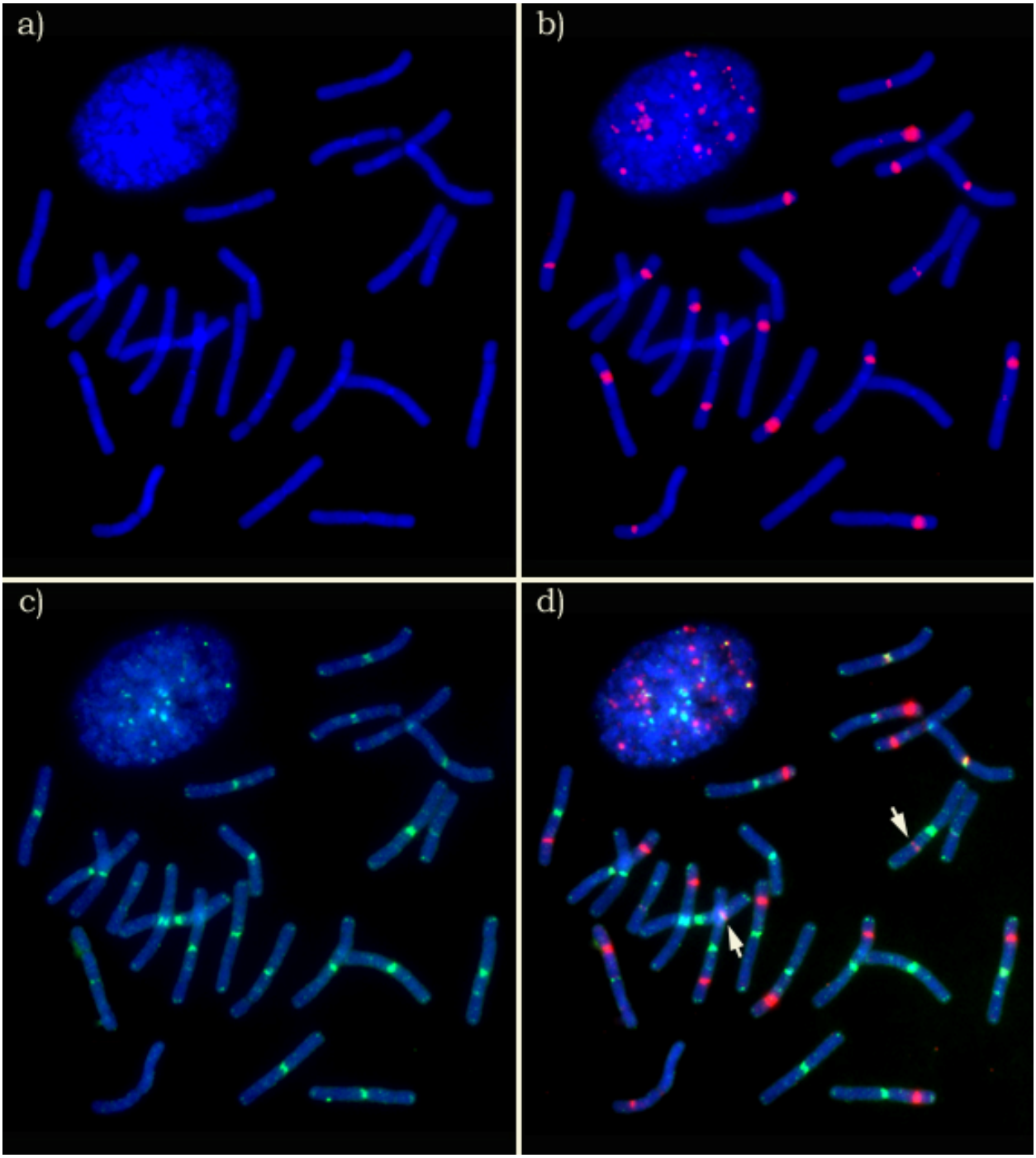

Figure 1. Longleaf pine chromosomes probed with 18S-28S rDNA and 5S rDNA clones (b and d, red signals) and *Arabidopsis*-type telomere repeat DNA sequence (c and d, green signals). Images "a" and "b" are from the first FISH experiment and Images "c" and "d" are from the second FISH experiment. Image "a" was taken under a DAPI filter and shows AT-rich bands; Image "b" was taken under DAPI and Cy3 filters, and then super-imposed; Image "c" was taken under FITC (green) and DAPI filters, and then super-imposed; Image "d" was taken under DAPI, Cy3 and FITC filters, and then super-imposed. Arrows point at the major 5S rDNA signals (d).

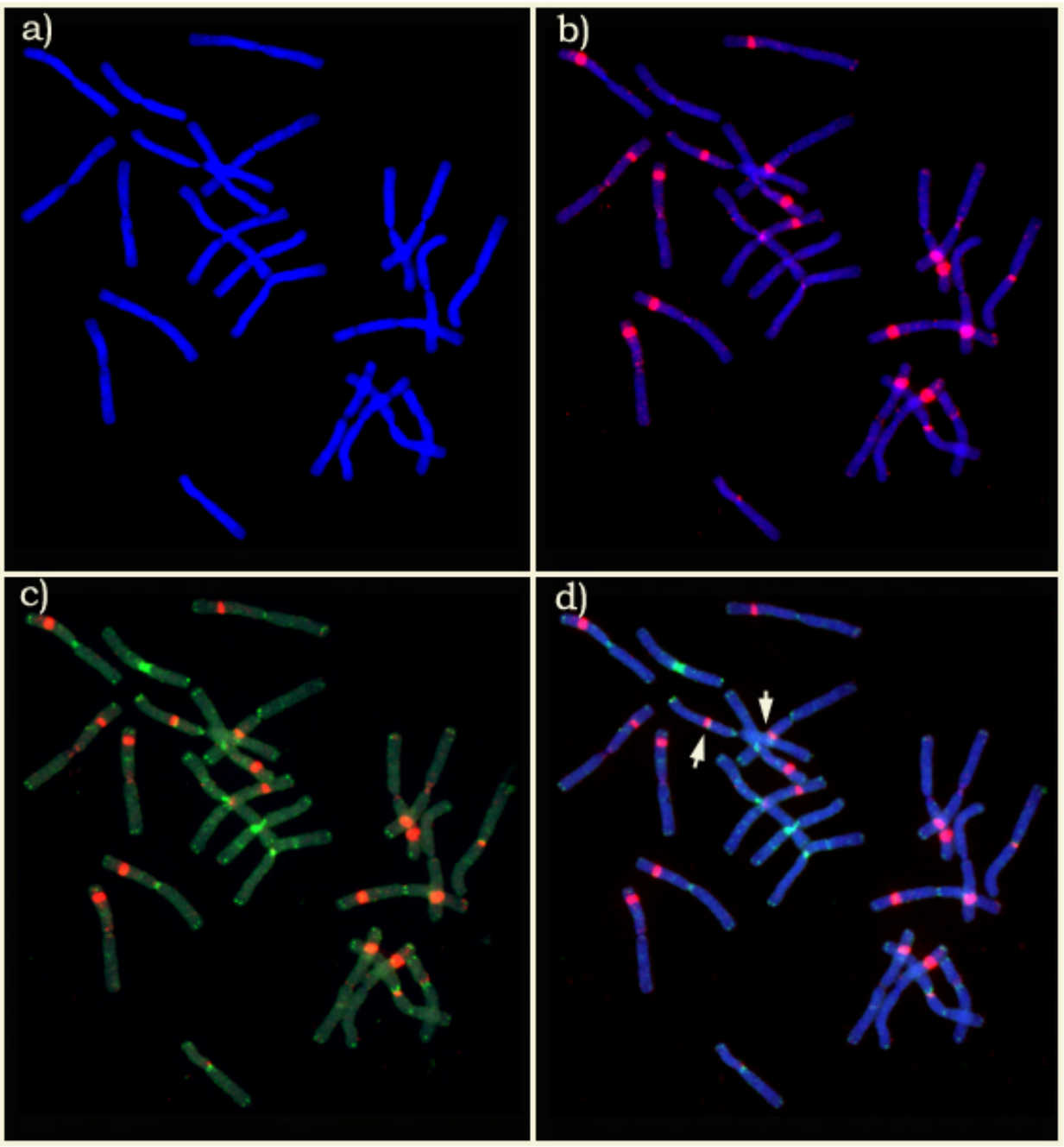

Figure 2. Shortleaf pine chromosomes probed with 18S-28S rDNA and 5S rDNA clones (b, c and d, red signals) and *Arabidopsis*-type telomere repeat DNA sequence (c and d, green signals). Images "a" and "b" are from the first FISH experiment and Images "c" and "d" are from the second FISH experiment. Image "a" was taken under a DAPI filter and shows AT-rich bands; Image "b" was taken under DAPI and Cy3 filters, and then super-imposed; Image "c" was taken under FITC (green) and Cy3 filters, and then super-imposed; Image "d" was taken under DAPI, Cy3 and FITC filters, and then super-imposed. Arrows point at the major 5S rDNA signals (d).

Strong to weak ATR signals were observed at or around the centromeric positions of all but one pair of chromosomes in both longleaf pine and shortleaf pine (Figs. 1c, 1d, 2c and 2d). Interstitial ATR signals were also observed on both chromosomal arms in longleaf pine and shortleaf pine. Similar results were reported in various pine species (Doudrick et al. 1995; Hizume et al. 2002; Islam-Faridi et al 2007), in contrast to other plant species where interstitial and centromeric telomere sites are rare (Fuchs et al. 1995). Finally, a pair of "snake-eyed" ATR signals was observed at the end of each chromosomal arm clearly revealing the telomere sequences at their usual terminal position.

Further analysis on measurements of chromosome lengths and distances between FISH signals are being carried out to develop a reference karyotype for both longleaf pine and shortleaf pine, which will then be compared to our loblolly pine reference karyotype (Islam-Faridi et al 2007) and two slash pine karyotypes (Doudrick et al. 1995; Islam-Faridi et al., unpublished data). It is our hope that cytogenetic analyses including karyotype comparisons between species will be useful in identifying structural rearrangements within and between species that may be used to infer evolutionary relationships, to inform gene conservation efforts, and to guide interspecies hybrid breeding projects.

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LITERATURE CITED

Borzan, Z. and D. Papes. 1978. Karyotype analysis in *Pinus*: A contribution to the standardization of the karyotype analysis and review of some applied techniques. Silvae Genetica 27:144-150.

Dorman, K.W. 1976. The Genetics and Breeding of Southern Pines. U. S. Department of Agriculture, Forest Service, Agricultural Handbook No. 471, 407 p.

Doudrick, R.L., J.S. Heslop-Harrison, C.D. Nelson, T. Schmidt, W.L. Nance and T. Schwarzacher. 1995. Karyotype of slash pine (*Pinus elliottii* var. *elliottii*) using patterns of fluorescence *in situ* hybridization and fluorochrome banding. Journal of Heredity 86:289-296.

Dvorak, W.S., A.P. Jordan, G.P. Hodge and J.L. Romero. 2000. Assessing evolutionary relationships of pines in the *Oocarpae* and *Australes* subsections using RAPD markers. New Forests 20:163-192.

Fuchs, J., A. Brandes and I. Schubert. 1995. Telomere sequence localization and karyotype evolution in higher plants. Plant Systematics and Evolution 196:227-241.

Hanson, R.E., M.N. Islam-Faridi, E.A. Percival, C.F. Crane, Y. Ji, T.D. McKnight, D.M. Stelly and H.J. Price. 1996. Distribution of 5S and 18S-28S rDNA loci in a tetraploid cotton (*Gossypium hirsutum* L.) and its putative diploid ancestors. Chromosoma 105:55-61.

Heslop-Harrison, J.S. 1991. The molecular cytogenetics of plants. Journal of Cell Science 100: 15-21.

Hizume, M., M. Arai and A. Tanaka. 1990. Chromosome banding in the genus *Pinus*. III. Fluorescent banding pattern of *P. luchuensis* and its relationships among the Japanese diploxylon pines. Botanical Magazine, Tokoyo 103: 103-111.

Hizume, M., F. Shibata, Y. Matsusaki and Z. Garajova. 2002. Chromosome identification and comparative karyotypic analyses of four *Pinus* species. Theoretical and Applied Genetics 105: 491-497.

Islam-Faridi, M.N. and A. Mujeeb-Kazi. 1995. Visualization of *Secale cereale* DNA in wheat germplasm by fluorescent *in situ* hybridization. Theoretical and Applied Genetics 90: 595-600.

Islam-Faridi, M.N., C.D. Nelson and T.L. Kubisiak. 2007. Reference karyotype and cytomolecular map for loblolly pine (*Pius taeda* L.). Genome 50:241-251.

Jacobs, M.D., R.C. Gardner and B.G. Murray. 2000. Cytological characterization of heterochromatin and rDNA in *Pinus radiata* and *P. taeda*. Plant Systematics and Evolution 223: 71-79.

Jewell, D.C. and M.N. Islam-Faridi. 1994. Details of a technique for somatic chromosome preparation and C-banding of maize. In: M. Freeling and V. Walbot, (eds) The Maize Handbook. Springer-Verlag, New York, pp 484-493.

Krupkin, A.B, A. Liston and S.H. Strauss. 1996. Phylogenetic analysis of the hard pines (*Pinus* Subgenus *Pinus*, *Pinaceae*) from chloroplast DNA restriction site analysis. American Journal of Botany 83: 489–498.

Leitch, I.J. and J.S. Heslop-Harrison. 1992. Physical mapping of the 18S-5.8S-25S rRNA genes in barley by *in situ* hybridization. Genome 35: 1013-1018.

Leitch, I.J., W. Mosgoller, M. Shi and J.S. Heslop-Harrison. 1992. Different patterns of rDNA organization at interphase in nuclei of wheat and rye. Journal of Cell Science 101: 751-757.

MacPherson, P. and W.G. Filion. 1981. Karyotype analysis and the distribution of constitutive heterochromatin in five species of *Pinus*. Journal of Heredity 72: 193-198.

Mergen, F. 1958. Natural ploidy in slash pine. Forest Science 4:283-295.

Sax, K and H.J. Sax. 1933. Chromosome number and morphology in the conifers. Journal of the Arnold Arboretum 15:356-375.

Wagner, D.B., W.L. Nance, C.D. Nelson, T. Li, R.N. Patel and D.R. Govindaraju. 1991. Taxonomic patterns and inheritance of chloroplast variation in a survey of *Pinus echinata, Pinus elliottii, Pinus palustris,* and *Pinus taeda.* Canadian Journal of Forest Research 22: 683–689.

Performance of Nuttal Oak (*Querqus texana* Buckl.) Provenances at Age 10 in the Western Gulf Region

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Abstract: Nuttall oak (*Quercus texana* Buckl.) is a member of the red oak family with a natural range restricted to the bottomlands of the Gulf Coastal Plain from Alabama to Texas and from Missouri to the coast. It is extremely hardy and fast growing and is therefore a highly desirable species for bottomland planting and restoration. Three series of three tests each of Nuttall oak were established by members of the Western Gulf Forest Tree Improvement Program at three locations transecting the central part of the range in a north-south direction. The three series included 28-42 different half-sib families from throughout the western and northern part of the natural range. These families were arbitrarily divided into provenances based on the river basin in which the parent originated. The primary recommendations on wild seed procurement made at age 5 were confirmed at age 10. The Red River provenance had the best growth performance in Series 3, but it was not represented in the other two test series. The best provenance in Series 2, the Ouachita River provenance, ranked second in Series 3. Family-mean heritability estimates were high ranging from 0.73-0.98 for height and 0.57-0.78 for diameter indicating that the potential for genetic improvement of growth in Nuttall oak is substantial. Orchard establishment with tested parents began in 2007 based on 10-year old performance estimates from 115 individuals. The 30 selected parents should produce seed with a 17% gain in 10-year-old volume over wild seed.

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

INTRODUCTION

Nuttall oak (*Quercus texana* Buckl. formally Q. *nuttallii* Palmer) is a member of the red oak group. It has a natural range restricted to the bottomlands of the Gulf Coastal Plain of the southern US from Alabama to eastern Texas, north in the Mississippi Valley to Arkansas, southeastern Missouri and western Tennessee (Figure 1, Filer 1990). It is the most tolerant of the red oak species to heavy, poorly drained, alluvial clay soils. Therefore, Nuttall oak is favored for bottomland planting because it exhibits good survival on a range of sites and is fast growing (Ducks Unlimited 2001). Nuttall oak is an important species because it produces high quality

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sawtimber and because it is beneficial to wildlife, producing large acorn crops at young ages. Like most oaks, it is shade intolerant and planting open areas following harvesting is a viable method of stand restoration. Previous studies have focused on natural regeneration, direct seeding, and comparison of growth performance of Nuttall oak with other species (Bonner 1966, Johnson 1975, Krinard and Johnson 1981). Genetic information is limited to the previously reported five-year results from the plantings in this study (Gwaze et al. 2003). Provenances along the Red and Ouachita Rivers appeared to be the best sources of wild seed. Family-mean heritabilities were high indicating that individual selection would be effective in improving height, diameter, and volume growth.

These plantings are part of the Western Gulf Forest Tree Improvement Program (WGFTIP) effort to establish seed orchards with progeny tested parents from this species. Open-pollinated seed were collected from 210 parents and established in five series of progeny tests across multiple locations. Collectively, the test series included samples from throughout the northern, central, and western range of Nuttall oak, providing an opportunity to determine provenance and within-provenance variation in the species. Scion material was collected from most of the parent trees and preserved in scion banks maintained by the Texas Forest Service and the Mississippi Forestry Commission. Progeny test information will be used to select tested parents for seed orchard establishment. This paper reports on 10-year survival and growth results from the first three test series. The primary objectives were to provide information on seed sources to those buying wild seed and to estimate expected gains from seed orchard establishment. Genotype by environment interaction, heritabilities and age-age phenotypic correlations were considered.

MATERIALS AND METHODS

Three series of three tests each were established at three locations: Desha and Lonoke Counties in Arkansas and Sharkey County in Mississippi (Figure 1, Table 1). Series 1 was established in 1994, series 2 in 1995 and series 3 in 1997. The three series included 28 to 42 different half-sib families. Families were arbitrarily divided into provenances based on the river basin in which they originated. The provenances were Black-White, Ouachita, Mississippi, Red, and Tallahatchie-Yalobusha Rivers. The sixth provenance (Western Region) originated in the western fringe of the main natural range of the species and was not representative of a single river drainage (Figure 2, Table 2). Some provenances were represented in all three tests series while others were represented in only one (Table 3). There were no families in common between test series.

The test designs were the same at each location, a randomized complete block design replicated ten times with four-tree row plots for families. Spacing was 3 x 3 m in all tests. All tests were assessed at 10 years of age for survival, height (HT, m) and diameter (DBH, cm). Height and diameter were used to calculate volume of each living tree using the following cone volume equation:

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

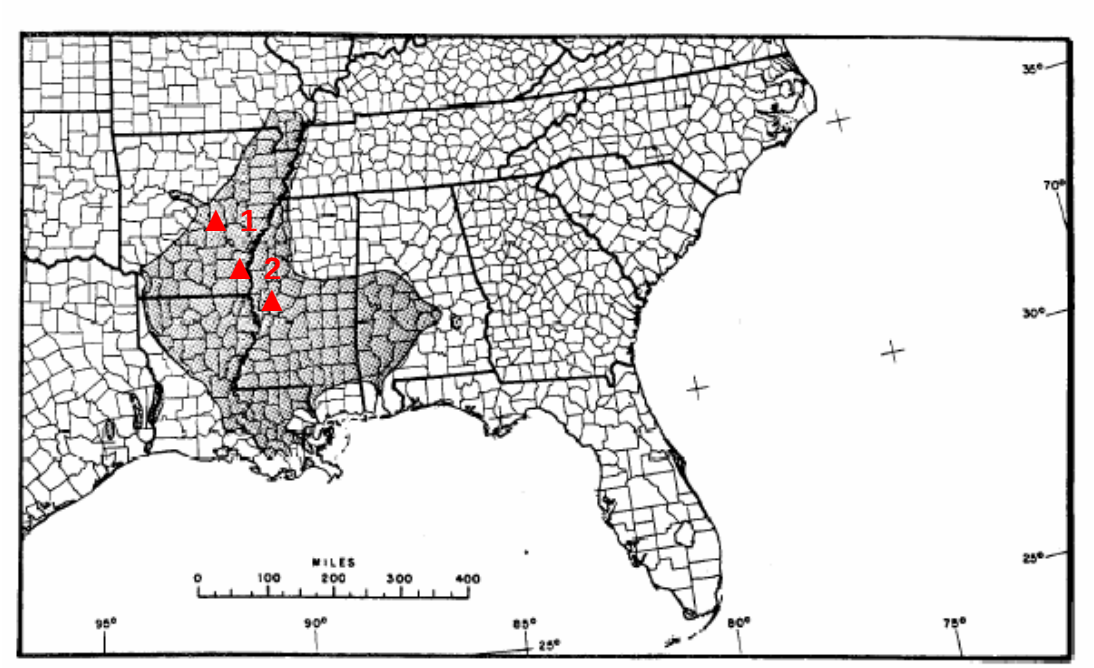

Figure 1. Natural distribution of *Quercus texana* Buckl. (formally Q. *nuttallii* Palmer) (Filer 1990). Progeny test locations are marked with ▲.

Table 1. Details of field tests of Nuttall oak provenances in the USA for all series.

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

Plot means were used for all analyses. Analyses were carried out for survival, height, diameter and volume for each series separately. In each series, data were pooled across the tests and the SAS PROC GLM procedure with a random statement for mixed models (SAS Institute 1988) was used to test for significant differences among sites, families, provenances, replications and the interactions between site and provenance, replication and provenance, and site and family. The following linear model was used for the pooled analysis across sites in each series:

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

where Y_{ijklm} is the observation on the m^{th} plot of the l^{th} family of the k^{th} provenance in the j^{th} replication in the i^{th} site, μ is the population mean, S_i is the random variable for site,

$R_{j(i)}$ is the random variable for replication nested within site, P_j is the fixed effect of provenance, $F_{l(k)}$ is the random variable for family nested within provenance, SP_{ik} is the random interaction site by provenance, $SF_{il(k)}$ is the random interaction site by family nested within provenance, e_{ijklm} is the error term.

Table 2. Details of seed origin of Nuttall oak provenances in the three series. Series number is in parenthesis.

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

Where significant differences were detected among provenances in the pooled data, Duncan's Multiple Range Test was used to compare means using site x provenance as the error term. This should be viewed as indicative only as the actual error term requires a pseudo F-test with a contribution from the family within provenance variance. Variance components were estimated using the VARCOMP procedure in SAS (SAS Institute 1988). Heritability estimates were determined using family variances for individual trees and at the family mean level. Since single-site heritability estimates are biased upwards because of genotype-environment interaction, only unbiased heritability was estimated using data pooled across the sites as for individual tree heritability [1] and family mean heritability [2] as

$$h^2_{F(P)} = 4 \times \sigma^2_{F(P)} / [\sigma^2_{F(P)} + \sigma^2_{SF(P)} + \sigma^2_e], \quad [1]$$

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

where:

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

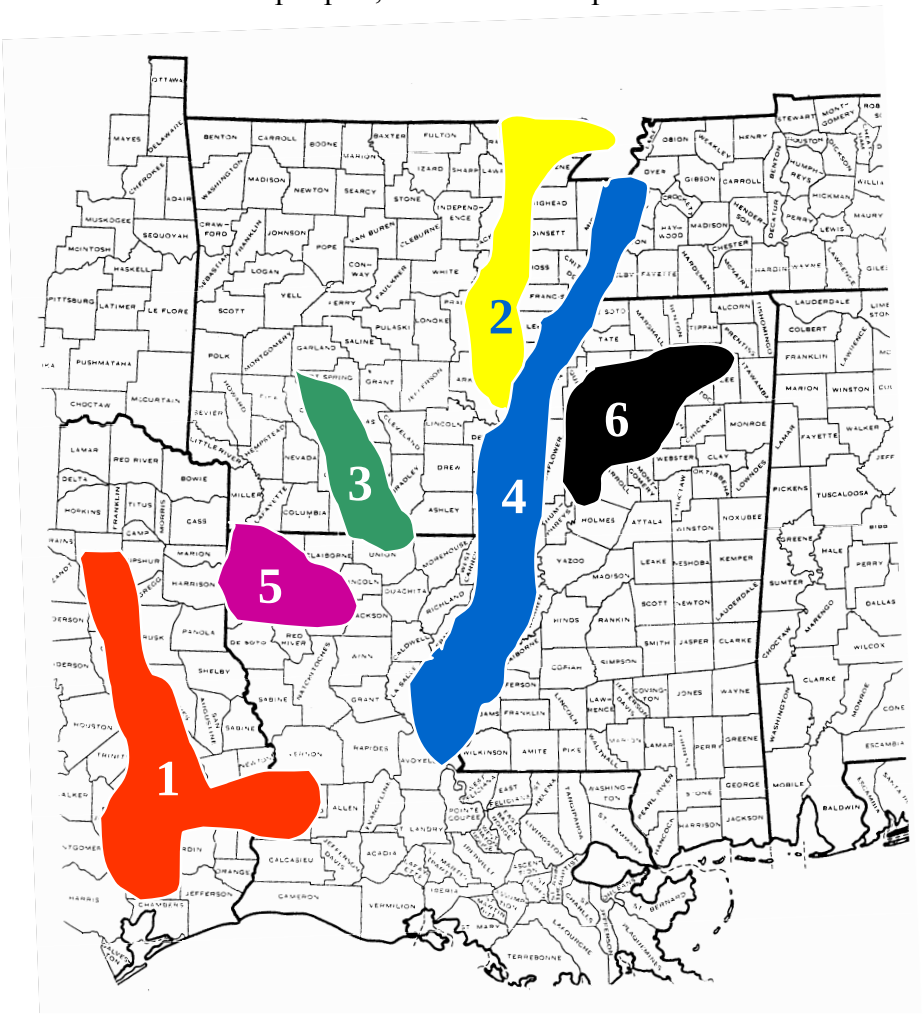

Figure 2. County/Parish locations of families used in the study are: Western Region (Provenance 1), Black -White Rivers (Provenance 2), Ouachita River (Provenance 3), Mississippi River (Provenance 4), Red River (Provenance 5) and Tallahatchie-Yalobusha Rivers (Provenance 6).

Table 3. The number of open-pollinated families representing each provenance by test series.

		Test Series			
	Provenance	1	2	3	Total
1	Western Region (WR)	15	3	12	30
2	Black – White Rivers (BW)		20	5	25
3	Ouachita River (OU)	6	2	6	14
4	Mississippi River (MS)	12	3	4	19
5	Red River (RR)			12	12
6	Tallahatchie – Yalobusha Rivers (TY)	8			8

Genotype by environment interaction (GxE) was evaluated for both the provenance and family level using GLM (SAS 1988). The family-mean correlations among different traits and among the same trait at different ages were estimated as product-moment correlations using PROC CORR in SAS (SAS 1988).

RESULTS AND DISCUSSION

Location means by test series are summarized in Table 4. Survival was outstanding at all locations with the exception of Test Series 1 at Desha County. Even there, survival was operationally acceptable at 73 percent. These survivals, which were obtained with bare root seedlings, emphasized the hardiness of this species and its suitability for reforestation and reclamation efforts. Growth rates were acceptable for oaks at all three locations ranging from 5.2 m to 8.1 m for 10-year height.

Table 4. Location means by test series.

	Survival (%)	Height (m)	Diameter (cm)	Volume (dm^3/tree)
Lonoke				
Series 1	96.0	8.1	9.8	21.6
Series 2	98.2	7.0	9.2	17.4
Series 3	93.4	7.7	9.5	19.5
Desha				
Series 1	73.4	5.5	6.6	5.9
Series 2	97.4	7.7	8.7	17.7
Series 3	91.5	5.2	6.3	6.6
Sharkey				
Series 1	80.5	5.7	7.2	7.7
Series 2	97.1	5.8	8.2	11.2
Series 3	92.0	5.2	6.5	6.6

Provenance Differences – Wild Seed Movement

There were significant differences ($P < 0.05$) among provenances for survival at age 10 in only the first series where mean performance ranged from 79.3 to 87.4 percent. The Ouachita River families had the highest survival while the Western provenance families had the poorest. Height differences were only marginally significant (alpha <0.10) in Series 2 and 3 while diameter varied significantly in all three test series (Table 5).

Volume per living tree was only marginally significant (alpha <0.10) in Series 3. The trend was for the Ouachita and Red River provenances to be the best while the Western provenance was poor to intermediate in performance.

Provenance by site interaction was significant for height, diameter and volume in both series 1 and 3 but only significant for survival in series 2. In only two cases did this GxE interaction term explain more of the variation than did the provenance effect. The first instance was survival in series 2 where the overall average exceeded 97 percent and any differences would be operationally meaningless. The second instance was for live-tree volume in Series 1 where the interaction resulted from changes in variance and rank changes were among intermediate sources (data not shown).

Table 5. Mean squares for analysis of variance for survival, height, diameter and volume at 10 years for three series of Nuttall oak provenance tests [1].

Source of variance	DF	Survival (%)	Height (m)	DBH (cm)	Volume (dm^3/tree)
Series 1					
Site (S)	2	22577.4***	318.8***	495.2***	9997.3***
Replication (Site) (R (S))	27	1966.0***	10.6***	23.0***	373.3***
Provenance (P)	3	4064.1**	5.5ns	63.2**	705.8ns
SXP	6	282.6ns	2.2**	10.2**	210.0***
PXR(S)	81	363.3ns	0.6ns	2.1ns	29.6ns
Family (F(P))	38	630.3ns	2.2***	7.6***	107.1***
SXF(P)	72	500.0**	0.8**	3.3***	49.1***
Residual	990	365.8	0.6	2.1	24.9
Series 2					
Site (S)	2	237.3ns	26.0***	7.1ns	431.4***
Replication (Site) (R (S))	27	830.3***	3.7***	6.3***	163.5***
Provenance (P)	3	567.9ns	23.3*	73.0*	555.1ns
SXP	6	426.4**	1.0ns	2.4ns	26.7ns
PXR(S)	81	122.3ns	0.5ns	1.2ns	23.0ns
F(P)	24	344.6**	9.5***	28.3***	270.4***
SXF(P)	47	165.0ns	0.8***	2.9***	43.1***
Residual	637	136.0	0.5	1.3	22.6
Series 3					
Site (S)	2	417.3ns	369.5***	576.5***	9920.2***
Replication (Site) (R (S))	27	960.5***	6.0***	11.3***	245.8***
Provenance (P)	4	714.4ns	18.9*	59.8*	815.9*
SXP	8	214.4ns	3.1***	12.7***	202.2***
PXR(S)	108	155.4ns	0.5ns	1.8ns	18.3ns
F(P)	34	1311.0***	5.0***	13.3***	132.8***
SXF(P)	66	233.3*	0.7ns	2.2ns	36.7***
Residual	898	180.1	0.5	1.8	22.7

[1] ns, *, **, *** = Not significant, significant at P $\leq$ 10%, 5%, and 1%

Table 6. Individual tree heritability, family-mean heritability and individual tree phenotypic correlations for survival, height, diameter and volume for Nuttall oak at age 10. All phenotypic correlations were significant at P<0.01.

Trait	h_i	h_F	HT	DBH	VOL
Series 1					
SUR	-	-	0.34	0.27	0.29
HT	0.38	0.73		0.90	0.90
DBH	0.23	0.57			0.95
VOL	0.15	0.32			
Series 2					
SUR	0.21	0.80	0.25	0.30	0.20
HT	1.00	0.98		0.75	0.86
DBH	1.00	0.94			0.90
VOL	1.00	0.92			
Series 3					
SUR	0.67	0.92	0.19	0.16	0.13
HT	0.71	0.75		0.91	0.90
DBH	0.63	0.78			0.95
VOL	0.55	0.77			

Recommendations to buyers of wild seed are to preferentially concentrate on collections from the central part of the range for planting in the areas represented by these progeny tests. This would include the Ouachita and Red River Basins. The differences among provenances tended to be minimal, however, and the only area that should clearly be avoided is the Western Region because of its demonstrated poorer survival and slower growth.

Family variation – Seed Orchard Establishment

There were strong differences among families for all of the traits analyzed in each of the series with the single exception of survival in Series 1. Individual and family mean heritabilities were high for all traits for which there were significant differences among families (Table 6). Heritabilities in Series 2 were clearly not reasonable but still support the assertion that genetic variance explains a large proportion of the total phenotypic differences. Such high heritabilities indicate that family selection will effectively improve performance and that GxE is a relatively unimportant part of the total variation. There were good families from all provenances validating the need for progeny testing. Positive and significant phenotypic correlations between traits implied that simultaneous gain can be made in all of the traits evaluated.

Family-mean phenotypic correlations were also calculated between early measurements and age 10 measurements (Table 7). This was age 5 to age 10 measurements in all cases except for Series 2 at Desha. This test's first measurement was not completed until age 7.

Table 7. Family-mean phenotypic correlations between early measurements (age 5 for all but Series 2 Desha County which was age 7) and age 10 measurements for traits with significant family differences at age 10. All phenotypic correlations significant at P<0.01.

Trait	SUR10	HT10	DBH10	VOL10
Series 1				
SUR5				
HT5		0.57	0.59	0.55
DBH5		0.49	0.52	0.54
VOL5		0.30	0.36	0.41
Series 2				
SUR5	0.76	0.32	0.45	0.31
HT5	0.07	0.70	0.26	0.47
DBH5	0.04	0.69	0.24	0.47
VOL5	0.08	0.68	0.25	0.47
Series 3				
SUR5	0.91	0.14	0.12	0.09
HT5	0.25	0.72	0.75	0.67
DBH5	0.12	0.10	0.26	0.19
VOL5	0.14	0.28	0.42	0.37

The highlighted diagonals represent the correlations among the same trait at different ages. Large correlations between the early and age 10 measurements for survival and height indicate that these traits could be selected for efficiently at the early measurement. Age-age correlations for diameter and volume tended to be lower and rank changes were observed among potential orchard candidates (data not shown) between measurement cycles. This implied that family selection should be delayed until at least age 10.

CONCLUSIONS

Nuttall oak had excellent survival and growth confirming it is a good choice for planting and restoring bottomlands. The provenance and family-within provenance variation and estimates of heritability indicated that genetic improvement of Nuttall oak would be successful. Provenance effects were moderate at best, but it would appear that seed collected toward the center of the

range (northern Louisiana or southern Arkansas) should be favored when purchasing wild seed for the areas represented by the progeny tests reported here. The better performing provenances tended to be from the Ouachita and Red River basins while the poorer provenances tended to be from the Western Region. It should be noted that sources from Alabama and southern Louisiana were not included in this evaluation.

Relatively high family heritabilities and the fact that good families could originate from any provenance validated the need for progeny testing. Furthermore, relatively moderate age-age correlations for diameter and volume implied that family selection should be delayed until at least age 10. The large family variation relative to the provenance effect suggest that the seed orchard approach will provide significant gain compared to wild seed collection, even from the best provenances. The top 30 families from the 115 families with age 10 progeny test data have an average phenotypic superiority of 26 percent in volume growth at age 10. If this value is multiplied by family heritability, these parents may be expected to produce seed with an expected value of 17 percent improvement. Members of the WGFTIP-Hardwood cooperative, led by the Arkansas Forestry Commission, began Nuttall oak orchard establishment in 2007.

ACKNOWLEDGEMENTS

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LITERATURE CITED

Bonner, F. T. 1966. Survival and first-year growth of hardwoods planted in saturated soils. USDA Forest Service, Research Note SO-183, 3 p.

Ducks Unlimited. 2001. Annual Report. 25 p.

Filer, T. H., Jr. 1990. *Quercus nuttallii* Palmer (Nuttall Oak). Silvics of North America.Volume 2: Hardwoods. USDA Forest Service, Agriculture Handbook 654. Washington, DC.

Gwaze, D.P., T.D. Byram, and E.M. Raley. 2003. Performance of Nuttall oak (*Quercus texana* Buckl.) Provenances in the Western Gulf Region. Proc. Of the 27th South. For. Tree Improv. Conf., pp. 126-137.

Johnson, R. L. 1975. Natural regeneration and development of Nuttall oaks and associated species. USDA Forest Service, Research Paper SO-104, 11 p.

Krinard, R. M. and R. L. Johnson. 1981. Description and yields of an 11-year-old hardwood stand on Sharkey clay soil. USDA Forest Service, Research Note SO 165, 2 p.

SAS Institute Inc. 1988. SAS/STAT® User's Guide Release 6.03 Edition. SAS Institute Inc., Cary, NC, 1028 pp.

Fusiform Rust Disease in East Texas Loblolly Pine: An Evaluation of Resistance and a Test of Two Hypotheses†

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Abstract: A set of 21 loblolly pine families produced by crossing trees from east Texas were tested for resistance to fusiform rust disease. The parents of these families were surviving trees in stands that experienced extensive mortality in the 1960s due to southern pine beetle infestation. Seedlings were artificially inoculated in the greenhouse with *Cronartium quercuum* (Cq) from five different sources of inoculum, each consisting of basidiospores derived from single gall collections of aeciospores. Four of the collections originated from galls on loblolly pine (*C. q. fusiforme* or Cqf), whereas the remaining collection was obtained from a shortleaf pine gall (*C. q. echinatae* or Cqe). Two collections of Cqf and Cqe were taken from round-shaped galls, while the other two Cqf collections were taken from typical fusoid-shaped galls. The design allowed for the testing of two long standing hypotheses in fusiform rust biology, namely (1) that Texas loblolly pine and shortleaf pine share genes for resistance to Cqf and (2) that Cqe and Cqf collected from round galls share genes for gall shape. No apparent relationship was observed between percent gall by the Cqe inoculum and that for any of the Cqf inocula, suggesting that different resistance genes are effective for Cqf and Cqe in these families and that Texas loblolly pine and shortleaf pine apparently do not share resistance genes. All galls produced by the Cqe inoculum were about round shaped with gall form values ranging from 0.88 to 1.54 and averaging 1.04. However, for the Cqf inocula (average gall form ranged from 1.85 to 2.38) no relationship was found between shape of source galls and the shape of galls formed on diseased seedlings, suggesting that gall shape is not strongly controlled by Cq genes and that these genes are not apparently shared between Cqe and round galled collections of Cqf. In addition, the variance observed among families in percent gall suggests that genetic gains in resistance to fusiform rust disease are possible within this Texas seed source.

INTRODUCTION

In general, resistance to fusiform rust disease in natural stands of loblolly pine increases from east to west in the southern pine region. The gradient starts near the Mississippi-Alabama state line, continues west across Mississippi and Louisiana, and ends with the highest levels of resistance in east Texas and Arkansas (Wells and Wakeley 1966, Grigsby 1973, Wells et al. 1982).

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The studies that documented higher levels of fusiform rust resistance in the western populations of loblolly pine were all conducted with bulk seed lots, i.e., a mixture of seeds from several trees was used for each area sampled. As a consequence, variation in resistance among individual trees could not be evaluated. The results seemed to imply that all the trees had some form of general resistance and were often interpreted this way (Wells et al. 1982). Studies with full-sib families of loblolly pine from Livingston Parish, Louisiana have shown that fusiform rust resistance is highly variable among individual trees and that the seed source's higher level of resistance is due to a high frequency of resistant genotypes (Snow et al. 1982). In addition our current understanding of this pathosystem suggests that trees do indeed differ in the resistance genes that they carry and that these genes interact specifically with corresponding genes in the pathogen to determine gall formation (Kubisiak et al. 2005; Nelson et al. 2008).

The Livingston Parish stock and those from even more resistant western seed sources, e.g., western Louisiana, Texas, and Arkansas are potentially valuable to tree breeders attempting to breed for fusiform rust disease resistance in loblolly pine (Powers et al. 1981). The present experiment was designed to obtain information on the rust resistance levels in a sample of east Texas loblolly pines and to test two long standing hypothesis in fusiform rust biology, namely (1) that Texas loblolly pine and shortleaf pine share genes for resistance to Cqf and (2) that Cqe and Cqf collected from round-shaped galls share genes for gall shape.

MATERIALS AND METHODS

The studied parent trees were originally selected as survivors of a southern pine beetle epidemic that occurred in east Texas in the 1960s (referred to as 'Coyne' trees for Jack Coyne, the entomologist who made the selections). The selection criteria were that the trees be surrounded by beetle killed trees, free of fusiform rust, and in the dominant or co-dominant crown class. This resulted in a sample of trees with a potential for resistance to both southern pine beetle and fusiform rust disease. Three experiments are described here in which the progeny from 7 of these trees were evaluated for fusiform rust resistance.

In the first experiment (Experiment 1), pine seedlings from each of 19 seed lots were artificially inoculated using five different inocula of *Cronartium quercuum* (Berk.) Miyabe ex Shirai (Cq) (Table 1). Seventeen of the seed lots were from controlled crosses produced in a half-diallel mating design (Table 2). Two check lots were also included in the test—bulk loblolly pine and bulk shortleaf pine each from south Mississippi. The Cq inocula consisted of one single gall aeciospore collection from shortleaf pine and four single gall collections from loblolly pine (Table 1). Two of the loblolly inocula were collected in Louisiana while the other two loblolly inocula and the shortleaf inoculum were collected in east Texas. One loblolly inoculum from each state was from a short, round-shaped gall and the other was from a long, fusoid-shaped gall.

Because of their origin on the two pine species, the inocula from loblolly pine were considered to be *C. quercuum*. f.sp. *fusiforme* (Cqf) and that from shortleaf pine *C. quercuum* f.sp. *echinatae* (Cqe) (Burdsall and Snow 1977). The two special forms of the pathogen were used to test a long standing hypothesis: Texas loblolly pine and shortleaf pine have common genes for fusiform rust resistance (Wells and Wakeley 1966, Wells et al. 1982). Approximately the same response of

the Texas loblolly pine and shortleaf pine progenies to Cqf and Cqe would support the hypothesis. Inocula from round- and fusoid-shaped galls on loblolly pine were used to test a second long standing hypothesis: Cqe and Cqf have common genes for determining gall shape. Similar reactions of inoculated pines to Cqe and to round gall Cqf collections would support this hypothesis and indicate relatedness in the fungal pathogen to match that which has been postulated for the pine host.

Table 1. *Cronartium quercuum* collections used to evaluate Texas loblolly pine for fusiform rust resistance.

Number	Source of spores	Gall Shape	Collection code
1	Shortleaf pine – Rusk County, TX	Round	MET-3
2	Loblolly pine – Trinity County, TX	Round	LT-3
3	Loblolly pine – Trinity County, TX	Fusoid	LT-1
4	Loblolly pine – Livingston Parish, LA	Round	LP-3
5	Loblolly pine – Livingston Parish, LA	Fusoid	WLP-6

Table 2. Diallel mating design and progeny code numbers for control-pollinated progeny of Texas loblolly pine.

Parent Trees	B11L	B123L	B134L	B142L	B144L	B145L
B7L	1	- -	3	4	5	6
B11L		7	8	9	10	11
B123L			12	- -	14	15
B134L				16	17	- -
B142L					19	20
B144L						21

The pine seedlings were grown in 4-inch plastic tubes with a 1:1 ratio of vermiculite and peat moss. When the plants were 6 to 8 weeks old, 10 seedlings from each seed lot were inoculated with each of the five inocula. This process was repeated to give three complete replications. The order in which pine families were inoculated and the order in which the inocula were used, were determined independently and randomly among and within replications. A forced air system (Snow and Kais 1972) with basidiospore counts maintained at 12-18 mm^2 was used for all pine inoculations. Afterwards, the pine seedlings were planted in nursery beds where they remained for 9 months until final evaluations were made.

A second experiment (Experiment 2) was established to compare the greenhouse inoculations with natural exposure. The seedlings were grown in the nursery in 1985 and were planted at a high rust hazard site on the Palustris Experimental Forest near Alexandria, Louisiana, in February 1986. The field experiment was designed as a randomized complete block with five replications. Each pine full-sib family and bulk seed source was represented by a 10-tree row-plot in each replication. A third experiment (Experiment 3) was established to conserve and

evaluate the genotypes of the pines that had remained gall-free after artificial exposure to the Cqf inocula. Twenty-five rust-free seedlings from each full-sib family were planted adjacent to Experiment 2 in February 1986 in five randomized complete blocks of 5-tree row-plots. In addition, a local Louisiana bulk source of loblolly pine was included as a third check lot.

Survival, height, DBH, and presence of fusiform rust galls were recorded for each tree at ages 4 and 20 years in both Experiments 2 and 3. In addition, resin yield (g / 24 hours, Roberds et al. 2003) was recorded on each of the surviving trees in the spring of year 20 (data not presented). The GLM procedure of SAS (SAS Institute, Cary, NC) was used to evaluate the significance ($p<0.05$) of the differences among full-sib families. Duncan's new multiple range test was used for comparing means.

RESULTS AND DISCUSSION

The full-sib families showed significant differences in resistance to all Cqf inocula (Inocula 2-5, Table 3) as measured by percent gall. Differences in resistance were also indicated among the pine families in response to Cqe inoculum (Inoculum 1, Table 3), but interactions across the replications apparently nullified their statistical significance. Consequently, a significant relationship could not be established between the Cqe inoculum and any one of the Cqf inocula. These results, therefore, cannot be used in support of the hypothesis that loblolly pine and shortleaf pine share common genes for resistance to Cqf. It is interesting to note, however, that the loblolly pine families were at least equally resistant (i.e., not significantly different) to Cqe as were the bulk shortleaf pine seedlings (Table 3) and that the bulk shortleaf were uniformly resistant to Cqf (0% gall for all Cqf inocula). Perhaps the two species or these specific trees have common genes for resistance to Cqe, but they are certainly different with respect to Cqf. This result is consistent with earlier data reported by Kraus et al. 1982. The uniform resistance of shortleaf pine to Cqf has been reported (Snow and Kais 1970; Powers 1972) as have exceptions (Kais and Snow 1972; Kraus et al. 1982).

All of the galls caused by Cqe were round shaped as are indicated by their low gall form values (Inoculum 1, Table 4). For Cqf (Inocula 2-5, Table 4), there was no relationship between the shape of galls from which the inocula were collected and the shape of galls that formed on inoculated seedlings. For example, Inoculum 3 consistently caused galls with lower gall form ratios than Inoculum 2, while the "parent" gall of Inoculum 3 was fusoid-shaped, and that for Inoculum 2 was round-shaped. Further, Inocula 4 and 5 were collected from round and fusoid galls, respectively, but the gall form values for galls caused by these inocula were not consistently high or low and varied by pine family. These results clearly fail to support the hypotheses that Cqe and Cqf have common genes that govern gall shape.

The data in Table 3 suggest the presence of interacting gene pairs between the full-sib families and the single gall rust collections (see Nelson et al. 2008 for further discussion on methodology). For example, both B7L and B11L do not appear to carry R genes (alleles) specific for the avirulence (Avr or A) genes (alleles) present in the four Cqf collections, since percent gall data for B7L x B11L exceed 75% for each inoculum. In addition, B123L may be alike in this regard as it responds similarly to the four Cqf inocula when crossed to B11L (note that B123L was not crossed with B7L for this experiment). On the other hand, parents B134L,

B142L, B144L, and B145L all appear to have R gene(s) that interact with one or more of the Cqf inocula. These interactions can be most easily seen by looking at the crosses of these trees with either B7L or B11L where several cells are in the 50%-60% range or lower. Also of interest is the suggestion by these data that the response of these trees differs among the Cqf inocula when crossed to B7L and B11L even though the latter two trees appear not to carry any R genes. This may be due to different background genetic effects (i.e., gene modifiers, enhancers, etc.) along with sampling effects given that only 30 trees were evaluated in each full-sib family-by-single gall inoculum combination.

Table 3. Percent of loblolly pine with fusiform rust galls 9 months after inoculation with five sources of *Cronartium quercuum*.

		Inocula[1]				
Family	Cross[2]	1 Cqe, R	2 Cqf, R	3 Cqf, F	4 Cqf, R	5 Cqf, F
1	7 x 11	0	83.3a[3]	91.7a	75.0abc	91.7a
3	7 x 134	6.7a	46.7abc	55.2abc	36.7c-g	70.0a-d
4	7 x 142	6.7a	60.0abc	70.0abc	43.3b-f	80.0abc
5	144 x 7	0	43.3abc	50.0bc	33.3d-g	40.0cd
6	7 x 145	5.6a	83.3a	72.2abc	38.9c-g	88.6ab
7	11 x 123	33.3a	66.7ab	91.7a	80.6ab	66.7a-d
8	134 x 11	0	58.3abc	78.3ab	51.6a-f	86.7ab
9	11 x 142	23.3a	60.0abc	56.7abc	90.0a	80.0abc
10	144 x 11	3.7a	46.7abc	60.0abc	33.3d-g	40.0cd
11	11 x 145	10.8a	49.2abc	67.8abc	79.6ab	70.0a-d
14	123 x 144	18.3a	22.2cd	40.4bc	32.9d-g	48.5cd
15	123 x 145	25.0a	38.9bc	50.0bc	91.7a	69.4a-d
16	134 x 142	17.4a	43.3abc	56.7abc	70.0a-d	90.0a
17	134 x 144	6.7a	45.0abc	36.7c	31.7d-g	30.5de
19	144 x 142	25.0a	66.7ab	72.2abc	27.8efg	30.5de
20	145 x 142	26.7a	66.7ab	56.7abc	85.6a	100.0a
21	145 x 144	10.7a	70.0ab	73.3abc	21.5fg	29.2de
22	MS Bulk Shortleaf	10.7a	0	0	0	0
23	MS Bulk Loblolly	7.0a	63.3ab	59.9abc	51.9a-f	62.7a-d
Means		12.5	53.4	59.9	51.9	62.8

[1]Inocula code is as sown in Table 1: Cqe is *C. quercuun* f.sp. *echinatae*; Cqf is *C. quercuum* f.sp. *fusifome*; R is round-shaped source gall; and F is fusoid-shaped source gall.

[2]Cross code is female parent x male parent. Parent codes used here omit the B prefix and L suffix shown in Table 2.

[3]For each column, means not followed by the same letter are significantly different at the 5% level.

Table 4. Form (gall length / gall diameter) of fusiform rust galls on loblolly pine 9 months after inoculation with five sources of *Cronartium quercuum.*

Family	Cross[2]	Inocula[1] 1 Cqe, R	2 Cqf, R	3 Cqf, F	4 Cqf, R	5 Cqf, F
1	7 x 11	- -	2.12ab[3]	1.95ab	1.88c-h	2.45a-e
3	7 x 134	0.89bc	2.33ab	2.02ab	2.41a-d	1.98cde
4	7 x 142	0.86bc	3.14a	1.87abc	2.2b-f	2.60a-d
5	144 x 7	- -	2.03ab	1.91ab	2.58ab	2.23a-e
6	7 x 145	1.41ab	2.35ab	1.96ab	2.53abc	3.05ab
7	11 x 123	0.88bc	2.73ab	1.62bc	1.30h	1.98cde
8	134 x 11	- -	2.19ab	1.68abc	1.77d-h	2.09cde
9	11 x 142	0.87bc	2.38ab	1.78abc	1.82d-h	2.22a-e
10	144 x 11	0.84bc	2.00b	1.63bc	1.64e-h	1.80cde
11	11 x 145	1.05abc	2.73ab	1.77abc	2.25b-e	1.78de
14	123 x 144	1.18abc	1.75b	2.11a	1.55fgh	2.11b-e
15	123 x 145	0.73c	2.69ab	2.01ab	2.96a	1.52e
16	134 x 142	1.02abc	2.74ab	1.85abc	2.22b-f	2.43a-e
17	134 x 144	0.80c	2.30ab	1.44c	1.84d-h	1.62e
19	144 x 142	0.95bc	2.60ab	1.87abc	1.50gh	3.11a
20	145 x 142	154a	2.44ab	2.02ab	2.99a	3.09a
21	145 x 144	1.27abc	2.16ab	1.85abc	1.97b-h	2.23a-e
22	MS Bulk Shortleaf	0.88bc	- -	- -	- -	- -
23	MS Bulk Loblolly	1.11abc	2.23ab	1.90ab	2.02b-g	2.75abc
Means		1.04	2.38	1.85	2.09	2.28

[1]Inocula code is as sown in Table 1: Cqe is *C. quercuun* f.sp. *echinatae*; Cqf is *C. quercuum* f.sp. *fusifome*; R is round-shaped source gall; and F is fusoid-shaped source gall.

[2]Cross code is female parent x male parent. Parent codes used here omit the B prefix and L suffix shown in Table 2.

[3]For each column, means not followed by the same letter are significantly different at the 5% level.

The variance in fusiform rust resistance observed among the Texas full-families demonstrates that gains in rust resistance are possible within this seed source (Table 3). Selection of individual resistant trees should be part of tree improvement practice, as has been recommended for other western sources of loblolly pine (Sluder 1973), but knowledge of specific R and A genes also needs to be considered. Candidate parent trees should be evaluated against a panel of Cqf collections (preferably single-spore isolates, Kubisiak et al. 2005) to determine their likely R gene composition. This information can then be used to determine useful crossing schemes to combine R genes originating in Texas with those from other areas of the south, if in fact they are found to differ. In any event, the long-term performance of such materials at many field sites should be determined before they are used on a large scale.

Survival and growth of trees on the Palustris Experimental Forest has been good through 20 years (Experiment 2, Table 5 and Experiment 3, Table 6). A few of the loblolly pine families grew as well, and in some cases, better than the south Mississippi or local Louisiana loblolly pine sources. This is encouraging, because the slow growth noted with some Texas seed sources (Wells and Wakeley 1966) is not clearly evident here. Rust incidence (percent gall) has been considerably lower than expected thus correlations between field and greenhouse data were not considered. Future plans for these plantings are to: (1) maintain the trees for genetic conservation; (2) maintain the galls for future experiments; and (3) monitor the trees for resistance to southern pine beetle attack should an outbreak occur.

Table 5. Survival, growth, and fusiform rust incidence for un-inoculated full-sib families (Experiment 2) of Texas loblolly trees planted on the Palustris Experimental Forest.

Family Code	Cross[1]	# Survivors 4 yrs	Height 4 yrs (m)	#Galled 4 yrs	Height 20 yrs (m)	DBH 20 yrs (cm)
3	7 x 34	41	2.86ab[2]	1	17.3abcd	20.9abc
4	7 x 142	45	2.72abc	1	18.4a	21.2ab
5	144 x 7	37	2.72cd	1	16.1de	18.0d
6	7 x 145	29	2.89ab	0	16.9bdc	22.6a
10	144 x 11	42	2.55bcd	2	15.7e	19.5bcd
11	11 x 145	45	2.76abc	2	17.5abc	21.9ab
12	134 x 123	23	2.99a	0	16.3cde	22.4ab
16	134 x 142	45	2.93ab	1	17.7ab	22.7a
17	134 x 144	39	2.76abc	1	16.3cde	18.7cd
21	145 x 144	28	2.29d	4	18.1ab	21.0abc
22	MS bulk Shortleaf	47	1.98e	0	14.31f	16.7d
23	MS bulk Loblolly	47	3.05a	1	18.0ab	22.3ab

[1]Cross code is female parent x male parent. Parent codes used here omit the B prefix and L suffix shown in Table 2.

[2]For each column, means not followed by the same letter are significantly different at the 5% level.

Table 6. Survival, growth, and fusiform rust incidence of rust-free survivors (Experiment 3) of Texas Loblolly pines inoculated in Gulfport, MS and planted on the Palustris Experimental Forest.

Family Code	Cross[1]	#Survivors 4 yrs	Height 4 yrs (m)	#Galled 4 yrs	#Survivors 20 yrs	Height 20 yrs (m)	DbH 20 yrs (cm)
1	7 x 11	16	2.59a-d[2]	0	15	17.2abcd	23.4bc
3	7 x 134	18	3.08abc	1	17	18.1abc	23.2bc
4	7 x 142	5	2.21cd	0	5	16.2bcd	22.1bc
5	144 x 7	3	2.21d	0	2	15.4d	25.7ab
6	7 x 145	19	3.14ab	0	16	18.9a	25.0abc
7	11 x 123	10	3.26a	0	9	17.4abcd	22.1bc
8	134x 11	17	3.01a-d	0	14	16.7abcd	21.1bc
9	11 x 142	16	2.81a-d	0	15	16.0cd	24.3abc
10	144 x 11	13	3.01a-d	0	9	17.4abcd	20.9bc
11	11 x 145	20	2.94a-d	0	20	18.7abc	23.1bc
14	123 x 144	7	2.79a-d	0	5	17.2abcd	22.1bc
15	123 x 145	15	3.12ab	1	15	17.9abcd	24.4abc
16	134 x 142	15	3.23a	0	14	17.6abcd	24.8abc
17	134 x 144	11	2.32bcd	0	11	17.0abcd	21.2bc
19	144 x 142	7	2.45a-d	1	7	17.1abcd	20.2c
20	145 x 142	23	3.11ab	0	23	18.5abc	24.4abc
21	145 x 144	14	2.87a-d	0	9	17.3abcd	25.0abc
22	MS bulk Shortleaf	9	2.33bcd	0	9	12.9e	16.1d
23	MS bulk Loblolly	14	2.91a-d	0	11	18.3abc	28.2a
24	LA bulk Loblolly	44	2.33bcd	0	37	17.1abcd	22.4bc

[1]Cross code is female parent x male parent. Parent codes used here omit the B prefix and L suffix shown in Table 2.

[2]For each column, means not followed by the same letter are significantly different at the 5% level.

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LITERATURE CITED

Burdsall, H.H. and G.A. Snow. 1977. Taxonomy of *Cronartium quercuum* and *Croanrtium fusiforme*. Mycologia 69:503-508.

Grigsby, H.D. 1973. South Carolina best of 36 loblolly pine seed sources for southern Arkansas. USDA Forest Service Research Paper SO-89, 10 p.

Kais, A.G. and G.A. Snow. 1972. Host response to pines of various isolates of *Cronartium quercuum* and *Cronartium fusiforme*. In: Biology of Rust Resistance in Forest Trees, USDA Miscellaneous Publication 1221:495-503.

Kraus, J.F., H.R. Powers, Jr. and G.A. Snow. 1982. Infection of shortleaf x loblolly pine hybrids with *Cronartium quercuum* f. sp. *echinatae* and *C. quercuum* f.sp *fusiforme*. Phytopathology 72:431-433.

Kubisiak, T.L., H.V. Amerson and C.D. Nelson. 2005. Genetic interaction of the fusiform rust fungus with resistance gene *Fr1* in loblolly pine. Phytopathology 95:376-380.

Nelson, C.D., T.L. Kubisiak and H.V. Amerson. 2008. Unraveling and managing fusiform rust disease: progress and plans. The 29th Southern Forest Tree Improvement Conference, June 20-22, 2007, Galveston, Texas, USA, in press.

Powers, H.R., Jr. 1972. Testing for pathogenic variability within *Cronartium fusiforme* and *C. quercuum*. In: Biology of Rust Resistance in Forest Trees, USDA Miscellaneous Publication 1221:505-511.

Powers, H.R., Jr., R.A. Schmidt and G.A. Snow. 1981. Current status and management of fusiform rust on southern pines. Annual Review of Phytopathology 19:353-371.

Roberds, J.H., B.L. Strom, F.P. Hain, D.P. Gwaze, S.E. McKeand and L.H. Lott. 2003. Estimates of genetic parameters for oleoresin and growth traits in juvenile loblolly pine. Canadian Journal of Forest Research 33**:**2469–2476.

Sluder, E.R. 1973. Open-pollinated progenies from six selected loblolly pines: 10-year performance in central Georgia. USDA Forest Service Research Note SE-194, 3 p.

Snow, G.A. and A.G. Kais. 1970. Pathogenic variability in isolates of *Cronartium fusiforme* from five southern states. Phytopathology 60:1730-1731.

Snow, G.A. 1985. A view of resistance to fusiform rust in loblolly pine. In: Proceedings of 34th Annual Forestry Symposium, Insects and Diseases of Southern Forests, pp. 47-51.

Snow, G.A. and A.G. Kais. 1972. Technique for inoculating pine seedlings with *Cronartium fusiforme*. In: Proceedings NATO-IUFRO Advanced Study Institute, USDA Miscellaneous Publication 1221, pp. 325-326.

Snow, G.A., W.L. Nance and E.B. Snyder. 1982. Relative virulence of *Cronartium quercuum* f. sp. *fusiforme* on loblolly pine from Livingston Parish. In: Proceedings Third International Workshop on the Genetics of Host Parasite Interactions in Forestry, pp. 243-250.

Snow, G.A., F.R. Matthews, W.L. Nance and G.S. Foster. 1990. Effects of pollen source on loblolly pine resistance to *Cronartium quercuum* f. sp. *fusiforme*. Forest Science 36:304-312.

Wells, O.O. and P.C. Wakeley. 1966. Geographic variation in survival, growth, and fusiform-rust infection of planted loblolly pine. Forest Science Monograph 11, 40 p.

Wells, O.O., G.L. Switzer and W.L. Nance. 1982. Genotype-environment resistance in Mississippi loblolly pine. Forest Science 28:797-809.

Zobel, B.J. 1953. Are there natural loblolly-shortleaf pine hybrids? Journal of Forestry 51:494-495.

The Potential of Acoustics to Determine Family Differences for Wood Quality in a Loblolly Pine (*Pinus taeda* L.) Trial

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Abstract: Acoustics have been used to determine wood quality attributes in both standing timber and sawn lumber. Sonic transmission data are collected non-destructively and can act as a surrogate for stiffness, they are directly related to modulus of elasticity (MOE) and closely related to differences in microfibril angle (MFA). Together with wood density, MFA and MOE are the most important wood characteristics that affect solid wood properties.

Breeding and selection for desirable wood properties will be a key factor in determining the global competitiveness of the forest industry in the United States. Breeders of loblolly pine (*Pinus taeda* L.) have been able to select for differences in wood specific gravity, MOE and MFA based on data collected from wood increment core samples. The use of acoustics in determining wood quality has merit in that it requires no direct wood sampling. However, before differences in acoustic transmission can be used in a breeding program, a number of questions need to be answered. Are there differences in transmission of sufficient magnitude and repeatability to allow heritable differences to be detected among families? How large a component is genotype by environment interaction? Is the equipment robust enough to be used by field crews and can data be collected efficiently?

To answer these questions, the Southern Institute of Forest Genetics in collaboration with the Western Gulf Forest Tree Improvement Program collected acoustic velocity data using the Fakopp Stress Wave Timer in three control-pollinated loblolly pine progeny tests established by International Paper Company in southeast Texas. Sampled trees were disease free and without forks. Acoustic velocity was measured at two radial directions on each tree over a length of 1.2 m spanning breast height. Measurements were averaged for each tree. Variance components were estimated for each location using the software packages DIALL and DIALLC. Then single location heritabilities were calculated. Heritabilities for the averaged acoustic velocity were moderate yet large enough to be useful in an applied breeding program. Phenotypic correlations among these traits were small, but positive suggesting that selection for growth and stiffness can be done simultaneously. Field protocols, however, need to be further refined to avoid data outliers and to arrive at easier methods of collecting observations on the large numbers of trees required to estimate parental breeding values.

Keywords: Acoustics, sonic velocity, wood quality, stiffness, MOE, MFA
Loblolly pine, *Pinus teada L*.

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INTRODUCTION

Acoustics have been used to determine wood quality attributes in both standing timber and sawn lumber (Brashaw and others, 2005; Grabianowski and others, 2006; Joe and others, 2004). Sonic transmission data are collected non-destructively and can act as a surrogate for stiffness. They are directly related to modulus of elasticity (MOE) and closely related to differences in microfibril angle (MFA) (Lindstrom and others, 2002). Together with wood density, MFA and MOE are the most important wood characteristics that affect solid wood properties. Whereas density determines the quantity of wood in a given volume, acoustic velocity with its inherent relationship with MFA can determine wood quality (Chauhan and Walker, 2006).

Breeding and selection for desirable wood properties will be a key factor in determining the global competitiveness of the forest industry in the United States. Breeders of loblolly pine (*Pinus taeda* L.) have been able to select for differences in wood specific gravity, MOE and MFA based on data collected from wood increment core samples (Isik and others, 2005). The use of acoustics in determining wood quality has merit in that it requires no direct wood sampling. Reasonable correlations have been found when comparing the acoustic velocity of standing trees and the mean stiffness of dried lumber cut from those trees (Chauhan and Walker, 2006). Associations between acoustic measurements taken in the outermost wood and from whole stems suggests that estimates of outerwood MOE are completely representative of whole stem MOE (Lassere and others, 2007).

This study was undertaken to develop answers to the following questions:

1. Are there differences in transmission of sufficient magnitude and repeatability to allow heritable differences to be detected among families?
2. How large a component is genotype by environment interaction?
3. Is the equipment robust enough to be used by field crews and can data be collected efficiently?

MATERIALS AND METHODS

The Southern Institute of Forest Genetics in collaboration with the Western Gulf Forest Tree Improvement Program (WGFTIP) collected acoustic velocity data in three six-year-old control-pollinated loblolly pine progeny tests established by International Paper Company in southeast Texas (Figure 1). Two of the tests contained three diallels from two different breeding groups, while the third had only a single diallel from each of the breeding groups. All tests were completely randomized single-tree plantings established on an 8’ X 8’ spacing with 40 replications. Survival, growth and form data were collected in all tests at the end of the fifth growing season (Table 1).

Figure 1. Natural distribution of loblolly pine in the western Gulf region. Progeny test locations are marked with ▲.

Table 1. Summary statistics of fifth-year survival, growth and form in the study tests. Straight score is a relative assessment of straightness on a scale of 1-4, with 1 being very crooked and 4 being very straight. Tests with average straightness score 2.5 for this trait.

Test	County	N	Survival (%)	HT (m)	DBH (cm)	Vol/tree (dm^3)	Straight Score	Forks (%)
1	Cherokee	46	87.8	5.9	9.0	11.6	2.6	7.2
2	Trinity	46	95.1	5.6	8.5	10.8	2.6	7.9
3	Polk	33	74.3	5.0	7.9	6.6	2.4	10.2

Acoustic time of flight (ToF) was measured at two radial directions on each tree over a length of 1.2 m centered at breast height using a Fakopp Wave Stress Timer (also known as a Tree Sonic). Sampled trees were from a single diallel that was common to all three tests. They were disease free and without forks. Time data were the average of three separate taps on the uppermost transducer. These data were converted to velocity using a time correction factor and formula provided by FAKOPP. Modulus of elasticity (MOE) was calculated using the formula:

$$MOE = \rho V^2$$

where ρ = green density and V is the velocity of sound. Since apparent wood density at fiber saturation point is theoretically a constant, V^2 is considered a surrogate for dynamic longitudinal MOE (Huber and others, 2007). MOE values for each tree were analyzed separately for each face and as an average of the two (aveMOE). A subsample of trees from each test was cored for specific gravity determination using the maximum moisture method.

Variance components at each location were estimated for ToF, velocity, MOE and aveMOE using the software packages DIALL and DIALLC (Schaffer and Usanis, 1968), where family sums of squares are partitioned into estimates of general (GCA) and specific combining abilities (SCA). Negative variance estimates were assumed to be zero. Narrow-sense heritabilities were calculated for all traits at each location using the following formula:

$$h^2 = 4\,\sigma^2_{GCA} / [2\sigma^2_{GCA} + \sigma^2_{SCA} + \sigma^2_{GE} + \sigma^2_r + \sigma^2_e],$$

where GE denotes the GCA by replication (r) interaction effect.

Data for aveMOE were pooled across locations and variance components estimated using the methods of Johnson and King (1998). Overall narrow-sense heritability was calculated using the previous formula and full-sib family heritability was calculated using the formula:

$$h^2 = 2\sigma^2_{GCA} / [2\sigma^2_{GCA} + \sigma^2_{SCA} + \sigma^2_{GE}/s + \sigma^2_r/lr + \sigma^2_e/lrn],$$

where s = number of test sites, r = number of test replications and n = number of sample trees. Phenotypic correlations among the growth, specific gravity and acoustic traits were estimated as product-moment correlations using PROC CORR in SAS (SAS 1985).

RESULTS AND DISCUSSION

Data for all acoustics variables were statistically significant in each of the individual tests. Individual site narrow-sense heritability estimates ranged from 0.18 to 0.49 for ToF, from 0.25 to 0.47 for velocity, from 0.27 to 0.46 for individual MOE estimates and from 0.31 to 0.44 for aveMOE (Table 2). The estimates for MOE are not very different from those observed in standing slash pine at age eight (Huber and others, 2007). The estimates for tests 1107 and 1109 were lower than those from test 1108 possibly due to outliers in the data sets for these two tests. However, removal of these outliers did not result in vastly different heritability estimates for any of the acoustic traits. In addition, correction for sample day was attempted with the data from test 1107, but resulted in little change in the estimates.

Table 2. Narrow-sense heritability estimates for each of the acoustic traits by radial direction ('Side') and test. aveMOE is the average of individual side MOE.

	Side 1			Side 2			
Test	ToF	V^2	MOE	ToF	V^2	MOE	aveMOE
1107	0.23	0.26	0.27	0.23	0.30	0.32	0.31
1108	0.49	0.47	0.45	0.41	0.39	0.37	0.44
1109	0.41	0.45	0.46	0.18	0.25	0.27	0.40

In the combined analysis family differences for aveMOE were significant but the interaction between families and sites was non-significant. The estimate of narrow-sense heritability was quite high at 0.94. The estimate of full-sib family heritability was also quite high at 0.97. GCA effects accounted for 16 percent of the total variance for aveMOE. With the large number of replications sampled in this study, the non-genetic components were only a small contributer to the total variance.

Family-mean phenotypic correlations among the growth traits, wood specific gravity and aveMOE were all positive (Table 3). However, the correlations between diameter and specific gravity and between volume and average MOE, while positive, were not significantly different from zero. The correlation between wood specific gravity and average MOE was also positive.

Table 3. Family-mean phenotypic correlations between growth, wood specific gravity and average MOE. Phenotypic correlations significant at P<0.01 in bold.

Trait	DBH	VOL	SPGR	aveMOE
HT	**0.72**	**0.83**	**0.18**	**0.23**
DBH		**0.96**	0.08	**0.18**
VOL			**0.23**	0.12
SPGR				**0.27**

CONCLUSIONS

The ultimate goal is to incorporate stiffness along with breeding values for volume, straightness, and wood specific gravity into a sawlog index for ranking candidates for inclusion in seed orchards. Results from this study suggest that acoustics may play a valuable role in the development of an additional wood quality selection trait. Family differences for all variables were statistically significant. Average MOE had moderate but positive correlations with growth variables, suggesting that simultaneous selection for growth and wood quality may be possible. However, to simultaneously improve volume growth and MOE, selection must be made first for specific gravity.

There was no significant genotype by environment interaction. However, this was not unexpected given the relatively small distance between tests. Single-location individual heritabilities for the averaged acoustic velocity were moderate and large enough to be useful in an applied breeding program. However, field protocols need to be further refined to avoid data outliers and to arrive at easier methods of collecting observations on the large numbers of trees required to estimate parental breeding values.

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LITERATURE CITED

Brashaw, B.K., R.J. Vatalaro, J.P Wacker, and R.J. Ross. 2005. Condition assessment of timber bridges: 2. Evaluation of several stress-wave tools. USDA Forest Service, Forest Products Laboratory General Technical Report 160. Madison, WI. 11p.

Chauhan, S.S. and J.C.F.Walker. 2006. Variations in acoustic velocity and density with age, and their interrelationships in radiate pine. For. Ecol. and Manage. 229:388-394.

Grabianowski, M., B. Manley and J.C.F. Walker. 2006. Acoustic measurements on standing trees, logs and green lumber. Wood Sci. Technol 40: 205-216

Huber, D., L. Parisi, G. Powell and X. Li. 2007. Cooperative Forest Genetics Research Program 49th Annual Progress Report. 22p.

Isik, F., B. Li and B. Goldfarb. 2005. Genetic Variation in MFA, MOE and wood density among clones of *Pinus taeda* L. Proc. Of the 287th South. For. Tree Improv. Conf., p. 95.

Joe, B., R. Dickson, C. Raymond, J. Ilic and C. Matheson. 2004. Prediction of *Eucalyptus dunnii* and *Pinus radiate* timber stiffness using acoustics. RIRDC Publication No. 04/013. 121 p.

Johnson, G.R., and J.N. King. 1998. Analysis of half diallel mating designs: 1- A practical analysis procedure for ANOVA approximation. Silv. Gen. 47: 74-79.

Lassere, J.-P., E.G Mason and M. S. Watt. 2007. Assessing corewood acoustic velocity and modulus of elasticity with two impact based instruments in 11-year-old trees from a clonal-spacing experiment of *Pinus radiata* D. Don. For. Ecol. And Manage. 239: 217-221.

Lindstrom, H., P. Harris and R. Nakada. 2002. Methods of measuring stiffness of young trees. Holz als Roh- und Werkstoff 60. pp. 165-174

SAS Institute Inc. 1985. SAS® Language Guide for Personal Computers, Release 6.03 Edition. SAS Institute Inc., Cary, NC, 558 pp.

Schaffer, H.E. and R.A. Usanis. 1968. General least squares diallel analysis. Department of Genetics, North Carolina State University, Raleigh.

Computer Simulation of Marker-Directed Population Improvement

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A wide range of commercially important traits in forest tree breeding may be referred to as complex, where a situation-specific approach will make genetic improvement as efficient as possible. The most common approach in current programs is to treat all traits as purely polygenic, assuming the classical "infinitesimal model" (Fisher 1918). The objective of our research is to quantitatively evaluate breeding strategies using marker-directed population improvement (MDPI) (Nelson and Echt 2004). In these strategies, the complex nature of commercial traits is reflected in predicting both polygenic and quantitative trait loci (QTL) genetic effects, and combining these into a single selection criterion. We review here the development of the computer simulation model that will enable this research. With the help of the model, it is possible to assess impact of several parameters, such as the density and information content of markers flanking the QTL, and the relative effect of the QTL on the trait's phenotype. The effects of these variables can be analyzed within the context of a regular recurrent selection strategy, where the main objective is the genetic response in a production population. Proportional reduction in gene diversity, cost, and time components can also be evaluated.

SIMULATION MODEL

The basic model features were derived from the stochastic simulation model POPSIM™ [initial reference by Mullin and Park (1995), and the review of the most recent version by Lstibůrek et. al (2005)]. We assume a single "breeding population" being managed over a number of successive, non-overlapping generations. The population consists of N_T individuals; the population size is held constant. Mating within each generation is declared, and any possible mating scheme can be implemented. Following mating, N_C individuals (progenies) are generated for each family. The full set of families is referred to as the "recruitment population", which then serves as a pool of candidates for forward selection. Environmental conditions are assumed homogeneous across generations.

Individual phenotypic values can be modeled as a function of independent genetic and environmental components. The genetic component is subdivided into two causal factors, polygenic and QTL, in what is commonly referred to as a "mixed-inheritance model" (Gomez-Raya and Klemetsdal, 1999). The polygenes are assumed to be distributed throughout the

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genome (following the infinitesimal model assumptions), while the QTL is assumed to reside in a particular genome position. A number of QTL loci can be declared. Individuals in the population are genotyped for any number of polymorphic loci flanking the QLT(s) (neutral markers with respect to the trait). Different types of markers can be simulated, such as SNPs or SSRs, where the variables of interest are the number of alleles per locus, their respective frequency, and the distribution of marker loci across the genome.

A QTL locus may reside anywhere within a given chromosome; the actual position is declared in terms of recombination distance from marker loci residing on the same linkage group. The recombination frequency is used when generating genotypes of individual progenies, using the random-walk algorithm, as implemented by Crosby (1973). Individual polygenic values are simulated assuming the infinitesimal model (the method is presented in detail by Mullin and Park, 1995). The effect of the QTL (α) is calculated (following Lstibůrek et al. 2005) as:

$$\alpha = \sqrt{\lambda h^2 V_p / 2p(1-p)} \qquad (1)$$

where λ is the proportion of the additive genetic variance explained by the QTL, h^2 is the narrow-sense heritability, V_P is the phenotypic variance, and p is the initial frequency of the A allele at the QTL (alleles in the founder population are sampled from a uniform distribution with mean p).

The dissection of the phenotypic observation into causal components is performed within a mixed-model framework introduced by Fernando and Grossman (1989). The following naming conventions as well as basic model assumptions are similar to those used by Villanueva et al. (2002). The mixed-model equations in the matrix formulation are as follows:

$$\begin{pmatrix} X'X & X'Z & X'W \\ Z'X & Z'Z + \gamma_1 A^{-1} & Z'W \\ W'X & W'Z & W'W + \gamma_2 G^{-1} \end{pmatrix} \begin{pmatrix} \hat{b} \\ \hat{u} \\ \hat{v} \end{pmatrix} = \begin{pmatrix} X'Y \\ Z'Y \\ W'Y \end{pmatrix} \qquad (2)$$

where X, Z, and W are known incidence matrices; A is the additive relationship matrix; G is a matrix of marker-QTL effects (genotypic relationship matrix); γ_1 and γ_2 are variance ratios in the founder population; $\hat{b}$ is a vector of estimated fixed effects; and $\hat{u}$ and $\hat{v}$ are vectors of predicted random effects of polygenes and QTL, respectively. We use the rapid method of Pong-Wong et al. (2001) for calculating the gametic IBD matrix, which is later converted to the genotypic matrix, G (see Nagamine, 2005) and supplied as input to the ASReml2 software (Gilmour et al. 2006) along with other required information. Data in advanced generations accumulate and full-pedigree information is used in the genetic evaluation (extending back to founders, as well as including all selection candidates in all generations) by Best Linear Unbiased Prediction (BLUP) (Mrode, 2005).

Under the MDPI strategy, both polygenic and QTL components enter the BLUP analysis, and the resulting breeding value (*bv*) of an individual *i* is the respective sum of both effects:

$$\widehat{bv}_i = \hat{a}_i + \hat{v}_i \quad (3)$$

When marker data is not utilized in the prediction, the breeding value is predicted in the regular fashion as:

$$\widehat{bv}_i = \hat{a}_i \quad (4)$$

In this latter strategy, the QTL effect is not properly separated from the polygenic genetic effect (therefore not properly accounted for). Thus, one can evaluate the difference due to the added value of marker information contributing to the selection criterion.

The best set of trees is then selected based on predicted breeding values and group coancestry, using group-merit selection (Lindgren and Mullin 1997), where a weighting is applied to group coancestry to control the proportional reduction in gene diversity. A full range of weight factors can be considered, resulting in two extreme situations (strong family selection and balanced within-family selection), and a number of values between these two extremes.

The approach introduced here makes use of the restricted maximum likelihood (REML) implemented by the ASReml2 software; variances are therefore considered unknown, and are estimated based on the phenotypic and marker data supplied with each ASReml2 call (generation). We believe that this approach is more realistic compared to assuming true variances, as performed in many other simulation models published in the scientific literature. In combination with a powerful method to calculate the inverse of *G* matrix, a proportion of the individuals may have missing records. This newly developed simulation model also offers the potential to assess the utility of early-age selection, which is likely where marker-aided selection can most economically enhance the efficiency of forest tree breeding programs.

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LITERATURE CITED

Crosby, J.L. 1973. Computer simulation in genetics. John Wiley & Sons, London.

Fernando, R.L., and Grossman, M. 1989. Marker assisted selection using best linear unbiased prediction, Genet. Sel. Evol. 21(4): 467–477.

Fisher, R.A. 1918. The correlation between relatives on the supposition of Mendelian inheritance. Trans. Roy. Soc. Edinburgh 52: 399-433.

Gilmour, A.R., Gogel, B.J., Cullis, B.R., and Thompson, R. 2006. ASReml User Guide Release 2.0 VSN International Ltd, Hemel Hempstead, HP1 1ES, UK.

Gomez-Raya, L., and Klemetsdal, G. 1999. Two-stage selection strategies utilizing marker-quantitative trait locus information and individual performance. J. Anim. Sci. 77(8): 2008–2018.

Lindgren, D., and Mullin, T.J. 1997. Balancing gain and relatedness in selection. Silvae Genet. 46(2-3): 124–129.

Lstibůrek, M., Mullin, T.J., Mackay, T.F.C., Huber, D., and Li, B. 2005. Positive assortative mating with family size as a function of parental predicted breeding values. Genetics 171(3): 1311-1320.

Mrode, R.A. 2005. Linear models for the prediction of animal breeding values. 2nd Edition. CABI Publishing, Wallingford, Oxfordshire, UK. 344 pp.

Mullin, T.J., and Park, Y.S. 1995. Stochastic simulation of population management strategies for tree breeding: a new decision-support tool for personal computers. Silvae Genet. 44(2-3): 132-141.

Nagamine, Y. 2005. Transformation of QTL genotypic effects to allelic effects. Genet. Sel. Evol. 37(5): 579–584.

Nelson, C.D., and Echt, C.S. 2004. Marker-directed population improvement. *In* Proc. IUFRO Joint Conf. For. Genet. (Div. 2), Nov. 1-4, 2004, Charleston, SC, pp 255.

Pong-Wong, R., George, A.W., Woolliams, J.A., and Haley, C.S. 2001. A simple and rapid method for calculating identity-by-descent matrices using multiple markers. Genet. Sel. Evol. 33(5): 453-471.

Villanueva, B., Pong-Wong, R., and Woolliams, J.A. 2002. Marker assisted selection with optimized contributions of the candidates to selection. Genet. Sel. Evol. 34(6): 679–703.

Integration of Crown Morphology and Leaf-level Physiology as a Tool for Predicting Differences in Aboveground Productivity Among Elite Families of Loblolly and Slash Pine

Daniel J. Chmura and Mark G. Tjoelker[1]

Current silvicultural systems increasingly involve the deployment of genetically improved planting material, together with high-input silvicultural treatments to enhance forest productivity. As forest plantations become increasingly uniform, factors that limit tree growth should be identified in order to effectively alleviate those limitations. To this end, an important task is the identification of the biological bases of within and between-species differences in growth and productivity to enable effective choices of selection criteria.

Forest production depends on CO2 assimilation, but is rarely solely a function of leaf-level photosynthesis. Crown characteristics may affect tree growth by altering light interception and photosynthesis at canopy level. Strong light gradients are present in forest canopies, which often result in parallel changes in leaf morphology and leaf nitrogen for efficient use of light in photosynthetic CO2 uptake. However, the genetic basis of crown and canopy trait differences among southern pine taxa are not well understood, but critical in predicting productivity differences for managing sustainable forest ecosystems.

MATERIALS AND METHODS

Three sites of the PPINES study (Pine Productivity Interactions on Experimental Sites) of the Forest Biology Research Cooperative are located in east Texas and Louisiana in the West Gulf area. At each site, two contrasting silvicultural treatments (control and high intensity) are assigned to main plots, and six selected families of loblolly pine and one of slash pine are assigned to sub-plots. Each treatment has five replications at each site.

In our study we investigated the effects of intensive silvicultural treatment on crown morphology and within-crown leaf-level physiology, and relationships to aboveground productivity in two families of loblolly and one slash pine family. At the end of the second growing season we calculated crown volume from measurements of branch lengths and angles along the tree stem (Chmura et al. 2007). During the fourth and fifth growing seasons we measured tree and crown growth, and within-crown variation in specific leaf area (SLA cm2 g-1, amount of leaf area per leaf dry mass) and leaf nitrogen (N) on a sample of trees representing the range in tree size within each family and treatment. In the mid-season of fifth growing period, we measured light-saturated rates of photosynthesis at the leaf-level in three canopy strata.

RESULTS AND DISCUSSION

In young stands, before canopy closure (age 2 years), we found significant among-family differences in aboveground biomass production and crown structure, and between-species

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differences in leaf area density per unit of crown volume. Loblolly pine produced more flushes and longer branches than slash pine, but branch angles varied little among taxa. Across the sampled range of tree sizes, loblolly pine had larger crowns than slash pine trees, but maintained lower leaf area per given crown volume. Thus, slash pine maintained similar leaf area per tree as loblolly pine of the same age and size, compensating for a smaller crown size. Cultural treatment had no effect on crown traits.

The two loblolly pine families had a higher tree volume index than slash pine at two of three experimental sites, and tree growth was enhanced by the high intensity treatment. The two pine species also differed in patterns of aboveground biomass partitioning, with slash pine having a smaller fraction of biomass in branches than loblolly pine. Bole biomass was positively correlated with leaf area per tree, and the slope of this relationship was similar among the examined families.

The most productive loblolly pine family had a different crown shape than the two other families, with longer branches in the middle of the crown. We suggest that these differences in crown shape and associated leaf area distribution influenced light interception and CO2 assimilation at the canopy level, leading to differences in aboveground biomass accumulation among the examined families at age 2 years.

Crown development during the fourth and fifth growing seasons reflected the pattern expected for stands at canopy closure. Crowns receded from the lower tree trunk, and crown length followed these changes. Crown diameter converged in both species at the end of fifth growing season. The two loblolly pine families intercepted significantly more light (PAR – photosynthetically active radiation) than the slash pine family at the plot level in both years (Fig. 1).

The two pine species differed in SLA and leaf N – loblolly pine families had higher values of both traits. SLA increased and leaf N decreased with canopy depth, reflecting leaf-level acclimation to light gradients within the canopy when stands approach canopy closure. Within-crown gradients of leaf-level photosynthesis rates were similar at both sites when expressed on a leaf-area basis, but not on a leaf-mass basis. Area-based photosynthesis was weakly, but significantly correlated with leaf N, though the correlation varied among sites and cultural treatments. Light-saturated leaf-level photosynthetic rates followed N distribution within crowns, decreasing from the upper to lower canopy. These changes reflected the acclimation of leaf physiological properties to increased shading. However, the two pine species did not differ in leaf-level photosynthesis rates at any crown position.

Tree growth, defined as a yearly tree volume increase, based on a stratified sample of trees was not directly related to leaf-level photosynthesis rates, but was correlated with plot-level spatially averaged PAR interception (Fig. 1). The slope of the relationship did not differ between the two pine species, but the intercept was significantly higher for loblolly than for slash pine in fifth growing season. Intercepts differed in both years between the two cultural treatments when fitted across families and sites.

High intensity treatment, although effective in increasing biomass accumulation in all examined families, did not affect leaf morphology or physiology. Aboveground biomass production differed among the tested families and was related more to accumulated leaf area and its display within crowns than to differences in rates of leaf-level photosynthesis. Therefore, examination of stand-level light interception and its implications for canopy photosynthesis is needed to relate stand growth with physiological properties. However, any modeling effort should take into account observed patterns of within-crown variability in leaf morphological and physiological adjustments to microenvironment within forest canopy.

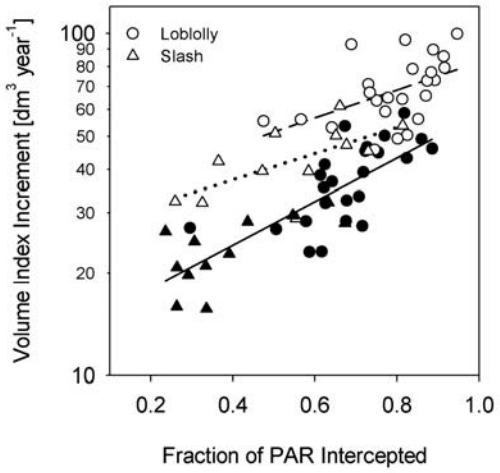

Fig. 1. Relationship of yearly tree volume index increment with plot-level interception of photosynthetically active radiation (PAR) in examined families of loblolly and slash pine. Shown are means for individual plots at two experimental sites in the West Gulf Coastal Plain area. Volume index was calculated for a stratified sample of trees; n = ranges from 4 to 6 for each point. Solid symbols with solid line represent the fourth growing season (2005) with a linear fit across two species and sites. Open symbols represent the fifth growing season (2006) with separate fits for loblolly pine (dashed line) and slash pine (dotted line).

RERERENCES

Chmura, D.J., M.S. Rahman and M.G. Tjoelker 2007. Crown structure and biomass allocation patterns modulate aboveground productivity in young loblolly pine and slash pine. Forest Ecology and Management. 243:219-230.

Is Randomization Necessary in Seed Orchards?

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Abstract: Randomization of clones in seed orchards is commonly practiced to promote cross-fertilization and minimize selfing. While it is practiced for the "right" biological reason, randomization comes with added managerial burden during crop management and harvest. Evidence for extremely low selfing rates in most conifers' seed orchards and natural populations has lead to a re-evaluation of seed orchard designs. The clonal-row seed orchard design represents a viable option for reducing management burden, but it comes with increased estimates of correlated matings between adjacent clones (known as "neighbourhood effect"). Staggering of clonal rows was proposed to double the number of adjacent clones to reduce correlated matings; however, it limits every clone to only four neighbours. We propose a modification to the staggering rows with a "randomized, replicated, staggered clonal-row" design to allow the simultaneous realization of randomization and clonal-rows orchard designs benefits. An interactive computer program was designed for this purpose that allows controlling orchard size and layout, number of clones, number of rows and their length, selection of the physical distance between repeated rows of the same clone, level of "anti-randomization" tolerance imposed by the design parameters, and clonal deployment mode (equal clonal size vs. linear deployment).

Keywords: Seed orchards designs, randomized, clonal-row, selfing, correlated matings.

INTRODUCTION

The genetic structures of seed orchards determine the type of mating events (El-Kassaby et al. 1986) while their layout (spatial arrangement of each clone's ramets relative to each other as well as to other clones' ramets) affects the magnitude and frequency of each mating type (El-Kassaby 1989; El-Kassaby and Askew 1998). In practice, randomization of parental ramets within a seed orchard is judicially practiced to minimize the impact of selfing/inbreeding and the permuted neighbourhood seed orchard design program was and still is the most commonly used for this purpose (COOL; Bell and Fletcher 1978).

The relative ease and access to genetic markers (El-Kassaby and Ritland 1998; Ritland and Ritland 2000), availability of powerful mathematical models for estimating mating system parameters (Ritland 2002), and the unique structure of conifers seed (haploid magagametophyte and diploid embryo) allowed the estimation of populations' and individuals' mating system parameters for many conifer species (see reviews by Adams and Birkes 1991; Mitton 1992).

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Results from studies conducted on natural and experimental populations (seed orchards) commonly highlighted, with very few exceptions (Perry and Knowles 1990; Xie et al. 1991; El-Kassaby et al. 1994; O'Connell et al. 2004), the fact that very little selfing is being observed and that the selfing rate of most natural populations is higher than that observed in seed orchards. These observations are not surprising considering conifers high inbreeding depression and the fact that, in most cases, selfing and most forms of mating among relatives do not develop to viable seed (Woods and Heaman 1989). In light of these biological facts and the added management burden caused by randomization, the development of new seed orchard designs is worth evaluation. The use of a clonal-row seed orchard design has been advocated and mating system parameters comparisons between clonal-row seed orchards and their randomized counterparts were conducted (El-Kassaby 2003; El-Kassaby et al. 2007).

Table 1. Outcrossing rate and correlated matings estimate comparisons between clonal-row and random seed orchard designs for seed samples collected from western hemlock and "interior spruce" seed orchards (95% confidence intervals).

Species	**Outcrossing rate**		**Correlated matings**	
	Random	**Clonal-row**	**Random**	**Clonal-row**
Western hemlock[a]	0.970±0.021	0.899±0.037	0.077±0.032	0.349±0.166
Interior spruce[b]	0.989±0.030	0.948±0.027	0.043±0.043	0.093±0.037

[a]El-Kassaby 2003.
[b]El-Kassaby et al. 2007.

These comparisons revealed that clonal-row orchards consistently produced higher selfing (i.e., lower outcrossing) and correlated matings estimates than that of the random design (Table 1). The observed differences in outcrossing rates are manageable and even if they persist, their impact is expected to have a very small effect on seed yield (Woods and Heaman 1989); however, it should be noted that common seed orchard management practices such as supplemental-mass-pollination (El-Kassaby et al. 1993) and/or bloom delay (El-Kassaby and Davidson 1990, 1991) could drastically reduce these slight differences. On the other hand, the observed disparities in correlated matings estimates are real and were double or higher by an order of magnitude for the interior spruce and western hemlock orchards comparisons, respectively.

PROPOSED DESIGN

El-Kassaby (2003) and El-Kassaby et al. (2007) advocated the use of clonal-row design and proposed two modifications that could substantially reduce the "neighbourhood effect" that is responsible for the production of higher selfing and correlated matings. In the classical clonal-row seed orchard design (Figure 1, left); each clone is spatially contiguous to two other clones, producing a complete side-by-side clonal flanking, thus promoting both within-row selfing and between-rows correlated matings. The first modification (Figure 1, center); involves staggering the adjacent rows, so that each individual clone will be flanked by four unrelated clones, thus promoting outcrossing and effectively breaking down the "neighbourhood effect" resulting in reduced correlated matings. The second modification (Figure 1, right), involves replicating and randomizing the staggered rows of a specific clone throughout the seed orchard, thus at every

replication, each clone is flanked by four different clones and these clones are different from those selected at the other replications.

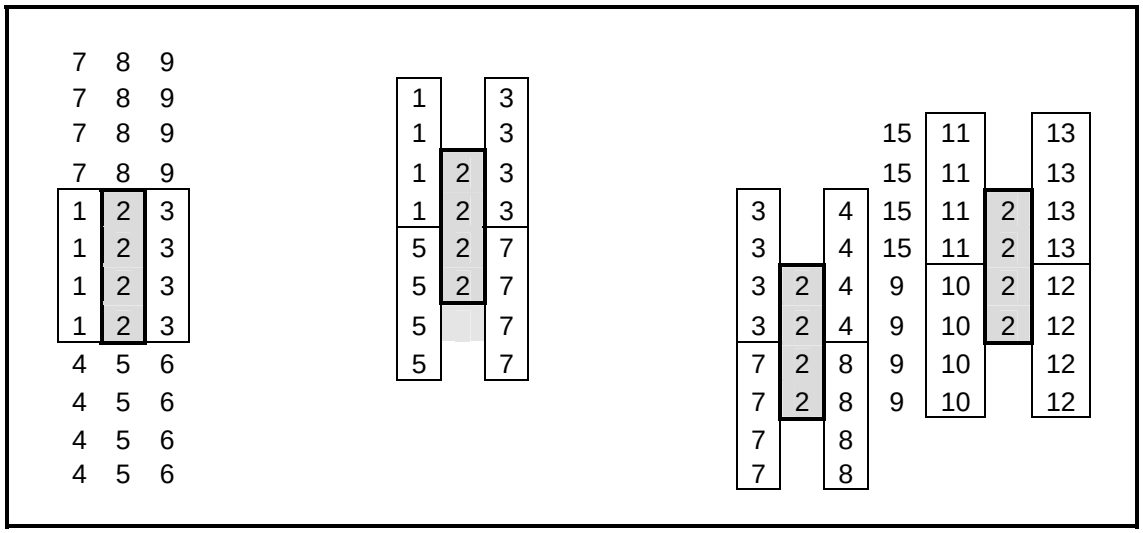

Figure 1. Illustration of three clonal-row seed orchards designs. The 1st (left) shows classical clonal-row with 50% flanking of a specific clone, the 2nd (center) shows the effect of row staggering where every clone is surrounded be four other clones (i.e., 25% flanking), and the 3rd (right) shows the effect of staggering and randomization of clonal-rows where each clone is surrounded by four different clones at each replication.

These proposed changes are expected to reduce both selfing and correlated matings observed in the classical clonal-row design (observations from the western hemlock and interior spruce studies) and the staggered clonal-rows orchards, creating a "compromise" between the systematic and randomized designs and the benefits of both clonal-rows and randomization are simultaneously realized. We propose designating this seed orchard design as "randomized, replicated, staggered clonal-row" (R^2SCR).

The generation of the proposed R^2SCR design layout is challenging since restrictions such as staggering of rows, row length, and the selection of the physical distance (i.e., number of rows) separating repeated rows of the same clone were imposed on the randomization process. An interactive computer program was developed and after each run, multiple orchard layouts are generated. The orchard's design parameters include: the number of clones, seed orchard size, length of the rows (single ramet which is equivalent to the COOL program or multiple-ramets row that must be in an even number to allow staggering), the physical distance separating rows of the same clone, seed orchard configuration (real estate barriers or lack of), level of "anti-randomization" tolerance (estimated using least square technique), and deployment mode (equal clonal size vs. linear deployment (Lindgren and Matheson 1986)).

LITERATURE CITED

Adams, W.T. and D.S. Birkes. 1991. Estimating mating patterns in forest tree populations. In: Biochemical markers in the population genetics of forest trees (Fineschi, S. et al., eds.). SPB Academic Publishing, The Hague, Netherlands. pp. 157–172.

Bell, G.D. and A.M. Fletcher. 1978. Computer organized orchard layouts (COOL) based on the permutated neighbourhood design concept. Silvae Genet. 27:223-225.

El-Kassaby, Y.A. 1989. Genetics of seed orchards: expectations and realities. In: Proc. of the 20th South. For. Tree Improve. Conf., June, 1989. Charleston, South Carolina, USA. pp. 87-109.

El-Kassaby, Y.A. 2003. Clonal-row vs. random seed orchard designs: Mating pattern and seed yield of western hemlock (Tsuga heterophylla (Raf.) Sarg.). For. Genet. 10:121-127.

El-Kassaby, Y.A. and G.R. Askew. 1998. Seed orchards and their genetics. In: Forest Genetics and Tree Breeding (Mandal, A.K. and G.L. Gibson, eds.). CBS Publishers abd Distributors. 4596/1 a, 11-Daryaganj, New Delhi-110002. Chapter 6: 103-111.

El-Kassaby, Y.A., S. Barnes, C. Cook and D.A. MacLeod. 1993. Supplemental-mass-pollination success rate in a mature Douglas-fir seed orchard. Can. J. For. Res. 23:1096-1099.

El-Kassaby, Y.A. and R. Davidson. 1991. Impact of pollination environment manipulation on the apparent outcrossing rate in a Douglas-fir seed orchard. Heredity 66:55-59.

El-Kassaby, Y.A. and R. Davidson. 1990. Impact of crop management practices on the seed crop genetic quality in a Douglas-fir seed orchard. Silvae Genet. 39:230-237.

El-Kassaby, Y.A. and K. Ritland. 1998. Isozymes: techniques, interpretation and applications to forest genetics. In: Forest Genetics and Tree Breeding (Mandal, A.K. and G.L. Gibson, eds.). CBS Publishers abd Distributors. 4596/1 a, 11-Daryaganj, New Delhi-110002. Chapter 12: 213-226.

El-Kassaby, Y.A., J. Russell, and K. Ritland. 1994. Mixed-mating in an experimental population of western redcedar, *Thuja plicata*. J. Hered. 85:227-231.

El-Kassaby, Y.A., M.U Stoehr, D. Reid, C.G. Walsh and T.E. Lee. 2007. Clonal-row vs. random seed orchard designs: interior spruce mating system evaluation. Can. J. For. Res. (in press).

El-Kassaby, Y.A., F.C. Yeh and O. Sziklai. 1986. Clonal and seedling seed orchards: a comparison of outcrossing rates and heterozygosity in coastal Douglas-fir using allozyme markers. In: Proc. IUFRO, Work. Parties, Williamsburg, Virginia, U.S.A. (A.V. Hatcher and R.J. Weir, eds.). pp. 410-421.

Lindgren D. and A.C. Matheson. 1986. An algorithm for increasing the genetic quality of seed from seed orchards by using the better clones in higher proportions. Silvae Genet 35:173–177.

Mitton, J.B. 1992. The dynamic mating systems of conifers. New For. 6:197–216.

O'Connell, L., J. Russel and K. Ritland. 2004. Fine-scale estimation of outcrossing in western redcedar with microsatellite assay of bulked DNA. Heredity 93:443-449.

Perry, D.J. and P. Knowles. 1990. Evidence of high self-fertilization in natural populations of eastern white cedar (*Thuja occidentalis* L.). Can. J. Bot. 68:663–668.

Ritland, K. 2002. Extensions of models for the estimation of mating systems using n independent loci Heredity 88:221-228.

Ritland, C.E. and K.M. Ritland. 2000. DNA fragment markers in plants. In: Molecular Methods in Ecology (Baker, A.J., ed.). Blackwell Scientific, Oxford, U.K. pp. 208-234.

Woods, J.H. and J.C. Heaman. 1989. Effect of different inbreeding levels on filled seed production in Douglas-fir. Can. J. For. Res. 19:54–59.

Xie, C.Y., B.P. Dancik,and F.C. Yeh. 1991. The mating system in natural populations of *Thuja orientalis*. Can. J. For. Res. 21:333–33.

A South-wide Rate Test of Esfenvalerate (Asana® XL) for Cone and Seed Insect Control in Southern Pine Seed Orchards

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Abstract: As many as five monthly applications may be required each year to protect southern pine seed orchards from coneworms, *Dioryctria* spp. Insecticides that control coneworms usually provide control of two other pests, the leaffooted pine seed bug, *Leptoglossus corculus*, and the shieldbacked pine seed bug, *Tetyra bipunctata*. Esfenvalerate (Asana® XL) is a pyrethroid insecticide that is effective for both coneworms and seed bugs. Aerial application of the maximum labeled rate of esfenvalerate can cause secondary outbreaks of scale insects and mealy bugs; and the honeydew they produce promotes growth of unsightly sooty mold that reduces tree vigor and growth. A South-wide study operationally evaluated the efficacy of reduced rates of esfenvalerate. Six orchards throughout the South were used in the study, five loblolly pine and one slash pine orchards. Each orchard had four treatment plots. A complete block design was used with each orchard serving as a replicate. The experimental unit was one treatment block in each orchard. The four study treatments were: Asana® XL at the labeled rate of 0.19 pounds active ingredient/acre (ai/ac), Asana® XL at 0.10 pounds ai/ac, Asana® XL 0.03 pounds ai/ac, and a control consisting of untreated trees. Aerial applications were made five times at monthly intervals (May-August). Efficacy data collected were crop survival, yields of healthy and damaged cones, and seed yield. Each treatment was surveyed for secondary insects the following year. All rates of esfenvalerate were effective in controlling seed bugs. First-year conelet survival, and percent good seed were significantly lower for the control when compared against the 0.03, 0.10 and 0.19 pound ai/ac application rates. The composite trait, good-seed per original-flower, gave the same results. However, the lower rates did not protect against coneworm damage. For the five loblolly pine seed orchards, coneworm damage at the 0.19 pound ai/ac was significantly lower than for the control or the two reduced rates. The two low rates did not result in secondary insect outbreaks. Reduced rates of esfenvalerate may be applied in combination with insecticides specific to coneworms, such as the growth regulator tebufenozide. This results in a combination of efficacy and reduced risk of secondary outbreaks.

INTRODUCTION

Cone and seed insects can severely limit production of genetically improved seeds in southern pine seed orchards. These seeds are vital for regeneration programs. Important insect pests include the pine coneworms, *Dioryctria* spp. (Ebel et al. 1980). Coneworm larvae feed in and destroy the flowers and cones of pines. Also, the leaffooted pine seed bug, *Leptoglossus corculus* (Say) and the shieldbacked pine seed bug, *Tetyra bipunctata* (Herrich-Schaffer), feed

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by sucking out the contents of developing seeds in cones and conelets causing conelet abortion and empty seeds in mature cones (Ebel et al. 1980). Without control, these insects can destroy as much as 90% of the potential seed crop (Fatzinger et al. 1980).

As many as five monthly applications may be required each year to protect southern pine seed orchards from coneworms. Insecticides that control coneworms usually provide control of the two seed bug species. Esfenvalerate (Asana® XL) is a pyrethroid insecticide that is effective for both coneworms and seed bugs (Lowe et al. 1994). However, aerial application of the maximum labeled rate of esfenvalerate can cause secondary outbreaks of scale insects and mealy bugs (Clarke et al. 1988); and the honeydew they produce promotes growth of unsightly sooty mold that reduces tree vigor and growth.

Previous ground application studies have shown that seed bugs can be controlled with much lower levels of esfenvalerate. Experience with other pesticides has shown that area treatments with aerial applications may require less pesticide than indicated in ground application studies which compare treatments on individual trees (Mangini et al. 1998). The amount of pesticide used may be reduced if lower application rates with aerial applications are shown to be effective. There is also the possibility that a lower application rate can be identified that will provide control of targeted insects while not promoting the buildup of secondary insects.

The objective of this study was to test the efficacy of several rates of Asana® XL for coneworm and seed bug control in loblolly and slash pine seed orchards across the South. The current labeled rate and two lower rates were compared against a control with no insecticide application using a protocol developed in previous South-wide operational efficacy tests (Lowe et al. 1994, Mangini et al. 1998).

MATERIALS AND METHODS

Initial Coordination

The Seed Orchard Pest Management Subcommittee (SOPMS) of the Southern Forest Tree Improvement Committee, was established to address the critical need for insect pest management in southern seed orchards (Lowe et al. 1994, VanBuijtenen 1981). Tests on an operational level require large areas of seed orchards to test the efficacy of aerially applied pesticides, no single organization has the necessary resources or expertise available. Consequently, the SOPMS coordinated the South-wide test of esfenvalerate.

Six companies supplied orchards for the study (Table 1). The orchards were in locations throughout the South; five were loblolly pine orchards and one orchard was slash pine. Previous experience in South-wide tests (Lowe et al. 1994, Mangini et al. 1998) indicated that careful planning and coordinated activities are essential to a successful test. Consequently, in February 2001, a meeting was held at Lyons, GA in which participating orchard managers and SOPMS members developed a study plan (Byram, T. D. 2001. Asana® XL Rate Study For Cone and Seed Insect Control in Southern Pine Seed Orchards. A Regional Cooperative Study Plan. Unpublished). A complete plan of activities was established (Table 2).

Table 1. Participating orchards used in the South-wide rate test of esfenvalerate (Asana® XL) for cone and seed insect control in southern pine seed orchards conducted in 2001.

Company	Orchard	Location	Tree Species
Florida Division of Forestry	Baker	Milton, FL	Slash
International Paper	Jay	Jay, FL	Loblolly
International Paper	Springhill	Springhill, LA	Loblolly
Mississippi Forestry Commission	Craig	Baxterville, MS	Loblolly
Temple-Inland Forest Products	Forest Lake	Jasper, TX	Loblolly
Weyerhaeuser	Lyons	Lyons, GA	Loblolly

Table 2. Timeline of activities for the South-wide rate test of esfenvalerate (Asana® XL) for cone and seed insect control in southern pine seed orchards conducted in 2001.

Times (2001-2002)	Activity	Responsible Group
February	Organizational meeting	Cooperative staff, orchard personnel, entomologists
March	Select and flag sample ramets Tag survival conelets and cones	Cooperative staff and orchard personnel
April	Apply first application	Orchard personnel
May	Apply second application	Orchard personnel
June	Apply third application	Orchard personnel
July	Apply fourth application	Orchard personnel
August	Apply fifth application	Orchard personnel
Cone Harvest (September/October)	Count survival conelets and cones Harvest ALL cones on sample ramets Separate and count damaged and undamaged cones Collect ten-cone samples from each ramet and send to respective Cooperative	Orchard personnel
Post-harvest	Examine and sort damaged cones Extract seed from ten-cone samples Radiograph and evaluate seed samples	Entomologists Cooperative staff TFS Pest Management
Summer 2002	Evaluate for secondary pest outbreaks	Orchard personnel and entomologists
2002 and later	Data analysis and report preparation	SOPMS Committee

Treatments

Three rate treatments of esfenvalerate were compared to an untreated control (Table 3). The timing of the applications was identical for all treatments. For loblolly pine seed orchards, the first application was within seven days of peak pollen flight (early April); the first application in slash pine seed orchards was made about April 1 (Lowe et al. 1994). In orchards of either

species, the initial application was followed by four subsequent applications made at monthly intervals (May, June, July and August).

Table 3. Treatment rates used in the South-wide rate test of esfenvalerate (Asana® XL) for cone and seed insect control in southern pine seed orchards conducted in 2001.

Treatment[1]	Amount of Asana® XL[2]
0.19	0.287 gallons or 36.8 fluid ounces
0.10	0.152 gallons or 19.4 fluid ounces
0.03	0.046 gallons or 5.8 fluid ounces

[1] Pounds of active ingredient per acre.
[2] To mix with 10 gallons of water for each acre to be sprayed (Asana® XL contains 0.66 pounds active ingredient per gallon of formulation).

Aerial Application Methods

The insecticide treatments were applied with either a fixed- or rotary-winged aircraft. To standardize the applications among orchards, each applicator conformed to the following application standards: effective swath width of 60 ft, five gal/ac of tank mix applied on each of two passes for a total of 10 gal /ac/treatment, spray droplet size of 350 microns volume mean diameter, and release height of 10-20 ft above the tops of the trees. A 30 ft row spacing in the orchards was assumed. Entomologist members of SOPMS were assigned to specific orchards to provide assistance with equipment calibration and spray deposition evaluation.

Field Layout

In each orchard four treatment plots were laid out in the test area. Each plot was at least five rows wide and comprised at least five acres in area. A buffer of at least four rows of ramets separated the treatment blocks.

A randomized complete block design was used with the experimental unit consisting of one treatment plot. Each seed orchard served as a replicate. Two sample ramets were selected from each of six clones in each plot for a total of 48 sample trees in each orchard. These same six clones were sampled in each plot within an orchard; however, clones differed among orchards. Treatments were randomly assigned to the plots within each orchard.

Efficacy Data Collection

Basic efficacy data included crop survival, yields of healthy and damaged cones, and seed yields for each sample tree. Each orchard/treatment block was surveyed during 2001 and in the following year for the presence and extent of secondary pests (scale insects and mealybugs).

Crop Survival. Orchard personnel counted and tagged a sample of at least 50 healthy conelets (2001 flower crop) and 50 healthy cones (2000 flower crop) from the south side of each sample ramet. These counts were made within one month of peak pollen flight in loblolly pine seed orchards and during April in the slash pine orchards. The tagged conelets and cones were

recounted in the fall just prior to cone harvest, to estimate crop survival (Lowe et al. 1994, Mangini et al. 1998).

Cone Yields and Damage. At harvest, all cones were collected from each ramet. Orchard personnel sorted the cones into healthy and damaged categories according to Nord et al. (1984). Each cone was examined carefully for holes, insect frass, discolored patches of scales, and dead tips. Cones with no visible damage were counted as healthy. Questionable cones were placed with damaged cones. The number of healthy and damaged cones were recorded for each sample ramet in the field. Damaged cones from each ramet were placed in an individual cloth bags and placed in cold storage (at least 45º C or below) until examination by entomologist. The entomologist made a second examination of the damaged/questionable cones and sorted them into damage categories including coneworm damage. The initial counts were adjusted for any cones deemed healthy at the second inspection.

Seed Analysis. Ten healthy cones were picked at random from each sample ramet at harvest. The ten-cone samples were placed in cloth bags and labeled by orchard, block, clone, ramet and treatment. These ten-cone samples were subjected to standard after-ripening procedures to ensure proper cone opening (Lowe et al. 1994, Mangini et al. 1998). Seeds (including second-year aborted ovules) were extracted. The aborted ovules were counted and removed. Counts of total, filled, empty and seed bug-damaged seed were determined from radiographs of the seeds from each ramet (Bramlett et al. 1977).

Secondary Pests. A critical part of the study was to estimate treatment effect on secondary homopteran pests. Ramets (including controls) were visually inspected for homopteran (scale insects and mealybugs) populations by Dr. Stephen R. Clarke (USDA Forest Service). Dr. Clarke inspected sample ramets in each orchard in March or April 2001, before the initial pesticide application; he made second inspection in the fall (September or October) of 2001 and a final evaluation in June 2002. When present, the relative population levels of the following insects were determined using the infestation scoring system of Cameron (1989): pine tortoise scale, *Toumeyella parvicornis* (Cockerell); striped pine scale, *T. pini* (King); wooly pine scale, *Pseudophillippia quaintancii* (Cockerell); mealybug, *Oracella acuta* (Lobdell); and the pine needle scale, *Chionaspis heterophyllae* (Colley).

Table 4. ANOVA and EMS for the South-wide rate test of esfenvalerate (Asana® XL) for cone and seed insect control in southern pine seed orchards. The ANOVA assumes the treatments are fixed and that orchards, clones and ramets within clones are random effects.

Source of Variation	Degrees of Freedom[1]	Expected Mean Squares
Orchards (O)	o – 1	$\sigma^2_\varepsilon + rt\sigma^2_{C(O)} + rct\sigma^2_O$
Clones within Orchards (C)	o (c – 1)	$\sigma^2_\varepsilon + rt\sigma^2_{C(O)}$
Treatments (T)	t – 1	$\sigma^2_\varepsilon + r\sigma^2_{C(O)T} + cr\sigma^2_{OT} + cro\sigma^2_T$
O x T	(o – 1)(t – 1)	$\sigma^2_\varepsilon + r\sigma^2_{C(O)T} + cr\sigma^2_{OT}$
C(O) x T	o(c – 1)(t – 1)	$\sigma^2_\varepsilon + r\sigma^2_{C(O)T}$
Sampling Error	otc(r – 1)	σ^2_ε
Total	otcr – 1	

[1] The letters o, c, t, r equal the number of orchards, clones within orchard, treatment and ramets within clones, respectively.

Data Analysis. Efficacy was evaluated by comparing treatment differences for crop survival, yields of healthy and damaged cones, and seed quality by analyses of variance (Table 3) using SAS software options PROC GLM or PROC MIXED (Littell et al. 2002). When necessary data were appropriately transformed (Zar 1999) prior to analysis.

RESULTS AND DISCUSSION

Crop Survival

Cone (2000 Crop) Survival. There were no differences among the treatments for survival of the cones (2000 crop) across all orchards (five loblolly pine orchards and the single slash pine orchard) (Figure 1.). The Baker Orchard (slash pine) had high survival (~99%) as did the Forest Lake Orchard (loblolly pine) orchard. When these two orchards were excluded, the analysis of variance still failed to show any differences among treatments and the control.

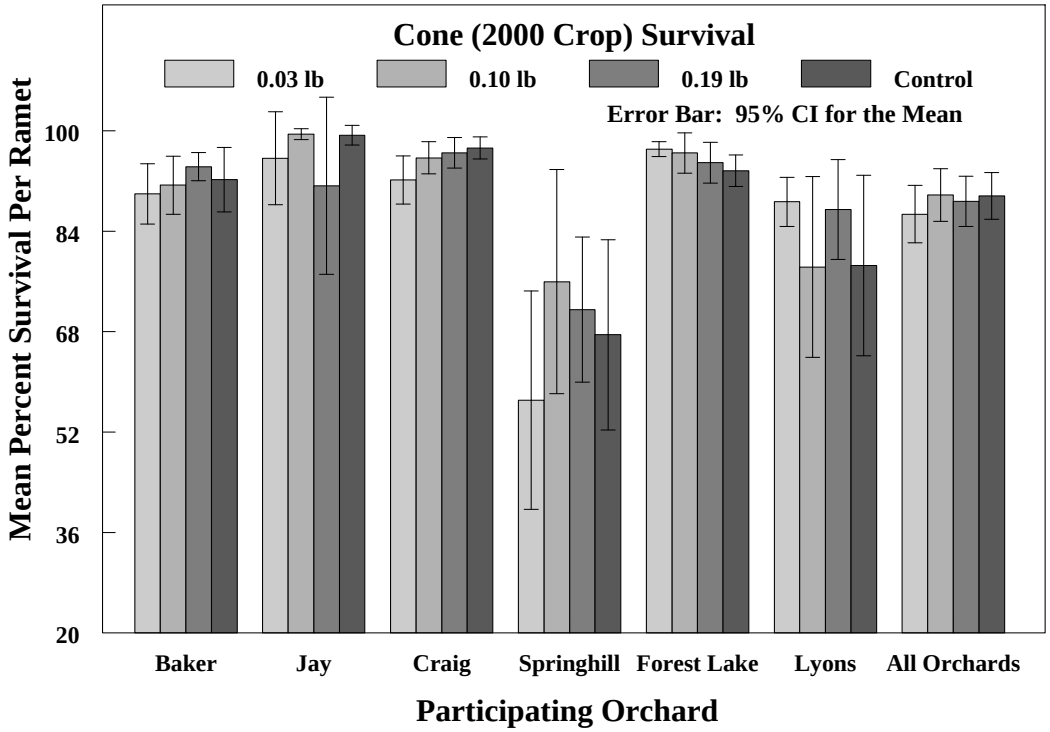

Figure 1. Mean percent survival per ramet of the cones (2000 crop) harvested in fall 2001 at the participating orchards the South-wide rate test of esfenvalerate (Asana® XL) for cone and seed insect control in southern pine seed orchards conducted in 2001. Estimate based on sample of 50 tagged cones per ramet. Loss factors include coneworm mortality and damage and loss due to other factors such as pitch canker. CI = confidence interval.

Conelet (2001 Crop) Survival. Survival of conelets in the control was significantly less than that in the Asana® XL treatments across all orchards (Figure 2). When conelet survival was weighted by the number of flowers per ramet, the analysis of variance (mixed model) was significant ($F = 6.45$, $p>F = 0.0051$). The resulting least-squares mean (Littell et al. 2002) were 0.03 lb = 0.8562, 0.10 lb = 0.8740, 0.19 lb = 0.8555 and control = 0.7767. There were no significant differences in survival counts among the three treatments; however, the average conelet survival of the three treatments was significantly different from and greater than that of the control.

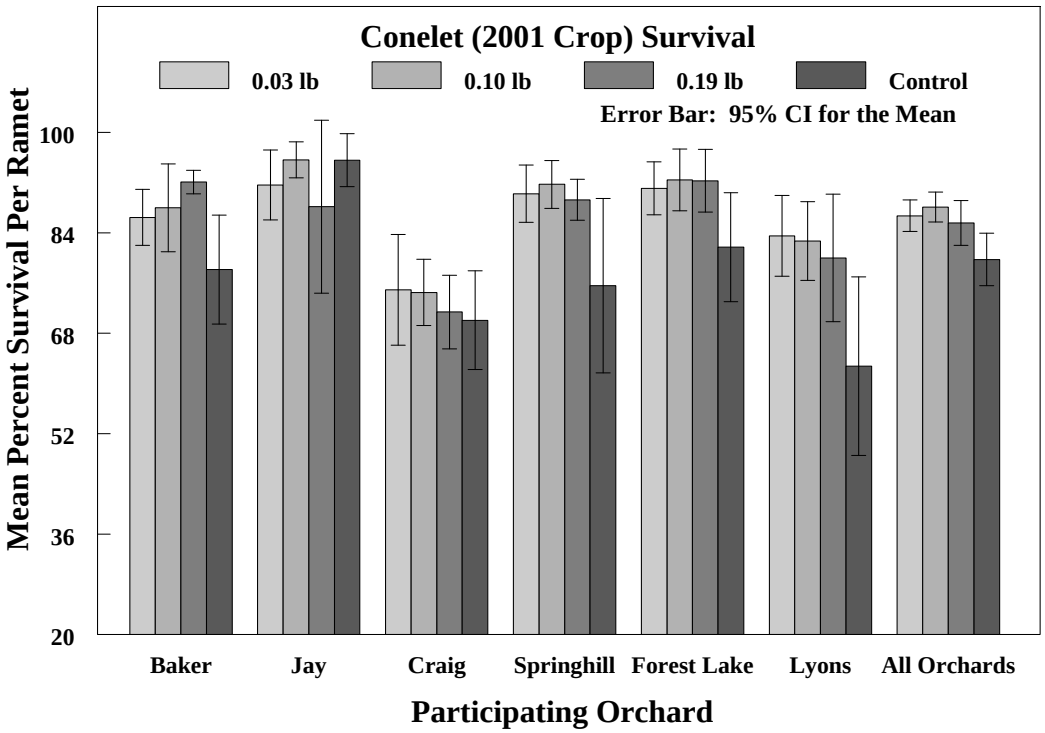

Figure 2. Mean percent survival per ramet of the conelets (2001 crop) harvested in fall 2001 at the participating orchards the South-wide rate test of esfenvalerate (Asana® XL) for cone and seed insect control in southern pine seed orchards conducted in 2001. Estimate based on sample of 50 tagged cones per ramet. Loss factors include coneworm mortality and damage and loss due to conelet abortion and unknown factors. CI = confidence interval.

Cone Yields and Damage

Healthy Cones. The number of healthy cones showed no significant effect of treatment (Table 5). However, the labeled rate of Asana® XL (0.19 lb) did result in the largest number of healthy cones. The other treatments had least-squares mean values (mixed model) about equal to that of the control (0.03 lb = 0.717, 0.10 lb = 0.722, 0.19 lb = 0.766, control = 0.717).

Coneworm-infested Cones. There were no significant differences for treatments versus control when all orchards were included in the analysis (Table 5). Two of the orchards (Springhill and Forest Lake) were heavily infested with coneworms. When these two orchards were analyzed exclusively, the resulting analysis of variance revealed significant differences (F=7.33, p>F=0.0680). However, these differences were between the 0.03 and 0.10 lb rates versus the 0.19 lb rate and not between the treatments and the control. The least-squares mean values (mixed model) for this analysis were 0.03 lb = 0.3807, 0.10 lb = 0.3458, 0.19 lb = 0.2325, and control = 0.2969.

Table 5. Cone quality and yield of loblolly and slash pine seed from the South-wide rate test of esfenvalerate (Asana® XL) for cone and seed insect control in southern pine seed orchards conducted in 2001. Estimates are based on whole-tree picks of the 2000 cone crop from each study ramet.

	Average Whole-tree Counts per Ramet[1]				
Treatment[2]	Coneworm	Healthy	Total	Percent Coneworm	Percent Healthy
0.03	58.4±25.6[3]	546.7±250.5	653.6±257.5	73.3±6.4	17.8±5.1
0.10	60.2±26.5	495.8±189.6	608.5±195.8	74.7±6.0	15.7±5.0
0.19	41.4±14.3	517.8±201.8	607.0±210.3	80.1±5.6	12.3±4.3
Control	57.6±20.0	407.3±159.2	517.3±170.4	74.1±5.9	17.1±4.4

[1]Averaged across all six orchards in the study.
[2]Pounds of active ingredient per acre of Asana® XL.
[3]Mean value ± 95% confidence interval about mean.

Seed Analysis

Based on quality estimates from radiography of seeds, all rates of esfenvalerate were effective in controlling seed bugs. Percent good seed was significantly lower for the control when compared against the 0.03, 0.10 and 0.19 lb rates (Table 6). The composite trait, good-seed per original-flower, gave similar results. The slash pine at the Baker Orchard did not have good control at the two lowest treatment rates.

Secondary Pests

Examinations of sample trees in spring before the first application indicated small numbers of secondary pests. Populations of mealybug, *O. acuta,* and the striped pine scale, *T. pini*, were sparse at all loblolly pine orchards. After the treatments, only the Craig and Forest Lake Orchards had noticeably larger numbers of these two pests. For example, at Forest Lake, on March 29, 2001, there were no *T. pini* present. However, on September 26, 2001, the sampling revealed 191 live striped pine scale on the samples from the 0.19 lb treatment and 260 on the 0.10 lb treatment sample. Similarly, at Craig, *T. pini* numbers were high in June but declined sharply by October. Examinations done in 2002 revealed sparse populations of these species.

Table 6. Percent seed bug damage to loblolly and slash pine seed from the South-wide rate test of esfenvalerate (Asana® XL) for cone and seed insect control in southern pine seed orchards conducted in 2001. Damage estimates are based on radiographic analysis of mature seed from a ten-cone sample of the 2001 cone crop taken from each study ramet.

	Participating Company[1]-Site-Tree Species						
Treatment[2]	IP-Jay-Loblolly	IP-Springhill-Loblolly	MFC-Craig-Loblolly	TI-Forest Lake-Loblolly	WEY-Lyons-Loblolly	All Loblolly Orchards	FDF-Baker-Slash
0.03	15.4±3.8**a** [3]	28.7±4.3**a**	12.1±2.8**b**	24.0±5.9**a**	26.5±6.1**ab**	21.2±2.2**a**	18.6±5.7**a**
0.10	16.6±3.9**a**	29.8±5.2**a**	9.3±2.0**a**	31.4±6.2**ab**	23.2±5.0**a**	22.2±2.3**a**	19.8±3.2**a**
0.19	16.6±3.8**a**	29.1±3.6**a**	10.6±1.9**ab**	26.7±5.8**ab**	21.7±5.1**a**	20.8±2.0**a**	14.6±1.8**a**
Control	19.2±4.1**a**	39.9±4.2**b**	12.7±2.4**b**	36.0±5.4**b**	32.5±4.8**b**	28.0±2.3**b**	20.6±1.4**a**

[1] IP = International Paper, MFC = Mississippi Forestry Commission, TI = Temple-Inland Forest Products, WEY = Weyerhaeuser Company, FDF = Florida Division of Forestry.
[2] Pounds of active ingredient (Asana® XL) per acre applied monthly April – August 2001.
[3] Mean ± Standard error of the mean. Means followed by the same letter in each column are not significantly different at the 5% level (Fisher's Protected LSD).

Implications

It is apparent that the reduced rates of Asana® XL were not effective against coneworms. The fact that cone (2000 crop) survival and number of healthy and coneworm-infested cones at the 0.03 and 0.10 lb rates were not significantly different from the control numbers indicates that these rates did not control coneworms. This is consistent with Nord et al. (1984) who show decreased cone damage with increasing pyrethroid dosage.

However, these reduced rates (0.03 lb and 0.10 lb) were effective in controlling seed bug populations. Again, the present study is consistent with past work. Seed bugs are controlled by lower rates of pesticides than those required for control of coneworms (Byram et al. 2003). Consequently, reduced rates of esfenvalerate may be applied in combination with insecticides specific to coneworms, such as the growth regulator tebufenozide. This results in a combination of efficacy and reduced risk of secondary outbreaks.

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LITERATURE CITED

Bramlett, D.L., E.W. Belcher, Jr., G.L. DeBarr, G.D. Hertel, R.P. Karfalt, C.W. Lantz, T. Miller, K.D. Ware and H.O. Yates III. 1977. Cone analysis of southern pines. A guidebook. USDA For. Serv. Gen. Tech. Rep. SE-13. 28p.

Byram, T.D., A.C. Mangini, and S.E. McKeand. 2003. Cone and seed insect pest research: The role of the southwide studies. Proc. 27th South. For. Tree Improv. Conf., pp. 116-125.

Cameron, R. S. 1989. Promising new pesticides for cone and seed insect control in the Southern United States. pp. 193-202 in Proc. Cone and Seed Insects Working Party Conf. IUFRO. For. Canada. Pac. For. Cent., Victoria, BC, Canada.

Clarke, S.R., G.L. DeBarr, and C. W. Berisford. 1988. Differential susceptibility of *Toumeyella pini* (King) (Homoptera: Coccidae) to pyrethroid and organophosphate insecticides: A factor in outbreaks in Southern pine seed orchards. J. Econ. Entomol. 81(5): 1443-1445.

Ebel, B.H., T.H. Flavell, L.E. Drake, H.O. Yates III, and G.L. DeBarr. 1980. Seed and cone insects of southern pines. USDA For. Serv. Gen. Tech. Rep. SE-8. 43p.

Fatzinger, C.W., G.D. Hertel, E.P. Merkel, W.D. Pepper, and R.S. Cameron. 1980. Identification and sequential occurrences of mortality factors affecting seed yields of southern pine seed orchards. USDA For. Serv. Res. Pap. SE-216. 43p.

Littell, R.S., W.W. Stroup and R.J. Freund. 2002. SAS® for linear models. 4th ed. SAS Institute Inc. Cary, NC 466 p.

Lowe, W.J., L.R. Barber, R.S. Cameron, G.L. DeBarr, G.R. Hodge, J.B. Jett, J.L. McConnell, A. Mangini, J. Nord and J.W. Taylor. 1994. A southwide test of bifenthrin (Capture®) for cone and seed insect control in seed orchards. South. J. Appl. For. 18(2): 72-75.

Mangini, A.C., L.R. Barber, R.S. Cameron, G.L. DeBarr, G.R. Hodge, J.B. Jett, W.L. Lowe, J.L. McConnell, J. Nord, and J. W. Taylor. 1998. A southwide rate test of azinphosmethyl (Guthion®) for cone and seed insect control in loblolly pine seed orchards. South. J. Appl. For. 22(2): 106-110.

Nord, J.C., G.L. DeBarr, N.A. Overgaard, W.W. Neel, R.S. Cameron, and J. F. Godbee. 1984. High volume applications of azinphosmethyl, fenvalerate, permethrin, and phosmet for control of

coneworms (Lepidoptera: Pyralidae) and seed bugs (Hemiptera: Coreidae and Pentatomidae) in southern pine seed orchards. J. Econ. Entomol. 77: 1589-1595.

VanBuijtenen, J.P. 1981. Insecticides for seed orchards – a case study in applied research. South. J. Appl. For. 5: 33- 37.

Zar, J.H. 1999. Biostatistical analysis. 4th ed. Prentice-Hall. New York. 663 p.

Genecological Responses in Western Conifers to Climate Changes Over the Past Two Millennia[1]

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Vegetation changes in western North American mountains are assumed to shift up and down in elevation in response to climate change. In addition, local populations are assumed to be optimally adapted to their environments. We review these assumptions in light of recent analyses of forest tree responses to climate changes over the past two millennia.

First, we analyzed well-preserved downed deadwood at 3000 m on Whitewing Mountain in the eastern Sierra Nevada. These were dated to 800-1350 CE and comprised whitebark, western white, sugar, Jeffery, and lodgepole pines, and western hemlock, identified by wood anatomical characteristics. Excepting whitebark pine, which is now largely in krummholz form, these species are currently 200 m or more lower in elevation; sugar pine is not locally native and is 600 m lower in elevation. Moreover, the lower elevation limit of whitebark is 100 m above the upper limit of sugar pine. Using the joint overlap of the climate spaces by discriminant analysis and based on downscaled PRISM data for these species, we estimated the climate for the period of sympatry to be warmer (+3.2^{o}C annual minimum temperature) and slightly drier (-24 mm annual precipitation) than present (Millar et al. 2006a); <http://www.fs.fed.us/psw/publications/millar/psw_2006_millar027.pdf>. This was a period of century-long droughts in the region (Stine 1994).

We next examine results from (Rehfeldt et al. 1999), who analyzed growth relationships in 120 lodgepole pine seed sources with the climate at 60 common garden locations in British Columbia, Canada. They found that the fastest-growing seed source at a location was from a warmer location and that growth of most seed sources was greater at a warmer location than the local one. In both cases, the difference between the local population's location and that for maximum growth increased with increasing latitude. This suggests that the populations not only lag current climate changes, but they did not fully adapt to the Little Ice Age climate (Westfall and Millar 2004); <http://www.fs.fed.us/psw/publications/westfall/psw_2004_westfall001.pdf>. However, because temperature changes have increased with increasing latitude, this will decrease the adaptational gradient (Cf. García-Ramos and Rodríguez 2002).

Next, we compared tree-ring growth between living trees with those that died in three eastern Sierran limber pine stands following a persistent drought during the 1980s. Both the mean and GARCH-modeled interannual variance in growth was greater in the dead tress than the living during the 18th and 19th centuries, but mean growth was greater in the living during the 20th

[1] A copy of the powerpoint presented at the 2007 SFTIC/WFGA meeting will be available at the senior author's web site (http://www.fs.fed.us/psw/programs/snrc/staff/westfall/)

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century Moreover, based on a second-order response surface model of station composites of maximum and minimum temperatures and precipitation versus tree ring widths, the dead trees were less responsive than the living to increasing winter precipitation under high minimum temperatures. This the dead trees were less water-use efficient, implying that these populations have undergone adaptation to current climate changes (Millar et al. In press); <http://www.fs.fed.us/psw/publications/millar/pub_limber_pine.pdf>.

Finally, we studied recruitment of limber and bristlecone pines in the White Mountains, in eastern California. Limber pine is recruiting in much greater numbers than bristlecone above current treeline, which is dominated by bristlecone, and about 300 m above current limber pine treeline and about 100 m above the bristlecone treeline. Recruitment peaked during the 1980s and was associated with higher minimum temperatures and a low phase of the Atlantic Multidecadal Oscillation (Millar et al. 2006b); <http://www.fs.fed.us/psw/publications/millar/posters/millar_etal_poster_agu2006.pdf>. In addition, recruitment was insensitive to minimum temperature under low winter precipitation, but increased with increasing minimum temperature under high precipitation. Additional analyses in correlations of recruitment with the NCEP/NCAR reanalysis 700 hPa data (http://www.cdc.noaa.gov/Correlation/) indicated that recruitment was enhanced by increased precipitation the September prior to the recruitment, a warm, relatively dry June in the current year, and relatively wet late summer, early fall, influenced by monsoonal moisture flow in the current and following two years.

Thus we find that episodic, threshold, and reversible changes are more common responses to late Quaternary climate changes in mountain ecosystems than are linear or gradual changes. These have resulted in non-analogous vegetation assemblages, like those at Whitewing Mt. Because species appear to respond to individualistic tragectories, such responses will complicate conservation planning. An additional complication is that populations will lag genetic (or adaptational) responses to not only current, but to past climatic changes.

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LITERATURE CITED

García-Ramos, G., and D. Rodríguez. 2002. Evolutionary speed of species invasions. Evolution 56:661-668.

Millar, C. I., J. C. King, R. D. Westfall, H. A. Alden, and D. L. Delany. 2006a. Late Holocene forest dynamics, volcanism, and climate change at Whitewing Mountain and San Joaquin Ridge,

Mono County, Sierra Nevada, CA, USA. Quaternary Research 66:273-287.

Millar, C. I., R. D. Westfall, and D. L. Delany. 2006b. Elevational Gradients and Differential Recruitment of Limber Pine (*Pinus flexilis*) and Bristlecone Pine (*Pinus longaeva*); White Mountains, California, USA. AGU, San Francisco.

Millar, C. I., R. D. Westfall, and D. L. Delany. In press. Response of high-elevation limber pine (*Pinus flexilis*) to multi-year droughts and 20th-century warming; Sierra Nevada, California, USA. Can J Forest Res

Rehfeldt, G. E., C. C. Ying, D. L. Spittlehouse, and D. A. Hamilton. 1999. Genetic responses to climate in *Pinus contorta*: Niche breadth, climate change, and reforestation. Ecol. Monog. 69:375-407.

Stine, S. 1994. Extreme and persistent drought in California and Patagonia during Mediaeval time. Nature 369:546-549.

Westfall, R. D., and C. I. Millar. 2004. Genetic consequences of forest population dynamics influenced by historic climatic variability in the western USA. Forest Ecology and Management 197:157-168.

Genetic Diversity and Hybridization in Seed Sources of Shortleaf Pine and Loblolly Pine

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Shortleaf pine (*Pinus echinata* Mill.) and loblolly pine (*Pinus taeda* L) are widely distributed over the southeast United States, thus it is reasonable to assume they possess a large amount of genetic variation due to wide adaptation. Southwide Southern Pine Seed Source Studies (SSPSSS) of both species were established in the 1950s, and provided much early range wide information. Schultz (1997), summarizing the SSPSSS and other studies of loblolly pine, reported the species possesses considerable natural variation for many morphological traits, and that this variation is generally clinal, extending both north to south and east to west. Some, but not all studies reported differences between populations east and west of the Mississippi River. Few studies of natural variation in shortleaf pine have been reported. The SSPSSS results, summarized at age 10 by Wells and Wakeley (1970), showed no geographic patterns, although northern sources survived best in northern plantings, and southern sources grew faster until moved too far north. In a Mississippi SSPSSS planting Wells (1973) noted that the only consistent genetic difference between east and west populations of shortleaf pine was in time of growth initiation, with sources west of the Mississippi River initiating growth earlier. Tauer (1980) reported that at age 20 two Oklahoma SSPSSS shortleaf pine plantings showed a north-south growth trend, but no east-west trend.

Early studies of natural variation relied on morphological traits, but later studies utilized monoterpenes and isozymes and they generally confirmed the morphological data, showing north-south and east-west gradients. However, some questions remained. There appeared to be some east verses west of the Mississippi River differences in loblolly pine (Wells and Lambeth 1983, Wells and Wakeley 1970), which Florence and Rink (1979) and Wells and Wakeley (1970) attributed to the presence of the river itself, while Schmidtling et al. (1999) and Wells et al. (1991) attributed the differences to separate east and west glacial refugia during the Pleistocene. There were few differences noted for shortleaf pine populations east and west of the river (Edwards and Hamrick 1995, Raja et al.1997), except that the frequency of heterozygosity of the *IDH* (isocitrate dehydrogenase) locus was higher west of the river. Heterozygosity at this locus indicates the tree is a shortleaf X loblolly pine hybrid (Huneycutt and Askew 1989), and the hybrid frequency in some western populations appears to be quite high (15% according to Raja et al. 1997 and Chen et al. 2004).

Current forest management favors loblolly pine, and many acres of shortleaf pine have been replaced with improved loblolly pine. The USDA Forest Service is one of a few organizations which regenerate shortleaf pine, usually relying on natural regeneration. As a result, naturally regenerated shortleaf pine stands are becoming surrounded by more and more loblolly pine. Raja et al. (1998) and Chen et al. (2004) reported a level of about 15% hybridization between these two species in west-central Arkansas. The long term effect of such a high hybridization

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level on species integrity is unknown. The samples collected in this study (from SSPSSS plantings) are from seeds collected in 1951 and 1952, when man's influence due to management was minimal. Thus, this study estimates genetic variation found in natural populations of shortleaf pine and loblolly pine approximately 50 years ago, prior to intensive management. These data will later be used as reference level data for addressing questions concerning diversity and hybridization level changes between these pine species from the 1950s to the present.

MATERIALS AND METHODS

More recently available DNA based markers can detect difference not easily discriminated by morphological traits or isoenzyme markers. Amplified fragment length polymorphism (AFLP) markers are easy to use for studying population genetics of trees as their use requires no previous sequence knowledge, has good repeatability and can detect multiple loci. In this study, to describe genetic diversity in natural stands of shortleaf pine and loblolly pine, needle tissue samples were taken from 93 shortleaf pine and 112 loblolly pine trees from 22 seed sources of SSPSSS plantings in Oklahoma, Arkansas and Mississippi. AFLP markers were developed and used to estimate genetic diversity and hybridization levels in these pine species.

RESULTS AND DISCUSSION

The 22 seed sources were grouped into 16 populations according to seed source geographic and physiographic region for the genetic diversity study. Of 48 primer pairs screened, 17 produced 794 AFLPs in shortleaf pine and 21 produced 647 AFLPs in loblolly pine. Analysis of these AFLP data showed high genetic diversity in both species with most of the genetic diversity within populations, in agreement with earlier studies discussed above. Analysis also showed gene flow is high among populations in both species, which explains the finding of no correlation between population genetic distances and geographic distances. Genetic differences between the east and the west regions were minimal. These results confirm the earlier studies based on morphology and isoenzymes and reinforce the appropriateness of current breeding strategies for both species in that most genetic variation lies within populations.

For the hybridization study, the 48 primer pairs screened revealed 17 primer pairs which produced 96 AFLPs polymorphic across loblolly pine and shortleaf pine. The *IDH* marker identified two loblolly pine and two shortleaf pine hybrids in the trees sampled. Two additional shortleaf pine hybrids were found by combining the 96 AFLPs with the *IDH* marker using software NewHybrids version 1.1 beta. Hybridization frequency varied geographically, ranging from 25% in Missouri to 0% in other sources in this study. The hybridization level was higher in populations west of the Mississippi River than east of the river (7.7% west vs. 0.7% east). If we consider five additional trees identified as having an average 36% probability of being hybrids, the rate would be 14.0% west vs. 4.0% east in shortleaf pine, 4.5% west vs. 2.2% east in loblolly pine and 10.8% west vs. 2.1% east in all populations. These results suggest that the existence of hybrids or the potential for creation of hybrids should be considered in forest management decisions, particularly for seed collection natural regeneration of shortleaf pine.

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LITERATURE CITED

Chen JW, Tauer CG, Bai G, Huang Y, Payton ME, Holley AG (2004) Bidirectional introgression between *Pinus taeda* and *Pinus echinata*: Evidence from morphological and molecular data. Can. J. For. Res. 34: 2508-2516.

Edwards MA, Hamrick JL (1995) Genetic variation in shortleaf pine, *Pinus echinata* Mill. (Pinaceae). For. Genet. 2: 21-28.

Florence Z, Rink G (1979) Geographic patterns of allozymic variation in loblolly pine. In: Proceedings of the 15th Southern Forest Tree Improvement Conference, June 19-21, 1979, Starkville, MS, pp. 33-41.

Huneycutt M, Askew GR (1989) Electrophoretic identification of loblolly pine shortleaf pine hybrids. Silvae Genet. 38: 95-96.

Raja RG, Tauer CG, Wittwer RF, Huang YH (1998) Regeneration methods affect genetic variation and structure in Shortleaf Pine (*Pinus echinata* Mill.) For. Genet. 5: 171-178.

Raja RG, Tauer CG, Wittwer RF, Huang YH (1997) Isoenzyme variation and genetic structure in natural populations of shortleaf pine (*Pinus echinata*). Can. J. For. Res. 27: 740-749.

Schmidtling RC, Carroll E, LaFarge T (1999) Allozyme diversity of selected and natural loblolly pine populations. Silvae Genet. 48: 35-45.

Schultz RP (1997) Loblolly Pine: the ecology and culture of loblolly pine (Pinus taeda L). USDA, Ag. Handb. 713.

Tauer CG (1980) Twenty-year results of a shortleaf pine seed source study in Oklahoma. OSU Ag. Exp. Sta. Bull. B-752. pp12.

Wells OO (1973) Variation among shortleaf pines in a Mississippi seed source planting. USDA For. Serv. Res. Note 3-SO-162. pp8.

Wells OO, Lambeth CC (1983) Loblolly pine province test in southern Arkansas 25th year results. South. J. Appl. For. 2:71-75.

Wells OO, Switzer GL, Schmidtling RC (1991) Geographic variation in Mississippi loblolly pine and sweetgum. Silvae Genet. 40: 105-119.

Wells OO, Wakley PC (1970) Variation in shortleaf pine from several geographic sources. For. Sci. 16:415-423.

Advances in American Chestnut Somatic Seedling Production

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Abstract: The American chestnut was one of the most important forest trees in the Appalachian Forest until the introduction of the chestnut blight fungus, which caused the death of virtually every mature American chestnut tree in the eastern United States. A system for mass propagation of blight-resistant material obtained through conventional breeding or gene transfer is still lacking. Thus, the goal of our project is to develop a high-frequency in vitro propagation system for American chestnut via somatic embryogenesis. Two bottlenecks in this approach are the low initiation rate of embryogenic cultures and the low production efficiency of plantlets (somatic seedlings) from the somatic embryos. To increase embryogenic culture initiation, we tested two plant growth regulators (2,4-D and picloram) at different concentrations and found that 2,4-D resulted in the highest frequency of embryogenesis (up to 3.5 %). This culture initiation experiment also demonstrated for the first time that highly productive embryogenic cultures could be initiated from immature seeds resulting from controlled crosses between known American chestnut parents. To increase plantlet production, we tested variations in cold (4° C) treatment duration (12, 15, and 18 weeks) and light quality (red, red + far red, and cool white fluorescent). For some genotypes, the longer cold treatments improved plantlet production and red light improved overall plantlet production frequency (up to 80% and 69%, respectively). Thus, by manipulating the cultural treatments, we were able to increase American chestnut somatic seedling production efficiency above the levels we previously reported. The first American chestnut somatic seedlings to be tested under nursery conditions were promising, growing up to 1.5 m in their first season. These advances in clonal propagation will aid in the restoration of the American chestnut to our forests.

INTRODUCTION

Prior to the early 20th century, American chestnut (*Castanea dentata*) was one of the most important forest trees in the United States, comprising up to a quarter of the trees in the southern Appalachian forest. The accidental introduction of the chestnut blight fungus, *Cryphonectria parasitica,* on Asian chestnut stock at the turn of the century resulted in the death of virtually every mature American chestnut tree from New England to Georgia within 40 years (Burnham 1988). Currently, the tree only exists in its natural environment as a minor component of eastern hardwood forests, surviving in the form of sprouts from old stumps and root systems, since the fungus does not penetrate the roots (Viéitez and Merkle 2005).

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While the blight remains an obstacle to the natural reestablishment of chestnut in our eastern forests, conventional breeding and gene transfer research currently underway should result in the production of blight-resistant American chestnut genotypes within the next 5-7 years. However, even once blight-resistant American chestnut genotypes become available, there exists no method for mass propagating the blight-resistant genotypes to meet the anticipated demand for planting stock. Somatic embryogenesis may offer the potential for mass clonal propagation of blight-resistant American chestnut genotypes, if somatic seedling production efficiency for those genotypes can be raised to useful levels.

Somatic embryogenesis in American chestnut (*C. dentata*) was first reported over a decade ago (Merkle et al. 1991). Embryogenic cultures were initiated at a low frequency (less than 1%) by culturing immature seeds on Woody Plant Medium (WPM; Lloyd and McCown 1980) with 2,4-dichlorophenoxyacetic acid (2,4-D), but no plantlets were produced. Carraway and Merkle (1997) found that initiation medium with indole-3-acetic acid (IAA) and 2,4-D induced an embryogenic response, but only cultures initiated on medium with 3 mg/L 2,4-D sustained production of somatic embryos for more than 2 months. In addition, for continued proliferation via repetitive embryogenesis, cultures had to be maintained continuously on medium with 2,4-D.

Plantlet production from American chestnut embryogenic cultures was problematic for several years. A 12-week cold (4º C) stratification period prior to germination tested by Carraway and Merkle (1997) improved development and germination of American chestnut somatic embryos, although conversion to plantlets was very rare, and none of the plantlets survived transfer to *ex vitro* conditions. Xing, et al. (1999) reported successful production of 20 American chestnut plantlets from somatic embryos without a cold treatment, but it was unclear how many of these plantlets were true somatic seedlings and how many embryos produced only shoots that had to be rooted in a separate step to produce complete plantlets. Robichaud et al. (2004) used a minimum of four weeks of cold (4º C) stratification and generated a few American chestnut somatic seedlings that survived and continued to grow following transfer to the greenhouse. Andrade and Merkle (2005) tested 0, 6, and 12-week durations for the cold (4º C) stratification period and found that 12 weeks of cold improved germination and conversion frequencies of American chestnut somatic embryos. While 12 weeks of cold stratification yielded the highest plantlet production frequencies and this treatment was incorporated into the standard protocol for plantlet production, longer cold treatments were not tested.

The standard protocol for American chestnut somatic embryo plantlet production employs a 16-h photoperiod under cool white fluorescent light (100 μmol m^{-2} s^{-1}) (Andrade and Merkle 2005). Red light improved germination and conversion frequencies for southern pine somatic embryos (Merkle et al. 2006). Kvaalen and Appelgren (1999) also reported that red light improved somatic embryo germination frequencies in Norway spruce (*Picea abies*), but did not improve germination of zygotic seedlings. Other light qualities have yet to be tested for their effects on chestnut somatic embryo germination and conversion.

The overall goal of the research reported here was to enhance our ability to generate embryogenic cultures of American chestnut and to produce plantlets from the cultures so that the system can ultimately be used for propagation of blight-resistant material from conventional breeding programs and for engineering with potential blight-resistance genes. The specific

objectives of the research were to: (1) Test different plant growth regulators and concentrations for their potential to improve the frequency with which new American chestnut embryogenic cultures can be initiated from immature seed explants, and (2) To test extended cold treatments and different light qualities for their potential to improve the germination of American chestnut somatic embryos and their conversion to somatic seedlings.

MATERIALS & METHODS

Culture Initiation Experiment

Immature American chestnut burs were collected from select mature trees, representing 11 families that were either open-pollinated or cross-pollinated with pollen from a known source tree. Altogether, burs were collected from eight source trees in Kentucky, Georgia, Connecticut, and Pennsylvania during late July and early August 2006. The burs were dissected to remove the nuts, which were surface-sterilized by agitation in the following solutions: 70% ethanol for 20 s; 10% Roccal (10% Alkyl dimethyl benzyl ammonium chloride, Winthrop Laboratories Div., Sterling Drug Co., New York, NY) for 1 min; 5.25% sodium hypochlorite for 5 min; sterile water for 3 min; 0.01N HCl for 3 min, and three subsequent rinses in sterile water for 3 min each. Then, the developing nuts were dissected to remove the immature seeds for culture. The number of seeds cultured for each family ranged from 45 to 994 (Table 1).

The immature seeds were cultured in 60x15-mm plastic Petri dishes on semi-solid initiation and maintenance medium (IMM; Andrade et al. 2005), modified with different auxins and concentrations. Two mg/l and 4 mg/l of both 2,4-dichlorophenoxyacetic acid (2,4-D) and 4-amino-3,5,6-trichloropicolinic acid (picloram) supplements were tested, for a total of four experimental plant growth regulator (PGR) treatments (2 mg/l 2,4-D for IMM1, 4 mg/l 2,4-D for IMM2, 2 mg/l picloram for IMM3, 4 mg/l picloram for IMM4). After autoclaving the medium and allowing it to cool, 0.5 mg l^{-1} L-glutamine was filter-sterilized and added.

All the live seeds from a given nut (ranging from 9 -18) were cultured together on a single Petri plate. The cultures were incubated in the dark at 25º C. Seed explants were transferred to fresh medium of the same composition after 5 weeks and the cultures were examined for signs of embryogenic proliferation after 10 weeks. The result for each seed explant was recorded, and the percentage of seeds that produced embryogenic material was calculated for each family and each PGR treatment. Those that appeared to be embryogenic after 10 weeks were maintained thereafter by subculture on fresh IMM1 or IMM3 every 3 weeks.

Somatic Embryo Germination and Conversion Experiments

Cultures used for somatic embryo germination and conversion experiments had been initiated in previous years from open-pollinated seeds collected from source trees in Virginia. Embryogenic clones WB569-37, WB569-61 and WB569-97 were initiated from seeds collected from one source tree in 2003, WB484-3 was initiated from a second tree in 2003, and AM54-1 and AM58-1 were initiated from a third tree in 2001. Suspensions were initiated from the American chestnut embryogenic cultures by inoculating approximately 0.5 g of proembryogenic masses (PEMs) into 125-ml Erlenmeyer flasks containing 30 ml of liquid IMM (with 0.5 mg l^{-1} L-glutamine). Suspensions were maintained by shaking on a gyratory shaker at 100 rpm at 25º C

in the dark. Cultures were fed every 2 weeks by aspirating out the old medium and adding 30 ml of fresh IMM.

After 45 days in suspension, the PEMs were collected by size-fractionating with stainless steel CELLECTOR sieves (Bellco Glass), based on the method described previously by Andrade et al. (2005). Suspensions were poured through nested sieves with pore sizes of 860 µm and 38 µm, such that cell clusters with diameters between the two pore sizes were collected on the 38 µm sieve. Using a pipette, the PEMs collected in the 38 µm sieve were backwashed from the sieve into a new, sterile 125 ml Erlenmeyer flask with 30 ml of liquid embryo development medium (EDM; Andrade et al. 2005). Re-suspended PEMs were incubated under the same conditions as described above for an additional 4 days. To plate the PEMs for embryo development, they were collected on Nitex nylon mesh with a 30 µm pore size (Sefar America, Depew, NY) using a Büchner funnel with mild vacuum. The mesh with PEMs was subsequently placed on semi-solid EDM in 60x15-mm plastic Petri dishes and incubated in the dark at 25º C.

Once the PEMs had developed into embryos reaching lengths of 2 to 4 mm, those possessing a morphology similar to that of zygotic embryos (i.e. with a radicle and two cotyledons) were selected using a dissecting microscope and transferred to semi-solid EDM in 100 x 15-mm plastic Petri plates. In cases where few embryos had the ideal form, those with a well-defined radicle and one to several "leafy" cotyledons were selected. Twenty embryos were placed in a grid pattern on each Petri plate. The embryos were oriented horizontally on the medium and were incubated at 25º C for 1 week in the dark prior to cold stratification treatments.

Cold treatment experiment

The effect of extended cold treatments on germination and conversion frequencies was tested by storing embryos from three embryogenic clones for 12, 15 or 18 weeks in a refrigerator at 4° C. The three embryogenic clones (WB569-37, WB569-61, and WB569-97) were derived from immature seeds of a single mother tree growing in Virginia, provided by TACF cooperators. Additional treatments to determine whether a transfer to fresh EDM during the cold treatment would improve germination and conversion frequencies were also tested. This variable was tested for only the 15-week and 18-week treatments. After 12 weeks of cold treatment, subsamples of the embryos in the 15- and 18-week treatments were transferred to plates of fresh EDM (Andrade and Merkle 2005) and returned to the refrigerator, while the other embryos remained on the same plate of EDM for the duration of the cold treatment. In total, there were 5 treatments, including 12-, 15-, and 18-week cold treatment duration treatments without transfer to fresh medium and 15- and 18-week cold treatments with transfer to fresh medium.

At the end of the cold stratification period, embryos were selected from the plates and transferred to GA7 vessels containing 100 ml of semi-solid GM (Andrade and Merkle 2005) with 5 g l^{-1} activated charcoal (EM Industries). Five embryos were placed in each GA7, oriented vertically (radicle down). Three GA7s (15 embryos total) were used for each genotype by treatment combination. GA7s with embryos were placed in an incubator at 25° C under cool white fluorescent light ($100 \cdot \mu mol \cdot m^{-2} \cdot s^{-1}$) with a 16-h photoperiod. Germination and conversion frequencies were tallied after 6 weeks of incubation.

Light quality experiment

To assess the effect of light quality on chestnut somatic embryo germination and conversion, different light qualities in the plantlet production stage were tested. The standard protocol was followed for embryo production for three embryogenic clones (AM58-1, AM54-1, and WB484-3), with the substitution of an 18-week cold duration instead of the standard 12 weeks. Using two Percival Model E-30LED incubators with light-emitting diodes (LEDs), we tested red light (600 nm) and red+far-red light (600 nm + 750 nm) wavelengths in comparison to our standard cool white fluorescent light ($100 \cdot \mu mol \cdot m^{-2} \cdot s^{-1}$) with 16-h photoperiod, for their effects on somatic embryo germination and conversion. We used three GA7s with 5 embryos each for a total of 15 embryos for each clone by light quality treatment combination. Germination and conversion frequencies were recorded after 6 weeks in the incubators.

Statistical analysis

Culture initiation data and somatic embryo germination and conversion data were analyzed by analysis of variance using PROC GLM and Duncan's Multiple Range Test in SAS (SAS Institute 1990).

RESULTS

Culture Initiation Experiment

Explants from 7 of the 11 families produced at least one embryogenic culture (Table 1). Of the 4,040 seeds that were placed in culture, only 97 produced embryogenic material, for an overall embryogenesis induction frequency of 2.4%. Embryogenesis induction frequency varied significantly among the successful families ($P<0.0001$) in the initiation experiment, with initiation frequencies ranging from 0.47% to 5.53% (Table 1). Two families with the same female parent (Adair x Kelly and Adair x Wayne3) gave significantly different initiation frequencies (5.53% and 2.00%, respectively), indicating a possible influence on embryogenesis due to the male parent.

Table 1. Embryogenesis induction results for seeds of 11 American chestnut families cultured in 2006.

Family	total # seeds	# embryogenic seeds	% embryogenesis
GAUNI3 x GAFAN2	211	1	0.47
GAUNI3 x OP	169	8	4.73
MY1 x ALA	541	4	0.74
RHR2T2	288	0	0.00
RHR2T7	45	1	2.22
RHR3T7	93	0	0.00
RHR4T3	126	0	0.00
Adair x Kelley	994	55	5.53
Adair x Wayne3	701	14	2.00
Stocker1 x Adair	414	0	0.00
McClosky x OP	458	14	3.06
OVERALL	*4040*	*97*	*2.40*

The 2 mg/l 2,4-D treatment yielded the highest average frequency (1.26%) of embryogenesis across the 7 families. However, Duncan's test indicated that this PGR treatment was only significantly superior to the 4 mg/l picloram treatment (0.24%) (Fig.1). While some families produced embryogenic material with all four PGR treatments, two families responded only to a single PGR treatment. Also, while the 2 mg/l 2,4-D treatment produced the highest average initiation rate among the five families that responded, the 2 mg/l picloram treatment produced a response from more families (six families). Both treatments that employed a PGR concentration of 4 mg/l produced embryogenic material in the same four families.

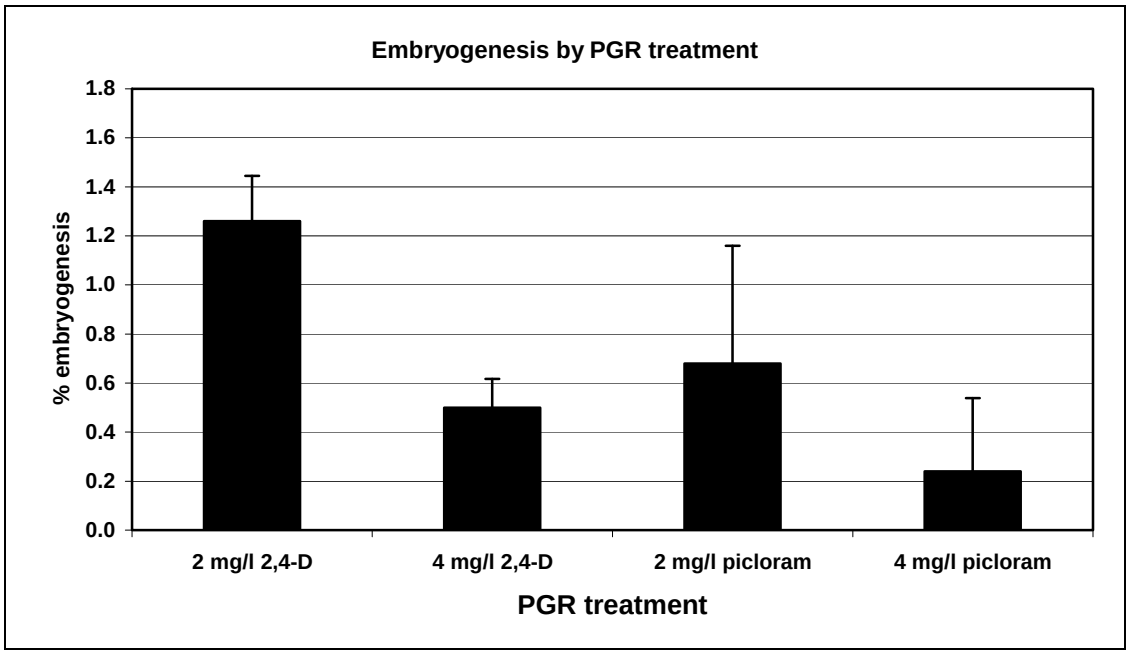

Figure 1. Effect of plant growth regulator treatments on embryogenesis induction from seeds of seven American chestnut families, averaged across all families. Bars indicate standard error.

Cold Treatment Experiment

Overall, analysis of variance indicated that both clone and clone x treatment interaction significantly affected germination frequency ($P<0.0001$), but only clone significantly affected conversion frequency ($P<0.0001$). However, according to the Duncan's test results, the 15-week with transfer and 18-week with transfer treatments yielded significantly higher germination rates (both 60.0%) than the 15-week treatment (44.4%). Duncan's test also indicated that the overall conversion rates were significantly different between the 18-week with transfer treatment (35.6%) and the 15-week treatment (15.6%) (Fig. 2).

When the results for each clone were examined individually, the nature of the interaction between clone and cold treatment on germination frequency became apparent, in that individual clones responded differently to the different cold treatments (data not shown). One clone (WB569-61) failed to germinate or convert to plantlets following the 15-week and 18-week cold

treatments without a transfer, but germinated and converted in the 15-week and 18-week cold treatments that included a transfer to fresh medium. This clone was consistently the lowest plantlet producer of the three clones across all cold treatments. A majority of the somatic embryos for this clone turned brown and stopped developing in the 15- and 18-week treatments that did not include a transfer to fresh medium.

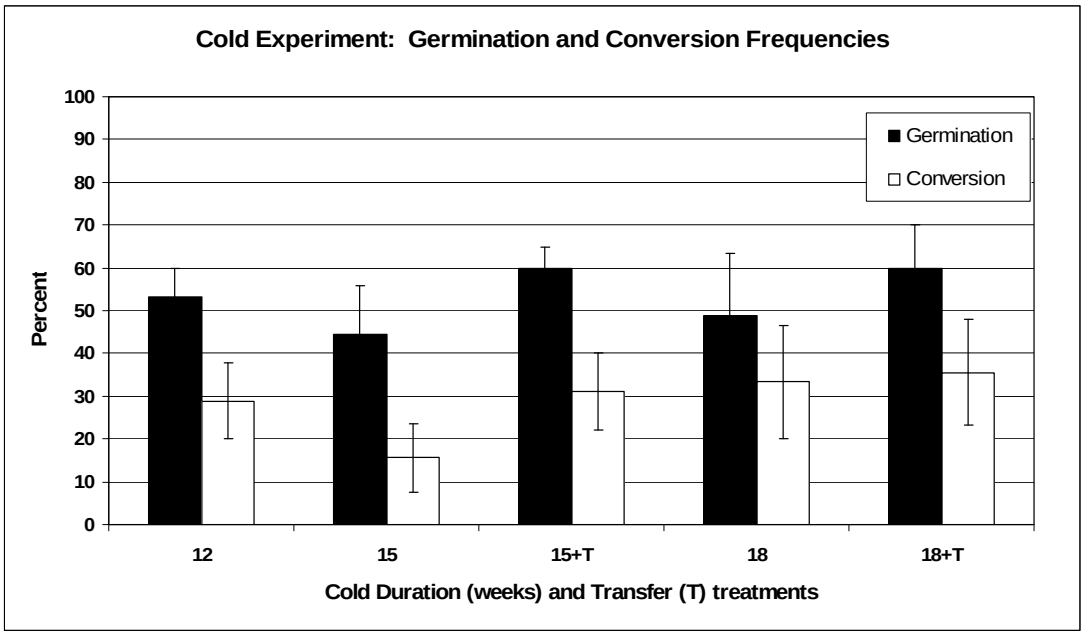

Figure 2. Results for the effect of cold and transfer treatments on average germination and conversion of American chestnut somatic embryos for three clones. Bars indicate standard error.

For clone WB569-97, there were no significant differences among the cold treatments for either germination or conversion. Embryos of this clone developed faster in the cold treatments that did not include a transfer, as indicated by greater radicle elongation by the somatic embryos that were not transferred compared to very little radicle elongation by those that were transferred to fresh medium. However, quantitative data for this observation were not recorded. For clone WB569-37, the 18-week and 18-week with transfer treatments yielded significantly higher germination frequencies (100% and 93.3%, respectively) than the 12-, 15-, and 15-week with transfer treatments (66.7%, 66.7% and 73.3%, respectively), according to Duncan's test. For this clone, the 18-week and 18-week with transfer treatments also yielded significantly higher conversion frequencies (80.0% for both treatments) than the 15-week treatment (33.3%).

Light Quality Experiment

Light quality treatment, clone, and clone x light quality treatment combination all had significant effects on germination frequency ($P = 0.0039$, $P = 0.0282$, and $P = 0.0069$, respectively). The light quality treatment also had a significant effect on conversion frequency ($P = 0.0022$). Duncan's test indicated that both germination and conversion frequencies were significantly higher under the red and red + far-red treatments (R germ: 86.7%, R+FR germ: 73.3%, R conv:

68.9%, R+FR conv: 57.8%), compared to the white light control (germ: 48.9%, conv: 31.1%) (Fig. 3).

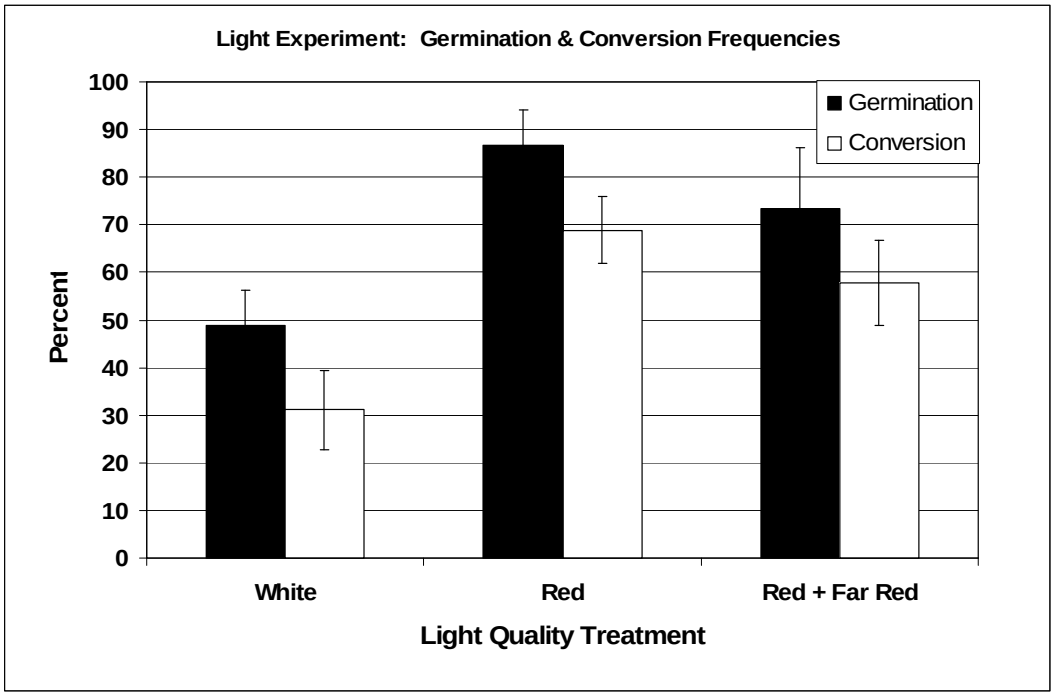

Figure 3. Effects of light quality treatment on germination and conversion of American chestnut somatic embryos, averaged across three clones. Bars indicate standard error.

As was the case with the cold treatment experiment, when the results for each clone were examined independently, the differential responses among the clones to light quality treatment became apparent (data not shown). In two of the three clones, red light significantly increased plantlet production. Light quality was especially critical for clone AM58-1. For this clone, Duncan's test indicated that germination ($P = 0.0016$) and conversion ($P = 0.0115$) frequencies were significantly higher for both the red and the red + far-red treatments than for the white light treatment. This difference is due to the fact that somatic embryos of AM58-1 completely failed to germinate and convert to plantlets under white light, but successfully produced somatic seedlings under red and red + far-red light, with germination rates of 86.7% and 80.0%, respectively, and conversion rates of 73.3% and 60.0%, respectively. For clone WB484-3, conversion frequency was significantly enhanced by red light (86.7%) compared to white light (53.3%), according to Duncan's test. For clone AM54-1, however, there were no significant differences in germination or conversion frequencies among the different light quality treatments. Somatic embryos also seemed to germinate more rapidly under red and red + far-red light compared to white light; however, these were only visual observations and no quantitative data were recorded.

DISCUSSION

Results from the experiment to test different PGRs and concentrations for their effect on embryogenesis induction indicated that each family reacted differently to each of the four PGR treatments. This is likely due to the genetic variation among the different families tested. Another possible explanation is that the seeds from the different mother trees may have been in different stages of development inside the nuts when they were harvested from the trees, and therefore may have reacted differently to the treatments. Unfortunately, it is impossible to schedule the harvest precisely for each bur on each tree and therefore, this is one source of uncontrollable variation. However, in general, 2 mg/l of either 2,4-D or picloram gave higher induction frequencies than 4 mg/l of either PGR. Based on these results, we concluded that there is no justification for replacing our standard 2 mg/l 2,4-D treatment for culture initiation with one of the alternative induction treatments. These results are similar to those of previous reports in which initiation medium with indole-3-acetic acid (IAA) and 2,4-D induced an embryogenic response, but only cultures initiated on medium with 3 mg/L 2,4-D sustained production of somatic embryos for more than 2 months (Carraway and Merkle 1997). It is possible that factors other than those that can be manipulated during the culture process (e.g. genotypic control over the embryogenesis response, low percentage of fertilized seeds) control embryogenesis induction in American chestnut, such that initiation frequencies will remain low regardless of what plant growth regulators or other culture variables are tested.

The results of the cold treatment experiment did not indicate that a cold treatment longer than 12 weeks would significantly improve germination and conversion frequencies for all clones of American chestnut that we tested. However, for some clones, a longer cold treatment (18 weeks) increased germination and conversion frequencies. If a longer cold treatment is needed, our results indicate that a transfer to fresh medium following the first 12 weeks of cold may produce higher rates of plantlet production. The variation among clones in response to the different cold treatments is likely attributable to genetic variation. We concluded that the optimal cold treatment duration will need to be determined for each American chestnut clone to maximize plantlet production efficiency. These conclusions agree with previous observations that various clones respond differently to a range of cold treatment durations (Andrade and Merkle 2005).

As with the cold treatment experiment, there was considerable variation in the responses of different lines to the different light quality treatments, which is again likely attributable to genetic variation among the lines. Overall, red light and red + far-red light improved germination and conversion rates compared to white light, and red light was superior to red + far-red light. The positive effects of red light on germination and conversion of somatic embryos have also been reported for southern pine species (Merkle et al. 2006) and Norway spruce (Kvaalen and Appelgren 1999). We concluded that somatic embryo germination under LED-supplied red light in a 16-h photoperiod at 25º C should be incorporated into our American chestnut plantlet production protocol.

While we were unable to improve culture initiation frequencies by manipulating plant growth regulator type or concentration, our results indicate that further enhancement of American

chestnut somatic seedling production efficiency is possible by manipulating cultural treatments such as cold stratification and light quality during the periods prior to and during germination. The ability to produce more plants from American chestnut cultures will be especially useful for propagation of trees from genetically transformed cultures, as recently reported by Polin et al. (2005) and Andrade et al. (2006). While plantlet production efficiencies still need to be greatly improved, we believe these enhancements will help move our embryogenesis system from a laboratory phenomenon towards a useful propagation system for blight-resistant American chestnut trees. Of course, the ability of these trees to grow in the field has yet to be determined. But the first American chestnut somatic seedlings from our research program to be planted in the nursery (following one year in the greenhouse) performed well in their first season, with some reaching heights over 1.5 m. A combination of plantlet production protocol improvements and genetic enhancement of fungal resistance in American chestnut will allow us to make substantial contributions to the restoration of this species to our forests.

ACKNOWLEDGEMENTS

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LITERATURE CITED

Andrade, G.M., and S.A. Merkle. 2005. Enhancement of American chestnut somatic seedling production. Plant Cell Rep. 24:326-334.

Andrade, G.M., C.J. Nairn, H.T. Le, and S.A. Merkle. 2006. Transgenic American chestnut trees: a novel approach for the restoration of the species. In: Plant Biology 2006 Program and Abstracts, August 5-9, 2006, Boston, MA. Abstract No. P46017.

Burnham, C.R. 1988. The restoration of American chestnut. Am. Sci. 76:478-487.

Carraway, D.T., and S.A. Merkle. 1997. Plantlet regeneration from somatic embryos of American chestnut. Can. J. For. Res. 27:1805-1812

Kvaalen, H., and M. Appelgren. 1999. Light quality influences germination, root growth and hypocotyl elongation in somatic embryos but not in seedlings of Norway spruce. In Vitro Cell. Dev. Biol. Plant 35:437-441.

Lloyd, G., and B. McCown. 1980. Commercially-feasible micropropagation of mountain laurel, *Kalmia latifolia*, by use of shoot-tip culture. Proceedings from The International Plant Propagators' Society 30:421-427.

Merkle, S.A., P.M. Montello, X. Xia, B.L. Upchurch, and D.R. Smith. 2006. Light quality treatments enhance somatic seedling production in three southern pine species. Tree Physiology 26:187-194.

Merkle, S.A., A.T. Wiecko, and B.A. Watson-Pauley. 1991. Somatic embryogenesis in American chestnut. Can. J. For. Res. 21:1698-1701.

Polin, L.D., H. Liang, R.E. Rothrock, M. Nishii, D.L. Diehl, A.E. Newhouse, C.J. Nairn, W.A. Powell, and C.A. Maynard. 2006. *Agrobacterium*-mediated transformation of American chestnut [*Castanea dentata* (Marsh.) Borkh.] somatic embryos. Plant Cell Tiss. Org. Cult. 84:69-78.

Robichaud, R.L., V.C. Lessard and S.A. Merkle. 2004. Treatments affecting maturation and germination of American chestnut somatic embryos. J. Plant Physiol. 161:957-969.

SAS Institute. 1990. SAS Procedures Guide, Version 6, 3rd Edn. SAS Institute, Cary, North Carolina.

Viéitez, F.J., and S.A. Merkle. Fagaceae. 2004. In: Biotechnology of Fruit and Nut Crops (R.E. Litz, Ed.). CAB International, pp. 263-296.

Xing, Z., W.A. Powell, and C.A. Maynard. 1999. Development and germination of American chestnut somatic embryos. Plant Cell Tiss.Org. Cult. 57:47-55.

Some Resistance Genes Against *Cryphonectria parasitica* May Be Strain-Specific

Timothy S. McKechnie

Abstract: The goal of The American Chestnut Foundation (TACF) is to produce hybrid American-type chestnuts (*Castanea dentata*) with adequate, long-lasting resistance to *Cryphonectria parasitica,* the fungus that causes chestnut canker disease. TACF is using two variations of a single backcross breeding scheme, which is designed to transfer resistance from Chinese chestnut (*Castanea mollissima*). Achievement of adequate, long-lasting resistance under either variation of the breeding scheme may depend on having knowledge of interactions between Chinese resistance genes and different strains of the pathogen. Third backcross hybrids derived from the "Clapper" source of resistance, along with Chinese, American, and F1 (Chinese x American) hybrid control trees were inoculated with mycelium of two fungal strains. Resulting cankers were measured at 11 weeks. Two independent lines of analysis suggest the existence of one or more strain-specific resistance genes. First, strain-specific phenograms show distinct inflection points and randomization of the second strain. Second, individual trees with extreme forms of strain-specific resistance phenotypes are more numerous than predicted by models lacking strain-specific genes. The existence of strain-specific genes can't be proven with the present data set because it was not an experiment designed to reveal strain-specific effects. However, if strain-specific genes are confirmed in studies of long-term resistance of TACF hybrids, there will be implications for the purpose and design of the central TACF breeding program in Meadowview, VA, the purpose and design of the regional breeding programs in eight states, and whether more than one breeding scheme may be advisable on the central and regional levels.

Keywords: Resistance, interactions, *Cryphonectria parasitica*, *Castanea dentata*, *Castanea mollissima*

INTRODUCTION

The goal of The American Chestnut Foundation (TACF) is to produce hybrid chestnut trees with many of the qualities of American chestnut (*Castanea dentata*) and also adequate, long-lasting resistance to *Cryphonectria parasitica,* the fungus that causes chestnut canker disease. TACF is using two variations of a single breeding scheme (Hebard 2005) one of which involves passing resistance from Chinese chestnut (*Castanea mollissima*) through a single BC1 hybrid followed by two or more backcrosses to American chestnut followed in turn by an intercross intended to create homozygous resistant hybrids that are expected to breed true for high resistance. The second variation passes resistance from a single Chinese chestnut through twenty F1 trees and is otherwise the same as the first variation. Evaluation of the resistance being developed under either variation of the scheme so far has not including testing for interactions between resistance genes and different strains of the pathogen.

Three studies potentially capable of detecting interactions between isolates of *C. parasitica* and individual Chinese chestnuts (*Castanea mollissima*) have been published. A phenotype-level study of aggressiveness of two *C. parasitica* isolates on Chinese chestnut (in China) reported that the two pathogen isolates had statistically identical aggressiveness and no isolate-by-tree interactions (Ling, Xiahong et al. 2002)[1]. However, it's not clear whether the detached-stem assay used was reliable[2]. In contrast to the study mentioned above, there are two published reports that support the existence of phenotype-level isolate-by-tree interaction between *C. parasitica* isolates and Chinese cultivars. One study found significant isolate-by-cultivar interactions at the seedling stage[3] (Huang, Carey et al. 1996) using three isolates and a whole-tree assay. The other report used whole trees at least 25 years old (Anagnostakis 1992) and two isolates but did not explicitly test whether the interactions observed were statistically significant. Turning attention to American chestnut, a genetic-level test on whole trees (saplings) indicated no isolate-by-tree-family interactions among half-sib Americans (Huang, Carey et al. 1996).

Remarkably, until the present report, there has been no published study of phenotype-level or genetic-level isolate-by-tree interactions on American chestnut hybrids. The lack of attention to strain-by-hybrid interactions is remarkable because interactions are more likely to be observed in hybrids than in pure species and because the existence of interactions is a significant cause for concern in a breeding program (Nelson 1978).

MATERIALS AND METHODS

TACF uses two strains of *C. parasitica* to test trees for resistance. Inoculation with the strain named "Ep 155" (hereafter "Ep") usually results in larger cankers than inoculations with the other strain, named "SG1 2-3", (hereafter "SG").[4]

The canker measurement data in this article are from a set of third backcross (BC3) progeny derived from "Clapper", which is a BC1 (Chinese X American) X American and is one of TACF's main sources of resistance. The BC2 parent is identified by TACF as "CL287". The Pennsylvania American[5] parent of the BC3s is known as "Ort". All trees were planted in the spring of 1997. They were grown[6] and inoculations performed according to standard TACF

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[1] Thanks to Bruce Levine of the Maryland Chapter and to Susan You, Duke University, 2005 summer intern with the PA Chapter of TACF, for the detailed translation.

[2] This report was based on mycelial inoculation of detached Chinese chestnut stem segments kept under (probably) anaerobic conditions in plastic bags, which were stored in an incubator at 25°C and 70% humidity. Not surprisingly, a fermented odor was noted for the larger cankers. There was no effort to demonstrate that the assay reflected resistance on whole trees.

[3] Huang *et. al.* used three isolates, fifteen seedlings per cultivar, one inoculation per seedling, and performed ANOVA on measurements taken at six weeks, which is after healing had begun, and the cankers were shrinking.

[4] "Ep 155" is American Type Culture Collection isolate number 38755 (http://www.atcc.org/). "SG1 2-3" was isolated near Meadowview, VA. It was chosen for testing purposes because it was one of the least aggressive isolates when inoculated on American chestnut.

[5] Possibly a European / American hybrid based on leaf characters. This is a point of controversy.

[6] The trees were grown by Ann and Dr. Robert Leffel near Brogue, PA.

procedures for backcross progeny[7], which involves two inoculations of each strain on every tree. All trees were inoculated on May 27, 2000 and measured between August 14 and 20, 2000, when the inoculations were 11-12 weeks old. Canker sizes reported in this article are the average of width and length measured without scraping the bark.[8]

Out of 149 BC3s originally planted, only 84 survived long enough to be inoculated. Only 76 of these provided two measurements for both SG and Ep and were included in the analysis. This was the first BC3 orchard at this location, and natural *C. parasitica* disease pressure appeared to be low (personal observation).

Controls trees were planted as same-age seedlings in a randomized fashion among the BC3s and were inoculated and measured at the same time as the BC3s. The eight Chinese chestnut control trees were half siblings, produced by open pollination on "Chinese D89" near Meadowview, VA. The American chestnut controls trees were open pollinated from various Pennsylvania trees. Three F1 controls were derived by controlled pollination of uncharacterized Chinese "Leffel North" x American "Ort". Five F1 control trees were derived by controlled pollination of Chinese "Meiling" KY175 x American TPE17.

RESULTS AND DISCUSSION

The existence of strain-specific resistance genes can't be proven with the present data set. The present data are based on canker measurements of a set of BC3 trees that were part of the TACF breeding program, not an experiment designed to reveal strain-specific resistance genes. The purpose of this paper is to argue that further testing is warranted.

Another important point is that the cankers discussed in this paper were only 11-12 weeks old when measured. Short-term observations of canker size do not always predict long-term resistance (Grente 1961). However, preliminary results (not shown) based on measurements of one year old cankers on a second Clapper BC3 family show patterns similar to those summarized by this paper.

With the above caveats in mind, phenograms are a good starting point for analysis because they provide a visual data summary which can often be used to formulate easily testable hypotheses.

When all four canker measurements, two for SG and two for Ep, are averaged for each BC3 tree, the resulting SG+Ep phenogram, Chart A, shows relatively little evidence for discrete gene effects. There are no dramatic inflection points or plateaus. The feature most suggestive of the effects of discrete genes is at the intersection of a small BC3 phenotype plateau with the upper limit of F1 control tree SG+Ep averages at 7.1cm, corresponding nearly exactly to a progeny ratio suggesting three unlinked equivalent[9] genes.

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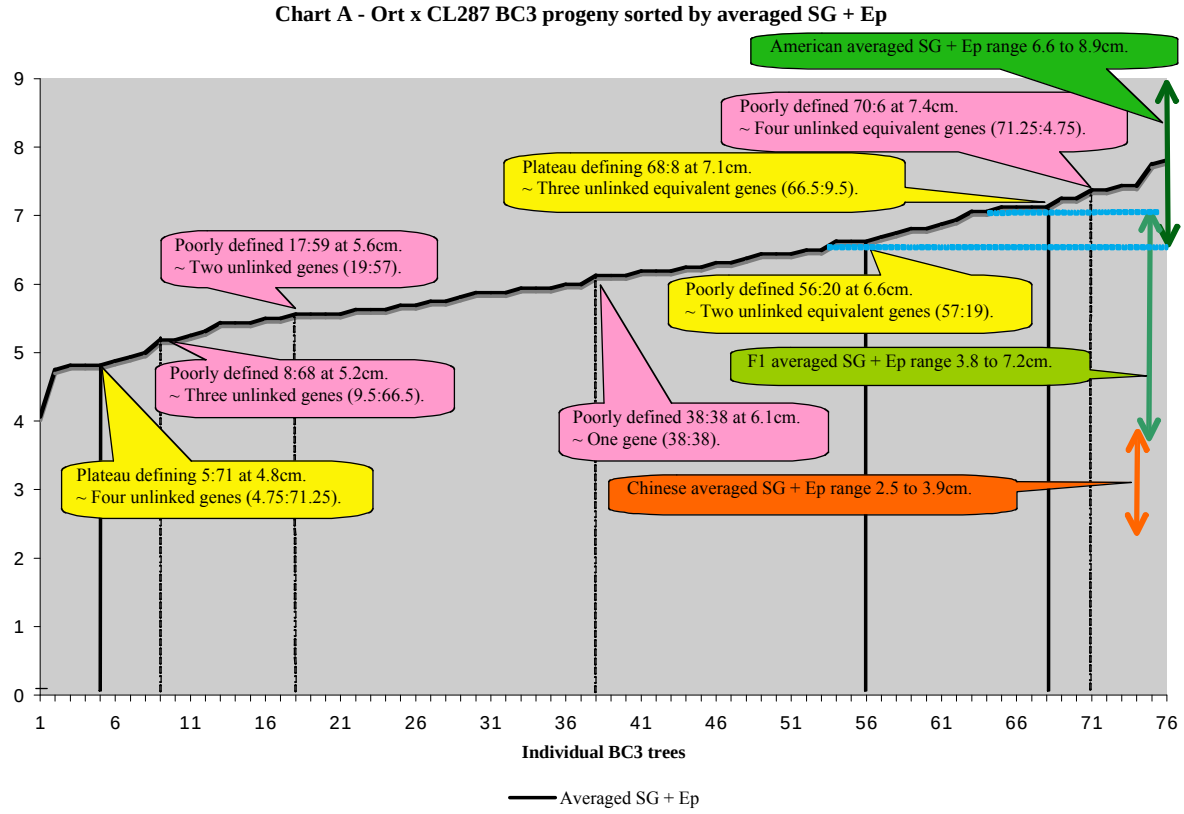
[7] See written procedures at http://chestnut.cas.psu.edu/Breeding.html.

[8] Thanks to Sara Fitzsimmons and Ann Leffel for their careful measurements.

[9] "Equivalent" here means that any one of the three hypothetical genes, acting alone, is capable of providing at least this level of resistance. Note that all three genes could be strain-specific.

When the two SG measurements are averaged for each BC3 tree, the resulting phenogram, Chart B, has at least four features that can be interpreted as the effects of unlinked genes acting alone. The largest plateau, consisting of eight trees with SG averages exactly at 4.5cm, ends nearly exactly where an inflection point defining a 1:1 ratio would be expected. The second largest plateau, consisting of five trees with SG averages exactly at 5.1cm, ends nearly exactly where an inflection point defining the effects of two unlinked equivalent[5] genes would be expected. Two inflection points are observed near the intersection of the phenogram with limits defined by the upper SG range of F1 control trees and the lower SG range of American control trees, nearly exactly at progeny ratios defining three and four unlinked equivalent genes respectively.



Assuming sources of non-genetic variation and gene x environment interaction are relatively small, if Ep canker size is governed by the same genes as the response to SG, one might expect the Ep phenogram to show similar features as the SG phenogram. However, Ep response is seen to fluctuate in an apparently random fashion between Chinese level resistance and American-level resistance across the entire range of SG response (dashed line, Chart B). These dramatic fluctuations in Ep phenotype, to the extent they are genetic, suggest assortment of at least one Ep-specific gene with large effect that is unlinked to any of the putative genes acting on SG.

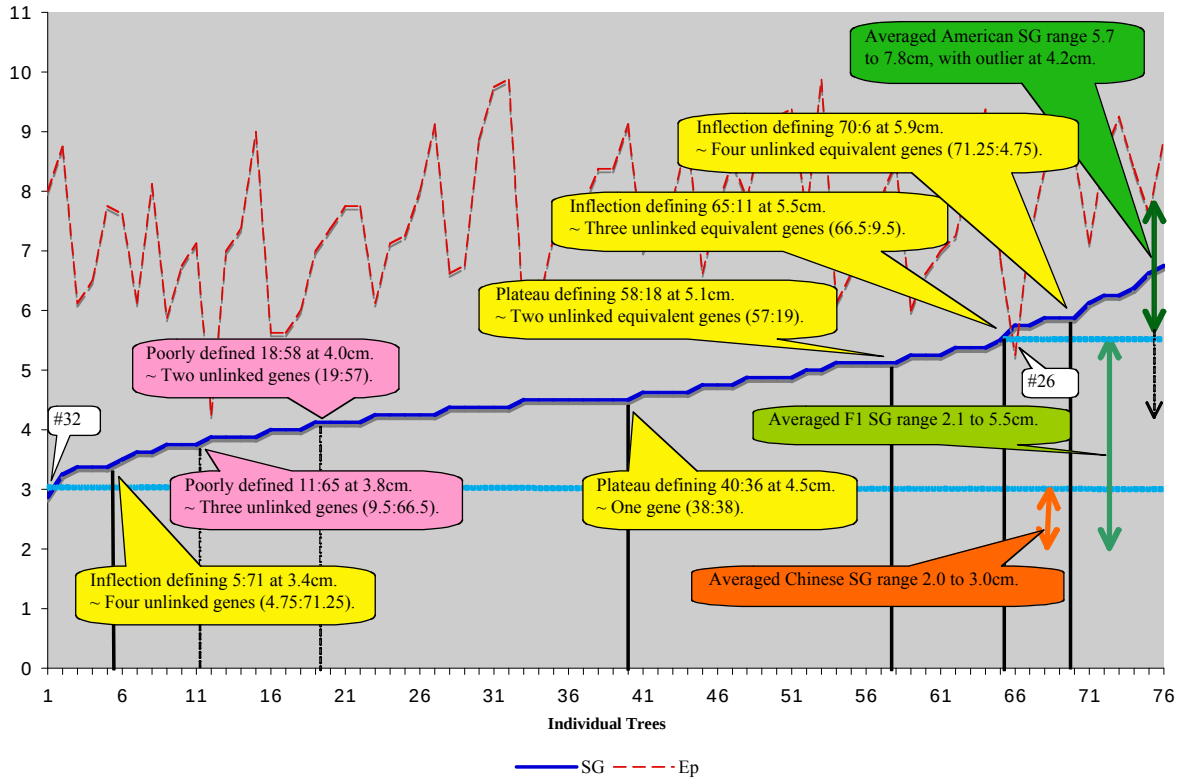
Limiting attention to BC3 trees ranked 19 to 38 in Chart B, the variation in their SG averages is easy to explain in terms of measurement error, Chart C.[10] In contrast, the variation of their Ep

[10] The standard deviation of all BC3 measurements (within strain, within tree) from their average is 0.44cm. The standard deviation of the average is $0.44/2^{1/2} = 0.31$. The deviation from the mean within this region is smaller: 0.22cm.

averages is too large to explain in terms of measurement error. The same observations apply to trees ranked 39 to 57 in Chart D. Whatever is causing the variation in Ep canker size in Charts C and D is affecting the whole tree, or at least a ~1.2 meter long section of the trunk.

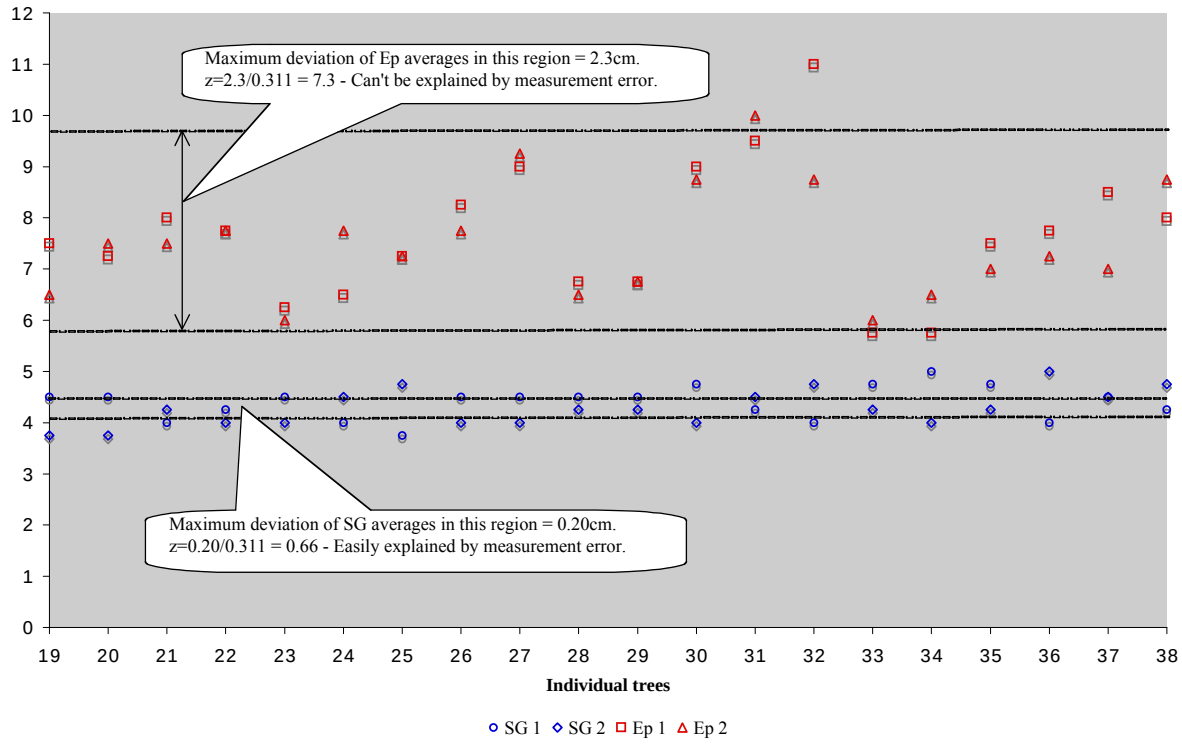
Because of their position in the SG phenogram and their low SG variation, it's reasonable to hypothesize that the sets of progeny considered in charts C and D might have the same genotype with respect to SG resistance. Because of the high variation in Ep canker size between trees and the reproducible behavior of Ep cankers within each tree, it's also reasonable to suppose that Charts C and D show the effects of one or more randomly assorting, Ep-specific resistance alleles.

Chart B - Ort x CL287 BC3 sorted by averaged SG



The Ep phenogram, Chart E, also shows features that can be interpreted as the effects of discrete genes. The largest plateau, consisting of six trees with Ep averages exactly 7.6cm, ends nearly exactly where an inflection point defining a 1:1 ratio would be expected. A plateau consisting of five trees with Ep averages exactly 8.4 cm ends nearly exactly where an inflection point defining a 3:1 ratio would be expected. The upper limit of F1 Ep response (8.9cm) intersects the BC3 phenogram nearly exactly where a 7:1 ratio would be expected. A dramatic inflection point adjacent to the plateau at 8.4cm suggests the action of a pair of linked resistance genes with large effect. Another dramatic inflection point adjacent to a plateau at 6.1cm suggests the action of three additive genes. The SG response fluctuates in an apparently random fashion between Chinese level resistance and American-level resistance across the entire range of Ep response (dashed line, Chart B). If those SG fluctuations have a genetic cause, they suggest at least one SG-specific gene that is unlinked to any of the putative genes acting on Ep.

Chart C - First subset of BC3 canker sizes, sorted by SG average.



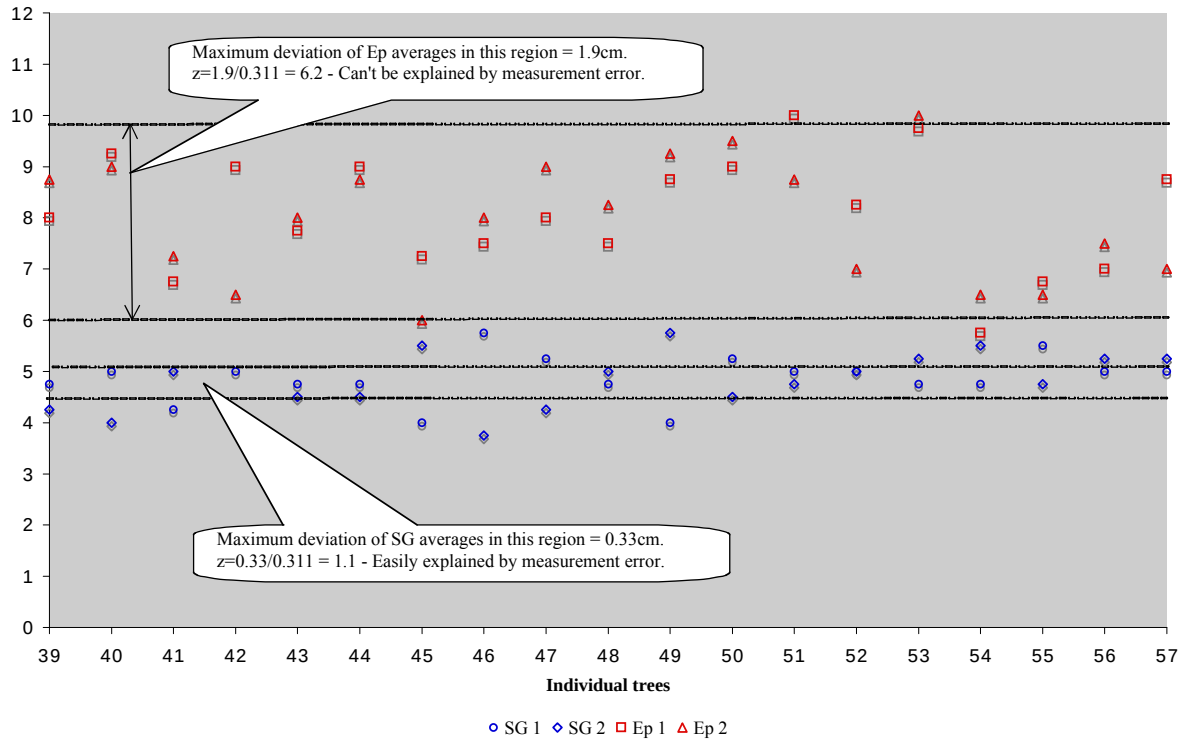
Compared to the SG+Ep phenogram, both the Ep and SG phenograms have more features suggestive of the action of discrete genes and these features are more dramatic. One interpretation might be that the SG+Ep average (over four measurements) removes the random effects present in the SG and Ep averages (over two measurements). However, as noted above, duplicate cankers for SG and Ep are highly reproducible. An alternative interpretation is that examining response to individual strains separately may clarify gene action, which in turn suggests that the resistance genes of largest effect may be strain-specific.

SG and Ep canker sizes in the BC3 are correlated (Pearson correlation of 0.84). That correlation could be interpreted in terms of one or more resistance genes effective against both SG and Ep. Alternatively or in addition, the correlation could be caused by environmental effects and/or pairs of strain-specific genes that are on the same chromosome, i.e., linked pairs consisting of one SG-specific resistance gene linked to one Ep-specific resistance gene.

Based on analyses like these, future crosses will be performed with the intent to isolate single strain-specific alleles.

A variety of other genetic modeling approaches also suggest the existence of strain-specific resistance genes in this cross. For example, it's possible to compare the observed number of trees with strain-specific resistance phenotypes with the number predicted under the assumption of random non-genetic phenotype fluctuations.

Chart D - Second subset of BC3 canker sizes, sorted by SG average.



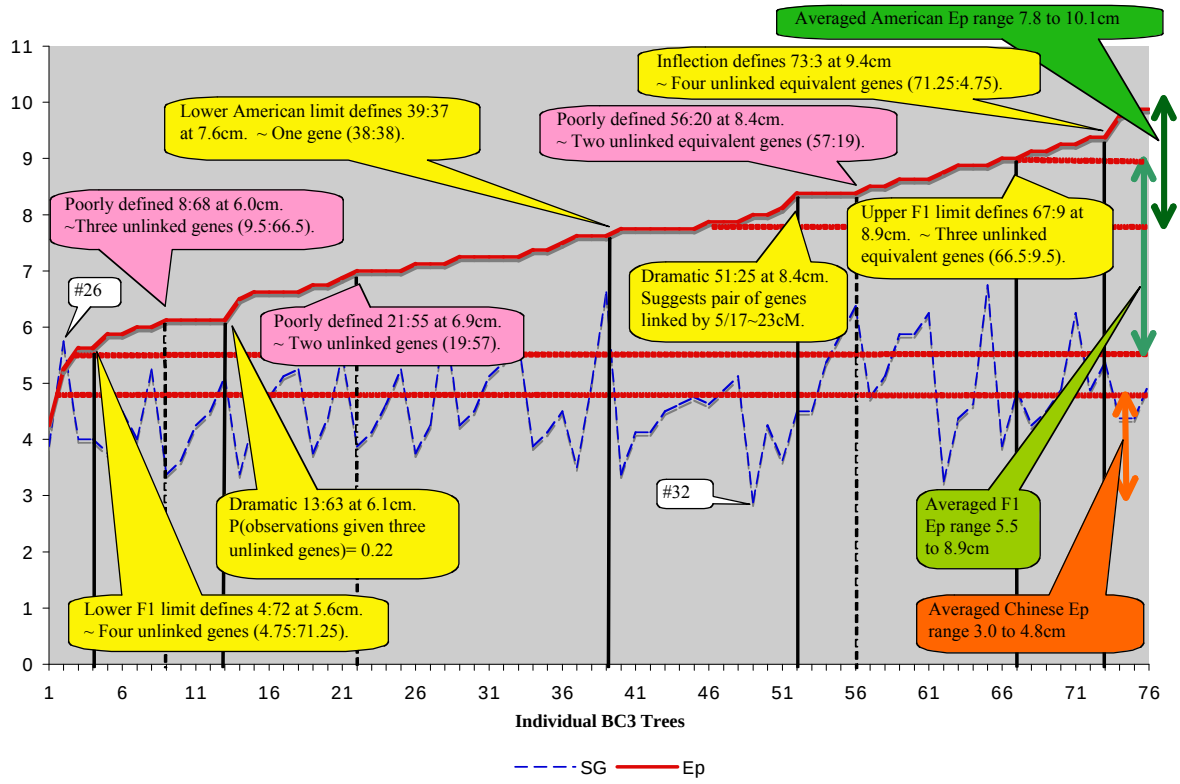
Suppose resistance is defined as any tree with cankers significantly smaller than the American controls.[11] In that case, ten out of the 76 BC3s could be considered to exhibit SG-specific resistance phenotypes[12] and seventeen to exhibit Ep-specific resistance phenotypes[13]. Prediction of the expected number of strain-specific phenotypes caused by non-genetic fluctuations involves three steps: (1) Assume a genetic model, preferably one that fits the over-all BC3 data. (2) Estimate the frequency of non-genetic strain-specific phenotypes for each genotype in the model using control tree data and the bivariate normal distribution. (3) Multiply the above frequency by the associated normalized Chi-square probability and integrate over all possible outcomes.

According to the modeling process described above, no simple genetic model based on non-strain-specific genes can fully explain the observed number of strain-specific phenotypes, Table A. The only simple model (considered here) providing a reasonable fit to the overall SG+Ep averages (two equivalent genes) predicts one-third as many Ep-specific phenotypes as were observed. An over-all numerical estimate of certainty for the above conclusion may be possible

[11] For z=1.65 at the 0.05 confidence level, SG<4.32cm and Ep<7.59cm. Under this definition, resistant trees will have phenotypes at the F1 or better level.
[12] That is, SG average less than 4.32cm and Ep average greater than 7.59cm.
[13] That is, SG average greater than 4.32cm and Ep average smaller than 7.59cm.

but is irrelevant. That's because the main source of uncertainty in this process lies in the assumptions made while constructing the models.[14]

Chart E - Ort x CL287 BC3 sorted by averaged Ep



The modeling process described above can be repeated based on a more extreme form of strain-specificity: Chinese-level resistance for one strain and American-level resistance for the other strain. Again, no simple genetic model based on non-strain-specific genes can explain the observed cases strain-specific phenotypes, Table B. In particular, all of the simple models (considered here) predict a very low probability (<0.008) for the Ep-specific phenotype that was observed in one BC3 tree (orchard #26), Charts B and E.

Note that mistakes at inoculation time, such as putting Ep in all four inoculation holes could explain the observations on tree #26 if it had Chinese-level resistance against Ep. However, the inoculation procedure is well-designed to avoid that kind of error. One person inoculates the SG strain and another person inoculates Ep: both people would have to miss such a mistake.

[14] The principle assumptions in Tables A and B are that SG/Ep variation and correlation within groups of control trees are not caused by variation in resistance genes and that these parameters apply to BC3 genotypes. "Lack of genetic variation within groups of control trees" may very well be a false assumption. However, that's not a problem for the over-all argument since the resulting low SG/Ep correlations would only serve to increase the estimate of non-genetic error rate.

Table A - Expected numbers of strain-specific phenotypes defined as: "Cankers of one strain the same as on American controls and cankers of the other strain significantly smaller than American controls, (p=0.05, z>1.65)".

	Predicted frequency among 76 BC3 progeny:		Overall model fit[b]
Models[a]	SG<=5.5cm, Ep>=9.0cm	SG>=5.7cm, Ep<=8.9cm	
Three equivalent genes predict: 66.5:9.5	9.0	5.7	0
Two equivalent genes predict: 57:19	8.3	5.4	0.29
One gene predicts: 38:38	6.6	4.7	0.0005
Two additive genes predict: 19:57	4.9	4.0	0
Three additive genes predict: 9.5:66.5	4.1	3.6	0
Actual cases of specificity observed:	10	17	---

a - Models assume that each resistance allele provides full (for equivalent genes) or partial (for additive genes) F1-level resistance.
b - Chi square fit to the 53:23 observed for averaged SG+Ep cankers significantly smaller than American.

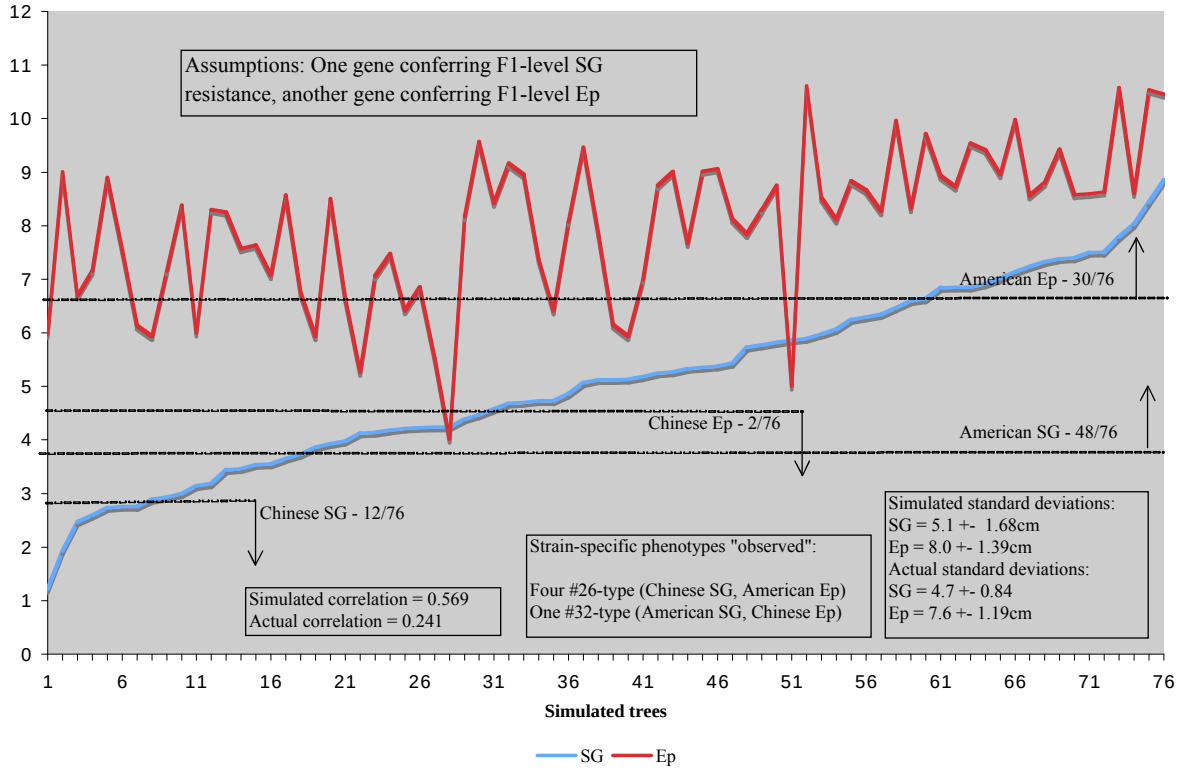
Table B - Expected numbers of strain-specific phenotypes defined as: "Cankers of one strain the same as Chinese controls and cankers of the other stain the same as American controls, (p=0.05, z>1.65)".

	Predicted frequency among 76 BC3 progeny:		Overall model fit[c]
Models[a]	SG<=3.23cm, Ep>=7.59cm	SG>=4.32cm, Ep<=5.17cm	
One gene predicts: 38:38	<0.19	<0.0076	0
Two additive genes predict: 19:57	<0.28	<0.0076	0
Three additive genes predict: 9.5:66.5	<0.33	<0.0076	0.0009
Four additive genes predict: 4.75:71.25	<0.35	<0.0076	0.024
Five additive genes predict: 2.4:73.6	<0.36	<0.0076	0.12
Six additive genes predict: 1.2:74.8	<0.37	<0.0076	0.27
Actual cases of specificity observed:	1 (#32)	1 (#26)	---

c - Chi square fit to the 0:76 observed for averaged SG+Ep smaller or equal to largest Chinese canker.

Data simulations such as that shown in Chart F suggest that just one Ep-specific gene and one SG-specific gene are sufficient to explain the observed cases of extreme strain-specific phenotypes noted in Table B. Over the course of ten simulations, an average of five cases of Chinese-level strain specificity like BC3 orchard #32 and 1.6 cases like orchard #26 were generated. In contrast, simulations without strain-specific resistance genes failed to produce any cases like orchard #26. Even adjustments to the SG/Ep correlations and the standard deviation of SG separately from Ep did not produce a strain-specific phenotype like orchard #26 or an otherwise reasonable model of observed data.

Chart F - Synthetic Ort x Clapper 287 data, sorted by SG.



The several methods of analysis used in this paper all suggest the existence of resistance genes with a moderate or high degree of strain-specificity. Phenogram analysis suggests that such strain-specific genes may have large effects on phenotype when acting alone. These are properties of R genes. Properties suggestive of R genes were also recently discovered in a canker disease of eucalyptus caused by *Cryphonectria cubensis* (van Heerden, Amerson et al. 2005).

Although never explicitly stated in print, the TACF breeding program has been based on the assumption that resistance in crosses between Chinese and American chestnut is not strain specific. It's possible to find broad claims in the literature that resistance to necrotrophic pathogens like *C. parasitica* is usually not strain specific, but such claims are rare. One such claim (Glazebrook 2005) is stated without explanation or reference. Another (Brasier 1987) presents an argument based on species-level resistance, which isn't a genetic test of strain-specificity. Resistance genes thought to be non-strain-specific do exist for necrotrophs, but such genes are found to be present with genes that are strain-specific (Newton and Crute 1989). Mechanisms for strain-specific susceptibility to necrotrophs have been studied on the molecular level (Thomma 2003; Ito, Tanaka et al. 2004). Resistance genes against specific strains of a necrotroph have been mapped (Cho and Muehlbauer 2004).

A literature survey of hardwood canker pathology suggests that belief in lack of specificity, to the extent such a belief exists, is probably based on long-term survival of wild genetic material, often clonally-propagated. Wild trees, because they are long-lived, can reasonably be expected

to harbor a diverse set of resistance genes against a wide spectrum of necrotroph strains adapted to that host. Therefore, long-term survival of such genetic material is not good evidence that resistance genes lack strain-specificity. A proper test for strain specificity involves crosses between resistant and non-resistant trees followed by tests with individual strains, as is being done in the TACF breeding program.

If strain-specific genes are confirmed in studies of long-term resistance of TACF hybrids, there will be implications for the purpose and design of the central TACF breeding program in Meadowview, VA, the purpose and design of the regional breeding programs in eight states, and whether more than one breeding scheme may be advisable on the central and regional levels. To the extent that TACF's present breeding method is based on incorrect assumptions, additional breeding methods should be considered which do not make such assumptions (Borlaug 2000; Leffel 2004). Apparently only silvicultural tests are planned before large-scale deployment of hybrids (Steiner, Ellingboe et al. 2004). Such silvicultural tests (no inoculations are called for) might or might not expose TACF hybrids to a wide variety of strains. The large-scale deployment itself is seen as the best way to test durability of resistance (Hebard 2004). If long-term resistance is strain-specific, this idea should be reconsidered.

LITERATURE CITED

Anagnostakis, S. L. (1992). "Measuring Resistance of Chestnut Trees to Chestnut Blight." Canadian Journal of Forest Research 22(4): 568-571.

Borlaug, N. E. (2000). "Norman Borlaug's response to the breeding program review." Journal of the American Chestnut Foundation 14(1): 25-27.

Brasier, C. M. (1987). Some genetical aspects of necrotrophy with special reference to *Ophiostoma ulmi*. Genetics and Plant Pathogenesis. P. R. Day and G. J. Jellis. Oxford, Blackwell Scientific: 297-310.

Cho, S. H. and F. J. Muehlbauer (2004). "Genetic effect of differentially regulated fungal response genes on resistance to necrotrophic fungal pathogens in chickpea (Cicer arietinum L.)." Physiological and Molecular Plant Pathology 64(2): 57-66.

Glazebrook, J. (2005). "Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens." Annual Review of Phytopathology 43: 205-227.

Grente, J. (1961). "Observations sur le comportement des plants de chataignier apres inoculation de l'endothia parasitica." Ann. Epiphyties 12: 65-70.

Hebard, F. V. (2004). "Research objectives of the American Chestnut Foundation 2004-2014." Journal of the American Chestnut Foundation 18(2): 13-19.

Hebard, F. V. (2005). The backcross breeding program of The American Chestnut Foundation. Proceedings of Conference on restoration of American chestnut to forest lands, http://chestnut.cas.psu.edu/nps.htm.

Huang, H., W. A. Carey, et al. (1996). "Evaluation of Chinese chestnut cultivars for resistance to *Cryphonectria parasitica*." Plant Disease 80(1): 45-47.

Ito, K., T. Tanaka, et al. (2004). "Dissection of the host range of the fungal plant pathogen Alternaria alternata by modification of secondary metabolism." Molecular Microbiology 52(2): 399-411.

Kubisiak, T. L., F. V. Hebard, et al. (1997). "Molecular mapping of resistance to blight in an interspecific cross in the genus Castanea." Phytopathology 87(7): 751-759.

Leffel, R. C. (2004). Strategies for Breeding Blight-Resistant, Timber-Type Chestnuts (*Castanea* Miller). Forest Genetics and Tree Breeding in the Age of Genomics: Progress and Future, Charleston, SC.

Ling, Q., G. Xiahong, et al. (2002). "Evaluation of the resistance of Chinese chestnut cultivars to *Cryphonectria parasitica*." Journal of Fruit Science 19(1): 39-42.

Nelson, R. R. (1978). "Genetics of Horizontal Resistance to Plant Diseases." Annual Review of Phytopathology 16: 359.

Newton, A. C. and I. R. Crute (1989). "A Consideration of the Genetic-Control of Species Specificity in Fungal Plant-Pathogens and Its Relevance to a Comprehension of the Underlying Mechanisms." Biological Reviews of the Cambridge Philosophical Society 64(1): 35.

Oliver, R.-P. and S.-V.-S. Ipcho (2004). "Arabidopsis pathology breathes new life into the necrotrophs-vs.-biotrophs classification of fungal pathogens." Molecular Plant Pathology 5(4): 347-352.

Sisco, P. H., T. L. Kubisiak, et al. (2004). An improved genetic map for *Castanea dentata* / *Castanea mollissima* and its relationship to the genetic map of *Castanea sativa*. Third International Chestnut Symposium, Portugal.

Steiner, K., A. H. Ellingboe, et al. (2004). "TACF adopts guidelines for testing blight-resistant American chestnuts." Journal of the American Chestnut Foundation 18(1): 7-11.

Thomma, B.-P.-H.-J. (2003). "Alternaria spp.: From general saprophyte to specific parasite." Molecular Plant Pathology 4(4): 225-236.

van Heerden, S. W., H. V. Amerson, et al. (2005). "Relative pathogenicity of *Cryphonectria cubensis* on Eucalyptus clones differing in their resistance to C-cubensis." Plant Disease 89(6): 659-662.

Improvements in Stem Form and Growth of Elite Genotypes in Loblolly Pine

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Improving the value of our forests in the southeastern United States is becoming increasingly important to the stability and long term productivity of Cooperative members as well as the industry as a whole. The North Carolina State University – Cooperative Tree Improvement Program has made significant gains in volume, rust resistance, and stem straightness through two generations of tree improvement (Li, McKeand et al. 2000). Increasing the value and amount of sawtimber from each acre of forest plantation is one opportunity for adding value to forest landowners in the South. With sawtimber prices nearly 5 times greater than pulp prices ("Timber Mart-South" 2007), even small increases in the proportion of sawtimber can significantly increase the value of timber. Previous research found that stem form and crown traits influenced the quality of sawlogs, but volume was the major influence in the value of sawlogs (Busby 1983). While it has been widely observed that variation exists among families, it has not been characterized within loblolly pine (*Pinus taeda* L.) breeding populations. Over the last year, the Cooperative has made efforts to assess the variation in traits thought to influence sawtimber quality.

The Lower Gulf Elite population was a joint effort between the North Carolina State University-Cooperative Tree Improvement Program, the Western Gulf Forest Tree Improvement Program, and the Cooperative Forest Genetics Research Project formed in the mid 1990's with elite material from the Atlantic Coastal Plain, Florida, and Livingston Parrish/East Texas provenances. At the time it was created, this was the most elite material from these provenances considered to be suitable for the development of a land race for the lower Gulf Coastal Plain of the United States. This combination of provenances provided an excellent population to quantify the variation in growth and sawtimber quality in loblolly pine. The objectives for this research effort were to 1) estimate genetic parameters for age 6 year growth and stem form traits and 2) determine potential gains in sawtimber quality by family selection.

The experimental design consisted of six 8-tree, disconnected diallels that resulted in approximately 128 crosses. Four tests in Alabama, Florida, and Georgia were measured for this; each test was a 20 replication single tree plot design. Data were analyzed using a mixed model approach performed in ASReml. A total of ten traits were measured in the LGE diallel tests: Height, DBH, Volume, Rust, Forking, Sweep, Branch Angle, Branch Diameter, Branch Frequency, and Sawtimber Potential (on a 1-4 scale with 1=high sawtimber potential, 4=cull).

Individual-tree heritability estimates ranged from 0.08 for sawtimber potential up to 0.24 for tree height (Table 1). The half-sib family-mean heritability estimates ranged from a low of 0.71 in branch angle to a high of 0.97 for branch frequency. Full-sib family mean heritability estimates were lower than half-sib estimates ranging from 0.66 for branch angle to 0.91 for rust incidence. While some individual tree estimates seemed low (below 0.10), the half-sib family mean

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heritability estimates suggest there is genetic variation at the family level that could be utilized for population improvement.

While branching characteristics were found to be heritable, it is perhaps most encouraging that the more subjective sawtimber potential score was heritable at the half-sib level at 0.85. The potential to grade a progeny test tree for multiple stem form characteristics with a single score could make sawtimber potential assessments rapid and cost-effective for tree improvement programs. Based upon correlations among parental breeding values for growth and stem form traits (Table 2), there are no relationships that would prevent the improvement of stem form and growth in the Lower Gulf Elite population. Sawtimber potential was most highly correlated (between breeding values) with volume and height (0.54 and 0.53, respectively) but was not highly correlated with any branching traits (Table 2). A relationship between growth and sawtimber potential was expected since above-average growth was a criterion for sawtimber grading. The modest correlations suggest that volume was not unfairly weighted for sawtimber evaluation. In addition to growth traits, sawtimber potential was also negatively correlated (favorable) to sweep (-0.52) and rust infection (-0.38). Both of these relationships are favorable as lower sweep values indicate straighter trees and rust is scored as a binary variable (0 = no rust, 1 = rust). These traits are also factors in sawtimber potential scoring since stem rust disqualified a tree from being a potential sawtimber tree and large sweep (>3") penalized a tree from being potential sawtimber. These relationships will be further explained through the development of a predictive model for estimating sawtimber potential from existing Cooperative breeding values.

In addition to the potential improvement of sawtimber quality in breeding populations, gains in sawtimber quality can also be captured through the deployment of select first- and second-generation parents. Figure 1 demonstrates the range in sawtimber and volume growth by full-sib family means. Since correlations were not high between growth and sawtimber potential it is important to quantify both the growth and sawtimber quality of each family. Selecting only on volume for deployment will not always result in the families with higher proportions of potential sawtimber trees. This consideration is also seen in Figure 2 where the top fifteen parents for volume show a range in sawtimber potential from less than 40% to greater than 70%. Selecting parents with high volume and sawtimber potential breeding values will be important not only for deployment decisions for reforestation now, but also for decisions in population management and the improvement of elite germplasm in the Cooperative for future breeding efforts.

Acknowledgements: Financial support for this research has been provided by members of the North Carolina State University – Cooperative Tree Improvement Program. We thank International Paper Company, MeadWestvaco, Plum Creek, and Rayonier for allowing access to the test sites; and special thanks to Phil Dougherty and Early McCall for their input.

Table 1. Heritability Estimates for traits measured in four Lower Gulf Elite tests.

Trait	h^2_i	h^2_{HS}	H^2_{FS}
Height	0.24	0.95	0.89
DBH	0.12	0.87	0.77
Volume	0.15	0.87	0.78
Rust	0.22	0.96	0.91
Forking	0.15	0.91	0.82
Sweep	0.16	0.94	0.78
Branch Angle	0.16	0.71	0.66
Branch Diameter	0.09	0.91	0.69
Branch Frequency	0.13	0.97	0.71
Sawtimber Potential	0.08	0.85	0.70

Where h^2_i is narrow-sense individual tree heritability, h^2_{HS} is narrow-sense half-sib family mean heritability, and H^2_{FS} is broad-sense full-sib family mean heritability.

Table 2. Parental Breeding Value Correlations (and p-value) among selected growth and stem form traits in four Lower Gulf Elite tests.

	Height	Volume	Sweep	Branch Angle	Branch Diameter	Branch Frequency	Rust	Sawtimber Potential	Forking
Height		0.86 <.0001	-0.06 0.68	-0.10 0.48	0.37 0.01	0.52 <.0001	0.00 0.99	0.53 <.0001	0.35 0.01
Volume			0.06 0.67	-0.28 0.04	0.53 <.0001	0.32 0.02	0.00 0.99	0.54 <.0001	0.28 0.04
Sweep				-0.25 0.07	0.35 0.01	0.16 0.26	0.22 0.11	-0.52 <.0001	0.10 0.48
Branch Angle					-0.40 0.00	0.03 0.85	0.11 0.44	-0.11 0.41	0.26 0.06
Branch Diameter						0.36 0.01	0.39 0.00	-0.04 0.79	-0.05 0.73
Branch Frequency							0.11 0.43	-0.01 0.95	0.26 0.06
Rust								-0.38 0.01	-0.01 0.96
Sawtimber Potential									-0.12 0.38

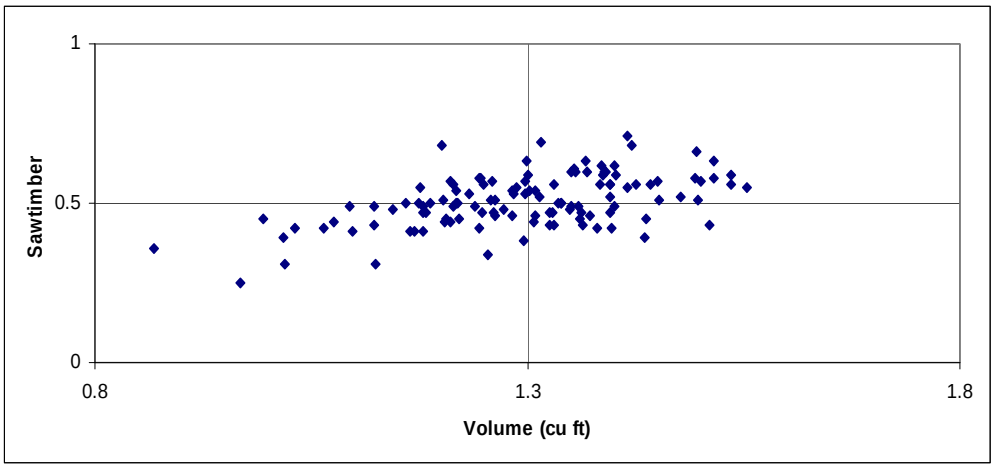

Figure 1. Plot of Sawtimber full-sib breeding values (proportion) vs. whole-tree 6-year volume full-sib breeding values (ft^3).

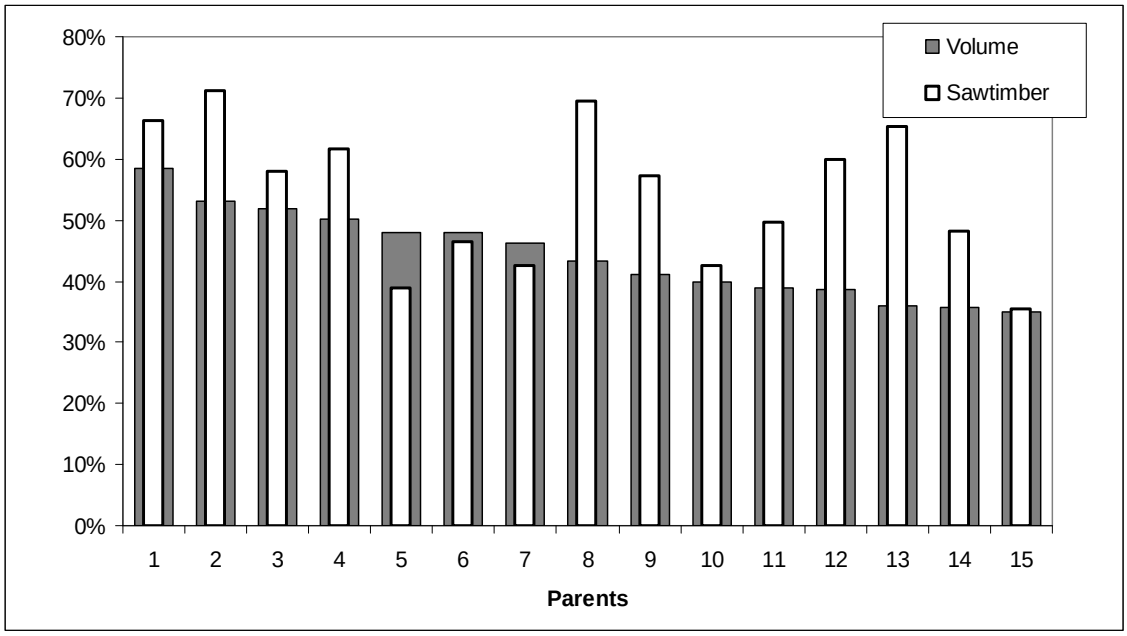

Figure 2. Half-sib breeding values for volume gain (%) and sawtimber potential (%) for the fifteen highest volume.

References

Busby, C. L. (1983). "Crown-quality assessment and the relative economic importance of growth and crown characters in mature loblolly pine." Proceedings of the 17th Southern Forest Tree Improvement Conference(39): 121-130.

Li, B., S. McKeand, et al. (2000). "Impact of forest genetics on sustainable forestry--results from two cycles of loblolly pine breeding in the U.S." Journal of sustainable forestry **10**(1/2): 79-85.

"Timber Mart-South."(2007). Retrieved June, 2007, from www.TimberMart-South.com/tmart/.

Poster Abstracts

Biomass-Based Monitoring of Heavy Metals

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New developments in biotechnology provide for heavy metal removal and monitoring. Hyper-accumulating plants such as *Thlaspi caerulescens* express genes amplifying heavy metal absorption and detoxification. These small plants are not practical for large-scale remediation. Current technology allows gene transfer and expression into high-biomass plants such as *Populus*. This would be beneficial since *Populus* has silvicultural systems for establishment, culture, protection, and harvest. Furthermore, Populus can be transformed into a real-time monitoring system by fusing heavy metal proteins with fluorescent proteins (FP). Metal concentration would be detected through FP-produced light changes measured by an optical sensor. Subsequently geographical positioning (GPS)/ global system for mobile communication (GSM) technology can be used to transfer this information to a central location for monitoring.

USDA Forest Service, Forest Health Protection Resistance Screening Center

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The Resistance Screening Center evaluates seedling materials for resistance to disease, primarily fusiform rust (*Cronartium quercuum* F. sp. *fusiforme*) and pitch canker (*Fusarium circinatum*), as a service to tree improvement specialists, seed orchard managers, scientists, and others in government agencies, research institutions, and private industry. Testing enables clients to obtain information on the relative resistance of materials in much less time than is possible in field progeny tests. Plant material is screened using protocols developed specifically for use at the screening center, utilizing native sources of inoculum. By using information from these tests, trees producing disease resistant progeny are identified. The Forest Service has an alternate interest in screening the material submitted for disease resistance to the Resistance Screening Center. The use of resistant materials on private land can reduce the base from which these diseases might spread to Federal, State, or other private land.

Scope of Forest Tree Breeding in the USFS

Marilyn Buford[1] and Randy Johnson[2]

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US Forest Service plant breeding programs function to meet the needs of federal land management agencies, state and tribal nursery programs, and private land owners and businesses. In the past, breeding objectives centered on improving productivity, wood quality and disease resistance in forest trees. More recently, breeding for disease and pest resistance has become more important as we combat diseases and pests that threaten US forests. Our genetics and tree breeding programs are critical components of developing innovative forest management opportunities to meet the Nation's needs.

These programs provide foundation material for research in the areas of biotechnology, genomics, disease resistance, and traditional population and quantitative genetics. In turn, these results are incorporated into our breeding programs and others around the world.

US Forest Service plant breeding programs involve Research & Development, National Forest System, and State & Private Forestry. Much of our work is conducted in collaboration with federal, university, industry, state, tribal and NGO partners.

Susceptibility of Southern Pines to Nantucket (*Rhyacionia frustrana*) Tip Moth Damage is Proposed to be Largely Controlled by a Single Dominant Locus

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Nantucket tip moth is the most serious insect pest of southern pines. Pine species vary greatly in their susceptibility to tip moth. For example, loblolly pine (*Pinus taeda* L) and shortleaf pine (*P. echinata* Mill) are susceptible, while slash pine (*Pinus elliottii* var. *elliottii* Engelm.) and longleaf pine (*P. palustris* Mill) are resistant. We measured the susceptibility (as % tip moth damage) of these species and their interspecific hybrids to investigate the genetic basis of susceptibility. We used a mixed planting of twenty-eight different pine families of various genetic origins that included two susceptible species used as parents (loblolly pine, and shorleaf pine,) along with resistant slash pine and longleaf pine parents. The interspecific hybrids were F1 and three-way crosses, as well as one test cross. Resistance ranged from 3 to 10 % tip moth damage, while susceptibility ranged from 76 to 90 %. Progeny of resistant parents appear resistant. Progeny of susceptible parents appear susceptible. Hybrids of susceptible x resistant crosses are susceptible. A three-way hybrid of a susceptible species x a hybrid of two resistant species is also susceptible. A testcross of a resistant /susceptible hybrid x a resistant parent is intermediate in susceptibility (35 to 40%). Based on these results, we propose that susceptibility in southern pine trees is controlled by a single dominant locus and that a simple major gene model may explain the genetic basis for resistance or susceptibility in these species. Further work is needed to confirm this hypothesis.

Clonal Variation in Flowering Abundance of *Pinus koraiensis*

I. S. Kim[1], K. S. Kang[2], S. D. Han[1], S. H. Han[1] and J. T. Kang[1]

In a seed orchard, unbalanced genetic contributions of clones were very important factors affecting seed quality and genetic flexibility of progenies. Generally, the superior clones had been selected to establish advanced generation seed orchard based on the progeny test. The flowering characteristics of clones have been relatively less considered and applied in a seed orchard management in Korea. Thus, this study was conducted to investigate the flowering characteristics of *P. koraiensis* clones and to supply information for advanced breeding.

MATERIALS AND METHODS

The clonal variation in flowering abundance was studied in *Pinus koraiensis* clone bank, established in 1983. It was located in mid Korea and consisted of 180 clones from mid and northern Korea. The flowering abundance was studied in 1991-2003 at annual, clonal and graft level. In 1996 and 2001, however, the flowering was very poor. Thus, the data of those years were excepted from genetic parameter estimation. The broad-sense heritability and genetic gain of male and female flowering was estimated. The clonal stability of male and female flowering was compared using clonal mean and coefficient of variation values of each clone. Additionally, the implications of the results to genetic improvement of *P. koraiensis* were discussed.

RESULTS AND DISCUSSION

The between-year variation was large in both female and male flowering. The average percentages of flowering graft of female and male were 54.3 % and 26.1%, respectively. The average number of flower per graft of female and male were 7 and 205, respectively. In both case, they were varied from graft to graft. The spearman rank correlation from year to year were usually positive and significant, and in general slightly higher in male than in female flowering. The proportion of clones that did not flower at all was larger when flowering was poor. The clonal variation of male flowering was larger than that of female flowering (Table 1).

The average broad-sense heritability values for female and male flowering were 0.32 and 0.44, respectively, but varied considerably from year to year (Table 2). The average of broad-sense heritability of female and male were relatively high. Thus, the flowering abundance of *P. koraiensis* might be under the strong genetic control, although the flowering was mainly affected by environmental conditions.

The genetic gain was estimated using average broad-sense heritability and selection differential of female and male flowering abundance. The genetic gain of female and male was 20.0% and 57.2% when the upper 30% clones were selected, respectively (Table 3). However, when the selelction intensity was increased to enhance the genetic gain, the genetic diversity was decreased. Thus, the more attention was required when the advanced selection breeding program was established.

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Table 1. The percentage of flowering grafts, clonal mean, minimum and maximum values for the number of flowers in different years.

Year	Female flower					Male flower				
	Flowering Graft (%)	Mean	Min.	Max.	CV(%)	Flowering Graft (%)	Mean	Min.	Max.	CV(%)
1991	39.7	2	0	45	202.3	20.9	51	0	2510	394.3
1992	55.8	4	0	53	167.2	30.8	27	0	1087	354.7
1993	70.7	8	0	83	141.1	41.3	46	0	3300	376.5
1994	73.0	6	0	46	126.2	42.7	77	0	1760	278.2
1995	52.6	3	0	34	150.4	32.4	47	0	3100	415.8
1997	54.4	4	0	45	162.5	5.7	10	0	502	488.6
1998	63.8	5	0	90	161.8	22.5	59	0	4000	443.5
1999	74.3	12	0	62	104.4	34.6	205	0	15000	525.8
2000	32.6	5	0	88	231.7	11.1	272	0	1500	472.9
2002	37.8	6	0	90	206.1	7.0	11	0	1000	642.1
2003	43.2	25	0	238	157.5	38.4	1700	0	30000	217.3
Average	54.3	7				26.1	205			

Table 2. The broad-sense heritability for the number of flowers in different years.

	’91	’92	’93	’94	’95	’97	’98	’99	’00	’02	’03	average
Female	0.38	0.40	0.58	0.35	0.42	0.45	0.32	0.45	0.05	0.07	0.00	0.32
Male	0.79	0.74	0.40	0.74	0.71	0.44	0.34	0.44	0.19	0.06	0.04	0.44

The clonal stability of flowering was compared with average number of flowering and CV value of each clone. Only considering seed production, a clone with large flowering abundance and high stability was ideal one. In female, the clones such as K16, K21, K23, K26 and K68 showed abundant flowering and high stability. In case of male, they were K96, K84, G11, K52 and K29 (Figure 1). However, the correlation coefficient between female and male flowering of clones was not high (r=0.43). Thus, different genetic contribution of female and male of a clone was also considered in a seed orchard management of *P. koraiensis*.

Table 3. Estimation of genetic gain for female and male flowering abundance.

	Parameter	Selection criteria				
		Upper 50% (90 clones)	Upper 40% (72 clones)	Upper 30% (54 clones)	Upper 20% (36 clones)	Upper 10% (18 clones)
Female	S	3	4	5	6	9
	ΔG	0.96	1.28	1.60	1.92	2.88
	%G	12.0	16.0	20.0	24.0	36.0
Male	S	320	428	563	759	1157
	ΔG	140.8	188.3	247.7	333.9	509.1
	%G	32.5	43.5	57.2	77.1	117.6

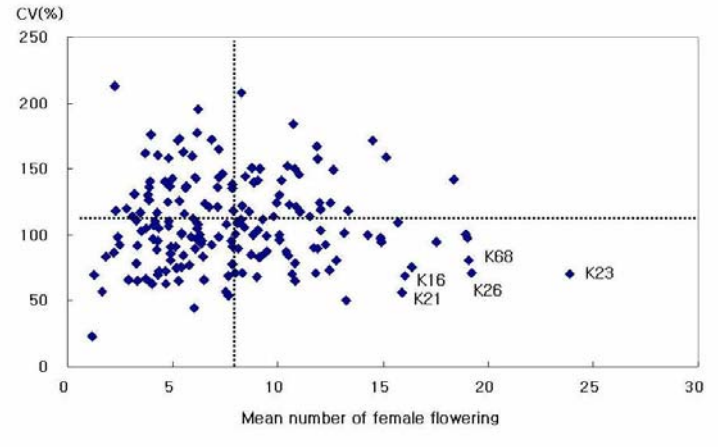

Figure 1. The biplot of mean number of female flowering and CV(%) of each clone

LITERATURE CITED

Han, S. S and S. B. Lee. 1990. Characteristics of flower of plus tree clones of Pinus koraiensis S. et Z. Jour. Korean For. Soc. 79: 290-301.

Nikkanen, T. and S. Ruotsalainen. 2000. Variation in flowering abundance and its impact on the genetic diversity of the seed crop in a Norway spruce seed orchard. Silva Fennica 34: 205-222.

Studies on the Pruning Methods for Seed Production in *Pinus densiflora* Seed Orchard

J. H. Kim, I. S. Kim, K. W. Jang, J. T. Kang and B. S. Lee[1]

Pinus densiflora is one of the most economically important timber species and the most widely distributed conifer species in Korea. To produce genetically improved seed of *P. densiflora*, the establishment of seed orchard was started from 1968. The cones of *P. denslfilora* were attatched at the top crown. As the height of trees get higher, we have faced on the difficulty of seed production. Additionally, the vitality of trees was a problematic, because the closed crown shaded the light. Thus, we decided to introduce crown pruning method to overcome these problems. This study was conducted to investigate the effect of crown pruning for seed production and seed quality in a seed orchard of *P. densiflora*.

MATERIALS AND METHODS

The test site was located in Anmyun-do, Chungnam province, which was established in 1977. From 1988 to 1995, the 1st crown pruning was executed in this test site. In 2001, the 2nd crown pruning was executed with several pruning methods such as (A) top pruning, (B) top + branch pruning, (C) top + branch pruning + branch trimming and (D) control (Figure 1). After 2nd crown pruning, the growth characteristics of pruned trees had been examined for five years. We investigated the number of male and female flowering per branch, cone survival rate seed production and seed quality at each treatment. Additionally, the implications of crown pruning on seed orchard management of *P. densiflora* were discussed.

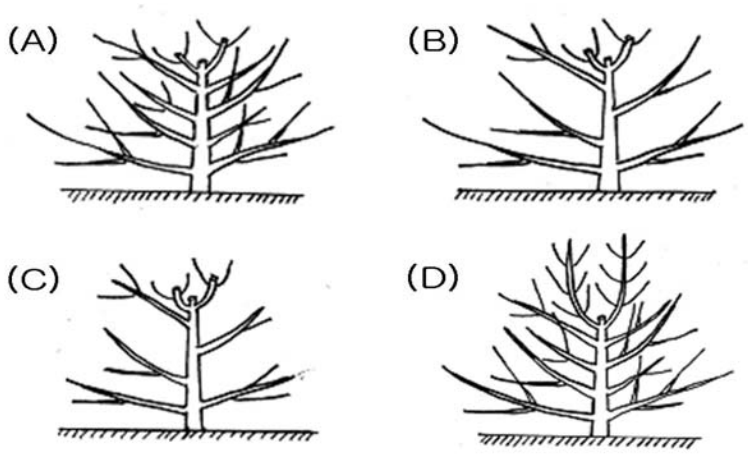

Figure 1. The crown pruning methods used in this study.

RESULTS AND DISCUSSION

The number of female flower per branch was largely decreased in 1st year after crown pruning, which was due to the diminishment of number of branch containing flower bud by pruning. In 2nd year after pruning, however, the number of female flowering and seed production of pruned

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trees were started to increase than that of control. Among the crown pruning methods, top + branch pruning + branch trimming showed the best promoting effect of female flowering at 3rd year after crown pruning (Figure 2). On the contrary, the number of male flowering per branch was largely decreased in all pruning treatment for five years investigated.

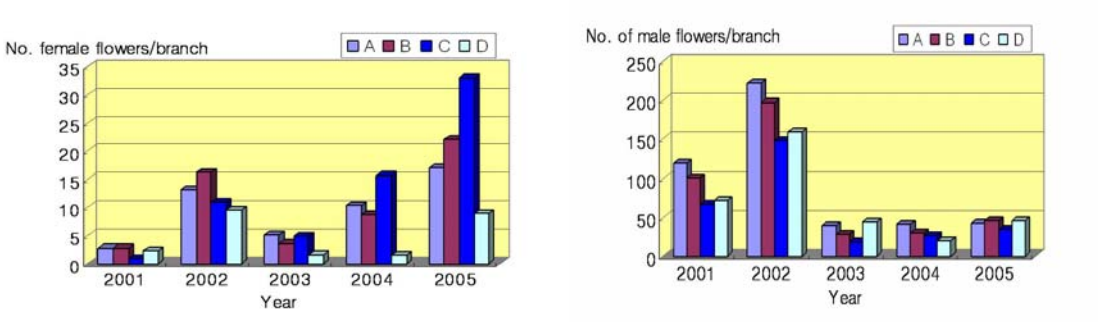

Figure 2. Flowering patterns of female and male for five years after pruning.

Cone survival rate and seed quality of pruned trees were higher than that of control (Table 1). Particularly, the top + branch pruning + branch trimming showed relatively high cone survival rate. Comparing seed quality, there were slight differences among treatments. The top + branch pruning + branch trimming showed highest seed production and seed quality (Table 2). Because crown pruning brought about the effect of cone density control per branch and affect the resource allocation among sinks, it was suggested that the cone survival rate and seed quality were enhanced through crown pruning treatments.

Table 1. Cone survival rate at different crown pruning treatments.

Treatment	Per cone				
	East	West	South	North	Average
A	89.4	62.3	65.4	83.2	73.7
B	78.4	68.5	89.0	73.2	76.0
C	75.5	69.7	76.8	80.8	75.9
D	60.7	70.2	75.7	73.2	73.3

Table 2. Comparison of seed quality at different crown pruning treatments.

Treatment	Per cone				
	Total seed weight (g)	Fulled seed weight (g)	No. of total seed	No. of fulled seed	Fulled seed rate (%)
A	0.45	0.43	38.0	32.7	86.1
B	0.34	0.33	34.5	29.9	86.7
C	0.55	0.54	47.3	43.0	90.9
D	0.58	0.56	47.9	41.6	86.8

According to above results, crown pruning was beneficial to seed orchard management of *P. densiflora* in relation to sustainable seed production, i.e. female flowering promotion at 3rd year after crown pruning. It might be a clue of artificial control of seed production. Particularly, top +

branch pruning + branch trimming treatment was a promising method for *P. densiflora*. However, more attention was required to apply this method for practice, because it is brought about male flower deficiency at early stage of treatment.

LITERATURE CITED

An, Z., X. Wang and W. Wang. 1992. A study on pruning in *Pinus koraiensis* seed orchard. *In* Seed Orchard Technique. Pp. 201-207.

Nienstaedt, H. 1981. Top pruning white spruce seed orchard grafts. Tree Plant. Notes 32: 9-13.

Melchior, Von G. H. and H. H. Heitmuller. 1961. Increasing the number of male flowers in grafts of *Pinus sylvestris* by pruning. Silvae Genet. 10: 180-186.

Metabolomics Complexity in Forest Trees Expected from Inron-Exon Gene Structure

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Computer analysis has been used to infer exon-intron structure from completely sequenced plant and animal genomes. Through modeling of alternative splicing the metabolomics complexity has been predicted for different species including forest trees.

Genetic Gain and Diversity in a Clonal Seed Orchard of *Pinus koraiensis* under Various Thinning Intensities

C.Y. Oh, K.S. Kang, W.Y. Choi, S.U. Han, and C.S. Kim[1]

There are various orchard management options to increase genetic gain while conserving genetic diversity, including selective harvesting, genetic thinning and combination of both. Genetic improvement is defined as a process that enhances the genetic value while giving deliberate consideration to the genetic diversity of deployed materials (Kang et al. 2001). The calculations of genetic gain and diversity in seed orchard populations are of great theoretical and of practical importance.

The objectives of this study were to evaluate the genetic gain and diversity of seed crops from a *P. koraiensis* clonal seed orchard under different thinning intensities, and to determine appropriate selection intensity.

MATERIALS AND METHODS

The clonal seed orchard of *P. koraiensis* is located in Dukduwon, mid-northern part of Korea (lat. 37° 52'N, long. 127° 37'E, alt. 500m and area 12ha) and was established in 1981. A total of 179 clones (total 5,268 ramets) were included at the stage of establishment. 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 were estimated by the method of best linear unbiased prediction (BLUP). Clonal fertility was estimated from assessments of strobilus production over twelve consecutive years from 1991 to 2003.

Genetic thinning was computed from 10% to 90% thinning intensities with 10% interval and compared with truncation (50%) thinning.. Since selection criteria for genetic thinning were based on clonal genetic values and flower production, clones with inferior general combining abilities (*GCA*) and poor seed production were targeted for ramet removal more than superior clones.

Genetic gain is the average of the genetic values of female and male parents. In the presence of pollen contamination, the genetic value of male parent is reduced due to the inferiority of contaminating pollen. Genetic gain (ΔG) was estimated (cf., GRIFFIN, 1982) as follows,

$$\Delta G = \sum_{i=1}^{N}\left(\frac{GCA_{female} + GCA_{male}}{2} \times q_i\right)$$

$$= \sum_{i=1}^{N}\left(\frac{GCA_{fi} + \{(1-2M)GCA_{mi} + (2M)GCA_{mc}\}}{2} \times q_i\right)$$

where GCA_{fi} and GCA_{mi} are general combing abilities of orchard female and male parents of the *i*-th clone, GCA_{mc} is the general combing ability of contaminating pollen, M is the rate of gene

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migration (i.e., half of pollen contamination), and q_i is the relative frequency of *i*-th clone, which considers ramet number and seed production.

Genetic diversity was measured by status number (N_s) that was defined as half the inverse of group co-ancestry by LINDGREN *et al*. (1996). For unrelated and non-inbred orchard clones, N_s can be estimated from the contribution of the clones. The status number was calculated (cf., KANG and LINDGREN, 1998) as,

$$N_s = \frac{1}{\sum_{i=1}^{N}(p_i \times r_i)^2} = \frac{1}{\sum_{i=1}^{N}\left(\frac{f_i + (1-2M)m_i}{2} \times r_i\right)^2}$$

$$= \frac{4}{\sum_{i=1}^{N} f_i^2 r_i^2 + (1-2M)^2 \sum_{i=1}^{N} m_i^2 r_i^2 + 2(1-2M)\sum_{i=1}^{N} f_i m_i r_i^2}$$

where p_i is the contribution of the *i*-th clone, f_i and m_i are the contributions of females and males of the *i*-th clone and r_i is the ramet proportion of the *i*-th clone. In the present study, the rate of pollen contamination was set to 30% ($M = 0.15$) and an additive variance of contaminating pollen (GCA_{mc}) was assumed to be –0.1.

RESULTS AND DISCUSSION

Genetic gain increased as thinning rates were set from 10% to 60% (Table 1). However, for the higher thinning intensities, the increase of genetic gain was not remarkable. Genetic thinning by means of truncation selection resulted in a greater genetic gain but a large decrease in status number. Status number was represented around 40 clones for 10% through 60% thinning intensities, but for the higher thinning intensities, it was a bit fluctuated.

In most first-generation seed orchard, it is difficult to establish with near-equal numbers of ramets for each clone. And the large variation of ramet numbers among clones always exists due to graft availability, graft incompatibility and etc. The average number of ramets per clone at the time of establishment was 29.4 but the range was 1 to 123. The clonal linear deployment concept applied in this study capitalizes on the differential variation of genetic gain among orchard clones. It can thus be beneficial to intentionally use an unequal number of ramet per clone, where clones with high breeding value contribute most to the seed orchard crop, thus gain is maximized without appreciable genetic diversity loss.

The present study has demonstrated the effective use of seed orchard's clonal information, such as genetic gain and fertility variation. Based on the present results, it could be concluded that thinning rate should not be stronger than 60% to optimize genetic gain while conserving genetic diversity. Consequently 50% or 60% thinning rate might be appropriate for genetic thinning in the clonal seed orchard of *P. koraiensis*.

Table 1. Census clone number (N), ramet number (n), genetic gain (G), status number (N_s) and relative status number (N_r) after the implementation of each genetic thinning intensities in a clonal seed orchard of *P. koraiensis*

	Initial	Linear thinning									Truncation
	establishment	10%	20%	30%	40%	50%	60%	70%	80%	90%	(50%)
N	179	165	165	165	165	165	165	165	165	165	87
n	5268	4741	4214	3687	3160	2634	2107	1580	1053	526	2634
G	-0.01	0.18	0.27	0.34	0.39	0.43	0.45	0.45	0.46	0.44	0.64
N_s	41.4	40.2	40.1	38.2	40.3	40.5	39.6	43.1	48.4	55.8	23.7
N_r	0.23	0.24	0.24	0.23	0.24	0.25	0.24	0.26	0.29	0.34	0.27

Note that ramet variation was considered to calculate genetic gain and status number. Rate of genetic thinning was based on the number of ramets/clone.

LITERATURE CITED

GRIFFIN, A.R. 1982. Clonal variation in radiata pine seed orchards. I. Some flowering, cone and seed production traits. Aust. For. Res. 12: 295-302.

KANG, K.S. and D. LINDGREN. 1998. Fertility variation and its effect on the relatedness of seeds in *Pinus densiflora*, *Pinus thunbergii* and *Pinus koraiensis* clonal seed orchards. Silvae Genet. 47: 196-201.

Kang, K.S., D. Lindgren and T.J. Mullin. 2001. Prediction of genetic gain and gene diversity in seed orchard crops under alternative management strategies. Theor. Appl. Genet. 103: 1099-1107.

LINDGREN, D., L. GEA and P. JEFFERSON. 1996. Loss of genetic diversity monitored by status number. Silvae Genet. 45: 52-59.

Gene Expression in an Association Population of Loblolly Pine

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As part of the Plant Genome project "Association Genetics of Natural Genetic Variation and Complex Traits in Pine" we are analyzing expression of approximately 200 genes involved in xylem development and disease responses in loblolly pine. An association population containing rooted cuttings of genotypes from across the natural range was developed at North Carolina State University. At Texas A&M University, real-time qPCR is being used to assay expression levels in 2 ramets of 426 genotypes. We plan to relate expression levels to the geographic origin of the parents, to look for genes that are coordinately expressed, and to associate variation in expression to the genotype and metabolite data being produced at UC-Davis and to phenotype data being produced at U. Florida and North Carolina State. Expression data for the first 23 genes involved in lignin biosynthesis has been gathered and will be presented. Other xylem-related genes to be analyzed include those involved in cellulose and cell wall biosynthesis, cell wall proteins, and genes involved in signal transduction.

Using Biotechnology to Conserve Eastern and Carolina Hemlock Germplasm

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Both hemlocks native to the eastern United States, eastern hemlock (*Tsuga canadensis*) and Carolina hemlock (*Tsuga caroliniana*), are threatened with extinction by the hemlock woolly adelgid (*Adelges tsugae*), a non-native insect that has now spread throughout the range of these two forest species. Although biocontrol measures for the insect are now being tested, the genetic diversity of both hemlock species is declining every year as populations of the trees are devastated by the pest. A system for long-term preservation of eastern and Carolina hemlock germplasm would help ensure that, even if biocontrol measures take decades to be optimized, the genetic diversity of these species could be maintained for restoration purposes. With the goal of aiding germplasm conservation, we have initiated the first embryogenic cultures of both eastern hemlock and Carolina hemlock to demonstrate the feasibility of conserving germplasm of these two species via cryopreservation of embryogenic cultures. In a preliminary study, we collected immature cones from eastern and Carolina hemlocks in North Carolina during July and August, dissected the cones to obtain immature seed explants, and cultured the immature seeds or embryos dissected from them on a pine embryogenesis induction medium containing 2,4-D. A low percentage of the explants produced callus that appeared similar to embryogenic callus reported for other conifers, and callus derived from one explant representing each species went on to produce cotyledonary stage somatic embryos following transfer to a maturation medium. Further research will determine the potential of these embryos to produce somatic seedlings and the ability of the embryogenic cultures to recover following cryostorage.

Resistant and Susceptible Molecular Host Responses to Hemlock Woolly Adelgid

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Hemlock woolly adelgid (*Adelges tsugae* Annand, HWA) infestations of Carolina (*Tsuga caroliniana* Engelm.) and eastern (*Tsuga canadensis* (L.) Carriere) hemlocks have lead to the decreased vigor and increased mortality of these ecologically important species. Approximately half the native range of hemlocks in the eastern United States is currently infested, with potentially significant consequences for the long-term survival of these species.

Since little is known about potential host tree mechanisms that may impact HWA resistance, we have focused on identifying hemlock genes involved in the host response to this pest. We are using laser capture microdissection followed by EST sequencing to identify genes whose expression levels are altered in response to HWA feeding in infested and non-infested *T. caroliniana* ray parenchyma cells, living cells embedded within the xylem of stems. We focus on these cell types since they are the feeding sites for HWA and are likely to react to the insect on a molecular level. In addition, because HWA is a relatively minor pest of *T. chinensis* and therefore considered resistant to HWA, we are generating EST libraries from needle samples of *T. chinensis* and *T. caroliniana* to compare gene expression patterns between these resistant and susceptible species. Analyses of the EST libraries will provide insights into the molecular mechanisms underlying HWA resistance and provide genetic information for developing molecular markers for breeding.

Male Genotype Influences Seed Set and Seed Size in Controlled Crosses of American Chestnut (*Castanea dentata* [Marsh] Borhk)

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Five American chestnut trees (*Castanea dentata [Marsh] Borhk)* located in Indiana were used as mother trees in a full diallel breeding design in order to determine the influence of pollen donor genotype on seed set and seed mass. Pollen from ten American chestnut or American chestnut hybrids was applied to the five mother trees. The seeds were harvested, counted, and individually weighed. A random effects model fit to the data showed that both female ($F= 18.10$, $p < .001$) and female by male cross ($F = 1.54$, $p = 0.0445$) contributed significantly to the variance in seed mass. Similarly, both female and female by male cross accounted for significant variation in seed set ($F = 10.98$, $p <.001$ and $F = 1.67$, $p = 0.0205$, respectively). A general association test showed male genotype and seed set to be statistically associated ($\chi 2 = 26.97$, $p = 0.0014$). A variance components model fitted to the data showed that individual female by male crosses explained 28.3% and 15.8% of the variation in seed mass and seed set, respectively. Individual crosses differed significantly in the amount of variation they explained, from 0% to 61.5% of variation in seed mass and from 0% to 67.6% of variation in seed set. These results demonstrate the influence of male genotype on seed mass and seed set and are the first to show differential male and female by male performance on seed characters of American chestnut. The seed from this experiment are currently being germinated to assess seedling vigor. The results will be combined with the results of the study above to present a fuller picture of the influence pollen donor genotype on seed and seedling characters in American chestnut.

Genetic Control of Forking in Diallel Tests of Loblolly Pine

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Forking defects are probably the most serious stem-quality problems in loblolly pine. Our assessment of forking includes forked stems and ramicorn or steep-angled branches, which greatly reduces wood yield and wood quality. Assessing forking in elite pedigrees will enable us to more successfully breed and deploy non-forked phenotypes. The genetic control of forking and the correlation of forking to growth, stem straightness and fusiform rust disease traits were investigated in 6-year-old diallel tests, located throughout the Southeast. There were 122 diallel series (12 parents, 30 crosses × 36 trees per cross per site × 4 sites) with sufficient forking (average forking between 20% and 80%) for genetic analysis. From preliminary analyses in a subset of these diallel series, the half-sib family mean heritability for percent forking / ramicorn branching was about 0.77, as high as the heritability for height, fusiform rust resistance, and straightness. High heritability will result in high response of selection against forking. A threshold model will be fit to dichotomous data to understand underlying additive and non-additive genetic effects controlling forking in loblolly pine. Results from all 122 diallel series will be presented.

Pre-Meeting Abstracts

Because submission of papers and extended abstracts was optional, the complete set of abstracts submitted for the program are included. Readers are encouraged to check the Presentation Section starting on page 1 for papers or extended abstracts submitted by some speakers.

Invited Papers

Southern Pine Tree Improvement – A Living Success Story

S.E. McKeand[1], B.J. Zobel[2], T.D. Byram[3], and D.A. Huber[4]

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The U.S. South can boast of the productivity, quality, and value gains realized from plantation forestry that silviculturists and tree breeders have developed the past 50+ years. From the beginning of tree improvement programs in the region, the focus has been on selecting, breeding, testing, and planting trees that provide landowners with the greatest return on their investments. The agrarian culture, available land, favorable political and social attitudes towards production forestry, productive soils, and a moderate climate all favor the growth of plantation forestry in the South. The trend in recent years has been for increasing intensity of forest management of these acres. With global demand for timber products increasing at the same time as the area of the world's forests is decreasing, increased productivity of southern plantations has local, regional, national, and global implications. These plantations help provide timber to meet increasing demands while simultaneously reducing the environmental footprint of industrial forestry by growing more wood on less area.

Foresters in the southern United States are responsible for over 75% of the nation's tree planting, and over 95% of these seedlings are genetically improved loblolly and slash pines. Deployment practices such as planting only the best open-pollinated (OP) families to the best sites are resulting in dramatic increases in productivity. Increased resistance to fusiform rust disease, especially in slash pine, has also had major impacts on plantation yields. Annually, 59% of the loblolly and 43% of the slash are being deployed as OP families by companies and small landowners. Over the last 10 years, seed orchard managers have had great success in developing methods to mass produce full-sib families for operational planting. The gains from improved quality and yield are very impressive when both the female and male parents are selected. Our estimate is that approximately 40 million full-sib seedlings have been planted each of the last 3 to 5 planting seasons. Propagation of selected clones has become a reality via somatic embryogenesis (SE), and the gains to be realized from planting these outstanding genotypes are tremendous. To date, almost 10 million seedling of somatic embryogenic clones have been planted, and the numbers are increasing annually.

Even with the changes in land ownership and the loss of the integrated forest products companies, we are optimistic that tree improvement and intensive silviculture will continue to be mainstays of forest management in the South. The challenges to the large tree improvement cooperatives are numerous, but support is still strong, and gains continue to be made.

The similarities and differences between breeding and plantation programs in the southern region and the Pacific Northwest of the United States will be discussed.

Douglas-fir Breeding: Past Successes and Future Challenges

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Breeding programs in Douglas-fir are among the most extensive in the world, with more than 4 million progeny from nearly 34,000 selected parents growing on almost 1,000 test sites in the Pacific Northwest (PNW). The PNW is an environmentally diverse, mountainous region with large tracts of public and private forestlands, and a public with strong environmental values. Not surprisingly, these factors influence forest management and tree breeding. Compared to the southern pines, Douglas-fir breeding programs typically maintain greater genetic diversity, make selections at older ages, use simpler mating designs, rely more heavily on open-pollinated seed orchards, and do not anticipate the widespread use of clonal forestry or genetically engineered trees. The primary breeding goals of increasing crop value and maintaining adaptability are generally met in different ways. Eight breeding zones in OR and WA are used to maintain adaptability to frosts and droughts, whereas breeding within these well-adapted populations is used to improve growth and (secondarily) stem quality and wood density. Most breeding occurs via a decentralized system of independent metacooperatives that are coordinated by an umbrella organization called the Northwest Tree Improvement Cooperative. A separate organization, the Pacific Northwest Tree Improvement Research Cooperative, focuses on tree breeding research. Other programs are managed by the British Columbia Ministry of Forests, Inland Empire Tree Improvement Cooperative, and a few private companies.

Historically, research focused on seed orchards (e.g., graft incompatibility, pollen contamination) and quantifying patterns of adaptive genetic variation. Other topics have attracted more recent attention, including: To what extent should wood properties be included in breeding programs? How can genetics be incorporated into growth models to predict gains over a rotation? Can miniaturized seed orchards be used to increase genetic gains and lower seed orchard costs? How will changes in ownership patterns and corporate structures affect tree breeding and forestry research? Other longer-term issues demand attention as well—particularly climate change. If climate change predictions are realized, the importance of plantation forestry will increase dramatically
within next 10 to 20 years. In the PNW, breeders must pay particular attention to maintaining forest health, including drought tolerance and resistance to native and introduced pests. Because native populations may become maladapted as climates change, we may need to replace native populations with better adapted seed sources using artificial regeneration. However, the information to do this wisely and confidently is not yet available for most species. To cope with climate change, we need sensitive systems for monitoring forest health, and the knowledge required to change seed source recommendations and breeding objectives. The ability to respond quickly will be enhanced by new genomic tools that will allow us to (1) monitor tree physiology at the gene level (i.e., via gene expression profiling), (2) characterize patterns of variation in

adaptive genes (i.e., via environmental and phenotypic association studies), and (3) practice marker-aided selection. Douglas-fir tree improvement and forest genetic research has been one of the best financial investments made by forest managers in the PNW, and may become one of the best environmental investments in the future.

Forestry in a Changing World: Will We Adapt or Be Left in the Woods?

John A. Helms
Professor Emeritus
University of California, Berkeley

The world has changed remarkably over the past decade. To be effective in ensuring that the broad field of forestry keeps pace with change we must constantly be adapting or else be "left in the woods" by others who are more effective in meeting the needs of a changing society.

The most important global issue is world population, the rise of Chinese and Indian economies, and the critical impact of increasing population on sustaining the world's wood resources in the face of massive deforestation. Recent global assessments such as the IPPC Reports, the Millennium Report, and the Stern Report all indicate that stresses on the world's ecosystems are reaching tipping points. Other global signals are changes in world trade of forest products, the severe impact of illegal logging, and the role of planted forests in both providing wood resources and in sequestering carbon. The important question is how should forestry adapt to these dramatic changes?

Changes in US forestry can be seen in the general performance of the forest products industry, the dynamics of international trade, and changes in US competitiveness in productivity and global markets. An astounding change is the rapidity in which forest industry has divested itself of timberlands. Similarly in the public sector, criticisms of the role and function of national forests have resulted in dramatic declines in harvests, reversal of fire suppression policies, and emphasis on threatened and endangered species. These concerns have caused the Forest Service to change management emphasis to restoration. Changes in the balance of political power in the 110th Congress is bringing changed focus on environmental issues, climate change, and a broader discussion of the role of forests in the 2007 Farm Bill. Public debate is increasing on such topics as the role of forests in providing ecosystem services, carbon sequestration and offsets, cap-and-trade carbon markets, production of cellulosic enthanol and wood pellets, and green building standards.

The profession of forestry is based on education and research. Despite increasing recognition of the importance of the world's forests, enrollment in undergraduate programs in forestry has declined dramatically in both the US and Canada. New programs in environmental science appear to be more attractive to prospective students. Research in forestry is marked by large decreases in federal research capacity, stagnation of funding for research and extension, and the demise of industrial research following industrial divestment of timberlands. The forestry profession is having difficulty in overcoming public perception that it is allied with exploitive practices.

Never-the-less, the future of forestry is bright. Whether the profession will adapt or be left in the woods, however, depends on convincing the public by communication and performance that it shares fundamental societal core values and visions for the future.

The Horizon of Forest Biotechnology

Bob Kellison
President Emeritus Institute of Forest Biotechnology

The three components of forest biotechnology (vegetative propagation, genomics. genetic engineering) are progressing erratically. Great strides are being made on vegetative propagation of forest trees through somatic embryogenesis to the point that commercial pine plantations are being established by use of the technology. In like vein, the technology has evolved in the mechanization of genomics that will allow the genome of conifers to be mapped in a quarter of the time that it took to gene map the human genome. That phenomenon will occur despite the size of the genome of loblolly pine, for example, being about seven times that of the human genome.

Genetic engineering of forest trees is progressing by fits and starts. A few years ago there was optimism that commercial plantations of forest trees would soon be dotting the landscape both in the United States and abroad. So far the only forest tree to be planted commercially is black poplar (*Populus nigra)* in China. Occupying an area of uncertain size, the species has been engineered with a *Bacillus thuringensis* gene that gives resistance to insect attack. Other genetically engineered trees, even those for which the technology has been perfected, are nowhere near being ready for commercialization because of environmental concerns about their potential adverse affects on the environment. It is my prediction that genetically engineered trees for value-added products will pave the way for commercial plantations of forest trees. The value added products will include those engineered for gene conservation of threatened and endangered species, pharmaceuticals, contaminated soils, carbon sequestration, pulping and bleaching and, most importantly, for energy production.

Promoting Science, Dialogue, and Stewardship in Forest Biotechnology

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Science and dialogue are prerequisites for positive social, economic, and ecological change. However, even with the very best science, and the most thoughtful discussions, there is no guarantee that the world will see any benefits unless an integrated approach to deliver results is in place. The Institute of Forest Biotechnology (IFB) provides that necessary infrastructure. IFB works for societal, ecological, and economic benefits from appropriate uses of biotechnology in forestry worldwide. IFB facilitates sound science to fill knowledge gaps. We create dialogue and creative thought to analyze the benefits forest biotechnology promises. Most importantly, we work closely with our partners to make the most advantageous of forest biotechnology outcomes a reality for society. Our partners are a diverse set of stakeholders that include researchers and planners from academia, industry, government agencies, and non-profits. Highlights of platform projects at IFB:

- Science. Heritage Trees – using biotechnology to restore threatened or endangered tree species such as the American chestnut.
- Dialogue. Ecological Impacts – promoting dialogue between our stakeholders and agencies such as the US Forest Service and APHIS to bridge the gap between research needs and regulatory policies.
- Stewardship. Pine Genome Initiative – understanding the biology of pines and other conifers to improve global forest health, provide sustainable bioproduct feedstocks, and advance carbon sequestration research.

Contributed Papers

Concurrent Session A1 - Marker Assisted Breeding/Molecular Genetics

Increasing the Efficiency of Breeding Without Breeding Through Phenotypic Preselection in Open Pollinated Progenies

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Unlike classical methods used by forest tree breeders that rely on pre-determined mating designs to construct pedigreed materials for testing and selection, the concept of Breeding Without Breeding (BWB: El-Kassaby et al., 2007) was introduced to allow the assemblage of full-sib (FS) and half-sib (HS) families from seed orchards' wind-pollinated offspring without conducting any crosses. The method relies on using highly informative molecular markers (e.g., SSRs) and pedigree reconstruction methods to unravel the genetic relationship among individual's offspring. Fingerprinted large wind-pollinated families are required to allow the assemblage of FS and HS families with reasonable size for field testing. To maximize the method's efficiency while minimizing methodological efforts, we propose the inclusion of phenotypic pre-selection from existing open-pollinated family tests to substantially reduce the number of fingerprinted individuals. The proposed application (merging mass selection with BWB) capitalizes on the efficiency of mass-selection in identifying groups of superior individuals and the use of pedigree reconstruction to delineate the paternal parents of the phenotypically selected individuals, hence complete pedigree tracking. Methods for expanding the BWB utility through either slight modification of the production populations' structure or the introduction of desired genotypes through pollen management techniques are presented. The most breath-taking possibility offered by BWB is offering opportunities to abandon not only clonal archives and crosses but also field testing. If both maternal and paternal pedigrees could be reconstructed in commercial plantations originating from a seed orchard, the same type of mass selection could be performed for the orchard's clones and long term breeding could be practiced in commercial plantations rather than of investing efforts and resources on specialized progeny test trials.

New Loblolly Pine Microsatellite Markers for Mapping and Population Genetics

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Select microsatellite (SSR) markers are being developed from enriched genomic libraries, EST databases, and published sources for uses in breeding programs, population genetic studies, enhancing pine genome maps, and DNA fingerprint kits. We tested 1264 primer pairs in loblolly pine (Pinus taeda) and selected 137 suitable markers, of which 111 are new. Primer sequences were obtained de novo and from external sources. For de novo primer development we used 18,498 P. pinaster EST sequences obtained from Christophe Plomion (INRA-UMR BIOGECO, France) and 179,433 P. taeda EST sequences from various public databases, combined to obtain 869 'sifg' markers. For a portion of the P. tadea EST primers we used assembled contig sequences generously provided by the Laboratory for Genomics and Bioinformatics at the University of Georgia. We also used P. taeda genomic sequences from SSR-enriched libraries to obtain 118 'ript' markers under a prior cooperative agreement with International Paper Company. Primers that we evaluated from extant external sources were those of Auckland et al. 2002 for 164 'PtTX' markers, Chagné et al. 2004 for 38 'SsrPt' and 5 'RPtest' markers, and Phil Wilcox (Scion LLC, New Zealand) for 70 'NZPR' P. radiata markers.

Our criteria for marker selection were, in order of precedence, strong amplification of single loci with a one-size-fits-all PCR protocol, easily interpretable chromatographic allelic profiles obtained from capillary electrophoresis, and gene diversity values above 0.30. Gene diversity was estimated from 14 P. taeda genotypes whose provenances were distributed across the species' natural geographic range. The proportions of SSR motif classes comprising the select set were 62% dinucleotide, 27% trinucleotide, with the rest being tetra-, penta-, or hexanucleotide repeats. EST-SSR markers made up 40% of the select set.

Ninety six percent of select markers were heterozygous in at least one of two sets of parents of the publicly distributed 'Base2' and 'Qtl2' reference mapping populations, with 72% heterozygous in both pedigrees. Genome mapping is underway and progress on the integrated framework reference maps will be reported.

Population genetic analyses comparing EST to genomic DNA origins of select marker loci in the 14 screening genotypes revealed gene diversities of 0.57 vs. 0.77, numbers of alleles/locus of 4.2 vs. 8.1, and inbreeding coefficients of 0.07 vs. 0.15, respectively. Although the EST-SSR markers were on average less diverse and more homozygous than the genomic markers they should work in a wider range of pine species because of the greater sequence conservation generally found in expressed genes. The EST-SSR markers generally had simpler allelic profiles than the genomic-SSR markers as characterized by fewer stutter patterns and shoulder peaks. Together these characteristics suggest that select EST-SSR markers would be well suited for construction of multi-species pine DNA fingerprinting kits.

Estimation of Population Structure in the Coastal Douglas-fir Association Mapping Study

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The population structure has been thoroughly studied in a range-wide sample of ~1300 coastal Douglas-fir trees from Washington and Oregon that are used for association mapping between cold-hardiness and phenology related phenotypes and SNPs in the adaptive trait related candidate genes. All trees have been genotyped for 25 isozyme and 6 SSR markers using individual megagametophytes. Population structure analysis has been done separately for isozyme and SSR markers, as well as for both data sets combined using the standardized measure of population differentiation that takes high levels of within population variation for SSR markers into account. Results based on isozyme and SSR data sets have been compared and discussed. The relatively low level of population differentiation has been found for both markers, which should help to avoid false associations between phenotypes and genotypes for pooled samples in association mapping due to the demographic or population structure. However, clinal variation has been observed for several loci that could be explained by ecogeographic adaptation and possibly by influx of genes from interior Douglas-fir.

Development of Reference Karyotypes for Longleaf and Shortleaf Pines using Fluorescence in situ Hybridization

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A reference karyotype (i.e., chromosome-specific description of the genome) is a pre-requisite for advanced genetic and genomic studies. A pilot project has been initiated at the Southern Institute of Forest Genetics' Forest Tree Molecular Cytogenetics Laboratory to develop reference karyotypes for each of the four major southern U.S. pine species— loblolly (*Pinus*

taeda L.), slash (*P. elliottii* var. elliottii), shortleaf (*P. echinata*) and longleaf (*P. palustris*) — using 18S-28S rDNA, 5S rDNA and Arabidopsis-type telomere repeat sequence probes and AT-rich banding. Reference karyotypes for loblolly and slash pines have been completed. Preliminary results for the rDNA genes show that both shortleaf and longleaf pines contain seven major intercalary 18S-28S rDNA sites. Shortleaf pine showed as many as eight medium-to-minor centromeric 18S-28S rDNA sites, and longleaf pine showed two major and six medium-to-minor centromeric 18S-28S rDNA sites. Both species showed one major and one minor site for 5S rDNA,. Strong AT-rich bands are found to flank the centromeres of most of the chromosomes in both species. Complete karyotypes for each of shortleaf and longleaf pines are being developed for comparison to each other and to the loblolly and slash pine karyotypes.

Strategies for Identification of QTLs Controlling Early Height Growth in Longleaf Pine

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Simple Sequence Repeat (SSR) markers are being used to map the genome and quantitative trait loci controlling the early height growth (EHG) in a backcross family (longleaf pine x slash pine) x longleaf pine. A total of 208 locus specific SSR markers have been screened against 6 longleaf pine recurrent parents and a sample of 7 slash pine parents. The SSR markers include 80 *PtTX* loci which were developed in Claire William's lab at Texas A&M, 56 *sifg* loci were developed by Craig Echt and Dana Nelson (Southern Institute of Forest Genetics) in collaboration with Daniel Peterson and Surya Saha (Mississippi State University), 6 *RPtest* loci were developed by C. Echt and 66 ***ript*** loci were developed by C. Echt and D. Nelson. Among the 13 parents, 132 markers (63.5%) show polymorphisms including 51 *PtTX* loci (63.8%), 26 *sifg* loci (46.4%), 5 *RPtest* loci (83%) and 50 *ript* loci (75.8%).

Based on the genetic variance in early height data, available sample size, and the number of SSR marker polymorphisms, 6 half-sib families with a common paternal parent (Derr488) were selected from 27 backcross families as the final mapping population. For these 6 half-sib mapping families, there are 97, 95, 89, 89, 99, 102 informative markers, respectively. Within each of the 6 families, the tallest and shortest 8 percent of seedlings (200 seedlings total) were selected for QTL detection (phase I). Then random selections of 8 percent of the seedlings from the rest of the population (100 seedlings) and 10 seedlings from both tails (120 seedlings total) of the within family distributions will be used for unbiased QTL verification and mapping (phase II). For data analysis, the residual which includes within-family genetic effect and specific-site environmental effect will be used as the phenotypic trait value. Progress on the project and results obtained will be presented and discussed.

ADEPT2 – Resequencing, SNP Discovery, and Association Studies for Loblolly Pine

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The ADEPT2 project aims to infer associations between natural genetic variation and complex phenotypic traits for loblolly pine (*Pinus taeda* L.). This is a collaborative project between the University of California at Davis (UCD), North Carolina State University (NCSU), the University of Florida (UF), and Texas A&M University (TAMU). Each partner is taking the lead on a part of the overall project, with UCD conducting all discovery and genotyping of single nucleotide polymorphisms (SNPs). Association analyses will subsequently be conducted on combined data sets consisting of our SNP data and the phenotypic data gathered at our partner institutions.

The resequencing of 7,850 EST unigenes in a diversity panel of 18 loblolly pine individuals has recently been completed by Agencourt Biosciences. All sequence data were generated from haploid DNA samples harvested from megagametophyte tissue, deposited within the TreeGenes database, and pushed through our sequence analysis, alignment, and SNP discovery pipeline. The average number of SNPs identified per amplicon is estimated to be approximately five, thus yielding a rough estimate of the average scaled population mutation rate (Q_W) of 3.44. We have also tested all primer pairs for single samples collected from *P. radiata* D. Don, P. lambertiana Douglas, *Picea abies* (L.) Karst., *Pseudotsuga menziesii* (Mirbel) Franco, and *Sequoia sempervirens* (D. Don) Endl. Observed rates of primer success across these taxa follow phylogenetic expectations, with success rates of 84%, 30%, 13%, 10%, and 2%, respectively.

Identified SNPs will then be evaluated for overall quality and ability to convert to standard genotyping platforms (e.g., Illumina GoldenGate Assay) using our automated SNP calling pipline. A subset of those SNPs will be used to genotype three loblolly pine association populations for which phenotypic data are or will be available from our partner institutions. Phenotypic data are grouped into five categories: wood properties (UF), disease resistance (UF), drought tolerance (NCSU), gene expression (TAMU), and metabolomics (UCD). A rangewide analysis of population structure and historical demography of loblolly pine using data from all three genomes is also underway to test assumptions of association-based analyses (i.e., lack of population structure), to provide appropriate null models for neutrality testing for the resequencing data, and to describe the phylogeography of this economically important species.

Our data provide an initial foundation for analyzing the diversity and structure of the loblolly pine genome and for the dissection of its complex phenotypic traits into their respective genetic components. Work at UCD has also resulted in a suite of bioinformatic tools and data available to the larger forest genetics community. We believe that these tools will help to answer the increasingly difficult questions arising in loblolly pine genetics and genomics.

Nucleotide diversity and neutrality testing in genes involved in adaptation in Douglas-fir

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Douglas-fir (*Pseudotsuga menziesii*) is one of the predominant timber-producing species in North America. One of the problems for wood production is the loss of annual growth through frost damage to actively growing tissues. Natural populations of Douglas-fir however exhibit tremendous variation in response to low temperature suggesting substantial underlying genetic control. This is supported by findings of QTL mapping studies over the last decade which have highlighted that multiple genomic regions contribute to the variation in genecological traits. The specific genes involved however are still largely unknown. In contrast, association genetics is proving to be a powerful approach for dissecting complex traits into their individual gene components. Our goal is to use this method to detect association between SNPs from candidate genes and the variation in cold tolerance phenotypes.

We have resequenced 128 Douglas-fir homologs to candidate genes for cold tolerance based on similarity to genes in Arabidopsis. These amplicons were analyzed using a panel of 24 diverse trees from across the Washington-Oregon region, and 684 SNPs were identified in total. We report the estimation of nucleotide diversity and tests for departures from neutrality in these candidate gene sequences. From these, 384 SNPs are being genotyped in the remaining association population of c.900 trees using the Illumina GoldenGate Assay platform.

The ability of plants to adapt to environmental fluctuation is critical to survival, and more so now in the face of global climate change. Douglas-fir is a good model for studying the molecular basis of adaptation, and resources are continuing to be invested to further understand the individual genes involved in this response. The information gained will also be transferable, helping to build genomic resources in less well-studied conifers, and enable a better understanding of adaptation in these important tree species.

Parent-Dependent Effects of the cad-n1 Mutant Allele on Height in Loblolly Pine Families

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The *cad*-n1 mutant allele encodes an inactive form of cinnamyl alcohol dehydrogenase, the enzyme that encodes the final step in the biosynthesis of lignin monomers in loblolly pine differentiating xylem. This mutant allele has dramatic effects on lignin composition and tree growth if present in the homozygous condition, and much more subtle effects if present in the heterozygous state with another allele that encodes normal levels of enzyme activity. Several authors have reported finding that trees heterozygous for the *cad*-n1 allele show differences in growth rate or wood properties, but not all studies that have tested for this effect have detected significant differences.

From a set of diallel progeny tests planted in the South Carolina Coastal Plain, we collected foliage samples from progeny of two parent trees, Parent A and Parent B, where Parent B is an offspring of Parent A. Both parent trees are heterozygous for the *cad*-n1 allele. The progeny were tested for the presence of the *cad*-n1 mutant allele, which was found in the expected 1:1 segregation ratio. Six-year height and volume data, standardized by replicate and site to minimize environmental contributions to variation, were used to compare performance of individuals that inherited the *cad*-n1 allele ("carriers") and those that did not ("non-carriers"). A combined analysis of all offspring of both parents shows no significant differences in height or volume between carriers and non-carriers. Separate analyses were conducted to compare height and volume between carriers and non-carriers by family, with the samples divided into offspring of crosses using the tested parents as pollen parent or as seed parent. Parent A was crossed to 13 other trees as a seed parent, and 8 other trees as a pollen parent; Parent B was crossed to 12 other trees as a seed parent, and 5 other trees as a pollen parent. A significant difference in height but not in volume was detected between carriers and non-carriers for the offspring of Parent A as seed parent, but not as pollen parent. The comparison among offspring of Parent B showed no significant differences in either height or volume, regardless of the direction of the cross.

A better understanding of the genetic basis for the difference in performance between parents within breeding programs can contribute to improved gains from selective mating of parents with complementary breeding values. Molecular genetic characterization of variation that underlies differences in breeding values is the first step toward this goal. Additional characterization of the effects of the cad-n1 mutant allele is needed to more fully describe the potential benefits and costs of using this variant in breeding for improved performance in loblolly pine.

Coastal Douglas-fir Wood Quality: Quantitative and Molecular Results

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Interior lodgepole pine (*Pinus contorta* Dougl. ex. Loud. var *latifolia*) is the most heavily planted tree species in British Columbia, Canada. The lodgepole pine breeding program is in its second generation of progeny testing which incorporates two breeds: (A) height growth improvement and (B) height growth and relative density improvement. An 'environmental map' free of genetic effects, was considered important to develop strategic deployment approaches for materials coming from the A or B breeding groups. To achieve this, environmental variation in wood relative density in lodgepole pine was examined by sampling from a 33 year-old provenance test planted at 60 test sites across the range of the species in British Columbia, using a set of six standard provenances.

Transfer curves (regression models with relative density as the dependent variable, and site climate variables as independent variables) were generated for each of the six standard provenances. Each regression equation was used to predict relative density at 370,000 grid points covering British Columbia. The predicted relative density values were converted to principal components and mapped using ArcView. Climate change scenarios were used to generate climate data for 2055, which was also used as input for the model.

Maximum temperatures in the summer months, and summer heat to moisture index accounted for most of the variation in relative density, with R2 ranging from 0.74 to 0.84. These relationships were mapped with Geographic Information Systems, which showed significant patterns of relative density variation across the province, but were geographically related to decreasing density with increasing elevation and increased density in areas of higher precipitation. This environmental map did not present any obvious approaches of how to currently utilize the two breeds with respect to deploying low or higher relative density individuals, however, considering one of many possible future climate change scenarios, relative density may be adversely affected across the entire planting range so that the higher density breed may become more important than previously considered.

Concurrent Session B1 - Selection, Breeding and Progeny Testing

Weyerhaeuser Douglas Fir Breeding Program

C. Dean

VP Timberlands Technology R & D, Weyerhaeuser Company

Whiskey and Trees: Seed Orchards at the Jack Daniel Distillery

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In 1998, the Jack Daniel Distillery decided to support the creation of seedling seed orchards to sustain local wood production of species used in the distilling process. In addition, the Distillery made land available for other species that are important in south central Tennessee for timber and habitat. Collections of open-pollinated progenies of *Acer saccharum* Marsh. (1998), *Quercus alba* L. (1999), *Quercus prinus* L. (1999), *Juglans nigra* L. (1999), and *Juglans cinerea* L. (2005) were made from local trees and planted at the Georgia Forestry Commission's Flint River Nursery. The resulting seedlings were grown for one year and planted as genetic tests that will eventually be thinned to produce seedling seed orchards. Survival, growth, reproductive maturation, and management are discussed.

Performance of Nuttall Oak (*Quercus texana* Buckl.) Provenances at Age 10 in the Western Gulf Region

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Nuttall oak (*Quercus texana* Buckl.) is a member of the red oak family with a natural range restricted to the bottomlands of the Gulf Coastal Plain from Alabama to Texas and from Missouri to the coast. It is extremely hardy and fast growing and is therefore a highly desirable species for bottomland planting and restoration. Three series of three tests each of Nuttall oak were established by members of the Western Gulf Forest Tree Improvement

Program at three locations transecting the central part of the range in a north-south direction. The three series included 28-42 different half-sib families from throughout the natural range that were arbitrarily divided into provenances based on the river basin in which the parent originated. Provenance differences originally reported at age 5 were revisited after the 10-year measurements. Family heritabilities, genotype by environment interaction, and age-age correlations were calculated. The orchard establishment strategy based on this information is discussed.

Current Results and Future Aspects of Oak Tree Improvement

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Legacy Westvaco's Central Forest Research Center located at Wickliffe, Kentucky established cherrybark and Nuttall oak provenance/progeny trials as well as oak species comparison studies on bottomland sites in western Kentucky and west Tennessee during the late 1980s and early 1990s. The oldest of the studies are now 20 years of age and have exhibited very high survival at early ages followed by intense tree-to-tree competition starting between the ages of 10 and 15 and increasing between ages 15 and 20. Efficient selection age of oaks seems to be approximately 10 years based on current available data from these studies. The best seed source material for western Kentucky proved to be material located some 350 miles south of the planting site. Information from such oak genetic trials needs to be brought together in determination of seed source zones and recommendations for use in conservation plantings. Currently, a variety of conservation programs such as the Wetlands Reserve Program (WRP), the Wildlife Habitat Incentive Program (WHIP), the Environmental Quality Incentive Program (EQIP) list a variety of hardwood species for outplanting but oak species dominate this list. In the lower Mississippi Alluvial Valley approximately 350,000 acres have been planted to WRP, alone. Although, the primary focus of these programs has been wildlife, recent indications are that landowners want the possibility of timber revenue.

Hardwood tree improvement programs have always faced a considerable amount of adversity ranging from low prices, limited markets for intermediate size material, to the lack of funding from private and industrial sources. In addition, the divesture of lands traditionally owned by the forest industry and the subsequent reduction in research efforts have only added to the problems facing hardwood tree improvement. The progress in tree improvements efforts in the more rapid growing species such as, eastern cottonwood (Populus deltoides Bartr.), sycamore (Platanus occidentalis L.) and sweetgum (Liquidambar styraciflua L.) has been the greatest. The ability of a single hardwood species to grow well over a variety of sites has also hindered work on hardwood species. However, there is enough genetic information concerning a variety of hardwood species including various bottomland oak species to draw some inferences concerning recommendations for seed source movement, outlining plans for use of selected material and the steps needed to conserve the genetic progress made to date.

Developing Disease Resistance to Non-native Pathogens: Status of Programs for Oregon and Washington

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Non-native, invasive pathogens continue to take large tolls on our native trees species and their associated ecosystems. In many cases, disease resistance programs that utilize the low frequency of natural genetic resistance present in our tree species offer the best opportunity to counter the invading pathogens. The Pacific Northwest Region (Region 6) of the USDA Forest Service encompasses both Oregon and Washington and currently has operational programs to develop resistance to two non-native, invasive pathogens. The programs are based at the regional forest genetics facility, Dorena Genetic Resource Center, in Cottage Grove, Oregon. The program to develop genetic resistance to white pine blister rust (caused by the pathogen *Cronartium ribicola*) in native white pines has been active for 50 years. The program to develop *Phytophthora lateralis* resistance in Port-Orford-cedar (POC) has been in the operational phase for just over a decade.

The blister rust program has evaluated progenies of thousands of western white pine (WWP) and sugar pine field (SP) selections and has recently begun to evaluate resistance of progeny of whitebark pine field selections. Breeding zones have been delineated for WWP and SP, and the first orchards have been established. Further breeding will be needed to increase the level and mix of rust resistance to be used in restoration and reforestation efforts. The operational program to develop populations of POC with resistance to P. lateralis has made rapid progress, and orchards for several breeding zones are now producing resistant seed.

Artificial inoculation of young seedlings and subsequent assessment for resistance responses is a key element to both resistance programs. Region 6 has developed extensive expertise in screening for resistance. Partners and cooperators also play an important role, and include federal, state, county, tribal, and private landowners. Field tests to validate the short-term screening results and to examine the durability of the resistance have been established. The Forest Service program primarily focuses on developing durable disease resistance while maintaining genetic diversity and adaptability. By their nature, resistance breeding programs are long-term, and further progress will require continuity of funding and staff. Challenges to proceeding with restoration on federal lands exist, as well as opportunities to enlist other landowners in increasing the deployment of resistant material in the Pacific Northwest.

Evaluation of Resistance to Fusiform Rust in Loblolly Pine from East Texas

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A set of 21 loblolly pine families produced by crossing trees from east Texas were tested for resistance to fusiform rust disease. The parents of these families were surviving trees in stands that experienced extensive mortality in the 1960s due to southern pine beetle infestation. Seedlings were grown in tubes in a greenhouse and artificially inoculated with *C. quercuum* from five different sources of inoculum, each consisting of single gall collections of aeciospores. Four of the collections originated from galls on loblolly pine (*C. q. fusiforme* or Cqf), whereas the remaining collection was obtained from a shortleaf pine gall (*C. q. echinatae* or Cqe). Two of the Cqf inocula were collected from trees in Louisiana, while the others were obtained from trees in east Texas. A single collection of Cqf in each state and Cqe were taken from round-shaped galls, while the other two Cqf collections were taken from typical fusiform-shaped galls.

Nine months following inoculation, seedlings were evaluated for presence/absence of rust gall(s) and gall form (gall length / gall diameter). Families showed significant differences in percent of trees galled (percent gall) by each of the Cqf inocula. No apparent relationship was observed between percent gall by the Cqe inoculum and that for any of the Cqf inocula, suggesting that different resistance genes are effective in these families for Cqf and Cqe. All galls produced by the Cqe inoculum were about round-shaped with gall form values ranging from 0.88 to 1.54. However, for the Cqf inocula no relationship was found between shape of source galls and the shape of galls formed on diseased seedlings. For example, Cqf inoculum collected from an elongated gall had an average gall form value of 1.85 compared to 2.38 for Cqf inoculum originating from a round gall. The variances observed among these families in percent gall and gall form suggests that gains in resistance to fusiform rust disease are possible within the East Texas seed source.

Fusiform Rust and Pitch Canker Resistance in Loblolly Pine Elite Varieties

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Loblolly pine elite clonal varieties of Atlantic Coastal Plains (ACP) origin were screened for fusiform rust and pitch canker resistance in tests involving artificial inoculations. The screenings were done by the USDA Resistance Screening Center (RSC) in Asheville, NC over the course of four years. Twenty two related and unrelated varieties were challenged with rust inoculum developed from aeciospores collected in three regions representing large sections of the eastern, central and western distribution range of loblolly pine. The inocula from the three regions were not mixed. Pitch canker resistance screenings involved eleven varieties challenged with mixed inoculum from three southern ACP sources.

RSC rust screening results were compared to field rust infection rates based on age five data from multiple test sites. There were very large differences among the varieties in rust and pitch canker resistance. In the rust screening tests the varieties ranged from more resistant than the resistant checklot to as susceptible as the susceptible checklot. The best varieties had less than 3% infection rates compared to 42% for the resistant checklot. The most susceptible and the most resistant variety came from the same family. The majority of varieties screened for rust resistance showed very little interaction with inoculum source. With very few exceptions, the RSC results were consistent from year to year. Levels of resistance based on field data and RSC data were highly correlated ($r = 0.80$) despite low overall levels of infections in the field. There was no correlation between growth and disease resistance. Two varieties with the highest growth rates were ranked as very resistant for both rust and pitch canker.

Wood Quality of Southern Pine Hybrids with Reference to Their Slash and Loblolly Parents

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Hybridization between closely and distantly related species is a valuable approach to genetic improvement, and has been successfully used in many plant species, including forest trees to improve yields and to introduce other desired traits such as cold tolerance and disease resistance. Loblolly and slash pine were hybridized and the F1s planted in replicated field trials. At year two, F1 hybrids were on average smaller than the parental species, indicating

negative hybrid vigor (Lopez-Upton et al., 1999, *Silvae Genetica* 48 (6): 303-313) for growth traits. This result was somewhat surprising given the two species have overlapping ranges and are highly related.

Given the negative hybrid vigor found for early growth, we wanted to evaluate wood properties of these slash and loblolly pine F1 hybrids to determine whether they also showed negative hybrid vigor. During the 11th year of growth, in-tree velocity stiffness was measured using Treesonic and the Director ST300. Our results show that the slash and loblolly parents had similar velocity stiffness; whereas, the F1 hybrids had significantly lower velocity stiffness. From two of the sites, 12 mm wood cores were taken at 1.3 m from a diameter at breast height stratified subsample of loblolly, slash and F1 hybrid trees. With these cores, the microfibril angle (MFA) of earlywood and latewood was determined with x-ray diffraction from rings 9 and 10. For both earlywood and latewood, the MFA of the parents where similar but the MFA of the F1 hybrids was significantly higher MFA. This increase in MFA agrees with the decrease in in-tree velocity stiffness. Thus, MFA and in-tree velocity stiffness show negative hybrid vigor, similar to the negative hybrid vigor found for early growth.

In other studies (Watt et al., 2006, *For. Ecol. Manage*. 229, 136-144; Roth et al., 2007, *For. Ecol. Manage*. In press), in-tree velocity stiffness was correlated with the ratio of height to diameter at breast height (H:DBH), with more slender stems having increased stiffness. We have investigated the correlation between H:DBH and MFA and in-tree velocity stiffness to test if this explains the negative hybrid vigor observed.

The Potential of Acoustics to Determine Family Differences for Wood Quality in a Loblolly Pine Trial

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Breeding and selection for desirable wood properties will be a key factor in determining the global competitiveness of the U.S. forest industry. Loblolly pine (Pinus taeda L.) breeders are currently able to select for differences in wood specific gravity, microfibril angle (MFA), and Modulus of Elasticity (MOE) by colleting and analyzing wood increment cores and bolts. However, the methodology required to do this can be time-consuming, cost-prohibitive and in the case of MOE, destructive. The use of acoustics in determining wood quality has merit as an alternative to traditional methodology in that it is non-destructive and relatively fast. Acoustic velocity is directly related to MOE and closely related to MFA so it can act as a surrogate for wood stiffness. Before acoustics can be used as a selection criterion, a number of questions must be answered. Can data be collected efficiently on the large numbers of trees required to estimate parental breeding values? Are there differences in transmission of

sufficient magnitude and repeatability to allow heritable differences to be detected among families? Are there significant genotype by environment interactions that need to be considered when making selections? Is the equipment robust enough to be used by different field crews?

To begin answering these questions, the Southern Institute of Forest Genetics, in collaboration with the Western Gulf Forest Tree Improvement Program, collected acoustic velocity data in three control-pollinated loblolly pine progeny tests. All three tests were established by International Paper Company and located in southeast Texas. Acoustic velocity was measured across breast height using the Fakopp Stress Wave Timer. Measurements were collected on two sides of each tree and then averaged together to estimate stiffness. Trees with forks and obvious signs of disease were excluded from measurement. Variance components were estimated for each location using the software packages DIALL and DIALLC. Results showed that single-location individual heritabilities for the averaged acoustic velocity were moderate yet large enough to be useful in an applied breeding program. Single-location individual heritabilities for volume were twice as large as expected but those for specific gravity were slightly smaller than expected. Multiple-location analyses still need to be completed to estimate the genotype by environment component and the relationship between acoustic velocity, volume, and specific gravity also needs to be determined. In addition, field protocols need to be refined to avoid data outliers and to arrive at easier methods of collecting observations. The ultimate goal is to incorporate breeding values for stiffness, volume, straightness, and wood specific gravity into a sawlog index so breeders can rank candidates for inclusion in seed orchards.

Concurrent Session A2 - Marker Assisted Breeding/Molecular Genetics/Physiology

Progress and Plans for Unraveling and Managing Fusiform Rust Disease

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Fusiform rust disease continues to be the most destructive disease in southern U.S. pine plantations. Our cooperative research program is designed to identify, map, and clone the interacting genes in both the host and pathogen. Several resistance (R) genes have been identified and genetically mapped using informative families and single-spore isolate inoculations. In addition we are mapping the first of many expected corresponding genes in the pathogen. These genes condition avirulence (Avr) and are required for an incompatible (i.e., resistant) reaction to take place within an inoculated host tree that carries the

corresponding R gene. We will provide an overview of our methodology for identifying and mapping R and Avr genes, an update of our current progress, and scenarios for use of this information by tree breeders and silviculturists to identify trees with prospective R genes and to determine their value in providing resistance against the pathogen in various environments.

Computer Simulation of Marker-Directed Population Improvement

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The wide range of commercially important traits in forest-tree breeding may be referred to as complex. The complex nature should in theory ask for a situation-specific approach to improvement to make the tree breeding as efficient as possible. However, the most common approach in real programs is to treat all traits as purely polygenic, assuming the infinitesimal model. The objective of our research is to quantitatively evaluate the marker-directed population improvement breeding strategy. In this strategy, the complex nature of commercial traits is reflected in predicting both polygenic and QTL additive effects, and combining these into a single criterion (BLUP value) prior to the selection. We first review the development of the method and challenges arising in outbred species, particularly forest trees. Later, we present the first results of computer simulation, where the main variables are the density of markers flanking to the quantitative trait loci, and the relative effect of QTL loci to the trait's expression. The strategy is compared to control scenarios under pure polygenic inheritance model. We focus on a single breeding population and respective genetic response and diversity over a number of consecutive generations. Finally, we discuss the future development of this strategy.

Regulation of Flowering in Poplar

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The control of flowering is of great scientific and commercial importance in trees. We have already identified the *FLOWERING LOCUS T2* gene that involves in "first-time" and "seasonal" flowering in poplar (Populus spp.). To elucidate the flower initiation networks and

pathways in poplar at the molecular level, we have taken functional and comparative genomics approaches that include physiological and genetic manipulations and high throughput platforms coupled with bioinformatics to identify genes and determine their expression patterns. We will present our recent findings and discuss their implications to tree improvement.

Phytochelatin Synthase Expression Effect on Zinc Interaction with Populus

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Heavy metal uptake and storage is necessitated for bioremediation of toxic metals in the environment. Heavy metal regulating genes have been isolated in small annual plants and have afforded those species with tolerant and accumulating phenotypes. One such gene codes for phytochelatin synthase and at high levels is correlated with increased heavy metal tolerance and accumulation in these annual plants. In perennial forest species, little is known about the genes involved in the heavy metal regulating pathway. However, identification and function of homologous genes in forest species is necessitated for their use as bioremediation agents. Thus the objective of this study is to isolate a *Populus* gene homologous to a known heavy-metal related gene and determine its role in heavy-metal regulation. A *Populus* gene homolog to a phytochelatin synthase (PC) coding gene in the zinc hyper-accumulator *Thlaspi caerulscens* was identified using a phylogenetic analysis. The gene transcript was isolated from *P. trichocarpa* tissue, sequenced, and cloned into an over-expression vector. Tissue was transformed with the gene construct and plants were generated from tissue culture. A 3x4 factorial design was used in which two lines of *Populus* with the over-expressed PC gene and a control were subjected to four concentrations of zinc in the growth medium. This paper will discuss the effect various levels of zinc had on the plants which will indicate the functionally of the homologous gene and if expression effects mimic those found in other species. Similar gene functionality with hyper-accumulators would afford *Populus* the ability to become heavy metal tolerant offering a new avenue for bioremediation.

Integration of Crown Morphology and Leaf-Level Physiology as a Tool for Explaining Differences in Aboveground Productivity among Elite Families of Loblolly and Slash Pine

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To a growing extent, current silvicultural systems involve the deployment of genetically improved planting material, together with high-input silvicultural treatments to increase forest

productivity. As forest plantations become increasingly uniform, factors that limit tree growth should be identified in order to effectively alleviate those limitations.

Forest production depends on CO_2 assimilation, but is rarely solely a function of leaf-level photosynthesis. Crown characteristics may affect tree growth by altering light interception and photosynthesis at canopy level. Strong light gradients are present in forest canopies, which often result in parallel changes in leaf morphology and leaf nitrogen for efficient use of light in photosynthetic CO_2 uptake. The genetic basis of crown and canopy trait differences among southern pine taxa are not well understood, but are critical for predicting growth and productivity differences for managing sustainable forest ecosystems.

In our study we investigated effects of intensive silvicultural treatments on crown morphology and within-crown leaf-level physiology, and the relationship to aboveground productivity of selected families of loblolly and slash pine. In young stands, before canopy closure, we found significant among-family differences in crown structure and between-species differences in leaf area density per unit of crown volume. Loblolly pine had larger crowns than slash pine trees of the same age or size, but maintained lower leaf area per given crown volume. The two pine species also differed in specific leaf area and leaf nitrogen at age four. These traits were highly variable within crowns, reflecting leaf-level acclimation to light gradients within a canopy when stands approach canopy closure. Leaf-level photosynthesis rates varied among different crown positions at only one of two experimental sites and was not affected by intensive silvicultural treatment. Area-based leaf photosynthesis increased from the lower to the upper portion of the canopy for loblolly pine, but was lower at the upper than in the middle crown position for slash pine. However, species did not differ in leaf-level photosynthesis rates at any crown position.

High intensity treatments, although effective in increasing biomass accumulation in all examined families, did not affect leaf morphology or physiology. Aboveground biomass production differed among tested families and was related more to accumulated leaf area and its display within crowns than to differences in rates of leaf-level photosynthesis.

Comparative Nutrient Economy, Stable Isotopes, and Related Adaptive Traits in *Picea rubens, Picea mariana,* and Their Hybrids

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Nutrient- and water economy-related traits in plants have significant implications for growth and fitness. We examined and compared nutrient concentrations, use efficiencies, assimilation, and informative isotopic elements in a seedling provenance experiment, and in seedling and mature tree controlled-cross hybrid experiments of red spruce (RS) (*Picea rubens* Sarg.) and black spruce (BS) (*P. mariana* (Mill.) B.S.P.). Provenance experiment results showed RS had consistently lower carbon (C), nitrogen (N), and N assimilation ratio (NAR), but higher N-use

efficiency (NUE), C:N ratio, water-use efficiency (WUE), needle calcium (Ca), and magnesium (Mg), than BS. Seedling hybrid experiment results showed similar results and additive inheritance for needle N, C:N ratio, NAR, Ca, and Mg, evident by a near linear progression from one species to the other. Within both species, seedling height showed a negative relationship with needle N and a positive relationship with NUE. However, across hybrid indices, seedling height showed a positive relationship with needle N and a negative relationship with NUE. Also across hybrid indices, seedling height showed a negative relationship to Ca and C:N, and a positive relationship with NAR and ^{13}C discrimination (without hybrid 25). Mature tree hybrid experiment results were similar to those of the seedling experiment, but with a dampening of differences caused by low nutrient availability and possibly age effects. The similarity was not true for ^{13}C discrimination as mature tree height showed a strong negative relationship to ^{13}C discrimination, indicating that BS had greater WUE. The reversal is most probably caused by the large difference in water availability.

Responsiveness to Fertilization of Diverse Families from two Provenances of Loblolly Pine

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The **S**outh**E**ast **T**ree **R**esearch and **E**ducation **S**ite–2 study (SETRES-2) was established in Scotland County, North Carolina adjacent to the USDA Forest Service / NC State University SETRES site in 1993. The objective of this study is to evaluate the response to nutrient stress of two very different provenances of loblolly pine, one from the "Lost Pines" region of Texas (LPT) and one from the Atlantic Coastal Plain of North Carolina and South Carolina (ACP). Five open-pollinated families from each provenance were used. A split-split-plot design was used with the two nutrient treatments as main plots, provenances as sub-plots, and families within provenances as sub-sub-plots. Fertilizer (including micronutrients) has been applied annually to maintain a balanced supply of all nutrients in the fertilized plots. All trees were measured annually for height and starting in year 4 for diameter at breast height.

Growth responses to fertilization were very large and significant at year 12. Through age twelve, height was 75% greater in the fertilized plots and stem volume per hectare was 173% greater, compared to the non-fertilized plots. Nutrient amendments dramatically increased uniformity within the 64-tree family plots. The average within-plot CV for 12-year height was 13.56% for the control plots and 6.06% for the fertilized plots. In the fertilized treatment, the LPT trees had higher volumes and basal areas than the ACP trees. Families within provenances also differed for growth traits. The family means at age twelve for the ACP families varied from 60 m3/ha to 133 m3/ha in the control plots and from 185 m3/ha to 262 m3/ha in the fertilized plots. The LPT families also differed in the control plots (55 m3/ha to 103 m3/ha) and in the fertilized plots (168 m3/ha to 306 m3/ha). Statistical differences were only found at the treatment level and replicate by treatment by provenance interaction for growth traits (ρ-value=0.05).

While many families have high growth rates, some of them have a high incidence of stem and branch deformities causing serious problem at wood quality level. For this reason, results from evaluations of straightness, sweep, rust, branch, fork, and stem and branch sinuosity will be also presented in this paper.

Towards a Comprehensive Proteome Analysis of Poplar Vascular Sap

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The long-distance transport system of xylem and phloem plays a pivotal role in growth/development of trees, long-distance signaling, and defense as it allocates water, nutrients, and hormones throughout the whole plant. We have discovered that the vascular sap from poplar (*Populus deltoides*) leaves contains a considerable amount of secreted proteins. Total proteins were extracted from the leaf sap and analyzed using 2-D SDS-PAGE MALDI TOF MS/MS and 2-D LC MS/MS followed by poplar protein database search to identify proteins. We will present our proteome analysis, functional classification, and potential applications of this information towards tree improvement.

Concurrent Session B2 - Seed Orchards/Climate Change

Is Randomization Necessary in Seed Orchards?

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Randomization of clonal ramets in seed orchards is commonly practiced to promote cross-fertilization and minimize selfing. While it is practiced for the "right" biological reason, randomization comes with added managerial burden during crop management and harvest. Evidence for extremely low selfing rates in most conifers' seed orchards and natural populations has lead to a re-evaluation of seed orchard designs. The clonal-row seed orchard design represents a viable option for reducing management burden, but it comes with increased estimates of correlated matings between adjacent clones (i.e., "neighbourhood effect"). Staggering of clonal rows was proposed to double the number of adjacent clones to reduce correlated matings; however, it limits every clone to only four neighbours. We propose a

modification to the staggering rows with a "randomized, replicated, staggered clonal-row" design to allow the simultaneous realization of randomization and clonal-rows orchard designs benefits. An interactive computer program was designed for this purpose that allows controlling the orchard' size and layout, the number of clones, rows and their length, selection of the physical distance between repeated rows of the same clone, the level of "anti-randomization" tolerance imposed by the design parameters, and the clonal deployment mode (equal clonal size vs. linear deployment).

Clonal Replacement as a Tool for Seed Orchard Managers – Year 3 Update

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Topgrafting scion from selected genotypes of loblolly pine (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. In fact, this practice has become routine in many southern tree improvement programs for use in breeding programs. The reduction in time for flower production is an economic advantage for breeding and could prove to be economically attractive for entire crown replacement of seed orchard or clone bank trees.

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. Data have been presented on graft survival, scion quality and male and female flower production one year after topgrafting. Interstock effects were significant for both male and female strobili production. Updates on female and male strobili production as well as seed production from the first cone harvest will be presented. Interstock effects on flowering will be examined to determine if early trends are maintained.

The big question remains – can we afford to do this? Using cost data compiled during this process, we will investigate the economic limitations to converting a small orchard from one genotype to another. How much a seed orchard manager can spend on this conversion will be dependent upon the marginal costs between conversion through topgrafting and establishing a conventional seed orchard. Therefore, the benefits of this strategy will be dependent upon the gain differential in the topgrafted orchard and the time to production realized from topgrafted trees. Both factors will be examined in determining the economics of entire crown replacement .

Using $GA_{4/7}$ to Induce Flowering in *Pinus ponderosa* Seed Orchards

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Ponderosa pine (*Pinus ponderosa* P.& C. Lawson) is an important tree species in much of the western USA. Members of the Inland Empire Tree Improvement Cooperative (IETIC) are interested in increasing seed crops from first generation ponderosa pine seed orchards to provide improved seed for operational planting programs. Beginning in 2003, IETIC members began establishing a series of trials to test the effectiveness of using $GA_{4/7}$ to promote flowering in both young and mature ponderosa pine seed orchards. Earlier experiments by one IETIC member suggested that flowering response could be obtained using $GA_{4/7}$ alone without the addition of stem girdling. Treatments were designed to 1) identify the optimal timing of $GA_{4/7}$ application to induce flowering, 2) compare single versus pulsed (repeated) dose treatments of $GA_{4/7}$, and 3) compare the results of standard versus reduced dose treatments. Results from several seed orchards treated over a four year period will be presented and discussed.

A South-wide Rate Test of Esfenvalerate (Asana® XL) for Cone and Seed Insect Control in Southern Pine Seed Orchards

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As many as five monthly applications may be required each year to protect southern pine seed orchards from coneworms, *Dioryctria* spp. Insecticides targeted to control coneworms usually provide control of two other pests, the leaffooted pine seed bug, *Leptoglossus corculus*, and the shieldbacked pine seed bug, *Tetyra bipunctata*. Esfenvalerate (Asana® XL) is a pyrethroid insecticide that is effective for both coneworms and seed bugs. Aerial application of the maximum labeled rate of esfenvalerate can cause outbreaks of secondary pests such as scale insects and mealy bugs. These secondary insect outbreaks promote growth of unsightly sooty mold and reduce tree vigor and growth.

Previous ground-application studies indicate that seed bugs can be controlled with much lower levels of esfenvalerate than the maximum labeled rate. If it can be demonstrated that these reduced rates are also effective against coneworms, the amount of pesticide used may be reduced. This will make management more economical while not promoting the buildup of secondary insects.

A South-wide study was conducted to operationally evaluate the efficacy of reduced rates of esfenvalerate. Six orchards throughout the South were used in the study, five loblolly pine orchards and one slash pine orchard. Each orchard had four treatment plots. A complete block design was used with each orchard serving as a replicate. The four study treatments were: Asana® XL at the labeled rate of 0.19 pounds active ingredient/acre (ai/ac), Asana® XL at 0.10 pounds ai/ac, Asana® XL 0.03 pounds ai/ac, and a control consisting of untreated trees. Aerial applications were made five times at monthly intervals (May-August). Efficacy data collected were crop survival, yields of healthy and damaged cones, and seed yield. Each treatment was surveyed for secondary insects the following year.

All rates of esfenvalerate were effective in controlling seed bugs. First-year conelet survival, and percent good seed were significantly lower for the control when compared against the 0.03, 0.10 and 0.19 pound ai/ac application rates. The composite trait, good-seed per original-flower, gave the same results. However, the lower rates did not protect against coneworm damage. For the five loblolly pine seed orchards, coneworm damage at the 0.19 pound ai/ac was significantly lower than for the control or the two reduced rates. The two low rates did not result in secondary insect outbreaks.

The resulting management recommendation is that reduced rates of esfenvalerate may be applied only in combination with insecticides specific to coneworms, such as the growth regulator tebufenozide. This allows an optimal combination of efficacy with minimal use of pesticide and reduced risk of secondary outbreaks.

Systemic Insecticide Injections: New Effective Option for Several Conifer Seed Orchard Pests

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The efficacies of systemic insecticides emamectin benzoate, fipronil, and imidacloprid have been evaluated in conifer seed orchards during the past 3 to 9 years for preventing damage and mortality to cones by cone and seed insects. Injection treatments of emamectin benzoate and fipronil have been found to be consistently effective in reducing cone damage and mortality (80 – 95%) by coneworms in both slash pine and loblolly pine orchards for two years compared to untreated checks. Both chemicals are only moderately effective against seed bugs; reducing damage by 10 – 25% compared to checks. In contrast, imidacloprid is effective against seed bug, but less effective against coneworms. A recent trial also showed that emamectin benzoate has some activity against slash pine flower thrips. Plans to test injections of imidacloprid and dinotefuran alone or combined with emamectin benzoate and fipronil in 2007 for protection of seed crops against seed bugs will be described.

Use of Genetic Variation to Adapt Climate Change

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An unprecedented global warming is predicted and observed. The concern about its impacts on forest adaptation and productivity is increasingly overwhelming. Forest geneticists are facing the challenge to better understand the relationship between forests/ecosystems and climate, and to use genetic variation to adapt to climate change. Through the development of a high-resolution climate model (ClimateBC), analysis of a comprehensive lodgepole pine provenance trial, and modeling of ecosystems and species distributions in future climates, we explored the possibility of selecting populations and species for changing climate. ClimateBC can provide 75 climatic variables for 30-year normals, each of the past 105 years and future periods (2020s, 2050s and 2080s) predicted by different GCMs. Growth response functions based on the lodgepole pine provenance trial allows us to predict the productivity of populations with different climate change scenarios and identify populations with wider adaptation range and greater growth potential in future climates. Modeling of ecosystems and species distributions enabled us to use inter-species variation to adapt to climate change.

Genecological Responses in Western Conifers to Climate Changes Over the Past Two Millennia

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Vegetation changes in western North American mountains are assumed to shift up and down in elevation in response to climate change. In addition, local populations are assumed to be optimally adapted to their environments. We review these assumptions in light of recent analyses of forest tree responses to climate changes over the past two millennia.

First, we analyzed well-preserved downed deadwood at 3000 m on Whitewing Mountain in the eastern Sierra Nevada. These were dated to 800-1350 CE and comprised whitebark, western white, sugar, Jeffery, and lodgepole pines, and western hemlock. Excepting whitebark pine, these species are currently 200 m or more lower in elevation; sugar pine is not locally native. Using the joint overlap of the climate spaces for these species, we estimated the climate for the period of sympatry to be warmer (+3.2oC annual minimum temperature) and slightly drier (-24 mm annual precipitation) than present (Millar, Westfall QR 06).

We next examine results from Rehfeldt et al (1999), who analyzed growth relationships in 120 lodgepole pine seed sources with the climate at 60 common garden locations in British Columbia, Canada. They found that the fastest-growing seed source at a location was from a warmer location and that growth of most seed sources was greater at a warmer location than

the local one, suggesting that the populations not only lag current climate changes, but they did not fully adapt to the Little Ice Age climate (Westfall, Millar FEM 04).

Next, we compared tree-ring growth between living trees with those that died in three eastern Sierran limber pine stands following a persistent drought during the 1980s. Both the mean and GARCH-modeled interannual variance in growth was greater in the dead trees than the living during the 18th and 19th centuries, but mean growth was greater in the living during the 20th. Moreover, the dead trees were less responsive than the living to increasing winter precipitation under high minimum temperatures, implying that these populations have undergone adaptation to current climate changes (Millar, Westfall CJFR In press).

Finally, we studied recruitment of limber and bristlecone pines in the White Mountains, in eastern California. Limber pine is recruiting in much greater numbers than bristlecone above current treeline, which is dominated by bristlecone, and about 300 m above current limber pine treeline. Recruitment peaked during the 1980s and was associated with higher minimum temperatures and a low phase of the Atlantic Multidecadal Oscillation (Millar, Westfall AGU 06). In addition, recruitment was insensitive to minimum temperature under low winter precipitation, but increased with increasing minimum temperature under high precipitation.

Thus we find that episodic, threshold, and reversible changes are more common responses to climate in mountain ecosystems than are linear or gradual changes. Such responses will complicate conservation planning.

Environmental Effects on Relative Wood Density in Lodgepole Pine and Strategies for Improved Growth and Density Breeds

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Interior lodgepole pine (*Pinus contorta* Dougl. ex. Loud. var *latifolia*) is the most heavily planted tree species in British Columbia, Canada. The lodgepole pine breeding program is in its second generation of progeny testing which incorporates two breeds: (A) height growth improvement and (B) height growth and relative density improvement. An 'environmental map' free of genetic effects, was considered important to develop strategic deployment approaches for materials coming from the A or B breeding groups. To achieve this, environmental variation in wood relative density in lodgepole pine was examined by sampling from a 33 year-old provenance test planted at 60 test sites across the range of the species in British Columbia, using a set of six standard provenances.

Transfer curves (regression models with relative density as the dependent variable, and site climate variables as independent variables) were generated for each of the six standard provenances. Each regression equation was used to predict relative density at 370,000 grid points covering British Columbia. The predicted relative density values were converted to

principal components and mapped using ArcView. Climate change scenarios were used to generate climate data for 2055, which was also used as input for the model.

Maximum temperatures in the summer months, and summer heat to moisture index accounted for most of the variation in relative density, with R2 ranging from 0.74 to 0.84. These relationships were mapped with Geographic Information Systems, which showed significant patterns of relative density variation across the province, but were geographically related to decreasing density with increasing elevation and increased density in areas of higher precipitation. This environmental map did not present any obvious approaches of how to currently utilize the two breeds with respect to deploying low or higher relative density individuals, however, considering one of many possible future climate change scenarios, relative density may be adversely affected across the entire planting range so that the higher density breed may become more important than previously considered.

Concurrent Session A3 - Genetic Diversity/Gene Conservation

Evolution of the Forest Service's National Forest System Genetic Programs

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The USDA Forest Service National Forest System (NFS) land base includes 45 states that are divided into nine Regions. Acreages total approximately 188 million and are managed within 155 Administrative Units. Seventy-eight percent of the land base is out West, where most of the catastrophic wildfires occur. The National Forests were established in the early 1900's. The original focus was to provide an adequate supply of timber products for a growing population. The Forest Service began tree improvement (TI) programs in the early 1960's to better manage the variety of commercial species and capture their associated geographic variation. Traditional activities included superior tree selection, seed orchard and seed production area establishment, breeding and progeny testing. First generation seed orchards were established for many of the species, and second generation orchards for only a few. All orchards would provide genetically improved seed needed for reforestation, following timber harvesting, on the National Forests.

In the late 1980s NFS goals and objectives shifted from timber production to ecosystem management and conservation of biodiversity. Timber harvesting decreased up to 90% in

some regions, resulting in a drastic reduction in seed needs. The TI programs' objective of genetic improvement for quality timber was no longer the top priority. As a result, some orchard components and most progeny testing were terminated. In 1992 the National Genetics Strategic Plan was penned and in 2002 was revised to focus on three main objectives: genetic conservation, ecosystem restoration and partnerships. Consequently TI programs shifted from traditional tree improvement to genetic resource management. Genetic diversity of tree species became a priority, rather than furthering genetic gains in volume. Currently the Genetic Resource Management Programs (GRMPs) focus on meeting the seed needs for operational reforestation and restoration. Genetic work related to disease resistance continues. Genetic conservation targets preservation of pine and hardwood tree species being impacted by disturbances (i.e. fire, pests, diseases, climate change). Partnerships have been formed with universities, other federal, tribal and state programs, private industry and Forest Service Research facilities to support and strengthen the GRMP goals. One critical partnership is with NFGEL, the NFS National Genetics Lab in Placerville, CA. Scientists provide valuable assistance with genetic diversity and population genetic questions related to conservation, reforestation and restoration.

Ecosystem restoration, and maintenance and sustainability of forested lands on National Forests are long-term commitments. The GRMPs continue to manage current species in the orchards and clone banks, incorporate new species into the orchards and establish seed production areas on National Forests. Ensuring a stable supply of seed that is well adapted is critical for species' perpetuation.

Genetic Diversity and Hybridization in Natural Stands of Shortleaf Pine (*Pinus echinata* Mill.) and Loblolly Pine (*Pinus taeda* L.)

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Ninety-three shortleaf pine and 112 loblolly pine trees from 22 seed sources were sampled from Southwide Southern Pine Seed Source Study (SSPSSS) plantings in Oklahoma, Arkansas and Mississippi. These sample trees were grown from shortleaf pine and loblolly pine seed formed in 1951 and 1952, prior to extensive forest management throughout their geographic range. Amplification fragment length polymorphism (AFLP) markers were developed and used to estimate genetic diversity and hybridization in these pine species.

The 22 seed sources were grouped into 16 populations for the genetic diversity study. Grouping was made according to seed source geographic and physiographic region. Forty-eight primer pairs were screened, of which 17 produced 794 AFLPs in shortleaf pine and 21 produced 647 AFLPs in loblolly pine. Analysis of these AFLP data shows high genetic diversity in both shortleaf pine and loblolly pine, and most of the genetic diversity is within

populations. The high values of unbiased measures of genetic identity and low values of genetic distance for all pair wise comparisons indicted that the populations have similar genetic structure. The estimated high inter-population gene flow may explain the high similarity among the populations. High gene flow exists between eastern and western populations. For both species there was no correlation between geographic distance and genetic distance.

For the hybridization study, the 48 primer pairs screened revealed 17 primer pairs which produced 96 AFLPs polymorphic across loblolly pine and shortleaf pine. Two hybrids in the loblolly pine samples and two hybrids in the shortleaf pine samples were found using the IDH (isocitrate dehydrogenase) marker. Two more hybrids in the shortleaf pine samples were found combining the 96 AFLPs with IDH markers using software NewHybrids version 1.1 beta. This study suggested that later generation hybrids could be found using molecular markers and confirmed that IDH is a useful marker to detect F1 hybrids between the two species. Hybridization frequency varied geographically, ranging from 25% in Missouri to 0% in other sources in this study. Also, the hybridization level was higher in populations west of the Mississippi River than east of the river. The results suggest that the potential for the existence of hybrids or the creation of hybrids should be considered in forest management decisions.

Hurricane Katrina and Evolution of Adaptive Traits in Slash Pine (*Pinus elliottii*)

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Hurricane Katrina was the most devastating storm ever recorded in the United States. While wind velocities were not as great as Hurricane Camille in 1969, the tidal surge was much higher and covered a much wider area.

Slash pine (*Pinus elliottii*) and live oak (*Quercus virginiana*) are common on the beach and on the barrier islands, and appear to be well-adapted to hurricanes. This storm, with its 30 foot tidal surge of brackinsh water has almost eliminated slash pine on the islands and within 500 yards of the beach. Preliminary estimates indicate that only one in a hundred slash pine survived on the islands. This will surely create a genetic bottleneck if they do in fact survive. Possible effects on evolution of the populations are discussed.

Effects of the Decline of the American Chestnut and Harvesting on Genetic Diversity of Northern Red Oak in Western North Carolina

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Sustainable forestry requires assessment and preservation of genetic diversity. The maintenance of genetic diversity within a species is important for three central reasons: genetic diversity is necessary to retain resistance to climatic change and pest invasion; protection of species is needed to promote a stable ecosystem; and the maintenance of allelic diversity is important for potential value in future breeding practices. *Quercus rubra* (L.) (northern red oak) (NRO) is the most economically important hardwood species in western North Carolina. Despite efforts to facilitate NRO regeneration, success in western North Carolina has been very poor and the decline in population sizes may be resulting in loss of genetic diversity. This study used nine microsatellite markers to determine if NRO populations in western North Carolina have experienced a change in genetic diversity following two different ecosystem changes; 1) after harvesting, and 2) after the disappearance of the American chestnut (*Castanea dentata* L.) overstory from the southern Appalachian forests. Cambial samples were collected from approximately 250 northern red oak individuals located in four sites that have been harvested and four sites that have not been harvested since the demise of the American chestnut in the Nantahala National Forest. All of the sites used in this study were historically American chestnut dominated, and each harvested site was located within a mile of an unharvested site of similar forest type. Sampled NRO trees include those that were established when American chestnut was still the dominant overstory species, individuals that have established since the loss of the American chestnut, and individuals established pre- and post-harvest of a stand. The hypotheses tested in this study were; 1) genetic diversity of pre-chestnut blight northern red oak trees does not differ from that of trees established after the decline of the chestnut; 2) genetic diversity of northern red oak stands before and after harvesting does not differ. Population genetic variation was analyzed using ANOVA, as well as autocorrelation analysis. The relative genetic diversity of the population was quantified in terms of allele frequencies, observed heterozygosities (Ho), expected heterozygosities (He), effective number of alleles (Ae), and fixation index (F). Genetic structure was quantified using F-statistics. This presentation will summarize preliminary results of this study, and will offer preliminary conclusions.

Conservation Genetics within the US Forest Service: From Quaking Aspen (*Populus tremuloides*) to Rocky Mountain Bristlecone Pine (*Pinus aristata*)

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The National Forest Genetics Laboratory (NFGEL) provides genetic testing and information for integrated solutions to on-the-ground problems faced by natural resource managers and policy makers. Solutions are provided for public agencies, non-government organizations, and private industries across the United States, often spanning geographical and organizational boundaries. As part of the National Forest System, the laboratory has a strong partnership with the Pacific Southwest Research Station, both of the USDA Forest Service. NFGEL works closely with land managers to provide key genetic information that is relevant and timely for management decisions. Society's ability to establish and sustain healthy forests and rangelands—especially in the face of current pressures such as habitat fragmentation, climate change, and degraded ecosystems—requires an understanding of genetics. Information about genetics helps assess past, current and future biological changes, and provides implications for management options in the future. NFGEL uses state-of-the-art technology to address genetic conservation and management of all plant species using various laboratory techniques including DNA analyses. The lab provides baseline genetic information, determines the effect of management on the genetic resource, supports genetic improvement programs, and contributes information in the support of conservation and restoration programs, especially those involving native and TES (threatened, endangered, and sensitive) species. This presentation will discuss in some detail our work to look at 1) the clonal structure of quaking aspen in the western United States, and 2) the conservation of Rocky Mountain bristlecone pine in Colorado.

Progress in *Juglans cinerea* Conservation: Genetic Diversity, Health, and Introgression

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The abundance of *Juglans cinerea* L. (butternut) is declining due to the exotic fungus *Sirococcus clavigignenti-juglandacearum*, which causes branch and stem cankers that ultimately girdle and kill host trees. Butternut freely hybridizes with two exotic species, namely Persian or English walnut (*Juglans regia* to form *Juglans* x *quadrangulata*) and Japanese walnut (*Juglans ailanthifolia* to form *Juglans* x *bixbyi*). These hybrids are vigorous, difficult to distinguish from butternut, produce large numbers of fruit and appear to be more resistant to butternut canker than butternut. A combination of nuclear microsatellite markers, RAPD (Randomly Amplified Polymorphic DNA) and morphology were used to distinguish

Juglans cinerea from hybrids. Results are discussed in terms of their implications for understanding the extent of introgression in extant butternut populations. Because small populations typically exhibit reduced genetic diversity which, in turn, reduces fitness, we investigated genetic diversity and population structure. DNA was isolated from leaf samples collected from 422 butternut trees in five populations from throughout the species' native range and polymorphic nuclear microsatellites were used to estimate the genetic diversity and structure of these populations. Loci were highly polymorphic and most local populations contained at least one private allele. Heterozygosity levels indicate that the species remains locally genetically diverse despite high rates of infection and generally low levels of regeneration in the studied populations. Wright's F statistics indicate a slight overall heterozygote deficit and a moderate degree of genetic differentiation among local populations, results that suggest a trend toward isolation and inbreeding. Scattered healthy butternut trees, often associated with an uncommon "dark-barked" phenotype, have been documented among diseased and dying trees, which suggests the possibility of resistance. The current plans for resistance breeding for this species will be presented and discussed.

Advances in American Chestnut Somatic Seedling Production

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The American chestnut was one of the most important forest trees in the Appalachian Forest until the introduction of the chestnut blight fungus, which caused the death of virtually every mature American chestnut tree in the eastern United States. A system for mass propagation of blight-resistant material obtained through conventional breeding or gene transfer is still lacking. Thus, the goal of our project is to develop a high-frequency in vitro propagation system for American chestnut via somatic embryogenesis. Two bottlenecks in this approach are the low initiation rate of embryogenic cultures and the low production efficiency of plantlets (somatic seedlings) from the somatic embryos. To increase embryogenic culture initiation, we tested two plant growth regulators (2,4-D and picloram) at different concentrations and found that 2,4-D resulted in the highest frequency of embryogenesis (up to 3.5 %). This culture initiation experiment also demonstrated for the first time that highly productive embryogenic cultures could be initiated from immature seeds resulting from controlled crosses between known American chestnut parents. To increase plantlet production, we tested variations in cold (4° C) treatment duration (12, 15, and 18 weeks) and light quality (red, red + far red, and cool white fluorescent). For some genotypes, the longer cold treatments improved plantlet production and red light improved overall plantlet production frequency (up to 80% and 69%, respectively). Thus, by manipulating the cultural treatments, we were able to increase American chestnut somatic seedling production efficiency above the levels we previously reported. The first American chestnut somatic seedlings to be tested under nursery conditions were promising, growing up to 1.5 m in their first season. These advances in clonal propagation will aid in the restoration of the American chestnut to our forests.

Some Resistance Genes against *Cryphonectria parasitica* May Be Strain-specific

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The goal of The American Chestnut Foundation (TACF) is to produce hybrid American-type chestnuts (*Castanea dentata*) with adequate, long-lasting resistance to *Cryphonectria parasitica*, the fungus that causes chestnut canker disease. TACF is engaged in a backcross breeding program designed to transfer resistance from Chinese chestnut (*Castanea mollissima*). Achievement of adequate, long-lasting resistance may depend on having knowledge of interactions between Chinese resistance genes and different strains of the pathogen. Third backcross hybrids derived from the "Clapper" source of resistance, along with Chinese, American, and F1 (Chinese x American) hybrid control trees were inoculated with mycelium of two fungal strains. Resulting cankers were measured at 11-12 weeks.

Three independent lines of analysis suggest the existence of at least one and possibly up to eight genes with a moderate or high degree of strain-specificity:
- Individual trees with extreme forms of strain-specific resistance phenotypes exist.
- Strain-specific phenograms show distinct inflection points and randomization of the second strain.
- Categorical genetic models (simplified phenograms) based on strain-specific genes provide superior fits to observed data.
- Quantitative genetic models based on strain-specific genes provide superior fits to observed data.

If strain-specific genes are confirmed in studies of long-term resistance of TACF hybrids, there will be implications for 1) the purpose and design of the central TACF breeding program in Meadowview, VA, 2) the purpose and design of the regional breeding programs in eight states, and 3) then number of breeding schemes advisable at the central and regional levels.

Male Genotype Influences Seed Set and Seed Size in Controlled Crosses of American Chestnut (*Castanea dentata* [Marsh] Borhk)

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Five American chestnut trees (*Castanea dentata* [Marsh] Borhk) located in Indiana were used as mother trees in a full diallel breeding design in order to determine the influence of pollen donor genotype on seed set and seed mass. Pollen from ten American chestnut or American

chestnut hybrids was applied to the five mother trees. The seeds were harvested, counted, and individually weighed. A random effects model fit to the data showed that both female ($F= 18.10$, $p < .001$) and female by male cross ($F = 1.54$, $p = 0.0445$) contributed significantly to the variance in seed mass. Similarly, both female and female by male cross accounted for significant variation in seed set ($F = 10.98$, $p <.001$ and $F = 1.67$, $p = 0.0205$, respectively). A general association test showed male genotype and seed set to be statistically associated ($\chi 2 = 26.97$, $p = 0.0014$). A variance components model fitted to the data showed that individual female by male crosses explained 28.3% and 15.8% of the variation in seed mass and seed set, respectively. Individual crosses differed significantly in the amount of variation they explained, from 0% to 61.5% of variation in seed mass and from 0% to 67.6% of variation in seed set. These results demonstrate the influence of male genotype on seed mass and seed set and are the first to show differential male and female by male performance on seed characters of American chestnut. The seed from this experiment are currently being germinated to assess seedling vigor. The results will be combined with the results of the study above to present a fuller picture of the influence of pollen donor genotype on seed and seedling characters in American chestnut.

Concurrent Session B3 - Selection/Growth and Yield/Valuation

Improvements in Stem Form and Growth of Elite Genotypes in Loblolly Pine

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For over fifty years the genetic improvement of loblolly pine for growth, form and disease resistance has produced significant gains for plantation forest production. Additional value has been added to forest plantations through the improvement of stem straightness, but other stem form traits such as branching and sweep are also important. While previous research showed that volume was the most important trait for economic return, there is increased interest in capitalizing on genotypes with desirable form for sawtimber production. The Lower Gulf Elite Population, a joint breeding effort among 3 cooperative breeding programs in the southeastern United States, provides a unique opportunity to assess the potential stem form improvement of elite loblolly pine selections. Forty-eight parents from three cooperative breeding programs were bred in a series of six 8-tree disconnected diallels, comprised of selections from the Atlantic Coastal Plain, Florida, and Livingston Parrish provenances. Previous results from a complementary polymix test series of the same parents demonstrated 6-year volume gains exceeding 40% over local checks. An analysis of six year growth, disease, and stem form traits will be presented.

Long-Term Selection for High and Low Oleoresin Production in Loblolly Pine

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In pines of the southeastern United States abundant flow of constitutive oleoresin is the primary course of action that provides trees with the means to successfully resist attack by southern pine beetle (*Dendroctonus frontalis* Zimmermann). This bark beetle is the most destructive insect pest of pine forests of this region, and currently, little is known about genetic properties of host resistance against attacks by this mid-bole inhabiting insect. Recent research, however, has revealed that substantial genetic variation exists in oleoresin yield in populations of loblolly pine (*Pinus taeda* L.), and that the phenotypic distribution for this trait is characterized by a large proportion of trees with low oleoresin yielding capacity. Given this information, it is reasonable to conjecture that the resin yielding ability of loblolly pines can be drastically changed in both positive and negative directions through a program of selective breeding. To test this hypothesis and to obtain information about the genetic characteristics of this form of resistance to bark beetle herbivory, geneticists at the Southern Institute of Forest Genetics together with entomologists in the US Forest Service's Southern Research Station have initiated a long-term genetic selection experiment to determine whether high and low oleoresin yielding lines can be developed in loblolly pine. Initial selections have been made from a base population that includes progeny from trees that survived earlier southern pine beetle infestations. Crosses among these selections are planned to produce individuals for the second generation of selection. In this report, we describe this selection experiment as well as predictions concerning outcomes that could result from the first generation of selection.

Genotype by Environment Interaction Under Optimal and Deficient Nutrient Regimes in Loblolly Pine at Age 12

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Year 12 growth data from the SETRES-2 study site were analyzed for growth differences due to nutrition, provenance, and family effects. Established in the winter of 1993-94, SETRES-2 is a loblolly pine (*Pinus taeda* L.) genotype by environment interaction study located in the Sandhills of Scotland County, NC. The study is a split-split plot experimental design, with

nutrition (annual optimum fertilization and non-fertilized) main plots, provenance (Atlantic Coastal Plain and "Lost Pines" Texas seed sources) sub-plots, and family (five OP genetic families per provenance) sub-sub-plots nested in provenance.

Differences in growth traits due to nutrition were highly significant at age 12. Fertilized plots showed a 66.2% increase in height over the non-fertilized plots. Diameter at breast height was 51.2% greater in the fertilized plots. Mean tree volume for fertilized plots was increased 183.0% over non-fertilized, and as a function of the above effects, volume per acre was 157.2% greater for fertilized plots. Genotype by environment interactions in height and diameter at breast height were limited to differences in magnitude of response to fertilization. Current annual increment (CAI) for volume per acre, however, showed a cross-over interaction between the provenances in the fertilized nutrient regime plots beginning at age 10. Prior to age 10, CAI for volume per acre was greater in the Atlantic Coastal Plain (ACP) provenance than in the 'Lost Pines" Texas (LPT) plots; after age 10 the volume per acre CAI was larger in the LPT source than the ACP source. This interaction continued through age 12. As survival was beginning to drop more rapidly in the fertilized ACP plots, likely due to competition induced mortality, the increase in individual tree volume growth rates was insufficient to match the volume produced by a greater number of remaining stems in the LPT plots.

Family mean rankings over time were very stable for height and diameter. The greatest rank changes occurred in the CAI for volume, with most families changing rank at least once between ages 5 and 12 in the fertilized treatment. This study is beginning to give insight into the behavior of improved loblolly pine genetic stock in block plots, and as the stand matures, will continue to provide valuable insight into the issues associated with modeling gain in operational improved loblolly pine plantations in the Southeastern U.S.

Genotype X Environment Interaction in Florida Sources of Loblolly Pine Across the Lower Coastal Plain of the Southeastern USA

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Florida sources of loblolly pine (*Pinus taeda* L.) have previously been reported to exhibit a high level of genotype x site interaction (GxE) when compared to other sources of loblolly in one set of progeny tests established by the Cooperative Forest Genetics Research Program (CFGRP). This may indicate the need for regionalization of breeding and deployment by separating sites on the coastal plains of the Atlantic and Gulf costs to minimize the level of within-region GxE. The study presented here aimed to elucidate patterns of GxE in Florida loblolly pine utilizing data on individual tree volume at 10-15 years of age collected from a total of 36 tests (27 open-pollinated and 9 control-pollinated tests) established by the CFGRP and its members across a diverse range of sites on the lower coastal plain of the south-eastern

USA. These tests were established over a 19-year period from 1970 to 1988 and comprised: five, largely genetically disconnected series of progeny tests; and, progeny from 117 plus-trees selected in a number of Florida counties, but chiefly from the Marion (46), Levy (22) and Nassau (19) counties. Climatic data was collated for each of the test-sites for the period from planting to the age of measurement. This climatic data, along with site characteristics calculated from the growth data (i.e. site index, and rust hazard) were used to classify the environment types represented by the progeny tests. Sites spaned a broad range of environment-types: Greene Co., MS to Levy Co., FL and Laurens Co. GA; site indexes ranging from 15.5 to 37.1m; and, rust hazard varying between 2 and 98%.

Heritability estimates for individual tree volume varied considerably between tests – average of 0.16 ± 0.19, and range between 0.00 and 0.62. Type B genetic correlations estimated within each series, and varied between a low of 0.39 ± 0.095 to a high of 0.84 ± 0.092. If data were available only from an individual test series, this would have indicated that GxE for volume in Florida loblolly was variously either very high or inconsequential. By contrast, rust incidence generally demonstrated much less GxE with estimated type B genetic correlations varying between 0.71 ± 0.16 and 1.0 ± 0.00 across the test series. Within each test series, multivariate analyses of variance were conducted to estimate the genetic correlation between each pair of tests. Results tended to indicate that particular tests or test combinations were largely responsible for the low, pooled type B genetic correlations across all tests within a test series. Pattern analysis (i.e. a combination of ordination and classification) of the climatic data, indicated that it was possible to delineate environment-types based on a set of minimum temperature variables, which broadly (with some exceptions) followed geographic location of the tests. On the basis of these results it is believed that the observed GxE in volume of Florida loblolly pine can largely be explained by: a) testing across frost-hardiness zones, b) inclusion of a few atypical test sites (e.g. calcareous soils or Phosphorus-deficiency), c) large differences in rust hazard, and possibly also, d) problems with test establishment and management.

Integer-Programming Approach to Group-Merit Selection

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The ultimate goal of artificial selection is to achieve high genetic response in a given trait(s). In real-world populations, the unavoidable effect of selection is the build-up of genetic similarities within and among individuals (reduction of diversity). This in turn limits the attainment of genetic response in later generations. In reality, the approach is to select n individuals out of N candidates, where $n < N$. In order to alleviate the contradictory effects, one may impose certain relationship restrictions to these selections (e.g. maximum tolerable number of half-sibs in the selected set). More efficient approaches have been developed so far. One of them is the group-merit selection, where the goal is to maximize group merit of a

selected set, with particular importance assigned to the diversity. The approach to this maximization taken so far consisted of a series of numerical iterations that have been relatively robust in many practical situations. However, as tree breeding approaches to advanced generations and pedigrees become more complex, finding the best set of individuals becomes more challenging. Therefore, we rephrase (without giving the theoretical development) the problem to the integer programming, and show how to search for solutions using commercially available optimization software tools. We focus on demonstration of the tool using small examples, particularly how to identify the best set of n individuals given the pedigree information and breeding values of N candidates. Finally, we discuss the applicability of the proposed approach in solving real-world examples in forest tree breeding programs, and outline the future development.

Diallel Crossing in *Pinus cembra*: V. Age Trends in Genetic Parameters and Genetic Gain for Total and Annual Height Growth across 16 Years of Testing

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A full diallel mating design (p=10 parents) was carried out in a natural population of Swiss stone pine (*Pinus cembra* L.) from the southern Carpathian Mountains. At age six, after nursery testing, the material was field planted in one site, using a completely randomized block design with 100 families, four replicates and 15 tree row-plots per replication, spaced 2.5 x 2.5 m. Total and annual height growth were assessed at successive ages across a 16 year testing period (i. e. nursery test between ages one to six and field test between ages seven and 16). Plot means of the measured traits were analyzed using the general least-squares method by means of the computer DIALL programme prepared by SCHAFFER and USANIS (1969).

Across the testing period, significant ($p<0.05$) and highly significant ($p<0.01$; $p<0.001$) differences occurred in total height growth for general and specific combining ability effects. These results suggest that the two traits were controlled by nuclear additive and non-additive genes. In an ascendant trend, the additive variance, as a percent of the total genetic variance, ranged between 5 % at age six to 67 % at age 16 for total height growth while that for annual height growth ranged between 33 % at age four to 78 % at age 16. In a descendent trend, the dominance ratios $\square^2$SCA / $\square^2$GCA for total height growth ranged between 8.2 at age two to 0.3 at age 16, suggesting that the additive variance could be used in a breeding programme. Parents with significant general combining ability effects for the two traits were found. For total height growth, the narrow-sense family mean heritability estimates varied in an ascendant trend between 0.06 at age two and 0.65 at age 16 while the narrow-sense individual tree heritability varied between 0.02 and 0.37. Age-age additive genetic correlations for total height growth rose from 0.85 at age two to 0.95 at age six and then leveled off across the field test indicating that if the goal is to improve 16-year height, early selection can be considered at age six. By selecting the best 20 families and the best 20 % of individuals within families, a genetic gain in total height growth of 8.8 % and 9.9 %, respectively, could be achieved at age

16. The improvement of growth using both family and individual selections could be applied. The very high age-age and trait-trait genetic correlations suggest that both early and indirect selection could be applied effectively.

Modeling Genetic Gain in Douglas-fir Growth Models

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Growth modeling of genetic gain has typically taken one of three approaches: site index adjustment, effective age computation, or growth modifiers. Each method involves certain assumptions that may or may not be easily tested with available data. We used the growth multiplier procedure to estimate the impact of genetic tree improvement on long-term growth. The reason for using this method was that we were limited to single-tree-plot progeny test data which can be used in individual tree / distance independent growth models. Such models, such as ORGANON and FVS, are frequently used in the Pacific Northwest. While growth modifiers can be used in other model constructs, the progeny test data provide sufficient information to modify the individual tree model.

Genetic-gain multipliers were calculated based on the BLUP breeding values of parent trees and the incremental growth measurements of the progeny tests from which the BLUP values were obtained. Results from our study suggest that constant genetic-gain multipliers can be used for stands 10 years and older; however, multipliers need to be increased for younger stands. How the multipliers are used depends on stand age and whether actual stand data or a "typical" tree list is used. The genetic gain reflected in the input tree list is as important as the multiplier in many situations. Growth projections for some example stands indicate that the percentage of volume gain in improved stands will decrease sharply between age 10 and 20 years as higher early gains give way to lower gains later in the rotation; however, absolute gains continue to increase during this time period. Gain projections are sensitive to the maximum stand density allowed by the model, raising the question of whether tree improvement increases maximum density or only allows the maximum to be reached earlier in the rotation.

Probing the Potential Value of Enhanced Genetic Stands

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Mass Controlled pollinated (MCP) and varietal somatic embryogenesis (SE) technology loblolly pine (*Pinus taeda* L.) seedlings are becoming available on a large scale. These seedlings are superior to the best open-pollinated (OP) seedlings in both production and stem quality. MCP and SE seedlings cost more than OP seedlings, but the higher degree of growth uniformity, lower inherent tendency to allocate growth into branch development, and lower rust infections permit decreasing the initial planting density and adjustments in silvicultural cost. To permit potential buyers of MCP and SE seedlings to determine if the extra seedling cost is a wise investment, an estimate of their impacts on yields and stand value are needed. Unfortunately block plantings of MCP and SE material are few, and most of the growth data is less than eight years old. However, these young stands do provide us with early estimates of growth gains, improvements in percent sawtimber trees, and the amount of reduction in fusiform rust that can be expected. What is needed are realistic projections of the yields and value of rapidly growing, high quality stands to the end of the rotation (23-25 years). This presentation utilizes the actual production of a 13-year-old varietal screening control-cross and a selected variety from within this cross to serve as a solid platform to project:

- The expected value of the control-cross stand (exhibited SI-100)
- The expected value of a stand of the best selected variety (exhibited SI of 105)
- The expected value of the control-cross stand but with improvements in the percent sawtimber that is being reflected in MeadWestvaco (MWV) young block plantings.
- The expected value of a varietal stand with current observed improvements in percent sawtimber.
- An estimate of the previous rotation stand yield and values.

The basis for and results of these simulations will be presented and discussed.

Genetic variation and control of chloroplast pigment content in *Picea rubens, Picea mariana,* and their hybrids, under ambient and elevated CO2 environments

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Traits related to light-energy processing have significant ecological implications for plant fitness. Our objective was to examine and compare chloroplast pigment content traits from a red spruce (RS) (*Picea rubens* Sarg.) – black spruce (BS) (*P. mariana* (Mill.) B.S.P.) genetic

complex under ambient and elevated CO2 conditions. We used two genetic experiments: 1) a comparative species' provenance experiment from across the near-northern part of the RS range, and 2) an intra- and interspecific controlled-cross hybrid experiment. Results from the provenance experiment showed total chlorophyll content (a and b) was, on average, 15% higher under ambient than elevated CO2 ($P < 0.001$). Under ambient CO2, BS populations had, on average, 11% higher total chlorophyll and carotenoid content than RS populations ($P < 0.001$). There were significant species, CO2, and species x CO2 interaction effects, where chlorophyll content decreased on average 7% and 26% for BS and RS, respectively. Results from the hybrid experiment showed hybrid index 25 (25% RS) had the highest total chlorophyll content, and hybrid indices 75 and 100 had among the lowest. Initial analysis of the hybrid experiment supported a more additive model of inheritance; however, parental analysis showed a significant and predominant male effect for chlorophyll content. Crosses with BS males had 10.6% and 17.6% higher total chlorophyll content than crosses with hybrid and RS males under ambient and elevated CO2 environments, respectively. Our results show a strong genetic control of chlorophyll content, and that these traits have a positive correlation with productivity within and across species. A significant positive correlation between chlorophyll content and nitrogen assimilation rate was also found ($r = 0.872$). Results from a light x water environment effects will also be presented. Results indicate that RS would most probably be at a competitive disadvantage in a higher CO2 environment.

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