Proceedings

32nd Southern Forest Tree Improvement Conference

Clemson, SC
June 10 – 13, 2013

Proceedings of the 32nd Southern Forest Tree Improvement Conference

Edited by

Michael W. Cunningham
ArborGen Inc
Tallahassee, FL 32318

The papers and abstracts in these proceedings were submitted by the authors as electronic files. Modifications were made in format to provide for consistency and to allow best possible placement of figures and tables. The authors are responsible for the technical content of their respective papers.

Proceedings of the 32nd Southern Forest Tree Improvement Conference

Clemson, SC
June 10 – 13, 2013

Sponsored Publication No. 54 of the Southern Forest Tree Improvement Committee

Foreword

The 32nd Southern Forest Tree Improvement Conference (SFTIC), marking 62 years of biennial technical conferences, was held June 10-13, 2013 in Clemson, South Carolina under the auspices of the Southern Forest Tree Improvement Committee and in cooperation with Clemson University's College of Agriculture, Forestry and Life Sciences and ArborGen Inc. The theme for this year's meeting was "Advancing the Value of Forest Plantations". For three days foresters, tree breeders, scientists and practitioners met and listened to invited and volunteer presentations describe the many ways that forest genetics adds value to our region's and our nation's forests.

Three awards were presented for outstanding contributions to the conference, and the Southern Forest Tree Improvement Committee thanks these individuals for their contributions:

The **Tony Squillace Award** is given for the best oral presentation based on content, style, and use of visual aids. There was a tie this year and the co-winners were Ross Whitten of North Carolina State University for his talk entitled "Impacts of cost-effective high-throughput genotyping of loblolly pine on applied tree breeding programs" and Katherine Smith from the University of Florida for her talk entitled "Fungal Effectors of Cronartium quercuum f.sp. fusiforme".

The **Bruce Zobel Award** is given for the best oral presentation by a student. The winner was Christine Holtz from the University of Georgia for her talk entitled "The influence of Chinese chestnut genome proportion on the success of somatic embryogenesis in chestnut".

The **Belle Baruch Foundation Award** is given for the best poster. This year we had a tie and the co-winners were Laura Townsend of North Carolina State University for her poster entitled "Identifying Genetic Variation in Site Adaptability in Loblolly Pine" and Kelsey Dunnell of North Dakota State University for her poster entitled "A medium throughput greenhouse phenotyping assay of Populus spp. for Septoria canker resistance". The third place poster award went to Xinfu Zhang of Clemson University.

The 32nd SFTIC was a success, in part, due to the talent and effort of Susan Guynn, Extension Associate of Clemson University's School of Agricultural, Forest and Environmental Sciences. Our sincere gratitude goes to her. The SFTIC Committee also thanks all the staff, graduate students and faculty in the School of Agricultural, Forest and Environmental Sciences who contributed to the success of the conference.

The 32nd SFTIC Planning and Scientific Committee:
Pat Layton (Conference cochair), Clemson University; Michael Cunningham (Conference cochair), ArborGen Inc; Haiying Liang, Clemson University; Patrick Cumbie, ArborGen Inc; Chris Rosier, ArborGen Inc; Dana Nelson, SFTIC Chair USDA Forest Service; Tom Byram, Texas A&M University; George Askew, Clemson University; and Greg Reighard, Clemson University

CONFERENCE SPONSORS:

RAYONIER

Rayonier

Value From The Ground Up

PLUM CREEK TIMBER CO.

PlumCreek

Growing Value from Exceptional Resources

CLEMSON UNIVERSITY

CLEMSON

School of AGRICULTURAL, FOREST, AND ENVIRONMENTAL SCIENCES

ARBORGEN

ARBORGEN

USDA FOREST SERVICE

CONFERENCE PATRONS:

NCSU TREE IMPROVEMENT

TREE IMPROVEMENT PROGRAM

THE WESTERVELT COMPANY

the Westervelt Company

INTERNATIONAL FOREST COMPANY

In Memoriam
Daniel Martin Schmitt
February 19, 1924 – March 24, 2011

Dan Schmitt had a distinguished career in forest genetics research doing early work on the reproductive biology of sweetgum (Liquidambar styraciflua). He was born in Chicago, and served in the US Army in WW II with an artillery unit in France. He received a forestry degree from Utah State University in 1949 and cruised timber for the old Office of Blister Rust Control from 1947 to 1950. After receiving a master's degree in forestry from the University of Toronto in 1952 he worked for the Bureau of Land Management in interior Alaska from 1952 to 1957. Dan told me that this was often a very isolated existence, but Tom Conkle recalls that some time after he arrived in Raleigh he received a mysterious package of smoked salmon wrapped in newspaper from an Eskimo woman in Alaska. Dan said he did not mind the isolation.

When the BLM wanted to move him up to an administrative position, Dan decided it was time to move on. He resigned, and started graduate studies at the University of Florida under Dr. Tom Perry. When Dr. Perry moved to North Carolina State College, now University, Dan moved with him and finished his PhD there in forest genetics.

In the early 1960's, Dan took a research position at the Southern Institute of Forest Genetics in Gulfport, Mississippi. When I arrived in June of 1967, the current project leader, John Barber, was preparing to leave to take a staff position in Washington, DC, and Dan was appointed to take his place as PL. Dan was a great Project Leader, leading with an unassuming and non-combative style, yet managing effectively. I certainly thrived under his non-invasive supervision. He assigned a senior Research technician to work for me, Norm Scarborough, arguably one of the best forestry technicians in the Southern Forest Experiment Station. I once asked Dan why he transferred Norm to my supervision rather than to one of the more senior scientists (with more clout), and he replied: "Because you came to us in woeful ignorance". This was typical Dan Schmitt. He knew my background was in botany, with no experience in forestry, and my last position was teaching biology at Inter American University in Puerto Rico. I learned a great deal from Norm.

Dan did research on sweet gum, as well as southern pine hybrids at Gulfport, but the weight of administering the project finally caused Dan to make another change. I recall him announcing "If I'm going to be a (#%@*) administrator, then I'm going to be an administrator". He took a job as Assistant Director in the Northeastern Forest Experiment Station in Upper Darby, PA, in 1972. His later duties included managing the Spruce Budworm Research Program.

While Dan was working for the Forest Service, he carried on a long-term, long-distance romance and then marriage with Valda Elga Lammas, a technician in Dr. Tom Perry's lab. Valda escaped to Germany from Latvia when the Russians invaded, and later immigrated to the US. When Dan retired from the Forest Service, he moved back to Raleigh, and he and Valda enjoyed their retirement traveling. Valda passed away about 1 ½ years after Dan's death.

Ron Schmidtling
Research Geneticist (Emeritus)
Southern Institute of Forest Genetics

In Memoriam
E. Bayne Snyder
1920 – 2012

 Bayne Snyder was one of the true pioneers in tree improvement research in the South. He originally came from the East, where his early childhood was spent in Pennsylvania and Maryland. He attended high school in St Petersburg, Florida, and returned to Pennsylvania to attend Dickinson College. He did graduate work at Iowa State and finished a PhD degree at the University of Wisconsin in Crop Science and Mathematics, with a dissertation on sorghum genetics. After graduate school, Bayne conducted pioneering research on the genetics of rubber trees in Sumatra, Indonesia.

When he began his Forest Service research career in the early 1950's at the Southern Institute of Forest Genetics (SIFG) in Gulfport, Mississippi, intense interest in forest genetics and tree improvement in southern pines was just developing. At a time when most tree improvement people originated in forestry, his experience with breeding crops and rubber trees gave him a unique perspective for breeding southern pines.

Organizing and codifying was Bayne's forte'. One of the first articles he wrote after arriving at SIFG was a glossary of terms for use in forest tree genetics and tree improvement. He was tireless in developing improved techniques for various tasks such as pollen handling and controlled crossing, and even wrote the standard manual for safely climbing trees. When I began my tenure at SIFG in 1967, Bayne had just finished organizing a system for filing all of our reprints in the library. He later developed a system for breed-tree tracking and trait identification using a needle-sort system.

Left to right – Bayne Snyder, Gene Namkoong, Ron Schmidtling.   SIFG Harrison Experimental Forest 2001.

Early in his tenure at SIFG he became interested in longleaf pine, arguably the most valuable of the southern pines, but neglected because of silvicultural problems. One could see the influence of his education in the crop sciences, as he initiated a diallel crossing study in longleaf pine. The study included 13 random parent trees crossed in all combinations with reciprocals and selfs. Because of the vagaries of pollen and female strobilus availability year-by-year, one can only imagine how many years were required, and how many times the trees were climbed to make the required 169 crosses (plus 13 self-pollinations). Bayne did a lot of the tree climbing himself. Of all the scientists and technicians, he was actually the best tree climber. He continued to climb trees until he injured his neck surfing at Destin, Florida in 1967.

    The longleaf diallel study was planted in 1960, and when I arrived in 1967, it had just been measured for the second time. The size and complexity of the design for the study was giving the computer people at N.C. State University heartburn, because computer hardware and software was so much more limiting at that time.

    Bayne's experience with longleaf pine lead him to disagree with the application of mass selection for tree improvement programs of all southern pine species, which was the accepted orthodoxy at that time. His work with genetics of the duration of the "grass" stage and brown-

spot resistance convinced him that progeny testing followed by selection was the logical path for improvement of longleaf pine. But Bayne was no salesman. His views were not accepted until much later.

Bayne mentored a number of younger scientists, including Gene Namkoong, who started his career at SIFG in 1958 (Bayne's oldest daughter Elaine was the Namkoong's babysitter). At Bayne's urging, Gene left Gulfport in 1960 to finish his graduate studies at North Carolina State University, but remained attached to SIFG. Gene eventually won the Wahlenburg prize for his work in the genetics of forest trees.

Bayne had a great appreciation for the out-of-doors. He was an avid hiker and camper and often hosted boat trips to the barrier islands. He was an environmentalist. When he retired in 1979, he moved to Fairhope, Alabama, and built a solar-powered home. He was a member of the Sierra Club, the Natural Resources Defense Council, the Union of Concerned Scientists, and the Population Institute.

He was preceded in death by his wife, Annabelle, in 2006, and is survived by four daughters, Elaine Snyder Conn, Janet Carol Snyder, Marilyn Budzynski (Ed), and Gwen Snyder (Phil Strniste); six grand children, Chris and Carissa Budzynski, Paul and Rachel Conn, and Christopher and Jeff Cundey; one great granddaughter, Allison Conn.

Ron Schmidtling
Research Geneticist (Emeritus)
Southern Institute of Forest Genetics
USDA Forest Service, SO
Saucier, MS

In Memoriam
John Clyde Adams
December 11, 1947 - November 14, 2012

Dr. John Adams retired as Director of the School of Forestry at Louisiana Tech University in 2011. He served as a professor in the school since 1976, where he taught such courses as introduction to forestry, fire, and silviculture. His research interests were in hardwood genetics and silviculture. He was an active member of the Southern Forest Tree Improvement Committee, where he served several terms as Forestry School representative during the mid-2000s. He attended Louisiana State University where he earned a B.S, M.S., and PhD in forestry. Prior to his forestry education and career, John served as an officer in the Marine Corps and served a tour of duty in Vietnam.

John was a big man with a big heart. He loved and dutifully served his family, his faith and his profession. His son, Joshua, is currently an Assistant Professor in the School of Forest Resources at the University of Arkansas- Monticello, and continues his role on the Southern Forest Tree Improvement Committee. To further the development of future forestry students, the Louisiana Tech University School of Forestry has established a Scholarship fund in John's name. The tree improvement community may have lost a giant, but his legacy will live on.

Table of Contents

x

GROW IT AND THEY WILL COME

Gene Kodama

South Carolina Forestry Commission, Columbia, SC

Forest industry is a leading driver of state economies across the South. In South Carolina, forestry is the number one manufacturing industry in terms of the number of jobs and labor income. Our 20/15 Initiative is focused on growing our $17.4 billion economic impact to $20 billion by 2015. To accomplish this aggressive goal, we must implement strategies that encourage landowners to reforest after harvest and afforest idle acres. Tree improvement programs have resulted in more alternatives in planting stock than ever before. Silvicultural treatments enhance the potential of advanced genetics to make dramatic improvements in site productivity on timberland. Active management on more acres will increase the amount of wood grown across the South and result in continuing growth of forest industry. The role of the South Carolina Forestry Commission and other state forestry agencies is to support tree improvement efforts and to promote tree planting and active management as a way to grow jobs and improve the overall economic impact of forestry in our state. Reliable sources predict global wood demand to exceed supply in the near future. Therefore, the South is in a favorable position to grow more wood and expand on its strong position as the world's wood basket.

PERSPECTIVES OF INSTITUTIONAL TIMBERLAND OWNERS ON MODERN TREE IMPROVEMENT TECHNOLOGIES

Anthony Cascio

Resource Management Service, LLC, Birmingham, AL

Owners of timberland having financial objectives for ownership have historically employed genetically improved seedlings. While the projected financial rate of return from the utilization of improved genetics is a driving factor in the decision process, various risks exist that temper the decision. Such risks include assessing: the 'gain' projections; the markets' ability to model the improvements with respect to future potential buyers of the timberland and appraisers who periodically value the timberland; and susceptibility to catastrophic loss due to reduced genetic diversity. Given these risks, the strategy for inclusion of advanced genetics becomes more complex and will likely vary among owners.

ANTICIPATING THE FUTURE OF FORESTS AND USES IN THE SOUTH: IMPLICATIONS FOR FOREST MANAGEMENT

David N. Wear

Center for Integrated Forest Science and Synthesis, USDA Forest Service Southern Research Station, Raleigh, NC

Contemplating the future of forests can involve either the study of historical trends or the modeling of the variety of social and environmental forces that reshape forests and the services they provide. A combination of the two can be used to inform not only what the future might hold, but also where the future is least certain. Using the recently completed Southern Forest Futures Project and Forest Service Resource Planning Act Assessment as a foundation, this talk explores the potential futures of forests in the South as a series of alternative trajectories and identifies some key uncertainties. One key issue behind forest forecasts is the demand for land in other uses. These assessments indicate that a long period of forest accumulation in the United States may be reversed over the next decade as urbanization continues apace but also as returns to cropland have and are expected to grow. A declining forest area could encourage more intensification and concentration of forest management, especially in the South. The area of planted pine forests would likely expand—as it has over the past three decades—with a consolidation of output from these forests. Expanding human populations in many areas of the South lead to concerns about the ability to actively manage forests in many areas, but management consolidation provides an offsetting effect. These and other anticipated changes may indicate that southern forests are on the cusp of a period of rapid change.

MODELING GENETIC IMPROVEMENT EFFECTS ON GROWTH AND VALUE OF FOREST PLANTATIONS

Harold E. Burkhart

Department of Forest Resources and Environmental Conservation, Virginia Tech, Blacksburg, VA

Genetic improvement has resulted in increased growth rates, improved tree-stem quality, and enhanced value of forest plantations. In the case of loblolly pine, genetically improved material ranging from open pollinated to control pollinated to clonal is available, but the cost of the seedlings varies significantly. Landowners and forest managers need reliable information on expected productivity – in terms of both wood quantity and quality – to make informed decisions on selection of planting stock, silvicultural treatments, and rotation ages. Early work with data from provenance and open pollinated plantings indicated that an adjustment in site index was sufficient to account for changes in stand basal area, volume and mortality (Buford and Burkhart, 1987). As genetic improvement has advanced, modification of site index alone is not adequate due to changes in tree allometry (height-diameter relationships), tree stem form and quality, disease resistance, and other factors.

Analyses were carried out to examine height-age relationships in a loblolly pine genetics screening trial. Further analyses of height-diameter relationships, height and diameter distributions, and stem quality were conducted using data from block plantings of open-pollinated (OP), control-pollinated (CP), and clonal stock established at two initial planting densities.

MATERIALS AND METHODS

The genetics screening trial consisted of 120 clonal genetic varieties in 10 single-tree blocks (Sabatia and Burkhart, 2013a). The clones were rooted cuttings developed by MeadWestvaco Company from a controlled cross between two half-sib families. The study was established in the spring of 1994 on an old-field site near Summerville, South Carolina, in the Atlantic Coastal Plain physiographic region. Initial spacing was 2.74 m by 2.74 m with two rows of buffer trees around the study. Height data used were obtained from clones with at least five ramets that had no history of damage or extreme suppression by age 15. Eighty-six of the 120 clones in the study satisfied these criteria.

Data were also obtained from a block-planted experiment established with four genetic varieties in spring of 2002 to investigate the effects of planting density (PD) on stand level growth and development. The study, near Summerville, South Carolina, in the Atlantic Coastal Plain

physiographic region, was established on a cutover site and was a completely randomized factorial design with four levels of genetic variety treatment of (OP, CP, and two clones) and two levels of PD treatments (680 trees/ha (TPH) and 1360 TPH). The study consisted of 24 contiguous 0.1781 ha plots as experimental units on which the inner 0.0526 ha was the measurement plot. Site preparation and management of competing vegetation and nutritional deficiencies on the study plots was done according to operational management prescriptions. Each 0.1781 ha experimental plot had 10 beds each 48.2 m long and spaced 3.7 m apart. Trees along the beds were spaced 4 m from each other in the 680 TPH PD treatment and 2 m from each other in the 1360 TPH plots. The 0.0526 ha measurement plot was made up of the inner six beds with the inner 6 trees per bed in the 680 TPH PD and the inner 12 trees per bed in the 1360 TPH density. Diameters and heights were measured during the 2009-10 dormant season when the stands were eight years old.

RESULTS AND DISCUSSION

The effects of genetic variety on the parameters of the height-age relationship were investigated for the 86 clones using the Chapman-Richard's height-age equation

$$H_{iA} = \beta_1 \left(1 - \exp\left(-\beta_2 A\right)\right)^{\beta_3} + \varepsilon_{iA} \tag{1}$$

where H_{iA} is the total height of the ith genetic variety at age A, β_1 is the asymptotic height parameter, β_2 is the rate parameters, β_3 is the shape parameter, and ε_{iA} is a normally distributed zero-expectation random error due to the total height observed at age A. The effect of clone on the height-age relationship was modeled by incorporating random effects on parameters of Equation (1).

Although the best fit was obtained by including random effects for both the asymptote and the shape parameters, there was no practical effect of varying the shape parameter on the resultant site index curves. Hence one can assume that the site curves are anamorphic and that an adjustment in level alone is sufficient for accounting for differences in site index of these clones.

Using data from the block planting at two densities with four genotypes, effects of genetic improvement on the tree height-diameter relationship were investigated using the equation form

$$H_i = \beta_0 \times \exp\left(\beta_1 D_i^{-1}\right) + \varepsilon_i \tag{2}$$

where H_i is the total height and D_i the diameter at breast height of the ith tree; β_0 is the upper asymptote parameter and β_1 is the rate parameter; and ε_i is the random stochastic error due to the ith tree ($\varepsilon_i \sim N(0, \sigma_\varepsilon^2)$). Genetic variety exhibited a significant effect on both the rate and

asymptote parameters. Planting density had a significant effect on the rate parameter but not on the asymptote parameter. Due to the effect of the PD on the parameter β_1, the effect of genetics on the parameters was investigated separately for the two PDs. Data from the three replicates of each genetic variety in a PD level were combined for use in fitting the equations. The two clones were taller for a given diameter and the variance of height within a given diameter was less than that of OP and CP material (Sabatia and Burkhart, 2013b).

When fitting two-parameter Weibull distribution functions to the diameter data for the four genotypes, the means and variances were similar. However, as expected from the foregoing results on height-diameter relationships, the height distributions for the two clones were shifted to the right (Sabatia and Burkhart, 2013b).

Improvements in stem form and straightness were clearly evident in the two clones. Furthermore, forking and fusiform rust incidence was essentially eliminated in the clones while occurring, respectively, at rates of 8 and 11 percent in OP and 2 and 9 percent in CP trees.

When incorporating genetic improvement relationships in growth and yield models, analysts must take the model architecture into account. Interrelationships among model components are complex and changes to any of the equations can have effects on other components.

REFERENCES

Buford, MA and HE Burkhart. 1987. Genetic improvement effects on growth and yield of loblolly pine plantations. For. Sci. 33:707-724.

Sabatia, CO and HE Burkhart. 2013a. Modeling height development of loblolly pine genetic varieties. For. Sci. 59:267-277.

Sabatia, CO and HE Burkhart. 2013b. Height and diameter relationships and distributions in loblolly pine stands of enhanced genetic material. For. Sci. 59:278-289.

A TRANSGENIC AMERICAN CHESTNUT SYSTEM FOR EVALUATION OF PATHOGEN RESISTANCE GENES

Campbell J. Nairn[1], Lisheng Kong, Haley Houke, Scott A. Merkle

[1]Warnell School of Forestry and Natural Resources, University of Georgia, Athens, GA

Forest tree species are increasingly exposed to novel pathogens introduced as invasive species outside their native ranges. The rapid spread of plant pathogens facilitated through anthropogenic activities circumvents natural evolutionary processes that produce resistance or tolerance in host species. A goal of the Forest Health Initiative is to explore the potential role of biotechnologies, including transgenics, for addressing existing and emerging threats to forest health. With the large number of plant genes that have been implicated in pathogen resistance, a robust system for gene identification, vector construction, plant transformation and regeneration of tree species is needed. We have developed an efficient embryogenic tissue culture, transformation, selection, and regeneration system and combined that with a modular expression vector to rapidly clone and express candidate pathogen resistance genes (PRGs) in American chestnut (*Castanea dentata*). The system facilitates cloning of PRGs from genomic analyses within and between species as well as the incorporation of PRGs from diverse heterologous sources. Approximately 30 PRGs have been cloned and transformed into each of several American chestnut (AC) genotypes. With a goal of producing 10-20 independent transgenic lines (events) for each PRG and AC genotype combination, dozens of transgenic lines for each PRG are screened for transgene integration and expression. Embryogenic cultures representing over 2,000 independent transgenic lines have been screened approximately 6-8 weeks post-transformation for transgene integration. Individual lines are then screened prior to plant regeneration by qRT-PCR to characterize transgene expression levels. Expression profiling reveals a wide range of PRG expression levels in independent transgenic lines, which can vary by more than 100 fold in AC lines harboring the same PRG vector. Using these data, independent lines that represent the full range of moderate to high expression levels are selected for plant regeneration and eventual testing for in vivo pathogen resistance.

ENHANCING *Septoria Musiva* RESISTANCE IN POPLAR WITH MOLECULAR GENETIC APPROACHES

Haiying Liang[1], Margaret Staton, Shivegowda Thammannagowda, Yi Xu, Tao Xu, Jared LeBoldus

[1] Department of Genetics and Biochemistry, Clemson University, Clemson, SC

Populus species and their hybrids are among the fastest-growing temperate trees. The primary commercial uses of poplar trees include pulpwood, engineered lumber products, and bioenergy. In anticipation of shrinking petroleum reserves and a reduced land base to produce forest products, interest in short-rotation intensive poplar culture as an alternative fuel and fiber resource has increased. However, widespread adoption of short-rotation intensive poplar production is hindered by the occurrence of several important foliar and stem infecting pathogens. Among them, leaf spot and stem canker caused by the necrotrophic fungus *Septoria musiva* Peck is the most serious disease affecting hybrid poplar production in North America (1). Severe leaf spot outbreaks can reduce the photosynthetic area and cause premature defoliation; thereby decreasing annual growth. Stem cankers reduce growth, predispose the tree to colonization by secondary organisms, and cause severe girdling and breakage of the main stem. Biomass losses due to this pathogen vary among clones and have been reported to be as high as 63% of total yield (2). Furthermore, Ostry et al. (3) found that 86% of the clones in a plot located near infected plantations in Michigan had cankers five years after plot establishment. Chemical and biological control of Septoria leaf spot and canker has been attempted. For example, spraying disease-suppressive *Streptomyces* strains can also significantly reduce leaf disease caused by *S. musiva* (4). However, all these approaches are expensive and problematic. In the case of *Streptomyces*, spore application needed to be done at least monthly but preferably weekly during periods of *S. musiva* spore release. As a result, planting resistant clones appears to be the best means of managing Septoria diseases. In this report, we evaluated three candidate resistance genes by genetic engineering. We also conducted the first global analysis of the *Populus* defense transcriptome dynamics in response to *S. musiva* leaf spot using the RNA-Seq method. Differential expression analyses were performed between resistant and susceptible clones and infected and uninfected leaf tissues.

MATERIALS AND METHODS

Poplar transformation: *Agrobacterium tumefaciens* strain EHA105 carrying candidate gene(s) that are listed in Table 1 were used to transform a *S. musiva* susceptible hybrid clone OGY (*P. deltoides × nigra*). Transformation and selection were conducted according to Liang et al. (5).

Host Propagation, pathogen propagation, and inoculations: Dormant branch cuttings from 4 clones of hybrid poplar, DN34, NM6, NC11505, and DN164, representing 2 resistant and 2 susceptible poplar clones, were cut into 10-cm-long sections and planted into 21-cm-deep Ray Leach cone-tainers TM with only the top-most bud exposed above the growing medium surface. Following the initial 90 days of growth trees were transplanted from the cone- tainers into 1 gallon plastic pots. Three isolates of *S. musiva*, isolated from Septoria cankers, were used in the experiment. Inoculations were conducted according to LeBoldus et al. (6).

Table 1. Candidate genes for poplar transformation (provided by Dr. William A. Powell at SUNY-ESF)

Gene(s) in construct	Promoter	Number of transgenic events	Notes
ESF39	A vascular promoter from American chestnut stem tissue (ACS2)	3	• ESF39: an antimicrobial peptide gene having a similar amphipathic α-helix found in magainins
Laccase	A vascular promoter from American chestnut stem tissue (ACS9A)	2	• Lac: a laccase gene from Chinese chestnut
ESF39+Laccase	Same as individual gene	3	• Kanamycin as selection agent for all constructs
OxO+Laccase	Same as individual gene	4	
OxO+ESF39+Laccase	Same as individual gene	14	

Illumina sequencing and data processing: Four days after inoculation, leaves were collected and immediately frozen in liquid nitrogen. Total RNA was extracted with a CTAB method. The integrity and quality of RNA were verified with an Agilent Technologies 2100 Bioanalyzer before subjecting the RNA to cDNA synthesis and library construction. A total of 16 cDNA samples were individually bar-coded and run in two lanes of HiSeq 2000 v3. The *Populus trichocarpa* genome and gff annotation file were downloaded from Phytozome, while the *Septoria musiva* genome and gff annotation file were from JGI. The RNA-Seq reads were aligned to both reference genomes using the software package TopHat (7). The resulting alignments were analyzed by Cufflinks (8) to assemble the transcripts and estimate their abundance in each indexed sample.

Identification of differentially expressed (DE) genes: A statistical package, DESeq, was used to analyze the transcript abundances (9) with default parameters. For the comparisons with combined genotypes, a multifactor design was used to account for the genotype differences as well as the sample condition ("Septoria challenged" vs "control"). An adjusted p-value of less than .05 was considered significant across all comparisons.

RESULTS AND DISCUSSION

As shown in Table 1, several transgenic lines have been obtained for the candidate genes as evidenced by PCR with gene-specific primers. The expression levels of transgenes have also been determined. We are currently propagating the plants for whole-plant *S. musiva* assays. Individual expression of the wheat oxalate oxidase gene and several antimicrobial peptide genes having a similar structure as ESF39A was able to enhance resistance in OGY to *S. musiva*, although the plants ultimately succumbed to the disease (5). By combining 2 or 3 resistance genes with different action mechanisms, we expect to achieve a higher level of and more durable resistance.

In total, 511 million reads were generated, constituting 22.8 Gb of sequences. An average of 78% of the reads was successfully aligned to the *P. trichocarpa* genome, 28% of which was

uniquely aligned. Functional annotation of differentially expressed genes based on comparisons between resistant and susceptible clones revealed that there were significant differences in the expression of genes involved in disease/stress resistance and oxidation reduction in mock treated leaves. Four days post inoculation of *S. musiva*, differentially expressed genes were most enriched with GO terms of leucine-rich repeats and disease defense. Among them were 23 loci involved in plant-type hypersensitive responses (GO:0009626), corresponding to 8 kinds of proteins: NB-ARC domain-containing disease resistance protein (11 loci), flavin-dependent monooxygenase 1, phospholipase A 2A, MAC/Perforin domain-containing protein, MutT/nudix family protein, EF-TU receptor, mitogen-activated protein kinase 3, and elicitor-activated gene 3-1. In particular, Potri.001G426500 locus, encoding a homolog of NB-ARC domain-containing disease resistance protein, was up regulated in the relative resistant clones of all compressions, suggesting a strong candidate R gene in response to *S. musiva* perception. The results from this study indicate that strong defense mechanisms involved in the pathogen perception, oxidation reduction, and accumulation of defense-related gene products may contribute to Septoria resistance in DN34 and NM6. We will use qRT-PCR to validate the expression data from RNA-Seq.

REFERENCE

1. J.E. Weiland, et al., Prediction of long-term canker disease damage from the responses of juvenile poplar clones to inoculation with *Septoria musiva*. Plant Dis 87 (2003) 1507-1514.

2. Jr. H.J. McNabb, et al., The effect and possible integrated management of *Septoria musiva* in intensive, short-rotation culture of Populus in the north central states. In: Zavitkovski WIJ, Manhattan EH, eds. Proceedings of the North American Poplar Council Meeting, July 20–22, Rhinelander. Wisconsin: Division of Extension, Kansas State University, pp. 51–58, (1982).

3. M.E. Ostry, et al., Impact and control of *Septoria musiva* on hybrid poplars. General Technical Report NC-133. Washington, DC: USDA Forestry Service, (1989).

4. L. Gyenis, N.A. Anderson, M.E. Ostry, Biological control of *Septoria* leaf spot disease of hybrid poplar in the field, Plant Dis, 87 (2003) 809-813.

5. H. Liang et al., Enhanced resistance to Septoria musiva in transgenic hybrid poplar leaves expressing an oxalate oxidase gene. Plant Molecular Biology 45 (2001): 619-629.

6. J.M. LeBoldus, et al., A method to induce stem cankers by inoculating nonwounded *Populus* clones with *Septoria musiva* spore suspensions, Plant Dis 94 (2010) 1238-1242.

7. C. Trapnell, et al., TopHat: discovering splice junctions with RNA-Seq. Bioinformatics 25 (2009) 1105-1111.

8. C. Trapnell, et al., Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation, Nat. Biotech. 28 (2012):511–515.

9. S. Anders, et al., Differential expression analysis for sequence count data. Genome Biol. 11 (2010) R106.

TOWARDS A GENOME SEQUENCE OF THE BROWN SPOT NEEDLE BLIGHT PATHOGEN (*Mycosphaerella dearnessii)* INFECTING LONGLEAF PINE

B.D. Bartlett[1], J.H. Roberds, J.A. Smith, D.G. Peterson, C.D. Nelson

[1] Southern Institute of Forest Genetics, Southern Research Station, USDA Forest Service, Saucier, MS

Longleaf pine (*Pinus palustris* Mill.) is the dominant species in one of the most diverse terrestrial ecosystems in the temperate US. Acreage devoted to growing longleaf pine has diminished to less than three percent of that at the time of European settlement (Ware *et al.* 1993). America's Longleaf Restoration Initiative is currently coordinating an effort involving a number of agencies to increase the area occupied by longleaf pine forest to 8 million acres (8% of the original range). Widespread incidence of brown-spot needle blight is a potential threat that can hinder progress in this restoration program. This disease results from infection by the fungus *(Mycosphaerella dearnessii)*, which can disrupt development in longleaf seedlings. This disruption can slow growth, delay stand development, and cause an increase in seedling mortality (Siggers 1934). While prescribed fire is recommended management practice for minimizing disease incidence, its use is problematic for many land managers. The absence of a proper fire regime will result in inadequate management for this needle disease on many sites, suggesting that approaches other than prescribed fire are needed for establishing longleaf pine stands. At the same time, few resources exist for elucidating the mechanisms by which this fungus is able to infect pine species. Obtaining an understanding of the systems involved in the infection process should prove valuable in efforts to select and breed trees resistant to brown-spot needle blight. Here we outline research that has been initiated to address these shortcomings.

MATERIALS AND METHODS

Samples of *M. dearnessii* from 25 different longleaf pine seedlings were collected from the Harrison Experimental Forest near Saucier, MS. Lesions from infected needles were dissected and placed in potato dextrose agar (PDA). Examination of conidia from embedded lesions required careful observation of the cultures, to avoid transferring contaminating fungi. Acervuli produced from the lesions appeared as a black speck on the needle and was typically noticed within 10-14 days. After 14 days, contaminating fungi dominated the media. A sterile toothpick was manipulated to capture spores for microscopic identification (400X magnification), and sequentially transferred to clean media after positive identification. Cultures were then grown at 21 C for 21 days prior to DNA extraction. Genomic DNA was isolated from each of the cultures using a CTAB protocol. To achieve optimal DNA yield, fungal tissue was frozen in liquid nitrogen and macerated with a pestle and mortar. For DNA analysis, standard PCR conditions were sufficient for amplification. Annealing temperature for the microsatellite primers (MD1, MD2, MD9 isolated from *M. dearnessii* and provided by Josef Janoušek, Irene Barnes, Michael Wingfield), was 56 C, while the internal transcribed spacer (ITS) primers required an annealing

temperature of 51 C. Capillary electrophoresis was carried out on an ABI 3730xl DNA Analyzer to evaluate the PCR products. Sixteen unique haplotypes were identified and selected for testing vegetative compatibility. Each haplotype pair was tested on being compatible or incompatible, or possibly intermediate (Figure 1). To form the pairings, conidia from each haplotype was placed on PDA media 1 cm from another haplotype and allowed to grow for 21 days prior to scoring.

RESULTS AND DISCUSSION

A location within the historic range of longleaf pine was chosen to obtain infected needles (Boyer 1990). Upon isolation of the fungus, definitive identification was needed to confirm *M. dearnessii.* Universal fungal primers from the ITS region were employed to verify this identification. Three samples were chosen to sequence the ITS region and compare those results to the nucleotide database maintained by the National Center for Biotechnology Information (NCBI). Two samples exhibited 100% homology to the ITS region of *M. dearnessii*, while the other exhibited 99.8% homology. The ITS PCR products of these three samples were determined by capillary electrophoresis to be 580bp. All isolates of the fungus demonstrated a sequence length of 580bp of the ITS region, supporting our visual (microscopic) identification of *M. dearnessii.*

Microsatellite analysis revealed that each of the three markers studied showed polymorphism among our 25 isolates, and our collection contained 16 unique haplotypes. A representative culture of each haplotype was selected for vegetative incompatibility testing. Of the 120 different combinations observed, 61 (51%) were considered to exhibit intermediate compatibility (Figure 1). Due to a high proportion of intermediate compatibility combinations observed, these results suggest that multiple loci are involved in vegetative compatibility. A haplotype pair was interpreted as incompatible if a zone of exclusion was detected, so that the two cultures could not fully merge. With these criteria, 68 (57%) of the pairs were found to be incompatible, while 47 (39%) were judged as compatible.

The microsatellite results were additionally applied to select two isolates for whole genome sequencing. These two isolates were determined to be genetically distinct from each other, as a result of neither having a similar microsatellite marker allele. The genome of *M. dearnessii* is estimated to be around 50 Mb, based upon other *Mycosphaerella* genome sizes (Santana 2012, Goodwin 2011). Using this estimate, a single lane from an Illumina MiSeq will provide enough data to assemble two haploid individuals. Using paired-end reads, a strategy will be employed to essentially double the length of the reads, instead of producing a single 250bp read, paired-ends will merge two 250bp reads that overlap by 50bp, providing a continuous 400bp read. This strategy will produce 40X coverage of each haploid genome. These sequence data will be combined to assemble a draft genome, followed by gene predictions to obtain gene ontology (GO) associations. Overall, this information will be applied to studying pathogenesis of *M. dearnessii* on *P. palustris,* leading to improved measures for controlling the incidence of the

brown-spot needle blight and developing genetic resistant longleaf pines.

Haplotype	15	1	16	4	13	18	8	12	14	6	5	3	9	17	2	10
15		NA	I	C	I	I	C	C	C	C	C	C	C	I	C	C
1			I	C	I	I	I	I	C	I	I	I	C	NA	C	NA
16				I	I	I	I	I	I	I	C	C	I	C	I	I
4					I	I	I	I	C	I	NA	C	C	I	I	I
13						I	I	I	I	I	C	I	I	I	I	NA
18							I	I	I	I	C	I	C	I	C	C
8								I	I	I	C	I	C	I	I	C
12									C	C	I	I	I	C	C	C
14										C	I	C	C	C	I	C
6											I	I	C	I	I	C
5												I	C	I	C	C
3													C	I	I	I
9														I	C	I
17															C	I
2																C
10																

Figure 1. Vegetative compatibility matrix; illustrating all the combinations of haplotype pairs. The grey background represents intermediate compatibility. Incompatibility is represented by an I, while compatibility is represented by a C.

REFERENCES

Boyer, WD. 1990. *Pinus paulustris,* Mill. Longleaf pine. In *Silvics of North America,* technical coordinators R.M. Burns and B.H. Honkala. Conifers Washington, DC: USDA Forest Service.

Goodwin, SB et al. 2011. Finished genome of the fungal wheat pathogen *Mycosphaerella graminicola* reveals dispensome structure, chromosome plasticity, and stealth pathogenesis. *PLoS Genetics*. 7(6).

Santana, MF et al. 2012. Abundance, distribution and potential impact of transposable elements in the genome of *Mycosphaerella fijiensis*. *BMC Genomics*. 13:720.

Siggers, PV. 1934. Observations on the influence of fire on the brown-spot needle blight of longleaf pine seedlings. *Journal of Forestry*. 32:556-562.

Ware, S et al. 1993. Southern mixed harwood forest: The former longleaf pine forest. In Biodiversity of the Southern United States: Lowland Terrestrial Communities. pg. 447-493. *New York: John Wiley & Sons.*

RECONSTRUCTION OF REGULATORY NETWORKS IN *FUSARIUM GRAMINEARUM* USING A SYSTEMS BIOLOGY APPROACH

Li Guo[1], Guoyi Zhao, Lixin Gao, Li-Jun Ma, Xiaoying Zhao, Jin-Rong Xu, Wilfried Jonkers, Corby Kistler

[1]Department of Biochemistry and Molecular Biology, University of Massachusetts Amherst, Amherst, MA

Regulatory networks (RN) function as information centers that transform signals into coherent cellular responses. The network plasticity achieved through evolutionary rewiring of regulatory networks is an important source of diversity among species. This study employs *Fusarium graminearum*, a mycotoxigenic filamentous fungus and causal agent of head blight diseases in cereal crops, as a model system. A robust searching algorithm using Bayesian networks model was developed and we tested the algorithm on a collection of *F. graminearum* transcriptomic datasets to infer the relationship between candidate regulators and their target genes. Preliminary validation of the inferred network using prior biological knowledge proofs the effectiveness of the program. Using a heuristic greedy approach, the program predicted in the network a set of top regulators, which interestingly control specialized and modularized gene regulation and cellular functions, providing exciting insights into the dynamics of transcriptional regulatory mechanisms orchestrating the fungal biology and pathogenesis. Combining comparative studies of sequenced *Fusarium* genomes with the network inference, the conservation of the regulatory modules will be identified and allow us to transfer the network knowledge gained from *F. graminearum* to other *Fusarium* species. The reconstruction of the regulatory networks will enable a comprehensive understanding of the complex biological processes related to pathogenesis and will have broad implications in disease controls.

CONSTITUTIVELY ELEVATED SALICYLIC ACID PROVOKES OXIDATIVE RESPONSE IN TRANSGENIC POPULUS VIA METABOLIC AND TRANSCRIPTIONAL REPROGRAMMING

C-J Tsai

Warnell School of Forestry and Natural Resources, and Department of Genetics, University of Georgia, Athens, GA

The phytohormone salicylic acid (SA) regulates many aspects of plant growth and adaptation. Exogenous applications of SA have long been shown to improve oxidative stress tolerance of several crop species. However, an in planta assessment of elevated SA effects on oxidative stress responses has been lacking, in part because constitutive overproduction of SA in transgenic or mutant Arabidopsis leads to severe growth retardation. We show that transgenic Populus can over-accumulate, by three orders of magnitude, SA and SA-conjugates without negative growth consequences. This has allowed a comprehensive characterization of constitutive SA overproduction in planta using a functional genomics approach. In the absence of oxidative stress, high-SA poplars exhibited a constitutively altered oxidative state, with transcriptional and metabolic responses resembling those of heat-stressed wild-type plants. Network analysis identified several metabolites and genes with strong SA-dependent regulation that have not been reported previously. Of particular interest were the increased expression as well as increased network connectivity of nucleoredoxins in SA-overproducing poplars. In contrast, orthologs of well-known Arabidopsis SA signaling components NPR1 and thioredoxins were absent in the reconstructed gene network of high-SA poplars. Together, the physiological, molecular and metabolite data reveal a sustained oxidative response in SA-hyperaccumulating Populus that is growth-compatible. This work advances previous studies reporting SA-homeostatic differences between Arabidopsis and Populus, and provides experimental evidence that components of the SA- and/or redox-mediated signaling pathway differ as well. The SA response in Populus involved a reprogramming of carbon uptake and partitioning during stress that is compatible with constitutive chemical defense and sustained growth, contrasting with the SA response in Arabidopsis which is transient and compromises growth if sustained.

BROAD-BASED TESTING COMPARED TO LOCAL TESTING OF FAMILIES OF LOBLOLLY PINE FOR RESISTANCE TO FUSIFORM

Jesse Spitzer[1], Steve Mckeand, Ross Whetten, Fikret Isik

[1]North Carolina State University Tree Improvement Program, Raleigh, NC

Breeding Loblolly pine *Pinus taeda* L. trees with resistance to fusiform rust is a central focus of Southeast tree improvement programs. Annual losses from this disease are estimated in the range of $24 to $135 million, making it the most economically important disease of loblolly pine. Identification and deployment of pine families with enhanced resistance to fusiform rust across a broad range of sites are critical for the full benefit of breeding programs to be realized. Interactions of families' susceptability/ resistance and specific pathogen avirulence/virulence among sites has been reported both in field trials and in controlled inoculations at the USDA Forest Service Resistance Screening Center in Asheville, NC, but the consistency and utility of these interactions have been limited. We will compare rust breeding values from the a series of trials planted across many sites (the Cooperative's Plantations Selection Seed Source Study - PSSSS) to breeding values from trials established in more narrow geographic regions The PSSSS used 80+ families that were also tested locally in Plantation Selection Diallel trials. By comparing the two breeding values for each family to see how much genotype by environment (e.g. family by virulence) interaction is occurring, we can elucidate any evidence for virulence variation in the locally tested breeding values. It is predicted that locally tested families of loblolly pine are reliable across a wide geographic range and little interaction is occurring.

FUNGAL EFFECTORS OF *CRONARTIUM QUERCUUM F.SP. FUSIFORME*

Katherine E. Smith[1,2], Daniel Ence, Nicolas Feau, Amanda Pendleton, Richard Hamelin,
C. Dana Nelson, Mark Yandell and John M. Davis

[1]School of Forest Resources and Conservation, University of Florida, Gainesville, FL; [2]Southern
Institute of Forest Genetics, USDA Forest Service, Southern Research Station, Saucier, MS

The genomic sequence of *Cronartium quercuum f.sp. fusiforme* (Cqf), the rust fungus that causes fusiform rust disease on southern pines, has been determined. The genome assembly contains 13,903 genes, 1,140 of which are predicted to be secreted from the fungus. Some of these proteins are secreted during infection stages and therefore potentially influence pathogenicity. Effectors are pathogen proteins that alter host cellular function and morphology to accommodate infection and fungal growth. They are at the heart of understanding how Cqf is able to overcome host defenses and live within plant tissues without killing the plant. Small secreted proteins (SSPs) fewer than 300 amino acids are considered to be good candidate fungal effectors since proteins must be secreted during the infection process to exert a direct effect on the host. Cqf gene expression during pine and oak infection was studied using microarrays. Proteins encoded by genes showing significant differential overexpression in one host over the other were greatly enriched for SSPs and included homologs of known effectors. A subset of effectors conform to predictions made by the gene-for-gene hypothesis, meaning they are avirulence genes with alleles that specifically interact with host resistance gene alleles resulting in a non-disease phenotype. A physical location for the Cqf avirulence gene 1, Avr1, was determined using two different genetic marker approaches. Exact genomic locations for avirulence genes allow perfect markers to be made that can precisely identify alleles present in fungal samples. This ability can lead to better studies aimed at understanding the nature of Cqf virulence and ultimately a greater level of control of fusiform rust disease. Experiments that measure the presence of virulent and avirulent alleles in the field can elucidate how virulence changes geographically and over time.

BEST PERFORMING COTTONWOOD AND HYBRID POPLAR VARIETIES IN THE SOUTHEASTERN UNITED STATES: THEIR BASIC SPECIFIC GRAVITY AND MOISTURE CONTENT

Bijay Tamang[1], Victor Steel and Jeff Wright

[1]ArborGen Inc, Tallahassee, FL

Cottonwood and hybrid poplars are among the preferred tree species for biomass production due to their fast growth and wide distribution. In the US, they are commonly planted as selected varieties in the Pacific Northwest as well as in the Midwest and Lake States. Trees are usually grown at 1482 trees ha^{-1} at pulpwood rotations which is usually 6-9 years. In the southeastern US, cottonwood commercial plantations are mostly concentrated along the Mississippi delta region in Arkansas, Louisiana and Mississippi but its range is gradually expanding in the other regions in the southeastern US.

Growth and productivity of cottonwood and hybrid poplars depend largely on genetics, site quality and silvicultural management. In general, productivity in the Pacific Northwest is higher than in the southeastern US as trees are intensively managed under irrigated conditions. In the Pacific Northwest, biomass assessment of hybrid poplar after six and eleven growing seasons show the mean annual increment around 13 MT ha^{-1} yr^{-1} (Berguson et al. 2010). Initial estimate for unimproved *Populus trichocarpa* was as high as 21 MT ha-1 yr-1 under two-year coppice rotations (Heilman et al. 1972).

Efforts are underway in the southeastern US to select highly productive varieties with better wood quality, resistance to diseases and suitability to grow across sites. In this report, we evaluate the growth performance of more than 422 cottonwood and hybrid poplar Varietals in the southeastern US and present basic specific gravity and moisture content of the top 30 Varietals.

MATERIAL AND METHODS

Five tests were established between 2003 and 2010 in the states of North Carolina, South Carolina, Georgia and Alabama. All tests were planted using randomized complete design with six replications except the Floyd site which only had four replications. Trees in the tests were measured at the end of 2011 growing season for height and diameter at breast height (DBH). Location of the tests, their genetic composition, age and tree size after the 2011 growing season are given in Table 1.

Since the tests were of various ages, height and DBH measurements were standardized to account for different ages. Standardized volume was estimated using the volume equation developed by Krinard (1983). Best linear unbiased prediction (BLUP) analysis was then done using the standardized data. Varietal S7-C8 was considered the check. Percent height, DBH and volume gain over the check S7-C8 was calculated for each Varietal.

Table1. Location of tests, age at the time of measurement, no. of Varietals and overall tree size (range in parentheses) after the 2011 growing season.

Test	Age (yrs)	# Varietals	Height (m)	DBH (cm)
Wooten Farm, NC	2	87	4.3 (1.8-5.7)	3.6 (2.0-5.3)
Eastover, SC	4	243	9.1 (3.8-11.5	7.9 (2.3-13.2)
Moultry, SC	3	161	3.6 (1.7-6.2)	3.0 (0.5-7.4)
Floyd, GA	8	120	17.0 (12.6-21.3)	15.7 (8.9-21.1)
Randolph-1, AL	3	162	5.5 (4.0-7.0)	4.8 (2.8-6.4)
Randolph-2, AL	3	124	5.2 (3.2-7.8)	4.1 (1.8-6.9)

Top 30 best performing Varietals based on volume gain as well as the check, S7-C8, were selected for basic specific gravity and moisture content determinations. Tests at Eastover, Moultry and Randolph were selected for increment core sampling as these were identical in age. Cores were extracted from three ramets per Varietal per test in October/November 2012 using an increment corer. Cores were immediately sealed in ziplock bags with pre-determined weight and stored in a cooler. Weight of the ziplock bags along with the increment cores was taken within three hours after extraction. Increment cores were soaked in water and their volume estimated using the water immersion method. Cores were then oven-dried at 101° C until constant weights were obtained. Core oven-dry weight was used to estimate basic specific gravity and moisture content.

RESULTS AND DISCUSSION

Check Varietal S7-C8 was ranked in the 151st, 24th and 15th positions when sorted by height, DBH and volume grain, respectively. Eight Varietals had at least 10% height gain over S7-C8 and six of them were statistically significant (α=0.05). Only five Varietals had more than 10% DBH gain over the check but none of them were statistically significant. There were seven Varietals that had at least 10% volume gain over the check but only two were statistically significant (α=0.05).

Average basic specific gravity ranged between 0.294 and 0.404 showing variation across genotypes. Klasnja et al. (2003) reported average basic specific gravity of 0.380 for eastern cottonwood. Specific gravity of *Populus* species in published studies across the globe ranged between 0.260 and 0.500 (Headlee et al. 2013). Our estimates are well within the published range.

Overall average moisture content was highly variable within genotypes ranging from 72% to 169% (dry basis). Average moisture content was also highly variable between genotypes within a site. Since the samples were collected within few hours from each site, the variation could be mainly due to difference in genotype and micro site differences to some extent. One of the other factors for low moisture content in some genotypes could also be due to sampling in late fall as some genotypes tend to go dormant early. Several other studies have also reported low and highly variable moisture content in poplar (Zhang et al. 2003, Pearson et al. 2010)

REFERENCES

Berguson, B., J. Eaton and B. Stanton. 2010. Development of hybrid poplar for commercial production in the United States: The Pacific Northwest and Minnesota experience. Ch. 17. Sustainable Alternative Fuel Feedstock Opportunities, Challenges and Roadmaps for Six U.S. Regions. Soil and Water Conservation Society. pp. 282-299.

Headlee, W.L., R.S. Zalesny Jr., R.B. Hall, E.O. Bauer, B. Bender, B.A. Birr, R.O. Miller, J.A. Randall and A.H. Wiese. 2013. Specific gravity of hybrid poplars in the north-central region, USA: within-tree variability and site x genotype effects. Forests 4:251-269.

Heilman, P.E., D.V. Peabody, D.S. DeBell, and D.F. Strand. 1972. A test of close-spaced, short rotation culture of black cottonwood. Can. J. For. Res. 2 456-459

Klasnja, B., S. Lopitovic and S. Orlovic. 2003. Variability of some wood properties of eastern cottonwood (Populus deltoides Bartr.) clones. Wood Sci. Technol. 37:331-337

Krinard, R.M. 1988. Volume equations for plantation cottonwood trees (*Populus deltoides*). Research Note SO-347, USDA Forest Service

Pearson, C.H., A.D. Halvorson, R.D. Moench and R.W. Hammon. 2010. Production of hybrid poplar under short-term, intensive culture in Western Colorado. Ind. Crops and Prod. 31:492-498

Zhang, S.Y., Q. Yu, G. Chauret and A. Koubaa. 2003. Selection for Both Growth and Wood Properties in Hybrid Poplar Clones. For. Sci. 49:901-908

LONGLEAF PINE GROWN IN VIRGINIA: A PROVENANCE TEST

Kurt Johnsen[1], Jerre Creighton, Chris Maier

[1]US Forest Service Southern Research Station, Research Triangle Park, NC

In 2006 the Virginia Department of Forestry established a longleaf pine provenance test on three sites near New Kent, VA, near the most northern native range of Longleaf pine. The provenances originated from native trees in Virginia, natural stands and a seed orchard in North Carolina, and natural stands in South Carolina, Georgia, Florida, Alabama, and Mississippi. The provenances were grown on three sites, two previous nursery sites (irrigated during year one) and a cut-over site (not irrigated). On average, survival ranged from 86 to 58% showing no clear clinal pattern although the Virginia source had the highest survival. At age three, growth was greatest for the VA trees and decreased in a mostly clinal pattern. By age 5, height of the provenances on the nursery sites had largely converged while the clinal pattern was maintained on the lower productivity cutover site. At the end of the fifth growing season, foliage was collected for carbon isotope discrimination (delta13C) and natural abundance of 15N. Water use efficiency (via delta13C) was greatest in the VA trees. A relationship between height and delta13C was only observed (r = -0.55) on the cut-over site and the negative relationship indicates variation in delta13C was due to differences in photosynthesis. Results will be discussed in terms of what longleaf pine sources might be deployed in VA the context of climate change.

HIGH RESOLUTION X-RAY MICRO COMPUTED TOMOGRAPHY FOR GENETIC ANALYSIS OF WOOD TRAITS

Alejandro Riveros-Walker[1], Jared Westbrook, Salvador Gezan, Gary Peter

[1]University of Florida – School of Forest Resources and Conservation, Gainesville, FL

Wood density is commonly measured in wood cores with a 2 dimensional x-ray scanning densitometer. An alternative is x-ray computed tomography (CT), which provides a higher resolution method that creates an image of wood which can be used to measure wood, annual growth ring, earlywood and latewood density, as well as cell wall thickness and lumen area. We have developed analytical methods to use x-ray CT to enhance our understanding of wood density, tracheid diameter and lumen area. In the winter of 2011-2012, three 2-mm diameter micro-cores per tree were collected from 5600 trees from 6 repetitions of the intensively managed treatments from the Nassau Co., FL; Cuthbert, GA and Palatka, FL sites of the CCLONES loblolly pine (*Pinus taeda L.*) clonal study. Using the freeware Image-J software, density profiles were generated from each image and later this data was processed based on the predicted value of density generated using a linear model where the gray-value of each pixel is strongly correlated with density ($R^2 = 0.99$). Using this technique, and a SAS program, we quantify density for each growth year and earlywood and latewood within each annual growth, latewood percentage, and tracheid radial dimensions. Using a quantitative genetic analysis approach we computed heritabilities and Type B genetic correlations for these traits. Heritabilities for density were in the range of 0.35 with SE of 0.05. Type B genetic correlations for density were found to be significantly high with low standard errors. Latewood percentages were low to moderate depending on the year and cell dimensions were also low. A secondary R code was developed to detect false rings and correct the transition point between earlywood and latewood and re-estimate means, heritabilities and Type B correlations. The result of this correction was very significant since some genetic estimates were actually improved.

MODELING CLIMATE CHANGE EFFECTS ON THE GROWTH OF LOBLOLLY PINE SEED SOURCES IN THE SOUTHEASTERN UNITED STATES

Alfredo Farjat[1], Fikret Isik, Ross Whetten, Steve McKeand

[1]Graduate Student, Department of Statistics and Cooperative Tree Improvement Program, Department of Forestry and Environmental Resources, North Carolina State University

Loblolly pine (*Pinus taeda L.*) is the dominant commercial species in the southeastern United States, with almost 1 billion seedlings produced annually. Genetic improvement through breeding and selection of loblolly pine since the 1950's has greatly improved the productivity, form, and disease resistance in the species. The establishment and analysis of provenance tests for investigating the genetic variation among forest trees has a long tradition in forestry. Such tests were meant to identify superior seed sources for planting at specific locations, but they can also provide valuable information for assessing the response of populations to environmental change. In this work, a statistical model to predict the responses of different seed sources to climate change using climate variables as predictors is presented. The approach integrates both genetic and environmental effects and is meant to overcome the critical limitations of population response function and transfer function methods by making full use of data from provenance trials. The model was developed and tested using data from the Plantation Selection Seed Source Study, a large replicated provenance test of loblolly pine.

Materials and Methods

The Plantation Selection Seed Source Study (PSSSS) was initiated in 1994 by the North Carolina State University Cooperative Tree Improvement Program to determine the patterns of geographic variation in plantation selections and to understand pine genotype interactions with the environment. In total 140 families were originally planted at 25 test locations throughout the Southeastern United States, however only 16 test sites were available for this study (Figure 1b). Figure 1a shows the location of the pine plantations from where the female parents were selected (seed source locations). In the same figure, 7 geographic regions of the natural range of loblolly pine are sketched: Virginia (VA), North Carolina Coastal Plain (NC), South Carolina Coastal Plain (SC), Georgia-Florida Coastal Plain (GF), Lower Gulf Coastal Plain (LG), Upper Gulf Coastal Plain (UG), and Piedmont (PD). Growth measurements (height and diameter at breast height), straightness score, fusiform rust incidence, forking, and survival were taken at tree age 8 years. The climatic variables were estimated using the PRISM (Parameter-elevation Regressions on Independent Slopes Model) climate mapping system (PRISM Climate Group, Oregon State University). The developed statistical model follows the Universal Response Function (URF) approach (Wang et al. 2010), which consists of a multiple linear regression that integrates both the environmental and genetic effects through climatic variables for the test sites, and climatic and geographic variables for the families.

(a) Geographic regions and seed sources. (b) Test site locations.
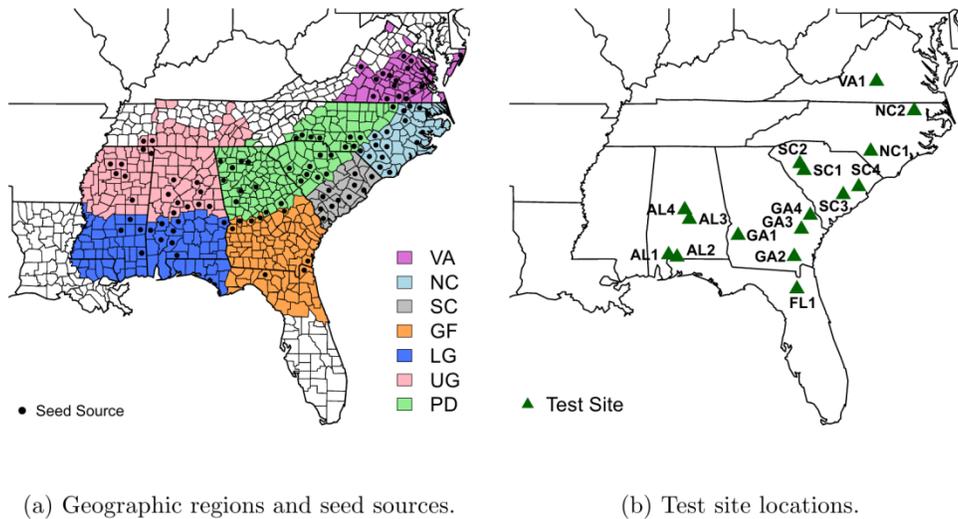
Figure 1. a) Geographic regions and seed sources of the selected families for the Plantation Selection Seed Source Study (PSSSS). **b)** Test site locations for the PSSSS available for this study.

Results and Discussion

Four examples are presented to illustrate possible applications of the proposed model. A hypothetical climate scenario was created from historical data, assuming a 10% decrease in precipitation, an increase of 1°C in maximum temperatures, and an increase of 1°C in minimum temperatures. The first example consists of predicting the 8-year height growth of loblolly pine growing in their native geographical range (e.g. planting only local seeds across the Southeastern U.S.). The predicted 8-year heights using local seeds are shown in Figure 2a.

(a) Predicted height using Local Seeds. (b) Climate change effect using Local Seeds.
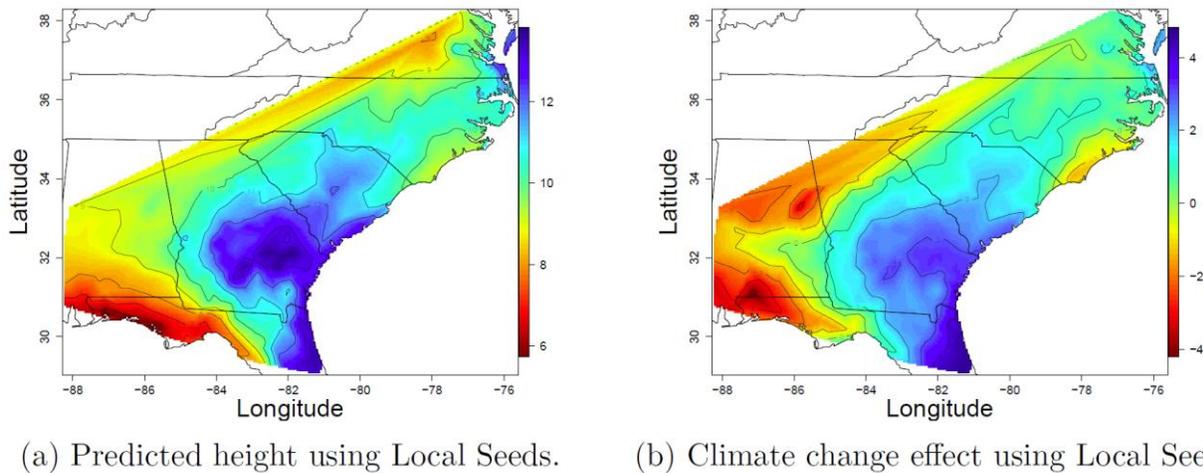
Figure 2. a) Model predictions for height (meters) at age 8 years using loblolly pine local seeds across the Southeastern U.S. for a hypothetical future scenario with 10% decrease in precipitation, an increase of 1°C in maximum temperatures, and an increase of 1°C in minimum temperatures, relative to historical average values. **b)** Difference of predicted height under the simulated climate scenario and current climate using loblolly pine local seeds.

The difference of predicted height under the hypothetical climate change scenario and the current climate using local seeds (Figure 2b) measures the effect of the simulated climate change scenario on the growth of these sources across the Southeastern United States. The Georgia-Florida coastal plain and South Carolina coastal plain regions exhibit the highest differences in growth relative to the current climate. It is interesting to note that local seed sources are predicted to perform much more poorly in the western part of the region under this climate scenario compared to its performance today. Figure 3a shows the model predictions for the 8-year height deviations from site mean for the same hypothetical climatic future scenario above described using loblolly pine seeds from Virginia (VA) throughout the Southeastern U.S. The blue colors in the predicted heights plot indicate that this seed source will only be superior in the most northern regions under the modeled climate change scenario. The last example corresponds to the 8-year height deviations from site mean using seeds from the Georgia-Florida coastal plain (GF) across the Southeastern U.S (Figure 3b). In this case, the trend is reversed relative to the previous example. The predicted heights plot indicate that this seed source will be superior in the southern regions under the modeled climate change scenario.

(a) Relative performance of VA seeds.   (b) Relative performance of GF seeds.
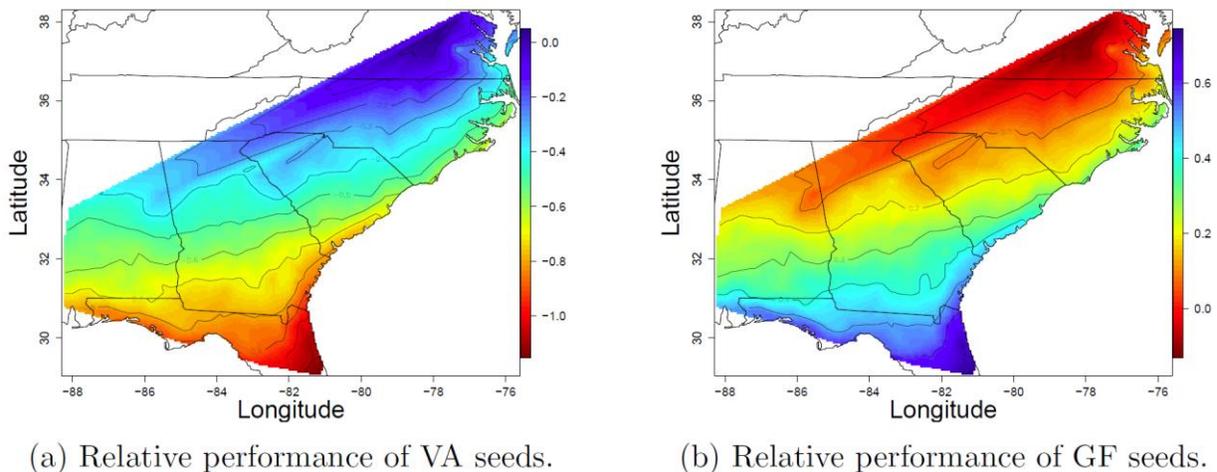
Figure 3. a) Model predictions for 8-year deviations (meters) from site mean using northern seeds from Virginia (VA) for the hypothetical future scenario under consideration. **b)** Model predictions for 8-year deviations from site mean using Georgia-Florida (GF) seeds for the simulated future scenario.

To conclude, the statistical model can be used as a quantitative tool to model the effect of climatic variables on the performance of loblolly pine seed sources. Furthermore, the model could be used to estimate growth potential for a given planting site under a given future climate.

References

Wang, Tongli, Gregory O'Neill, and Sally Aitken. "Integrating environmental and genetic effects to predict responses of tree population to climate." *Ecological Applications* 20, no. 1 (2010): 153-163

IMPACTS OF DIFFERENT LEVELS OF GENETIC HOMOGENEITY ON JUVENILE STEM CHARACTERISTICS AND POTENTIAL STAND-LEVEL VALUE IN LOBLOLLY PINE

J.J. Steiger[1], B.P. Bullock, R.C. Abt, R.W. Whetten

[1]NC State University Cooperative Tree Improvement Program, Department of Forestry and Environmental Resources, NC State University, Raleigh, NC

The recent, wide-spread deployment of more genetically homogenous genotypes drives the demand for better understanding growth and uniformity in stands with varying levels of genetic diversity. We hypothesized that stands planted with more genetically homogenous genotypes would be more uniform for growth traits and sawtimber potential in juvenile lobololly pine. Our results indicate that less genetically diverse genotypes are not more uniform than the more genetically diverse genotypes, and in most cases genetic homogeneity actually led to more variation in growth traits.

Furthermore, we assessed and compared the predicted values of 9 genotypes using a scenario based on growth and yield projections using the LobDSS software package. Although volume is the primary driver of value, the quality of stems in a stand also plays a significant role. By incorporating predicted product class proportions and sawlog quality measures, we aimed to accurately predict and assess the overall stand value over a range of genotypes. Having access to reliable stand value data will play an important role for land managers that aim to increase the value of forested land. Our results show that by incorporating more accurate product class allocation and sawtimber quality measures, the value of stands can change dramatically. These results suggest that if the landowner is paid for the quality of their timber, not just the quantity, then some genotypes are better options than others.

Materials and Methods

The study site was located at the Hofmann Forest in Jones and Onslow County, North Carolina (34°49.4'N, 77°18.2'W). The soil consists of a Pantego mucky loam (Fine-loamy, siliceous, semiactive, thermic Umbric Paleaquults). This soil series consists of very poorly drained, thick loamy deposits with a water table very near the surface. Due to the high water table, the site was bedded to give study trees optimum growing conditions. There are four levels of genetic control incorporated into the design (clonal, full-sib families (mass control pollinated), half-sib families (open-pollinated), and seed orchard mix). The following genetic entries were planted: two clones (C2 and C3); three full-sib families (FS1, FS2, FS3); three half-sib families (HS1, HS2, HS3); and one Seed Orchard Mix (SOM). The clonal material used in this study originated from top performing full-sib families and was propagated by somatic embryogenesis. Full-sib family seedlings were created by selecting and breeding superior well tested parents. The half-sib families (open-pollinated) were created by collecting seed from a single, well-tested orchard tree that was pollinated by wind-blown pollen. Finally, the seed orchard mix seedlings are second

generation selections from the coastal plain of Georgia and South Carolina that are a mix of parent trees that were wind pollinated. All full-sib and open-pollinated families in this study are among the top commercially available families, as verified by the NCSU Cooperative Tree Improvement PRS (Performance Ranking System) database.

The design was set up as a split-split-plot design with the two combinations of thinning being the whole-plots. Additionally, the two combinations of spacing were the sub-plots and each genotype was the sub-sub-plot. Each genetic entry was randomly assigned to each sub-plot within each whole-plot. Each measurement plot consisted of 68-108 measurement trees planted at either 1.5 m or 3 m within bed spacing.. The measurement trees span 4-6 beds, spaced 6.1 meters apart and run for 9 to 20 trees in each bed. Buffers were installed to reduce any adjacency effects. In sum, there are a total of 558 trees per rep per family. The plot-level size for the 1.5 m and 3 m spacing treatments was approximately 1.66 hectares and 3.04 hectares, respectively. Furthermore, an attempt was made to keep the rectangularity of the plots to a minimum. Total test size is 25.84 hectares.

All trees were assessed at ages 5 and 6 for total height, diameter-at-breast height and survival. Individual tree volume was calculated according to Sherrill et al. (2011) from measurements taken at age 5. To get stand-level volume for each genetic entry, average tree volume was calculated, and then multiplied by trees per hectare which was generated using overall family survival. Sawtimber potential was assessed and each tree was given a score of 1 to 4, with 1 being high-quality sawtimber, 2 being sawtimber, 3 being pulpwood/some sawtimber, and 4 as non-merchantable. Additionally, to estimate the value of various loblolly stands at harvest, we used the Loblolly Pine Silviculture Decision Support System of the North Carolina State-Virginia Tech Forest Productivity Cooperative. Analysis of variance (ANOVA) was used to test for effects on height, DBH, volume, and sawtimber potential. Additionally, ANOVA was used to test for differences in the coefficient of variation (CV) among blocks, treatments, genotypes, homogeneity type, and interactive effects.

Results and Discussion

At both planting densities, FS2 had the greatest total height while C2 had the shortest total height. Additionally, FS2 had the greatest diameter-at-breast height, 12.1 cm and 13.59 cm, respectively. Clones C2 and C3 had the smallest DBH in both spacing treatments. Moreover, the two clones in the study had significantly smaller DBHs than all other genotypes, with the one exception being C3 compared to the SOM in the wide-spacing treatment. FS2 had the greatest mean volume, but was only significantly greater than three of the other genotypes, two of which were the clonal families. In fact, compared to the top performing clone (C3), FS2 had a 41% greater volume when planted at 1.5 m x 6.1m and 26% more volume at 3 m x 6.1 m. Mean sawtimber scores were similar across genotypes and spacing treatments. However, the frequency of 1's and 4's varied dramatically. For example, 25.29% of trees in family FS2 had a sawtimber score of 1 in the tightly-spaced treatment, while only 9.77% had a score of 1 in the wide spacing

treatment. Interestingly, clone C3 had 19.88% of all sampled trees in the wide-spaced treatment receive a score of 1, more than any other genotype. On the contrary, 4.02% of trees from genotype FS2 in the tightly-spaced treatment were considered cull trees (score of 4) and only 1.72% of FS2 trees were culls in the wide spacing treatment. Clone C2 had the greatest percentage of cull trees across all genotypes in the tightly-spaced treatment, while C3 had the most cull trees in the widely-spaced treatment. Across genotypes, the most variable were clones, not the seed orchard mix, regardless of spacing or growth trait. Consequently, the clonal genetic type was almost always the most variable type for total height, possibly due to environmental effects overwhelming genetic uniformity.

Generating stand values by applying age 6 stand data to LobDSS and integrating both empirical harvest data and sawtimber potential data for each family, resulted in a range of adjustments across all families. The exception was the SOM, which did have harvest data applied to merchantable tonnage, but did not have adjustments for sawtimber scores applied as it was used as a baseline for comparison for the other eight families. Adjustments were two-fold, one being a price increase for sawlog tonnage and the other being an increase or decrease in non-merchantable wood. Compared to SOM, several families had an increase in sawtimber potential and received a premium increase of 25% of the current sawlog price to account for the higher quality sawlogs. Families C2, C3, FS1, FS2, and FS3 all received sawlog price increases when planted at 1.5 m spacing. In order, their increase in percent high-quality sawtimber (score of 1) over SOM was 9.22, 7.67, 8.26, 13.08, and 1.43%. When these same families were planted in the wider spacing, differences in sawtimber quality were not as pronounced. In fact, the overall proportion of trees that were scored as 1 for sawtimber potential decreased.. Only three families were given a price increase for premium sawlogs, C2, C3 and FS1. While C2 and FS1 had a slightly better sawtimber potential than SOM (2.22%, 1.33), C3 had a large increase, producing nearly 7% more premium sawtimber.
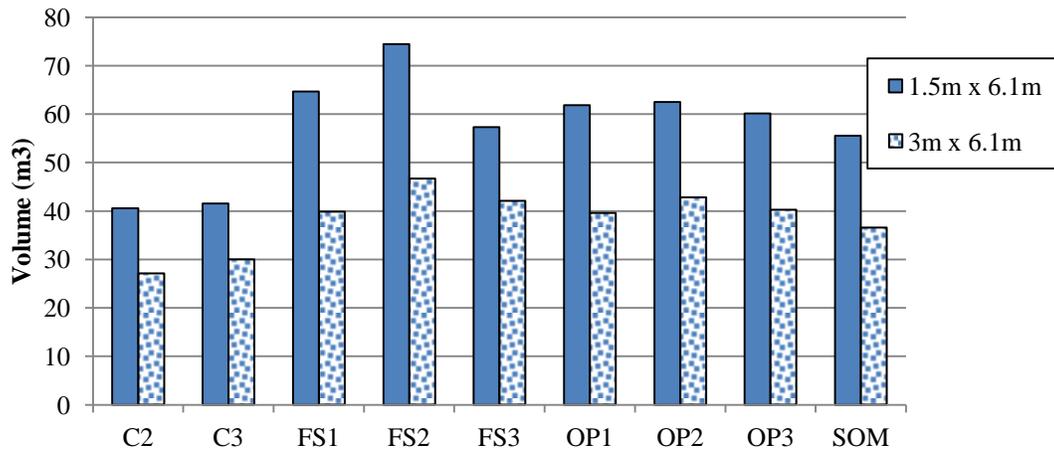
Figure 1. Mean volume per hectare of all 9 genotypes, at both the 1.5m x 6m and 3m x 6m spacing. Volume based on age 6 measurements.

GENETIC EFFECTS ON EARLY STAND DEVELOPMENT OF IMPROVED LOBLOLLY PINE (*PINUS TAEDA* L.) SEEDLINGS

S. Sharma [1], Joshua P. Adams, Jamie L. Schuler, Don C. Bragg and Robert L. Ficklin

[1]University of Arkansas at Monticello, School of Forest Resources, Monticello, AR

Abstract: This study was conducted to assess the effect of genotype on the early performance of improved loblolly pine (*Pinus taeda L.*) seedlings planted on the University of Arkansas at Monticello School Forest located in southeast Arkansas.We used a split- plot design consisting of two spacing treatments (3.05m×3.05m and 3.05m× 4.27m) randomly assigned as main plots and three loblolly pine genotypes (Arkansas Foresty Commission 3-Star half-sibling seedling, Cellfor® clone Q3802, and Cellfor® L3791) randomly assigned to the subplots. Survival, ground line diameter, height, and flush length were collected. Genetics had a significant effect on survival, height, ground-line diameter, and flush length. Cellfor clone L3791 showed greater growth (diameter and height) and survival compared to other seedlings. Survival and growth were not affected by the spacing as expected, considering the early stage of stand development. The high growth and survival of the clonal stock suggest that productivity can be enhanced through selecting the improved genotype.

Introduction

Over the last 50 years, southern pine management in the southern US has shifted from natural stands to intensively managed plantations (Prestemon and Abt 2002, Wear and Greis 2002). These plantations have been established with an increasing amount of genetic improvement (McKeand et al., 2003, 2006). This improvement has also coincided with increasing deployment of full-sib families and clones which could result in greater stand-level uniformity and enhanced productivity (Jansson and Li 2004). Loblolly pine (*Pinus taeda* L.) is the most commonly planted tree species in the southeastern United States (McKeand 2006) primarily because it responds well to silvicultural treatments. Selection of genetic sources and planting density are among the key decisions that must be made prior to plantation establishment.These initial decisions dictate future timing of other silvicultural treatments and directly impact productivity and the quality and type of wood products generated over a rotation.

In Arkansas, there are numerous options of commericially available loblolly pine seedlings for forest managers and landowners. Half-sib seedlings produced by the Arkansas Forestry Commision (AFC) are inexpensive (less than $ 0.1per seedling) and are widely used on private lands (www.ark.org/afc2/seedlingsales.php). Mass controlled pollinated and cloned loblolly seedlings from private companies represent the next generation of improved genetics and promise even better performance, but these are considerably more expensive and less tested in the region, and hence, are not as widely planted as half-sibling seedling stocks.

Thus, the objective of this study was to assess genetic effects on survival and various growth attributes of newly planted loblolly pine. First growing season observations of flushing traits suggested that the clonal stock may gain growth advantages partially due to early flushing; therefore, flushing was quantified during the beginning of the second growing season.

Materials and Methods
Study Area

The study area is located in Drew County, Arkansas, on the University of Arkansas-Monticello Teaching and Research School Forest (Latitude 33º37'1'' North, Longitude 91º43'9'' West). Mean annual precipitation is 53.5 inches, with an average January temperature of 43.3ºF and an average July temperature of 82.0ºF (Larance et al. 1976, NOAA 2013). Soils across the study area are mapped predominantly as Calloway silt loam (Fine-silty, mixed, active, thermic Aquic Fraglossudalfs) with gentle slope (1-3%). Prior to plantation establishment, the site was a mature pine stand of about 55 years when it was harvested. The site was cleared of most debris and was hand planted in January 2012.

Experimental Design and Plant Material
To assess the relative effects of both planting density and seedling stock, a split-plot experimental design was utilized.With relatively wide (3.05m x 4.27m) and narrow (3.05ft x 3.05ft) spacing treatment were randomly assigned to the main plots, and genotypes were randomly assigned to the split-plots. The three levels of genetics and two levels of spacing made six treatment combinations which were replicated three times.
The three seedling types consisted of one half-sibling and two clonal planting stocks. The half-sibling seedlings were the Arkansas Forestry Commission 3-star loblolly pine stock which were 1-0 bare root seedlings (several bulked families) produced from seed sources selected under the Western Gulf Tree Improvement Cooperative. These seedling are reported to have a 41-51% genetic gain over woods-run stock (http://forestry.arkansas.gov/Seedlings/Pages/default.aspx). The other two genotypes were ArborGen (formerly CellFor® clones Q3802 and L3791). Both were produced as 1-0 containerized seedlings. Q3802 clone was advertised as having exceptional tree form with small branches, narrow crown, outstanding stem straightness, excellent growth rate, and high resistance to fusiform rust (CellFor clone® 2010a). Clone L3791 was advertised as having an exceptionally high growth rates and being high resistance to fusiform rust and pitch canker, and possessing outstanding stem straightness (CellFor clone® 2010b).

Data Collection
First year ground-line diameter (GLD) and height (HT) of all seedlings were measured in December (2012) through January (2013) using caliper and meter stick respectively. Survival was also determined during this sampling period. We had observed an early flush in the clones during the spring of 2012, so we tracked flushing on random sample of all genotype in the spring of 2013 to determine if this behavior was repeated

Data Analysis

Effects of spacing, family and their interactions were analyzed using as split-plot with spacing as the main effect and family as the sub-plot effect. Effect of spacing, family, and their interaction on mean height, ground line diameter (GLD), survival, first flushing and flush length were analyzed using a mixed model approach (Proc Mixed, in SAS version 9.2) with the block and

genotype as a random effect with spacing as a fixed effect. Survival and presence of flushing was expressed as a percent per plot, we transformed these percentage using the arcsine function prior to running ANOVA (at α=0.05).

Result and Discussion

Effect on Survival and First Flushing

Survival was significantly affected by the genotype (*p*=0.03) (Table 1). At out site, Clone L3791 had a significantly higher survival rate (88%) than either clone Q3802 (78%) or the AFC three star seedling (73%). More time is needed to determine the reason behind this differential surviroship. Much of the region experienced drought during 2012-2013, which could have differentially impacted loblolly pine seedling survival. A Longer-term study (Adams et al. 2007) reported that the survival of loblolly pine at age of 9,13 and 17 was significantly affected by family and spacing, but given that these widely-spaced plantings have not yet reached canopy closure, spacing effects are not yet relevant in out study. Although we observed some differences in mean flushing among the families when we measured flushing rate in March (72 % for CellForclone L3791, 68% for Cellforclone Q3802, and 53 % for Halfsib), those differences were not statistically significant (Table 1).

Table 1: ANOVA table of arcsine transformed first-year survival and second year flushing in March.

Source	Survival					Flushing count			
	Num DF	MS	Error DF	F value	Pr>F	MS	Error DF	F value	Pr> F
Spacing	1	0.0014	1	0.35	0.6	0.000096	10.02	0.02	0.9
Block	1	0.0065	0.075	-4.44		0.11	0.65	1	0.5
Block*Spacing	1	0.004	10	0.41	0.5	0.004	10	0.04	0.8
Genotype	2	0.12	2	29.37	0.03[*]	0.16	2	0.71	0.5
Block*Genotype	2	0.0035	10	0.37	0.7	0.22	10	2.27	0.1
Residual	10	0.0097				0.99			

* Denotes significance at α=0.05

Effects on Diameter, Height, Flush Length

Overall, ANOVA results indicated that genotype-by-spacing interaction significantly affected diameter (p<0.01); however spacing did not have significant impacts on diameter (Table 2). Adams et al. (2007) reported the similar interaction effects between genotype and spacing on diameter at age of 17.The greatest diameter (1cm) occurred in the clone L3791in the narrow spacing; whereas least diameter (0.8cm) occurred in the halfsib with narrow spacing combination (Figure 1b). AFC stock (halfsib) diameter growth varied between the two spacing levels. Furthermore, AFC stock grown on narrow spacing was significantly smaller than the two clones.
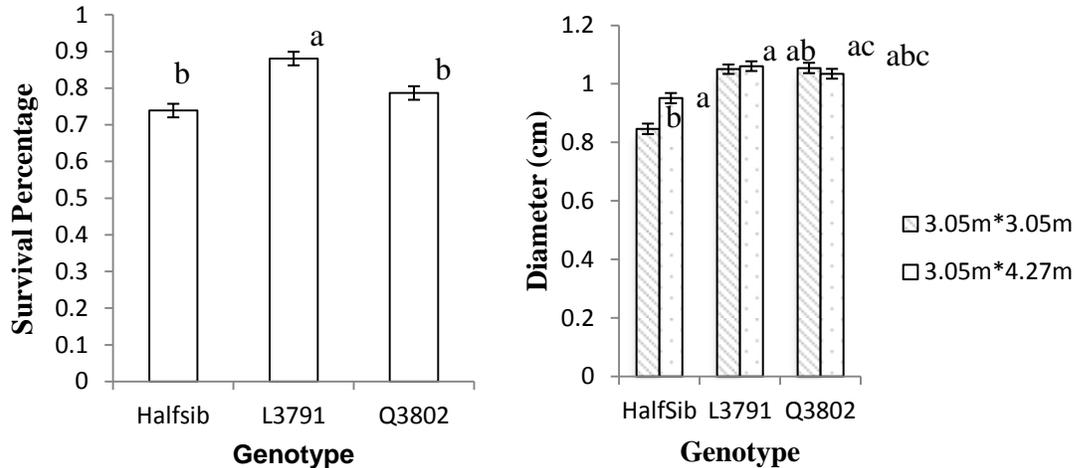
Figure 1. Mean (a) survival percentage by Genotype and (b) diameter by spacing and Genotype after one growing season. Means not followed by a common letter differ significantly (at α =0.05)

No significant effects of spacing or spacing-by-genotype interactions on height were observed, but height did vary significantly among genotype (p =0.05) (Table 2). The greatest height growth was 63.52cm for clone L3791 and the least was 41.66cm in halfsib (Figure 2a). Mulitiple comparisions with standard error and estimate indicated that clone L3791 had significantly greater height growth than the half sib seedling, but height growth was not significantly different between the two clones (L3791 and Q3802).

Also, genotype significantly affected flush length (p=0.01), while the effects of spacing and the interaction between spacing and genotype were not significant on flush length. In contrast to height growth, the flush length growth of clone Q3802 was significantly less than clone L3791 and the half-sib 3-Star stock(Figure 2b).

Table 2: ANOVA table of first year height and second year flush length.

Source	Diameter					Height					Flush length				
	Num DF	MS	Error DF	F value	Pr>F	Num DF	MS	Error DF	F value	Pr>F	Num DF	MS	Error DF	F value	Pr>F
Spacing	1	0.533	1.74	0.51	0.5	1	691.53	1	1.25	0.4	1	20.59	1	5.53	0.2
Block	1	0.0568	1.83	0.03	0.8	1	1761.91	2.34	0.44	0.5	1	29.49	77.136	-0.74	
Block* Spacing	1	0.000005	1924	0	0.9	1	553.64	1795	3.05	0.08	1	3.72	352	0.07	0.7
Genotype	2	4.63	3.59	1.48	0.3	2	69160	2	17.26	0.05[*]	2	127.15	2	88.97	0.01[*]
Block* Genotype	2	1.75	1924	23.64	<0.0001	2	4007.12	1795	22.11	<0.001	2	1.42	352	0.03	0.9
Genotype* Spacing	2	1.11	1924	15.08	<0.0001[*]										
Residual	1924	0.074				1795	181.27				352	52.19			

* Denotes significance at α=0.05
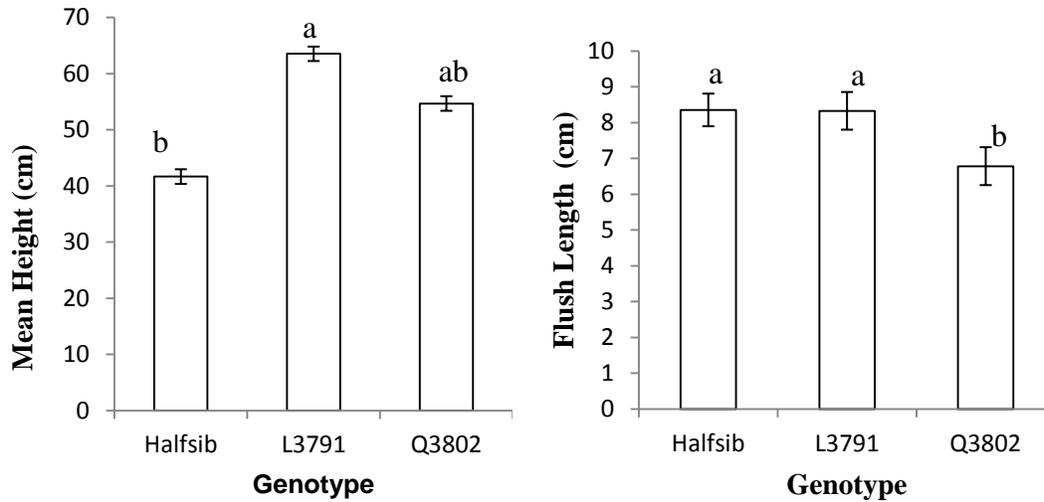
Figure 2. Mean(a) Height by genotype and(b) Flush length by genotype. Means not followed by a common letter differ significantly (at α =0.05)

Conclusion

One of the primary goals of genetic improvement is to enhance the productivity of loblolly pine plantations. Our preliminary results suggest that genotype differences appear very quickly under the conditions of our test site in southeastern Arkansas, and they provide further (albeit limited) support for the use of genetically improved planting stock. Clonal stock was found to have higher rates of survival as well as greater height and diameter growth when compared to half-sib improved stock; however, neither of the clonal varieties consistently outperformed the other in all measures. Across all parameters of interest, spacing/planting density did not vary by a statistically significant margin. These findings are not unexpected, given the early stage of stand development. More time is needed to determine if other factors, such as site conditions or intra- or interspecific competition, may change the outcomes of these measures of success. Although the clonal varieties did outperform the improved half-sibling 3-Star seedlings both in survival and growth, the lower cost and ready availability of the 3-Star stock make it a popular choice for landowners in southeastern Arkansas who want to plant improved loblolly pine. With additional data on relative improvements in stand productivity among clonal, full-sib and half-sib loblolly pine seedlings and with improvements in production and distribution of clonal stock, a greater proportion of forest landowners may seek to invest in genetically improved loblolly pine.

Literature Cited

Adams, J.P., S.B. Land, Jr., K.L. Belli, and T.G. Matney. 2007. Comparison of 17–year realized plot volume gains with selection for early traits for loblolly pine. For. Eol.Manag. 255:1781-1788.

"Arkansas Forestry Commission." Arkansas Forestry Commission.N.p., n.d. Web. 30 May 2013 (https://www.ark.org/afc2/seedlingsales.php).

Cellfor.2010a. Loblolly Pine Variety: CF Q3802 [Brochure].

Cellfor.2010b. Loblolly Pine Variety: CF L3791 [Brochure].

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

Larance, F.C., H.V. Gill, and C.L. Fultz.1976.Soil survey of Drew County, Arkansas.USDA Soil Conservation Service and Arkansas Agricultural Experiment Station.86 p.

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

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.

NOAA.2013.1981-2010 climate normals for Monticello, Arkansas.Online data from http://www.srh.noaa.gov/lzk/?n=wxcntl3.htm, last accessed 5 June 2013.

Prestemon, J.P. and R.C. Abt. 2002.The southern timber market to 2040. J.For.100:16-22.

Wear, D.N. and J.G. Greis (eds.). 2002.Southern Forest Resource Assessment. Gen.Tech. Rep. SRS-53. Ashville, NC: U.S. Department of Agriculture, ForestService, Southern Research Station.

A CLIMATE CHANGE RESPONSE FUNCTION FOR LOBLOLLY PINE (*PINUS TAEDA* L.) FROM THE WESTERN GULF REGION OF THE UNITED STATES

Thomas D. Byram[1], Tomasz E. Koralewski, Earl M. Raley

[1] Texas A&M Forest Service, 2585 TAMU, College Station, TX

The Western Gulf Forest Tree Improvement Program (WGFTIP) established a geographic seed source study for loblolly pine (*Pinus taeda L.)* between 1974 and 1978 to better delineate breeding and deployment zones within the Western Gulf region of the USA. A total of seventy-three plantings were established in two series with each series replicated over two years. Series I sampled 26 open-pollinated families representing a north-south transect, while Series II sampled 17 open-pollinated families in an east-west transect, that were planted at 43 and 30 test sites, respectively. The locations ranged from the maritime conditions of the Mississippi Gulf Coast to the more xeric and continental conditions encountered beyond the northern and western edges of the current natural range for the species in Arkansas and Oklahoma. The objectives of this study were to develop models that could be used to 1) guide future deployment decisions, 2) support projections of future forest productivity when combined with climate models, and 3) direct future breeding efforts by quantifying the variation among families for phenotypic plasticity exhibited in response to changing environments. Family responses to variation in climate were modeled using regression procedures in SAS. Age-fifteen height, diameter, and planted-tree volume were used as response variables. Mean minimum temperature of the coldest month (MMIN [°C]) and aridity index (AI) and its coefficient of variation, at both the provenance and the test site, were used as explanatory variables. Data show that the optimal performance is achieved when southern sources are moved to more northern locations by MMIN ≈ 2-4°C, which confirms earlier observations, and that a moderate level of AI is optimal. Both mean AI and its variance reached maxima near the western species boundary indicating their potential role as the range limiting factor. The data were insufficient, however, to delineate the adaptability-limiting factors in the east-west transect.

EFFECT OF ORGANIC SOILS ON TREE FORM AND QUALITY TRAITS OF OPEN-POLLINATED LOBLOLLY PINE IN THE COASTAL PLAIN OF NORTH CAROLINA

Christopher Rosier[1], Richard Bryant, Patrick Cumbie, Victor Steele

[1] ArborGen Inc, Bellville, GA

Loblolly pine (*Pinus taeda L.*) is the dominant pine plantation species in the southeastern United States. The productivity and product quality of these even-aged plantations can be influenced by a wide range of factors, including genetics, silvicultural practices, soil types, pests and disease. Genetically improved loblolly pine trees grown on highly organic soils have demonstrated significant increases in tree volume; however, the effects of genetics and intensive silviculture on tree form and wood quality is less understood on these soils. High incidences of stem and branch defects, forking, ramicorn and heavy branching have been observed in loblolly pine grown on high organic soils in the coastal plain of North Carolina. In addition, fusiform rust infection has been observed to be higher on organic soils than on mineral soils in the same geographic region, when studying the same genotypes. 3.In an effort to optimize performance for both growth and quality traits, fifteen open-pollinated loblolly pine families were grown on one organic soil site and one mineral soil site in Brunswick County, North Carolina, and evaluated at age seven. A row-plot design was used and a number of quantitative traits were measured, including tree height, diameter at breast height, sweep and fusiform rust infection. Quality traits assessed included forking, ramicorn branching, sweep, saw-timber potential, height to live crown, crown width and branch diameter.

INTEGRATING CLIMATE AND GENETIC EFFECTS OF LOBLOLLY PINE BY UNIVERSAL RESPONSE FUNCTIONS

Jianxing Zhang[1], Salvador A. Gezan, Gary F. Peter

[1] School of Forest Resources and Conservation, University of Florida, Gainesville, FL

Loblolly pine is adapted to grow across wide environmental gradients of soil and climate. The degree of genetic differentiation selected for by climate is understudied. Current knowledge suggests, like annual crops, that mean monthly minimum winter temperature, is a key variable for seed movement. Important questions remain about the risks associated with moving local germplasm sources to different climatic zones, and whether additional climatic variables such as rainfall will become more important. To address these questions we are analyzing the relationship between the geographic origins of loblolly germplasm. In 1982-1983, seven provenance-progeny tests wild seed originating from Florida, Gulf and southern Atlantic coast were established in Alabama, Florida, Georgia and Mississippi and tree height, stem diameter and volume and disease traits were measured at years 5, 10, and 15. In the winter of 2001-2002, ten Florida Wild-Seed tests were established in Florida, Georgia, South Carolina, and Alabama, and tree height, stem diameter and disease traits were measured at year 6. With data from both these tests, different statistical methods are being evaluated to develop new universal response functions. Our goal is to create a more robust set of seed deployment guidelines by creating universal response functions with different variable selection methods, and generate maps for matching optimum genetic materials with appropriate planting sites.

GENETIC CONTROL OF GROWTH AND SHOOT PHENOLOGY IN LOBLOLLY PINE (*PINUS TAEDA* L.) CLONAL TRIALS DURING THE SECOND AND SIXTH GROWING SEASONS

Tania Quesada[1], Liliana M. Parisi, Dudley A. Huber, John M. Davis, Salvador A. Gezan, and Gary F. Peter

[1] School of Forest Resources and Conservation, University of Florida, Gainesville, FL

Pine genetic improvement programs in the Southeast United States have resulted in the development of loblolly pine material with increased yield and disease resistance (Li et al., 1999). Most traits of interest are related to growth, wood properties or disease resistance and are usually multigenic. In addition, growth and crown traits have been very well characterized in loblolly pine and their genetic architecture is well known (Baltunis et al., 2006; Emhart et al., 2007; Hodge and White, 1992). In contrast, the genetic architecture of phenology traits is less well known, despite the increased interest in forestry research, particularly in anticipation of changes in global climate patterns (Hanninen and Tanino, 2011). There is evidence that growth phenology traits are under genetic control (Jayawickrama et al., 1998), but these have not been well characterized. In this study, we compare second-year growth and phenological traits from two loblolly pine sites from a clonal study consisting of 71 full-sib families, including some high-performing elite genotypes, and we estimate their genetic correlations with sixth-year growth measurements.

MATERIAL AND METHODS

Measurements of growth and phenological traits were taken from two populations of 71 full-sib families obtained from crosses among 36 selected parents and located in Palatka, Florida and Cuthbert, Georgia. The crosses were obtained using a circular mating design and consisted of rooted cuttings and seedlings from each family planted in a randomized incomplete block design with four replications. Growth and flush measurements from both sites consisted of total height at years 2 and 6, average crown width at year 6, diameter at year 6, number, average and sum of flush lengths at year 2, as well as number of stem units (NSU) and mean stem unit length (MSUL) at year 2. Phenology traits measured at the Palatka site at year 2 were: growth initiation, cessation, duration and rate, whereas only cessation was recorded during the second growth season in the Cuthbert site.

Broad-sense and narrow-sense heritability estimates were obtained for cuttings and seedlings for all traits in both sites and growing seasons. Genetic correlations were estimated among all traits within a site as well as across sites (type B correlations).

RESULTS AND DISCUSSION

Measurements for growth, flush descriptors and phenological traits were obtained for 3472 cuttings and 928 seedlings at the Cuthbert site, and for 3656 cuttings and 824 seedlings at the Palatka site. The mean heights observed during the second (2.24-3.27 m) and sixth-year (6.5-8.4) measurements in Palatka and Cuthbert were similar to those previously reported, whereas mean crown width and diameter ranges between 1.41 and 1.98 m and 10.1 and 13.5

cm, respectively. Mean flush lengths were 36.9 cm in Cuthbert and 24.9 cm in Palatka (Figure 1), whereas the number of flushes remained fairly constant between the two sites (5.39 and 5.35 for Cuthbert and Palatka, respectively, suggesting that differences observed in height were mainly due to flush length and not number of flushes. Higher values for growth traits in Cuthbert with respect to Palatka may be due to more favorable growth conditions in Cuthbert for loblolly pine cuttings and seedlings, as both sites have different soil types and environmental conditions (Parisi, 2006).
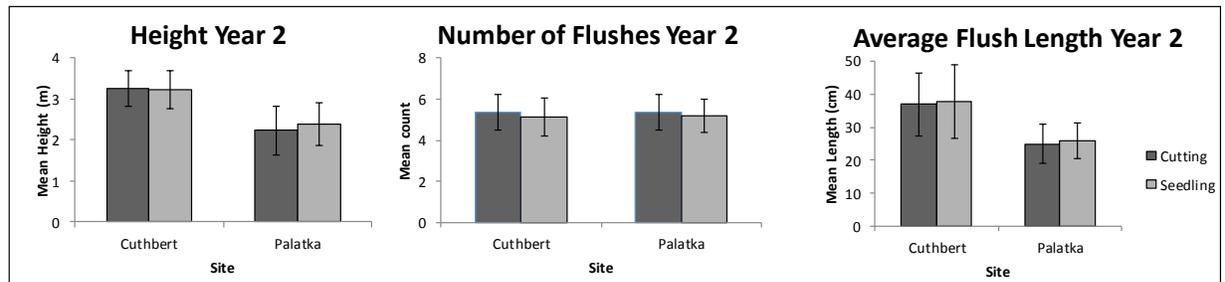
Figure 1. Mean height, number of flushes and average flush length in the second growth season for cuttings and seedlings in Palatka, FL and Cuthbert, GA. Error bars correspond to standard deviations from the mean.

Heritability estimates in cuttings from both sites were moderate to high for most growth and flush traits, with broad-sense heritabilities ranging between 0.35 and 0.68 in Cuthbert and 0.27 and 0.52 in Palatka. These estimates are consistent with those from previous reports in loblolly pine and other conifers (Hodge and White, 1992) and are suggestive of strong genetic control. For phenology traits, cessation showed the lowest heritability in both populations, with 0.0025 and 0.078 in Cuthbert and Palatka, respectively (Table 1), suggesting that there is a strong environmental influence for this trait, whereas shoot initiation is likely to be more under genetic control.

Table 1. Broad-sense heritabilities for different growth, flush and phenological traits in loblolly pine cuttings during the second and sixth growth seasons for the Cuthbert and Palatka sites. Numbers in parenthesis correspond to standard errors.

Site	Height year 2	Height year 6	Crown Width year 2	DBH year 6	Number Flush year 2	logAvFL year 2	Sum FL year 2	MSUL year 2	NSU year 2	Initiation year 2	Duration year 2	Cessation year 2	Growth Rate year 2
Cuthbert	0.68 (0.17)	0.35 (0.11)	0.67 (0.17)	0.53 (0.15)	0.49 (0.15)	0.39 (0.12)	0.49 (0.15)	0.37 (0.11)	0.27 (0.09)	-	-	0.0025 (0.0048)	-
Palatka	0.32 (0.088)	0.52 (0.047)	0.27 (0.078)	0.45 (0.12)	0.43 (0.12)	0.31 (0.97)	0.36 (0.07)	0.21 (0.076)	0.17 (0.062)	0.74 (0.17)	0.19 (0.058)	0.078 (0.028)	0.34 (0.046)

Strong genetic correlations (r>0.6) were observed in cuttings for a given site between second year growth traits (height, diameter and sum of flush length) and sixth year height and diameter in Palatka. Correlations were slightly lower in the Cuthbert site. This suggests

that such traits could be potentially important for early selection of material in breeding programs. Type B genetic correlations were also high (r>0.64) between Palatka and Cuthbert for a given trait. The high correlations suggest low genetic by environment (G x E) interactions and could represent an advantage for deployment in these two sites, as the material may likely have a similar performance in these locations.

Even though much is known on the genetic architecture of growth traits in loblolly pine, much less is known on shoot phenology. In this study, genetic analysis of shoot phenology in the Palatka site showed that most phenology traits have moderate to strong heritability values, and are thus likely to be under strong genetic control, with the exception of cessation, which showed very low heritability, and is therefore more likely to be under strong environmental influence. Phenology traits and their relationship to growth are useful for understanding how the timing of growth events is affected in different environments. Further knowledge on these relationships can be useful for modeling tree growth in new sites or under different environmental conditions, particularly as global climate is predicted to continue changing in the near future.

REFERENCES

Baltunis, B.S., Huber, D.A., White, T.L., Goldfarb, B., and Stelzer, H.E. 2006. Genetic analysis of early field growth of loblolly pine clones and seedlings from the same full-sib families. Canadian Journal of Forest Research **37**(1): 195-205.

Emhart, V., Martin, T., White, T., and Huber, D. 2007. Clonal variation in crown structure, absorbed photosynthetically active radiation and growth of loblolly pine and slash pine. Tree Physiology **27**(3): 421-430.

Hanninen, H., and Tanino, K. 2011. Tree seasonality in a warming climate. Trends in Plant Science **16**(8): 412-416.

Hodge, G., and White, T. 1992. Genetic parameter estimates for growth traits at different ages in slash pine and some implications for breeding. Silvae Genetica **41**(4-5): 252-262.

Jayawickrama, K., McKeand, S., and Jett, J. 1998. Phenological variation in height and diameter growth in provenances and families of loblolly pine. New Forests **16**(1): 11-25.

Li, B., McKeand, S., and Weir, R. 1999. Tree improvement and sustainable forestry - Impact of two cycles of loblolly pine breeding in the U.S.A. Forest Genetics **6**(4): 229-234.

Parisi L.M. 2006. Shoot elongation patterns and genetic control of second year height growth in *Pinus taeda* L. using clonally replicated trials. MSc Thesis. Forest Resources and Conservation. University of Florida Gainesville, Florida, p. 93.

TREE IMPROVEMENT AND SILVICULTURE, TEAMWORK IN ACTION

Marshall A Jacobson

Plum Creek Timber, Watkinsville, GA

Forest Management has matured from a time when tree improvement targeted seed supply, and silviculture targeted survival. Currently, when combined efficiently, the combination of genetics and silviculture has the ability to increase yield per acre by a factor of two or more. Tree Improvement brings more to the table than just increased yield, however, as stem quality, disease resistance, and seed quality are all improved to the point that we often take them for granted. Past practices have given way to advanced operations combining tree improvement and silviculture. The future will bring more opportunity to target specific sites, regimes, and outcomes. Challenges are to understand the impacts, and have the ability to model the impacts of the many practices to allow more accurate quantitative predictions of the future.

In the 1980s, first generation testing was catching up with early seed orchards, and slowly, all wild collections, and collections from thinned stands were phased out. In addition, significant gains were made in resistance to fusiform rust, as orchards were rogued, and excess seed supply allowed targeted collections. In the same time frame, silviculture adopted the use of herbicides, and overall production levels were improved. It was recognized that Site Index with a base age of 25 was being increased, often by 10-20 feet with the combined use of culture and genetics.

Nursery practices were advanced with the use of precision seeders, and the culture to provide a high quality seedling that made even better use of the silviculture in the field, adding more consistency to stand growth and development.

A system that utilizes all techniques is not inexpensive, and the need to predict the future is critical to continued investment in genetics and silviculture.

New options available to us looking forward include and array of genetic, including varietals. Options for regeneration include bare toot and containerized seedlings, and a wide array of herbicides to control competition.

Continuous improvements in genetics, silviculture, and seedling quality provide tools to allow real increases in overall growth, stand quality, and value. As we move ahead, matching prescriptions for future growth as well as markets will be challenging. We need the standard background, such as genetic potential, soils, disease, and some climate factors, but we will also need the ability to predict outcomes with better certainty to optimize performance.

PLANTED FOREST VALUE: TREE BREEDERS CAN ENABLE NEW MARKETS

Gary F. Peter[1], Rafael de la Torre

[1]School of Forest Resources and Conservation, University of Florida, Gainesville, FL

Since forest tree genetic improvement began in the 1950s, breeders have increased wood yields by selecting for fast early growth and resistance to fungal diseases. For southern pines, planting improved families and managing them with good silvicultural practices increases stem yields more than 10 fold. Widespread adoption of these systems has dramatically increased the wood supply and enabled traditional wood based industries to expand and also enabled new markets for wood. New markets such as engineered wood and most recently electricity, biofuel and chemicals have implemented new technologies to take advantage of the abundant supply of wood from young pine trees at predictable prices. Thus, breeders focus on increased volume has enabled new markets for wood. Given this success and the fact that current markets still only directly value stem diameter and volume/wet mass, make it difficult for breeders to focus on non growth traits like wood properties. Moreover, the long development and growth cycles, as well as large deployment scales complicate justifications for improving wood properties. To overcome these challenges, development cycle times need to be dramatically shortened and credible ways to value wood properties for growers and wood processors need to be developed. Recent advances in DNA marker technologies and analytical methods offer the potential to dramatically shorten breeding cycles. However, identifying specific wood properties that justify investment in accelerated breeding and biotechnology remains a significant challenge. Case studies for valuing changes in juvenile wood mechanical and chemical properties will be presented.

WILL ADVANCED LOBLOLLY PINE GENETICS DELIVER ADDED VALUE TO LANDOWNERS IN THE SOUTHEASTERN UNITED STATES?

W. Patrick Cumbie[1], Rafael De La Torre, Michael Cunningham, John Pait

[1]ArborGen Inc, Ridgeville, SC

Advanced generation loblolly pine (*Pinus taeda L.*) genetic trials have produced substantial gains in growth, stem form, and fusiform rust resistance at early ages, but the demonstration of stand level gains has been elusive.   As landowners consider the many genetic options for plantation establishment now available, demonstrated gains and increased financial value are critical for the adoption of elite genetics such as MCPÂ® and varieties. A trial of three open-pollinated and two full-sib families was established in 1998 in the lower coastal plain of South Carolina. Each family was planted in 100-tree block plots in each of four replicates at a single location. Mid-rotation data after twelve years of growth shows a value increase of over 100% in bare land value and a volume increase greater than 50% for the best full-sib family when compared to open-pollinated families. Financial analyses revealed that the increased cost of full-sib seedlings is justified with volume gains alone over open-pollinated seedlings but additional value is captured through stem quality which influences the potential number of sawtimber trees harvested. Volume growth and sawtimber potential will be reported before and after an operational thinning, and each family will be modeled to an age 24-year rotation. Revenue at rotation age, internal rates of return, bare land value, and marginal analysis for seedling price will be discussed.

PHYSIOLOGICAL APPROACHES TO SPECIES CONSERVATION AND RESTORATION

Rodney E. Will[1], John F. Stewart, C. Dana Nelson

[1]Department of Natural Resource Ecology and Management, Oklahoma State University, Stillwater, OK

Physiological processes link genotypic and phenotypic expression. As such, understanding the physiological response of individuals and species to environmental variation is important to achieve desired management objectives. Global change, e.g., climate change, land-use change, invasive species, fire suppression, urbanization, has intensified the need for species conservation and ecosystem restoration under changing and often novel conditions. Because of global change, current or historical species-site information are less reliable as a guide for conservation. Rather, predicting the best locations and environmental conditions to attempt restoration or conservation might be accomplished through understanding species-level physiological responses. At the level of the individual (within species), specific genotypes that exhibit desired physiological traits will need to be identified and selected based on their expected suitability to current and future conditions. Increased hybridization between shortleaf pine (*Pinus echinata*) and loblolly pine (*P. taeda*) is likely a response of global change (in particular fire suppression and climate change) and is affecting species identity, morphological and physiological traits, and possibly the future resilience of southern pine forests. The hybrids exhibit faster growth like loblolly pine, greater drought tolerance like shortleaf pine, intermediate sprouting ability following topkill, and lack a strong basal crook (a shortleaf pine adaptation to protect dormant buds from fire). A complete understanding of the physiological and morphological differences between the hybrids and the parent species will allow for more effective practices to maintain shortleaf pine stands and to focus conservation and restoration efforts on sites with the highest likelihood of success.

DNA MARKER APPROACHES TO SPECIES CONSERVATION AND RESTORATION

C. Echt[1], S. Josserand, V. Hipkins, and B. Crane

[1] USDA Forest Service, Southern Institute of Forest Genetic, Saucier, MS

Maintaining or increasing gene diversity must be an integral part of species conservation and restoration. Because we seldom, if ever, know which particular genes are essential for successful conservation and restoration efforts, we must monitor gene diversity by proxy, that is, by using a suitable sampling of random genetic markers from the genome. A properly selected set of DNA markers can provide accurate data about population inbreeding, gene flow, differentiation, and substructure. When applied to managed populations, DNA markers also can help assess the genetic diversity and level of inbreeding of seed orchards and restored stands. Currently, the DNA markers of choice are microsatellites (SSRs) because of their potential for efficient detection of multiple alleles. By way of example, we analyzed the distribution of longleaf pine SSR allele diversity in natural and managed germplasm sources at different spatial scales across longleaf's natural range. Specifically, we looked for population genetic differences among populations and ecoregions, evidence for inbreeding, maintenance of gene diversity in germplasm collections, and, at the northern extent of the range, localized spatial patterns of relatedness.

MATERIALS and METHODS

The 745 longleaf pine samples from several sources, represented in 17 populations (18 cohorts), were genotyped for 10 SSR marker loci: NZPR0143, PtTX052, PtTX4003, PtTX4058, PtRIP_0984, PtSIFG_0561, PtSIFG_0745, PtSIFG_3147, PtSIFG_4102, and PtSIFG_4218 (Echt et al. 2011a). Samples from Virginia were additionally genotyped for markers PtSIFG_6065 and PtSIFG_6067 (Echt et al. 2011b). We omitted samples that were missing data for more than one marker, yielding 709 samples analyzed. The institutional sources of samples were as follows: clonal archives at Southern Region National Forest System's Seed Orchards (prefix NFS, Table 1), clonal archives at North Carolina Forest Service Nurseries (NCFS), a seedling seed orchard at Berry College (BC), native Virginia trees (VDF), a Virginia Department of Forestry provenance trial established with OP seed from International Forest Company (IFC), and a coastal and piedmont clone mix obtained from NCFS (NCFS_NCmix). Kurt Johnsen and Chris Maier collected samples from the VDF provenance trial; Billy Apperson and Bob Eaton collected samples from native Virginia stands. Records show that trees from which scions or needles were collected, and native Virginia trees, were established before 1930 and therefore are presumed to be naturally regenerated from native germplasm. The same is assumed true for stands from which IFC collected seed.

The NFGEL lab isolated DNA and determined genotypes of sample groups NF, NCFS, and BC. The SIFG lab isolated DNA and determined genotypes of sample groups IFC, NCFS and VDF.

All DNA extractions were from needle tissue. Following PCR amplification, both labs used ABI DNA Analyzers (capillary electrophoresis) for allele fragment detection. We ran control DNAs of known longleaf pine genotypes in both labs to standardize allele assignments, which allowed merging of data sets for analyses.

Gene diversity statistics, N_a, N_e, and H_e, were calculated with GenAlEx v6.501 software (Peakall & Smouse 2012). Estimates of inbreeding, F, for each population were obtained by simultaneously estimating frequencies of null alleles (non-amplifying SSR alleles that can deflate observed heterozygosity) using an individual inbreeding model and a Gibbs sampler with 10,000 iterations, as described by Chybicki & Burczyk (2009) and implemented in their INEst v1 software. F in this context is reported as the probability of alleles at a locus being identical by descent; that is, not Wright's F, but Malecot's. Genetic differentiation among populations or regions was measured by Jost's D_{est} with bootstrap 95% CI calculated by the DEMEtics R-package (Gerlach et al. 2010); hierarchical AMOVA for F_{st} was calculated by GenAlEx using 9999 permutations. Principal component analyses were conducted in GenAlEx using pairwise D_{est} values. All the above metrics were calculated using genotypes from the first set of 10 SSR loci listed above. For the Virginia population samples only, using genotypes from all 12 marker loci, two-dimensional local spatial autocorrelation (2D-LSA) was conducted with GenAlEx and plotted as bubble charts in Excel; sibling groups were identified with their 95% CI by maximum likelihood analysis using ML-RELATE software (Kalinowski et al. 2006); estimations for proportions of seedling progeny contributed by specific parents were conducted using a full-pedigree likelihood method implemented in COLONY.v2 software (Jones & Wang 2010).

RESULTS and DISCUSSION

Marker allele diversity results are summarized in Table 1. For all but one population, genotypes from 25 to 60 individuals were analyzed. This level of sampling is sufficient to estimate population allele frequencies accurately (Hale et al. 2012). Diversity measures for the one exception, a clonal archive of 17 sampled genets representing, a southern Alabama population (NFS_ALs), did not contradict our overall conclusions. We saw no evidence for differences in genetic diversity (N_a, N_e, H_e) among germplasm sources, with but one exception. The exception, a native Virginia provenance (VDF_VAnative), is an interesting and instructive case that we discuss in detail below. (Consequently, this population was not included in our range-wide diversity and differentiation analyses.) Once null alleles were accounted for, we saw no evidence of inbreeding in any population (95% CI of F included zero). We conclude that the various germplasm collections adequately represent natural longleaf pine gene diversity.

Contrary to expectations for populations from different ecozones, we measured slight or non-existent population differentiation across the range. AMOVA showed 1% of genetic variation was between populations, the rest within ($F_{st} = 0.01$, $p < 0.000$). In pairwise population comparisons, the maximum differentiation in allele frequencies was between the two most

geographically distant populations from coastal North Carolina (NCFS_NCmix) and east Texas (NFS_TX) (D_{est} = 0.09, 95% CI = 0.07 – 0.11).

To better assess broad regional evolutionary and biogeographic trends, we pooled samples within the five main geographic regions (Figure 1, Table 1) and then calculate pairwise differences. We noted with interest that the montane longleaf region (Mtn in Figure 1) is evolutionarily closest to the central Gulf (S coast) region. Also of interest is that piedmont longleaf is equally diverged from the east coast and south coast regions; we cannot speculate on its biogeographic origins. The largest, though still relatively small, difference was seen between the east coast and the west regions. Based on their isozyme survey, Schmidtling and Hipkins (1998) hypothesized a Holocene migration of longleaf westward up through south Texas toward the east coast. Our current analyses do not contradict this scenario, but do present the alternative hypothesis of post-glacial migration northward and outward from the central Gulf region. In any event, we conclude that there has been historically high gene flow through longleaf's range; any genetic effects of recent population declines and habitat fragmentation are not yet evident.

The VDF_VAholland population was not included in the preceding discussion of range-wide analyses because it proved to be a special case. This population derived from seeds collected at a small site of ten trees, 80 to 100 year-old, near Holland, VA. It was used by the Virginia Department of Forestry as the native Virginia representative in a provenance trial. Initial principal coordinate analyses (not shown) demonstrated it to be a genetic outlier. It had considerably lower marker diversity than other populations, but no evidence of population level inbreeding (Table 1). Suspecting consanguineous matings at the site, we genotyped the candidate parent trees and others from the surrounding area (these made up the VDF_VAnative population) and conducted 2D-LSA to look for clusters of relatedness. This spatial analysis identified four related individuals within the Holland site. Subsequent sibship analysis showed that these four trees were a group of full- and half-sibs (one of several possible sibship groups at the site). Further, a separate parentage analysis indicated that up to 40% of the seedlings from the Holland site shared one parent and at least one tree at the site from which seed was collected did not contribute any seedlings to the provenance trial. Therefore, not only were there strong familial relationships among many of the ten potential seed source trees, their progeny had very skewed parental representation. What is particularly notable about the Holland, VA provenance is that, despite its narrow genetic base, it performed as well or better than the other seven south-wide longleaf provenances in the trial (Creighton and Johnsen, personal communication). In conclusion, we have seen that fine-scale spatial analysis of individuals can identify strong genetic relationships not evident in population level analyses. We recommend that local genetic structures should be determined to identify seed sources that can maximize genetic diversity in germplasm conservation and tree improvement programs.

TABLE 1. Summary statistics for longleaf pine population genetic diversity and inbreeding.

Population	Region	n	N_a	N_e	H_e	F (SE)
IFC_SC	E. coast	50	6.8	3.6	0.64	0.013 (0.010)
VDF_VAnative	E. coast	60	6.8	3.4	0.64	0.011 (0.010)
VDF_VAholland	E. coast	51	4.0	2.3	0.53	0.007 (0.008)
NCFS_NCcoast	E. coast	32	6.4	3.7	0.65	0.006 (0.007)
NCFS_NCmix	E. coast	51	6.0	3.9	0.65	0.009 (0.009)
IFC_NC	piedmont	44	6.8	3.6	0.66	0.011 (0.010)
NFS_NC	piedmont	39	6.8	3.7	0.66	0.009 (0.010)
NCFS_AL	montane	26	5.9	3.5	0.65	0.008 (0.009)
BC_GA	montane	30	6.4	3.8	0.67	0.012 (0.012)
IFC_AL	montane	52	7.2	3.8	0.66	0.013 (0.015)
NFS_ALs	S. coast	17	5.6	3.3	0.63	0.007 (0.009)
NFS_FL	S. coast	25	6.5	3.6	0.64	0.008 (0.010)
NFS_MS	S. coast	26	6.3	3.4	0.65	0.010 (0.012)
IFC_FL	S. coast	48	6.5	3.8	0.67	0.025(0.023)
IFC_GA	S. coast	50	7.3	3.5	0.64	0.014 (0.013)
IFC_MS	S. coast	52	6.8	3.7	0.66	0.048 (0.024)
NFS_TX	west	30	6.7	3.9	0.68	0.005 (0.005)
NFS_LA	west	26	6.0	3.4	0.65	0.013 (0.016)

Population name prefix denotes the institutional source of the germplasm, followed by the state were germplasm originated, ending with a suffix further specifying germplasm origin. N_a mean number of alleles per locus (allelic richness), N_e effective allele number, H_e expected heterozygosity, F null-corrected inbreeding coefficient and its standard error.

FIGURE 1. Evolutionary relationships among regional pools of longleaf pine populations. The neighbor-joining algorithm (Saitou & Nei 1987) was used to generate the tree of evolutionary distances, conducted in MEGA5 (Tamura et al. 2011). The optimal tree with the sum of branch length = 0.05 is shown. The tree is drawn to scale, with branch lengths in D_{est} units.
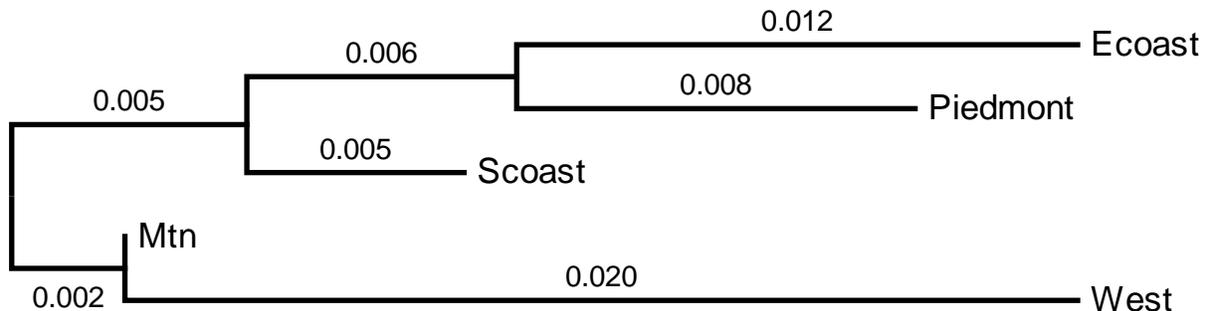
REFERENCES

Chybicki IJ and Burczyk J. 2009. Simultaneous Estimation of Null Alleles and Inbreeding Coefficients. *Journal of Heredity,* 100:106–113.

Echt CS, Saha S, Krutovsky K, Wimalanathan K, Erpelding JE, Liang C, Nelson CD. 2011a. An annotated genetic map of loblolly pine based on microsatellite and cDNA markers. BMC Genetics 2011, 12:17

Echt CS, Saha S, Deemer DL, Nelson CD. 2011b. Microsatellite DNA in genomic survey sequences and UniGenes of loblolly pine. Tree Genet. Genome 7: 773-780.

Gerlach G, Jueterbock A, Kraemer P, Depperman J, and Harmand P. 2010. Calculations of population differentiation based on G_{st} and D: forget G_{st} but not all of statistics! Molecular Ecology 19:3845-3852.

Hale ML, Burg TM, Steeves TE (2012) Sampling for Microsatellite-Based Population Genetic Studies: 25 to 30 Individuals per Population Is Enough to Accurately Estimate Allele Frequencies. PLoS ONE 7(9): e45170.

Jones OR, and Wang J. 2010. COLONY: a Program for Parentage and Sibship Inference from Multilocus Genotype Data. Molecular Ecology Resources 10: 551–555.

Kalinowski ST, Wagner AP, and Taper ML. 2006. Ml-relate: a Computer Program for Maximum Likelihood Estimation of Relatedness and Relationship. Molecular Ecology Notes 6: 576–579.

Peakall R and Smouse PE. 2012. GenAlEx 6.5: Genetic Analysis in Excel. Population Genetic Software for Teaching and Research—an Update. Bioinformatics 28: 2537–2539.

Saitou N and Nei M. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* 4:406-425.

Schmidtling RC, Hipkins V. (1998) Genetic diversity in longleaf pine (*Pinus palustris*): influence of historical and prehistorical events. Canadian Journal of Forest Research 28:1135-1145.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, and Kumar S. 2011. MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Molecular Biology and Evolution* 28:2731-2739

THE ROLE OF GENETICS IN AGROFORESTRY SYSTEMS

Gwendolyn Boyd

Alcorn State University, Lorman, MS

Agroforestry is the science and practice of intensive land-use management combining trees and/or shrubs with crops and/or live stocks. There are five integrated Agroforestry practices. They are alley cropping, forest farming, riparian buffers, silvopasture, and windbreaks. The trees in Agroforestry systems are the main emphasis. We can use genetics to improve the longevity, quality, and insect resistant of each species. Genetic enhancements will improve the longevity of each tree species by cultivating the desired genes. Gene pools for genetically altered trees can be produced in the laboratory. Finding the correct combination of genes will be the limiting factor. Genes determine the quality as well as the ability of tree species to resistant insect pests. Several pests have found trees as food sources which in turn causes tree mortality. Producing insect resisting trees through the use of genetics can enhance tree quality. High quality tree generates increased income to the landowner. Landowners desire to produce a profit from enterprises that are money-making endeavors. The Agroforestry practices have the ability to diverse the landowners land use. Genetics can be used in any aspect of these practices. It can play a major role in not only the trees which were previously mentioned, but also any other areas such as crops or even live stocks. Genetics has many venues to be directly incorporated into Agroforestry practices.

MULTIPLE PEDIGREES ALLOW CONSTRUCTION OF A DENSELY POPULATED REFERENCE LINKAGE MAP IN LOBLOLLY PINE (*PINUS TAEDA* L.)

V.E. Chhatre[1], M.F.R. Resende, P. Munoz, G.F. Peter, J.M. Davis, M.M. Kirst, C.S. Echt, K.V. Krutovsky, and C.D. Nelson

[1]Genetics Graduate Program, Texas A&M University, College Station, TX

A linkage map remains an indispensible tool for QTL analysis, application of marker-assisted selection (MAS), comparative mapping, and genome assembly. In loblolly pine, several high-density linkage maps are presently available (e.g. Eckert et al. 2010), including a reference map (Echt et al. 2011) and a recently released high-density linkage map (Martinez-Garcia et al. 2013). Most of these maps are based on one or two pedigrees and their map-distance resolution is limited by the size of the mapping populations. A densely-populated, high-resolution reference map, integrating as many as possible of available genetic markers in loblolly pine, is essential not only for QTL mapping but also for a better genome assembly now that a genome sequence has been released.

An integrated, framework map was constructed using about 3,000 SNP and 472 other (SSR, ESTP, RFLP) markers and the following populations: (1) two three-generation outbred pedigrees (QTL and BASE) consisting of 674 progeny-clones; (2) a pseudo-backcross family, consisting of 490 progeny-clones; and (3) a family-structured population (CCLONEs), consisting of 34 parents and 948 progeny-clones. Our goal is to define and map approximately 50 framework loci per linkage group, separated by intervals of up to 5cM. The remaining unmapped loci will be assigned to the framework map bin intervals using the multiple families in CCLONEs. Results of this work and the utility of the final map for QTL analysis of traits will be discussed.

IMPACTS OF COST-EFFECTIVE HIGH-THROUGHPUT GENOTYPING OF LOBLOLLY PINE ON APPLIED TREE BREEDING PROGRAMS

Ross Whetten[1], Will Kohlway, Laura Townsend

[1]Department of Forestry & Environmental Resources, NC State University, Raleigh, NC

Advances in DNA sequencing technology over the past several years have increased the yield of DNA sequence per dollar by several orders of magnitude. Over the same time period, strategies have been developed for combining multiple DNA samples together to minimize labor costs and maximize the transfer of effort from researchers to computers. The combined result is that it is now possible to discover and describe genetic variation at far higher resolution than ever before, at a cost comparable to that of establishing and maintaining field trials for progeny-testing candidate selections. Costs of establishing, maintaining, and measuring field trials are likely to increase in the future, as labor and operating costs increase with inflation, while the cost of genotyping is likely to continue decreasing for at least the next few years. This is likely to lead to a situation in which obtaining progeny phenotypes to use in estimation of parental breeding values will be much more expensive than high-density genotyping of the parent trees. This change in the cost structure of phenotyping versus genotyping will lead to a new and unfamiliar set of opportunities for tree breeders to apply molecular markers in applied breeding programs. Exploiting these new opportunities in the most effective and efficient way is likely to require changes in strategies for breeding, selecting, and field testing. This presentation will describe the methods for tissue collection, DNA isolation, and cost-effective genotyping, and present data regarding the suitability of the resulting markers for genetic analysis in breeding and testing populations of loblolly pine.

THE INFLUENCE OF CHINESE CHESTNUT GENOME PROPORTION ON THE SUCCESS OF SOMATIC EMBRYOGENESIS IN CHESTNUT

C.T. Holtz[1], A.R. Tull and S.A. Merkle

[1]Warnell School of Forestry and Natural Resources, University of Georgia, Athens, GA

The American chestnut (*Castanea dentata*) was one of the most economically important angiosperm forest tree species in the eastern United States up until the early 1900s (Carraway and Merkle 1997). The range of the species extended from northern Georgia and Alabama through southern Maine and parts of Canada (Burnham 1988). Comprising up to 25 percent of the trees in the Appalachian forest and with a natural life expectancy of 500-1000 years, the American chestnut could reach up to 150 feet tall and 10 feet in diameter. The tree was fast-growing and its wood was highly rot-resistant and did not warp or shrink, thus making it a major timber tree for construction lumber and furniture (Andrade and Merkle 2005; Vieitez 1995; Anagnostakis 1987). The nuts that the tree produced were an essential cash crop. A single tree could produce over 100 kilograms of nuts annually at maturity (Vieitez 1995).

Around 1904, the chestnut blight fungus (*Cryphonectria parasitica*) was introduced to the United States on Asian chestnut nursery stock. *C. parasitica* is necrotrophic, producing a sunken canker that eventually girdles the tree. The fungus spread approximately 200 miles every 10 years, and it took only 45 years for 9 million acres of American chestnut trees to be either infected or dead (Anagnostakis 1987; Burnham 1988; Johnson et al. 2008; Vieitez 1995). Today, the tree can still be found in the understory due to its stump sprouting ability, but rarely does it survive beyond a few years. Natural reestablishment of the American chestnut is highly unlikely, as the blight remains an obstacle.

As a part of the considerable effort to re-introduce the American chestnut to its native range, a significant amount of research has been put forth to produce and propagate American chestnut trees with blight resistance. One such approach is hybridization with Asian chestnut trees, which carry resistance to the fungus. Burnham (1988) suggested that crossing an American chestnut with a Chinese chestnut and then backcrossing multiple times to American chestnut would result in an individual with both American chestnut characteristics and the genes from Chinese chestnut responsible for blight resistance. The American Chestnut Foundation (TACF) was established to accomplish the plan that Burnham proposed. Since a system has already been developed to clonally propagate pure American chestnut trees via somatic embryogenesis (SE; Carraway and Merkle, 1997; Andrade and Merkle 2005), we believe it also has great potential to be used to multiply blight-resistant trees produced by TACF's hybrid backcross breeding program.

Since it is well-known that genotype exerts a strong influence in embryogenesis induction, it is possible that Chinese chestnut (CC) genes in the hybrid backcross material could affect the success of SE using the protocol established for American chestnut (AC). To date, there have been no published reports of somatic embryogenesis in either CC or hybrid backcross material. Over three years of culture initiations, we tested the effects of CC genome proportion on the success of SE induction using our standard protocol for culturing AC and, subsequently,

protocols based on a published protocol for SE in European chestnut (Vieitez 1995). A total of 145 different chestnut genotypes were used to assess the impact of hybrid genotype on the success of embryogenesis induction. As shown in Table 1, along with American chestnut and Chinese chestnut, tested genotypes included the following CC x AC hybrid and backcross types: F1 (½ Chinese and ½ American), B1 (¾ American and ¼), B2 (7/8 American and 1/8 Chinese), B3F3 (15/16 American and 1/16 Chinese). In 2010, all of the species and hybrids were cultured using the standard protocol. Briefly, burs were dissected to remove the nuts and the immature seeds were cultured in on semi-solid induction-maintenance medium (IMM; Andrade and Merkle 2005), which is a modified woody plant medium (WPM; Lloyd and McCown 1980) with 4.0 mg l^{-1} 2,4-D, 0.5 g l^{-1} L-glutamine, and 3% sucrose. All cultures were transferred to fresh IMM of the same type after one month, and at the end of the second month, they were transferred to secondary medium, which was the same as IMM, but with only 2.0 mg l^{-1} 2,4-D. Cultures were scored for signs of proliferation of embryogenic material after 10 weeks and the percentage of seeds that produced embryogenic material was calculated for each source. None of the CC or F1 cultures produced embryogenic tissue; however, the B3F3 and American chestnut explants had inductions of 0.99% and 1.60% respectively.

Table 1. Hybrid types cultured each year of the experiment

2010	2011	2012
American chestnut	American chestnut	American chestnut*
B3F3	B3F3	B3F3*
F1	Chinese chestnut*	B2*
Chinese chestnut		B1*
		F1*
		Chinese chestnut*

* Species and hybrids cultured using both the standard protocol and experimental treatment

In 2011, 3 new treatments were tested for the CC initiation, along with the standard protocol. Research conducted on the phylogeny of *Castanea* shows that the genus originated in Asia, migrated west to Europe, and then to North America (Lang et al. 2007). Based on these data, we theorized that CC is more closely related to European chestnut (*Castanea sativa*) than AC and that induction protocols based on the one reported for European chestnut by Vieitez (1995) may be successful for CC embryogenesis induction. Nuts of each genotype were randomly divided into four groups and seeds were cultured using one of four treatments (Table 2). CC cultures initiated with Treatment 4 were the only cultures to successfully produce embryogenic tissue with a percent induction of 1.19. In 2012, only two treatments were used for the initiation of all species and hybrid types: the successful experimental treatment (Treatment 4) from 2011 and the standard protocol. With the standard protocol, induction percentages were as follows: AC, 2.35; B3F3, 0.47; B2, 0.80; B1, 0; F1, 0.36; CC, 0. Cultures initiated with the introduced treatment had the following induction percentages: AC, 0; B3F3, 0; B2, 0.77; B1, 0; F1, 0; CC, 2.04.

Analysis of variance results for all three years of initiation data combined, for the standard protocol only, indicated there were significant differences among the species and hybrids (P = 0.0037). Tukey's test revealed that AC and B3F3 were not significantly different from each other, but both were significantly different from CC. When all hybrid types as well as the AC

and CC cultures were compared, using both the standard protocol and Experimental Treatment 4, analysis of variance results showed that species and hybrids were significantly different (P <0.001), and the interaction between species/hybrid and media treatment was also significant (P= 0.0221). The results of these experiments indicate that the proportion of Chinese chestnut genome does have an effect on the induction of SE using the standard protocol for American chestnut. The significant interaction between species/hybrid and treatment is due to the fact that the standard SE protocol works for pure American chestnut, and B3F3 and B2 hybrids, but not Chinese chestnut, B1, or F1 hybrids, while the European chestnut SE protocol was successful for Chinese chestnut, but not for other Chinese x American hybrids.

Table 2. Experimental treatments used for Chinese chestnut culture initiations in 2011

Primary medium	Secondary medium
*1. WPM with 4 mg/l 2,4-D	WPM with 2 mg/L 2,4-D
2. WPM with 4 mg/l 2,4-D	WPM with 0.1 mg/l BAP and 0.05 mg/l NAA
3. WPM with 1 mg/l 2,4-D and 1mg/l BAP	WPM with 1 mg/l 2,4-D and 1 mg/l BAP
*4. WPM with 1 mg/l 2,4-D and 1mg/l BAP	WPM with 0.1 mg/l BAP and 0.05 mg/l NAA

* Treatments also used in the 2012 culture initiations

REFERENCES

Anagnostakis, S.L. 1987. Chestnut blight: The classical problem of an introduced pathogen. Mycologia 79(1):23-37.

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

Burnham, C.R. 1988. The restoration of the American chestnut. American Scientist 76(5):478-487.

Carraway, D.T. and S.A. Merkle. 1997. Plantlet regeneration from somatic embryos of American chestnut. Canadian Journal of Forest Research 27:1805-1812.

Johnson, S.B., H.T. Lee, T.L. Kormanik and S.A. Merkle. 2008. Advances in American chestnut somatic seedling production. In: Proceedings of the 2007 Joint Meeting of the Southern Forest Tree Improvement Conference and the Western Forest Genetics Association, June 19-22, 2007, Galveston, TX, pp. 85-95

Lang, P., F. Dane, T.L. Kubisiak and H. Huang. 2007. Molecular evidence for an Asian origin and a unique westward migration of species in the genus *Castanea* via Europe to North America. Molecular Phylogenetics and Evolution 43:49-59.

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

Vieitez, F.J. 1995. Somatic embryogenesis in chestnut. In: Jain, S.M., P.K. Gupta and R.J. Newton (eds) Somatic Embryogenesis in Woody Plants, Vol. 2 (pp. 375-407). Kluwer Academic Publishers, Dordrecht, Netherlands.

THE PEACH GENOME: A COMPARATIVE GENOMICS REFERENCE FOR IDENTIFICATION OF GENES CONTROLLING TRAITS OF IMPORTANCE IN FRUIT AND FOREST TREES

Albert G. Abbott[1], Ignazio Verde, Meg Staton, Tetyana Zhebentyayeva, Veronique Decroocq, Sook Jung, Dorrie Main, C. Dana Nelson

[1]Dept. of Genetics and Biochemistry, Clemson University, SC

With the advent of molecular marker based technologies in plant breeding, researchers in fruit and forest tree genetics have explored their application to genetically map and genotype key tree species genomes. Much of the evolution of these technologies within these tree species has gone on in parallel between the forest and fruit tree genetics research communities with little exploration of the interface between the two. Culminating with the completion of whole genome sequences for key tree species in particular peach (*Prunus persica (L) Batsch*), significant advances in gene discovery and our understanding of the gene networks underlying characters of importance for tree sustainability and improvement are coming to the forefront. Because there are a number of common breeding targets for fruit and forest trees, comparatively merging the genetics and genome sciences of each provides opportunities to significantly build our understanding of the fundamental biology of tree species while integrating marker assisted breeding approaches for fruit and forest tree improvement. The peach genome serves as a key tree genome in this regard. In this presentation a brief history of the development of the peach genome as a resource for tree genetics and genomics is presented and data from comparative genomics studies among forest and fruiting trees will be highlighted to demonstrate the importance of this genomics resource for integrating the forest and fruit tree genomics research communities into a common forum for the continued study and preservation of our tree resources.

FISHING FOR A CYTO-MOLECULAR MAP OF CHESTNUT (*CASTANEA* SPP.) USING GENETICALLY AND PHYSICALLY MAPPED BACS

Nurul Islam-Faridi[1], M.A. Majid, T. Zhebentyayev, L.L. Georgi, M.E. Staton,
F.V. Hebard, P.H. Sisco, **C.D. Nelson**

[1]USDA Forest Service, Southern Research Station, Southern Institute of Forest Genetics, Forest
Tree Molecular Cytogenetics Laboratory, College Station, TX

The American chestnut (*Castanea dentata*, $2n = 2x = 24$), once thriving on 200 million acres in eastern North America and known as the "King of the Forest", was eliminated as a foundation species by chestnut blight (caused by *Cryphonectria parasitica*) during the first half of the 20th century. Chinese chestnut (*C. mollissima*) is relatively resistant to chestnut blight and efforts are being made to transfer this variation into American chestnut through backcross breeding and genetic engineering. To facilitate both of these improvement approaches, the Chinese chestnut genome has been mapped and is being sequenced. In general, plant genomes contain large amounts of repetitive DNA causing difficulties in genetic mapping, gene discovery, and genome sequence assembly. Fluorescence *in situ* hybridization (FISH) is an important technique for assigning markers and/or gene sequences to specific chromosomes. To overcome some of the mapping and sequencing limitations we are using genetically and physically mapped BAC clones from all 12 linkage groups in FISH to directly locate their positions to respective chromosomes. The resulting cyto-molecular map will be valuable in assisting the assembly of the sequence contigs into scaffolds that will provide a draft genome for chestnut. For example, one results show that BAC contig 3296 on linkage group E is located distal to BAC contig 1231, not proximal as it was positioned on the integrated genetic/physical map. Details of our BAC FISH results will be presented, and discussed in the context of genome analysis and tree improvement.

EVALUATING THE POTENTIAL OF BLACK WILLOW AS A VIABLE BIOMASS SPECIES FOR THE LMAV

R.J. Rousseau[1], **T.D. Leininger**, B.L. Herrin, and E.S. Gardiner

[1]Mississippi State University, Mississippi State, MS

Black willow (*Salix nigra* Marsh.) is commonly found on poorly drained sites that are unsuitable for most other hardwoods species in the lower Mississippi Alluvial Valley (LMAV). These sites are considered marginal for agriculture and forestry production, due to limited accessibility and frequent flooding. The ability of black willow to not only survive but grow well on these sites makes it a good candidate as a biomass species. In addition, other major advantages that black willow possesses are the ease of vegetative propagation using cuttings and the ability to regenerate a stand following harvest via coppice.

While there has been very little research activity into growing black willow in the southeastern United States, there has been considerable work on shrub willows since the 1980's at the State University of New York College of Environmental Science and Forestry (SUNY-ESF). As a result, over 20 organizations have formed a Salix Consortium to develop a willow biomass program for the northeastern United States. Unfortunately, the success of this program has not been adopted in other parts of the United States (Volk et. al 2004, Wright et.al 2000). This may be due in part to the inability of hybrid shrub willows to survive and grow in different environment types. In 2009, Mississippi State University and the U.S. Forest Service Center for Bottomland Hardwoods Research entered into a joint venture agreement to determine the feasibility of black willow as a biomass species. A primary objective of this venture is to determine growth rates and genetic variability within the southern population of black willow when grown on heavy clay soils.

MATERIALS AND METHODS

In the winter of 2008-09, we collected five geographic sources of black willow that included the Atchafalaya River, the Brazos River, the Trinity River, and two geographic areas along the Mississippi River. Four stands were located at each of the five geographic sources and from each stand a total of five to six one or two-year-old seedlings (i.e. whips) were collected and brought back to Mississippi State University for processing into nine-inch cuttings. Cuttings were stored until the spring of 2009 then planted on a 0.91 x 0.31 m spacing into a cutting orchard established in Stoneville, MS. Clonal material raised in the cutting orchard was used to establish a second cutting orchard in 2010, two clonal screening trials in 2010, and two clonal screening trials in 2011.

The clonal screening trials were located at Stoneville, MS and Prairie, MS in 2010, and Hollandale, MS and Prairie, MS in 2011. Each of the four screening trials per year were established with 38-cm unrooted cuttings that had a bottom diameter no larger than 2.5 cm and no smaller than 1.9 cm. Spacing at all four locations was 2.74 by 1.83 m. At each location, a nested design was employed using four blocks, with stands and clones nested within the respected geographic area. Each clone was represented by a two-tree row plot, thus eight ramets per clone were included in each screening trial.

In 2013, we established clone tests located at Stoneville, MS and Holly Springs, MS. The selected material included in these tests was the top 25 clones based on age-three volume performance in the 2010 Screening Trials. A clone test differs from a screening trial in that each clone is represented by a greater number of ramets per clone. Each clone test was arranged as a randomized complete block design consisting of 12 blocks and 25 to 30 clones, which was planted in two-tree row plots.

In 2013, we increased the test population with the inclusion of four geographic sources along the Mississippi River at Baton Rouge, LA, Fort Adams, MS, Vicksburg, MS, and Osceola, AR and another geographic source on the Red River near Shreveport, LA. Sampling protocols defined during the initial phase remained the same for this collection. A new cutting orchard using only material from the second collection was established in May 2013 on the Mississippi Agricultural and Forestry Station near Holly Springs, MS. This orchard will provide cuttings for future screening trials planned for establishment in 2014 and 2015.

RESULTS AND DISCUSSION

Initial collections and establishment of the cutting orchard went exceptionally well with excellent survival and growth. The 0.91 by 0.31 m spacing used in the cutting orchard proved to work very nicely and produced a high number of quality cuttings. Black willow form was quite different in the cutting orchard from the form observed in the 2009 Cutting Length Study, established by Mississippi State University, where the cuttings were planted at a 3.05 by 1.82 m. There the cuttings produced multiple shoots that expressed considerable sinuosity. However, the extremely high density of 35,864 stems/ha employed in the cutting orchard resulted in either a single or double stem having excellent straightness. The extraordinary rooting characteristics of black willow, even when taken from young native stands, resulted in a cutting orchard with very few missing individuals and high uniformity in the cutting orchard.

2010-2011 Black Willow Screening Trials

Since our biomass production rotation is currently aimed at three to five years, only information concerning the 2010 Black Willow Screening Trials will be presented at this time. Our goal is to select superior clones as early as possible because of the short rotation length however it is important to thoroughly evaluate seed source and clonal performance across sites and not to jump to conclusions that are inappropriate.

Survival of the 2010 Stoneville, MS test site averaged 90.4 %, 90.3 %, and 89.4 %, respectively at year one, two, and three. At the 2010 Prairie, MS test site survival averaged 99.7 %, 99.2 %, and 99.0 %, respectively at ages one, two, and three years. Although survival at both test sites was good, the lower age-one survival at the Stoneville site may be attributed to the combination of drought conditions and the heavy clay soils. Although the drought conditions at the Prairie and Stoneville sites were very similar, the lower clay content of the soils at the Prairie site allowed the black willow to extract the moisture needed to survive.

Plants differed in height between the two sites at age one with willow established at Stoneville averaging 18.3 cm shorter than those at the Prairie site. However, by age two this trend was reversed with plants established at the Stoneville site being 74.2 cm taller than the Prairie site. By age three this difference was much larger with the willow at Stoneville site averaging 259 cm

taller than the willow at the Prairie site. The extreme difference in soil pH and texture as well as more normal precipitation are factors that likely affected height growth at the two sites. Soil pH at the Prairie site averaged 4.6 while pH at the Stoneville site was much more basic averaging about 7.2. Since black willow is adapted to more neutral to basic soil pH, we would expect it to perform better at Stoneville site. Indeed it did when more normal precipitation returned during ages two and three. While source differences for height were significant at all ages at both sites, we did not observe a strong clinal trend especially as age increased. In fact, we observed only minor differences among sources at each site. At the Stoneville site, the height difference at age three between the tallest source (Brazos River) and the shortest source (Trinity River) was 109 cm. At the Prairie site the tallest source (Atchafalaya River) and the shortest source (Brazos source) showed a height difference of 42.7 cm. As an illustration of the substantial site by source interaction, the Trinity River source grew the tallest at the Prairie site, but was the shortest at the Stoneville site.

The greatest variation in growth was noted among clones within sources. This can be seen in the age-three volume index (D^2H) scores at the Stoneville test site. At this site volume index ranged from 187.92 m^3 for a clone originating from the Tunica source to 22.77 m^3 for a clone originating from the Trinity River source. The top five percent of the test population showed clonal volume index scores that ranged from 187.92 to 145.01 m^3. These five clones originated from three sources, Tunica, MS, the Brazos River, and the Atchafalaya River. In terms of selection efficiency, early–age selection using age-one height to predict age-three volume was rather disappointing but not unexpected. Based on these results we selected the top 25 performing clones and established our first highly replicated clone test in 2013 on two sites in Mississippi.

CONCLUSION

The initial steps in our Black Willow Tree Improvement Program have been taken with a limited population sampling scheme and multiple screening trials established on sites in Mississippi. Early results indicate good gains can be made through the first stage selection. Increased intensity of clonal testing and selection should provide estimates of genetic gain leading to the production of genetically superior black willow clones that can be deployed for growing biomass on marginal agricultural sites.

LITERATURE CITED

Volk, T.A., T. Verwijst, P.J. Tharakan, L.P. Abrahamson, and E.H. White. 2004. Growing fuel: a sustainability assessment of willow biomass crops. Frontiers in Ecology and the Environment, 2: 411–418.

Wright, L.L., M.A. Walsh, M.E. Downing, L.A. Kszos, J.H. Cushman, G.A. Tuskan, S.B. McLaughlin, V.R. Tolbert, J. Scurlock, and A.R. Ehrenshaft. 2000. Biomass feedstock research and development for multiple products in the United States. 1st World Congress and Exhibition on Biomass for Energy and Industry of the 11th European Conference on Biomass for Energy and Industry and the Biomass Conference of the Americas, Conference and Exhibition Centre, Seville Spain.

BEYOND HYBRID BACKCROSS BREEDING: THE INTERSECTION OF THE AMERICAN CHESTNUT FOUNDATION'S BREEDING PROGRAM AND VARIETAL FORESTRY

S.A. Merkle[1], A.R. Tull, C.T. Holtz, A. Newman and C.J. Nairn

[1]Warnell School of Forestry and Natural Resources, University of Georgia, Athens, GA

Since its inception in the early 1980s, The American Chestnut Foundation (TACF) has made substantial progress on developing blight-resistant chestnut trees through its hybrid backcross breeding program with American chestnut (*Castanea dentata*; AC) and Chinese chestnut (*Castanea mollissima*; CC). Following the generation of the original F1 hybrids, the hybrids were backcrossed with AC for three generations, with selection at each generation for blight resistance and AC form, to obtain the BC3 generation, followed by two generations of inter-crosses to obtain the BC3F3 (or B3F3) generation. B3F3 trees, which should average 15/16 AC genes and 1/16 CC genes, are known as the "Restoration 1.0" chestnuts. B3F3 nuts are currently being produced in open-pollinated (OP) seed orchards, and thus make use of "half-sib" technology. While the broad range of phenotypes produced by half-sib technology may be useful for restoration purposes, we believe there will likely be an increasing demand for B3F3 or similar hybrid chestnut trees by landowners who wish to grow them on a commercial scale for production of durable (i.e. decay-resistant) timber for outdoor uses (decks, play structures), short-rotation biomass energy plantations and/or nuts. Since landowners will want to plant the best genotypes, rather than a collection of variable genotypes, TACF may want to consider other means of producing planting stock besides open-pollinated seed orchards (i.e. full-sib or varietal technologies) for this market. Southern pine breeding programs have already moved from OP seed orchards significantly in the direction of full-sib technology, producing millions of seedlings per year via mass controlled pollination (MCP) and varietals are also beginning to be deployed in southern pine plantations (McKeand et al. 2007). We know of no studies in which MCP has been tested with chestnuts, so the applicability of this tool to producing elite families of chestnuts is unknown. Thus, varietal (i.e. clonal) approaches may be a more realistic option for production of elite chestnuts.

The advantages of deploying clonal trees in plantations (compared to half-sib or even full-sib seedlings) include the capture of all components of genetic variance of the ortets, the ability to apply very high selection differentials and enhanced product uniformity. Disadvantages of applying this approach include relatively high labor and operating expenses, the requirement for specialized equipment and facilities, the fact that propagules may need to be containerized and the high variation in success rate, depending on species, ortet age and other factors. Varietal approaches that might be applied for propagation of elite chestnuts include rooted cuttings and *in vitro* propagation via axillary shoot multiplication (micropropagation) or somatic embryogenesis (SE). Compared to the other two methods, SE has a number of advantages, including potentially very high multiplying power and the amenability of embryogenic cultures to cryostorage. Propagation of American chestnuts by all three of these methods has been reported over the years, with varying levels of success, but to our knowledge, B3F3 chestnuts have not been clonally propagated previously. We conducted a preliminary experiment testing different

hormone treatments for rooting stump sprouts collected from TACF B3F3 stumps in 2012, with a very low (0.6%) success rate (unpublished data).

For the past three years, as part of our lab's participation in the Forest Health Initiative (FHI), we have tested our chestnut SE protocol for its applicability to propagate a range of chestnut hybrids (Holtz et al. 2013). One particular goal of the FHI was to establish embryogenic cultures of TACF B3F3 material to facilitate clonal testing. Over the past two decades, we have developed a scalable, suspension culture-based system for production of chestnut somatic embryos and somatic seedlings (Andrade et al. 2005, Merkle et al 2011). However, prior to the beginning of the FHI project, only pure AC material had been propagated via SE, so the potential to propagate advanced generation hybrid material using this approach was unknown. Open-pollinated B3F3 seeds, representing both the Clapper and Graves lines of blight-resistance, were collected from B3F2 seed orchard parents by TACF cooperators and used to initiate cultures in 2010 and 2011. Average embryogenesis induction percentages were 0.85% for nine OP B3F3 families in 2010 and 1.63% for 11 OP B3F3 families in 2011. These "capture" percentages were not significantly different from those for AC cultures initiated in those years. The first of these B3F3 somatic seedlings were planted in a clonal field test by Virginia Tech cooperators in Virginia in June 2013. Of course, in order to take full advantage of SE for production of elite varieties, it needs to be combined with full-sib breeding. Selected B2F3 parents were crossed to for this purpose in 2012. While the average embryogenesis induction percentage for the full-sib material (0.5%) was lower than for OP seeds, at least one embryogenic culture was produced for eight of the nine crosses. Now that we have confirmed that SE can be applied to propagate B3F3 material, all the pieces are in place to apply the varietal forestry approach to blight-resistant chestnuts. Somatic seedlings derived from B3F3 cultures initiated from crosses between the best B3F2 parents can be rigorously field tested while the cultures from which they were produced are held in cryostorage. Once the best varieties are identified, those cultures can be recovered from cryostorage and scaled-up for mass somatic seedling production of elite planting stock. The decision as to whether to apply this approach to produce elite chestnut varieties for purpose-grown trees is, of course, up to TACF.

With additional research, we hope to be able to directly clone elite, blight- and Phytophthora-resistant chestnuts by initiating embryogenic cultures from mature tree tissues. This has been accomplished using leaves from epicormic shoots of mature trees of cork oak (*Quercus suber*; Hernandez et al. 2003) and English oak (*Quercus robur*; Toribio et al. 2004). Since oaks and chestnuts are both in the Fagaceae, a similar approach may be applicable to clone elite chestnut trees.

REFERENCES

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

Hernandez, I., C. Celestino, J. Alegre and M. Toribio. 2003. Vegetative propagation of *Quercus suber* by somatic embryogenesis II. Plant regeneration from selected cork oak trees. Plant Cell Rep. 21:765-770.

Holtz, C.T., A.R. Tull and S.A. Merkle. 2013. The influence of Chinese chestnut genome proportion on the success of somatic embryogenesis in chestnut. In: Proceedings of the 32nd Southern Forest Tree Improvement Conference, June 10-13, 2013, Clemson, SC (in press).

McKeand, S.E., B.J. Zobel, T.D. Byram and D.A. Huber. 2007. Southern pine improvement – a living success story. In: Proceedings of the 29th Southern Forest Tree Improvement Conference. Tree Improvement in North America: Past, Present and Future, WFGA/SFTIC Joint Meeting, June 19-22, Galveston, TX. Pp. 3-6.

Merkle, S.A., L. Kong, R. Tull, G. Andrade, P.M. Montello, C.T. Holtz, R. Sakowski, S. Bryson and C.J. Nairn. 2011. Scaling up transgenic American chestnut somatic seedling production for the Forest Health Initiative. In: Proceedings of the 31st Southern Forest Tree Improvement Conference, June 13-16, 2011, Biloxi, MS, pp. 58-60.

Toribio, M., C. Fernandez, C. Celestino, M.T. Martinez, M.C. San Jose and A.M. Vieitez. 2004. Somatic embryogenesis in mature *Quercus robur* trees. Plant Cell Tiss. Org. Cult. 76:283-287.

EARLY EFFECTS OF GENETIC IMPROVEMENT ON CHERRYBARK OAKS (*QUERCUS PAGODA* RAF.) IN SOUTHERN ARKANSAS

Nicholas A. Mustoe[1], Joshua P. Adams

[1] University of Arkansas at Monticello, School of Forest Resources, Monticello, Arkansas

Abstract: Our current study attempts to quantify the growth and survival difference between second generation half-sib (improved) and woods-run cherry bark oak (*Quercus pagoda* Raf.) seedlings in operational settings. One-year-old seedlings of both cherrybark oak varieties were planted at two sites in southern Arkansas at 2.43 meters x 3.04 meters spacing using a randomized block design. There were no statistical differences among site or seedling type for ground line diameter. However, there was a signification interaction effect between site and seedling type. Specifically, seedling type differences were present at the Monticello site with improved seedlings having 20% greater average height and 14% greater survival when compared to the unimproved seedlings.

INTRODUCTION

Cherrybark (*Quercus pagoda* Raf.) is currently the only oak species that the Arkansas Forestry Commission (AFC) offers as an improved planting stock through their selective breeding efforts with Western Gulf Tree Improvement Program (WGTIP). Improved cherrybark oak seedlings are predicted by the AFC and WGTIP to have increased survival and rates of growth. The commercial success of hardwood tree improvement programs depends in large part on the ability to produce high quality seedlings with early gains in height and diameter that outweigh the added cost of production.

Early growth in oaks is important due to their susceptibility to being outcompeted by other species. This has led to substantial research in manual and chemical control of competing vegetation (Dubois 2000, Miller 1993, Ezell et al. 2007). Ideally, successful artificial regeneration of oaks should come from planting vigorous and competitive seedlings (Duryea 1985). The ability of oak seedlings to put on early and rapid growth is critical in reducing the time competing vegetation poses a risk to the success of regeneration (Dey et al. 2008). Tree improvement offers one avenue in which to produce seedlings with the qualities that can allow for greater growth and survival in artificially regenerated stands with costs savings from fewer herbicide treatments.

MATERIALS AND METHODS

Two sites in Arkansas, one near Hope (33° 43' 9.76", -93° 31' 49.92") and another near Monticello (33° 37' 12.31", -91° 44' 0.38") were planted with 1-0 seedlings of improved and woods-run cherrybark oaks grown at the AFC's Baucum Nursery in North Little Rock. Prior to planting, the Monticello site was a pine-dominated forest salvage logged following wind damage and then cleared of slash. Soils on the Monticello site are silt loams with a 50-year site index

(SI$_{50}$) of 85 feet for cherrybark oak. The Hope site, a former old field, is on a silty clay loam soil with a SI$_{50}$ = 90 feet for cherrybark oak.

Each site has two blocks planted with replications for a total of 480 randomly sampled improved and unimproved cherrybark oak seedlings split between the two sites and blocks. Seedlings were planted at 2.438 meters x 3.048 meters spacing. Both received 59.14 milliliters per acre of Oust® XP at the time of planting. Survival, height, and ground line diameter (GLD) were measured for a random subset of each replication at the time of planting and at the end of the first growing season (October 2012). Survival and re-sprouting was measured again at the start of the second growing season (May 2013).

A general linear model using fixed effects was conducted on the data according to a general randomized block design. Survival was analyzed using a general linear mixed model with a specified binomial distribution and a logit link function. Additionally, a logistical regression model was used to determine relationships between sprouting and GLD. In all analysis, an alpha of 0.05 was used as a significance threshold. Means separation was conducted using Least Significance Difference procedures.

RESULTS AND DISCUSSION

GLD for the first year results differed across the two sites but not between treatments (Table 1). At the end of the first growing season, neither planting stock had obtained a mean GLD of 8-10 mm; which has been found to be more competitive at planting time (Johnson 1984, 1992, Stroempl 1985, Johnson et al. 1986, von Althen 1990, Kennedy 1993, Pope 1993, Smith 1993, Dey and Parker 1997). The end of the first growing season GLD measurements were significantly different between sites.

Table 1. General linear model ANOVA for first year height and ground line diameter measurements.

Source of variation	df	Sum of squares	Mean square	F	P
Ht (R=.12)					
Model	5	12318.85	2463.77	8.75	<.0001
Site	1	6461.78	6461.78	22.95	<.0001
Block(site)	1	2086.64	2086.64	7.41	0.0068
Seedling type	1	1026.54	1026.54	3.65	0.0571
Site*Seedling type	1	2654.34	2654.34	9.43	0.0023
Block*Seedling type (site)	1	89.54	89.54	0.32	0.5732
Error	336	94606.41	281.57		
Corrected Total	341	106925.25			
GLD (R=.15)					
Model	5	3.14	0.63	12.21	<0.0001
Site	1	2.25	2.25	43.66	<0.0001
Block(site)	1	0.7	0.7	13.55	0.0003

68

Source of variation	df	Sum of squares	Mean square	F	P
Seedling Type	1	0.17	0.17	3.4	0.0662
Site*Seedling Type	1	0.01	0.01	.22	0.6406
Block*Seedling Type (site)	1	0.02	0.02	.32	0.5743
Error	336	17.29	0.05		
Corrected Total	341	20.43			

After the first year of growth, improved and unimproved seedlings were not significantly different in regards to height and survival (Table 1). The improved cherrybark oaks had significantly greater survival at the Monticello site compared to the unimproved at the Monticello site and both seedling types at Hope. Total survival is relatively low in comparison to previous findings with bareroot cherrybark oak seedlings (Kormanik et. al. 1976). Low overall survival is likely related to the significant drought experienced by southern Arkansas in the summer of 2013. The unimproved seedlings at the Monticello site had a significantly lower height when compared to the improved planting stock and both seedling types at Hope.

The mean seedling height for both improved and unimproved seedlings at the end of the first growing season is only now exceeding the range, 40 – 50 cm, recommended in literature for seedling shoot length at time of planting (table 2) (Foster and Farmer 1970, Johnson 1981, von Althen 1990, Kennedy 1993).

Table 2. Mean height, ground line diameter, and survival for two levels of genetic improvement of cherrybark oak seedlings in October, 2012.

Variety	Site	Sample Size	Height (cm)[1]	GLD (cm)[2]	Survival (%)[3]
Improved	Monticello	56	54[a]	0.6[a]	68.75[a]
	Hope	110	57[a]	0.7[b]	60.56[b]
Unimproved	Monticello	60	43[b]	0.5[a]	55.23[b]
	Hope	116	58[a]	0.7[b]	63.33[b]

1 Values in this column followed by the same letter are not significantly different at the 0.05 level for site by seedling type interaction.
2 Values in this column followed by the same letter do not have significantly different means at the 0.05 level.
3 Values in this column followed by the same letter are not significantly different at the 0.05 level for site by seedling type interaction.

Oaks are known to put a great deal of energy into root growth in early stages of development (Gardiner and Hodges 1998). This energy allocation helps explain the prolific sprouting seen at the beginning of the second year of growth. Sprouting that occurred during the start of the second growing season increased the rate of seedling deemed to be surviving for both seedling

types (Table 3). Our further investigation of re-sprouts found no statistical evidence for a relationship between planting GLD and sprouting ($p=.3669$) that occurred after the end of the first growing season. The effects of this sprouting will likely have an impact on height and ground line diameter measurements to occur at the end of the second year growing season.

Table 3. Mean survival for two levels of genetic improvements of cherrybark oak following leaf-out in May 2013.

Variety	Site	Survival (%)[1]
Improved	Monticello (N=56)	72.50[a]
	Hope (N=110)	67.84[a]
Unimproved	Monticello (N=60)	61.25[b]
	Hope (N=116)	69.01[a]

1 Values in this column followed by the same letter do not have significantly different means at the 0.05 level.

CONCLUSIONS

The significant drought that occurred in the first summer following planting is likely the cause of relatively low survival for both seedling types and may have confounded differences, or lack of thereof, between the improved and unimproved planting stocks in regards to height and diameter measurements. Still, these results do represent the conditions seedlings would face when planted in years with less than ideal conditions.

Sprouting at the start of the second growing season was similar for both planting stocks. It will undoubtedly cause an impact on height and diameter estimates that are planned for the end of the second growing season. At the present, the differences in terms of early gains in height, survival and GLD from improved cherrybark oak planting stock are inconclusive.

LITERATURE CITED

DEY, D.C., J. DOUGLASS, K. MCNABB, G. MILLER, V. BALDWIN, AND G. FOSTER. 2008. *Artificial Regeneration of Major Oak (Quercus) Species in the Eastern United States – A Review of the Literature*. Forest Science 54(1): 77-106.

DEY, D.C., AND W.C. PARKER. 1997. Morphological indicators of stock quality and field performance of red oak (*Quercus rubra* L.) seedlings underplanted in a central Ontario shelterwood. *New For.* 14:145–156.

DUBOIS, M.R. A.H. CHAPPELKA, E. ROBBINS, G. SOMERS, AND K. BAKER. 2000. "Tree shelters and weed control: effects on protection, survival and growth of cherrybark oak seedlings planted on a cutover site." *New Forests* 20.2: 105-118.

DURYEA M.L. (ED.). 1985. *Evaluating seedling quality: Principles, procedures, and predictive abilities of major tests.* Forest Research Laboratory, Oregon State University, Corvallis, OR. 143.

EZELL A.W., J.L. YEISER, AND L.R. NELSON *2007*. Survival of Planted Oak Seedlings is Improved by Herbaceous Weed Control. Weed Technology 21(1): 175-178.

FOSTER, A.A. AND R.E. FARMER, JR. 1970. Juvenile growth of planted northern red oak: Effects of fertilization and size of planting stock. *Tree Planters' Notes* 21(1):4 –7.

GARDINER, E.S. AND J.D. HODGES 1998. Growth and Biomass Distribution of Cherrybark Oak (Quercus Pagoda Raf.) Seedlings as Influenced by Light Availability. Forest Ecology and Management 108(1–2): 127-134.

JOHNSON, P.S. 1981. Nursery stock requirements for oak planting in upland forests. P. 2–19 in *Proc. of Northeastern area nurserymen's conf.* US Forest Service, Broomall, PA.

JOHNSON, P.S., C.D. DALE, K.R. DAVISON. 1986. Planting northern red oak in the Missouri Ozarks: A prescription. North J. Appl. For. 3:66-68.

JOHNSON, P.S. 1984. *Responses of planted northern red oak to three overstory treatments. Can. J. For. Res.* 14:536 –542.

JOHNSON, P.S. 1992. *Underplanting northern red oak in Missouri without herbicides.* US For. Serv. Gen. Tech. Rep. NC-152. 4 p.

KENNEDY, H.E., JR. 1993. Artificial regeneration of bottomland oaks. P. 241–249 in *Proc. of Oak regeneration:* Serious problems, practical recommendations, Loftis, D.L. and C.E. McGee (eds.). US For. Serv. Gen. Tech. Rep. SE-84.

KORMANIK, R.P., R.P. BELANGER, AND E.W. BELCHER 1976. Survival and Early Growth of Containerized and Bareroot Seedlings of Cherrybark Oak. Tree Planters' Notes. 27(3):9-10,23.

MILLER, J. H.   1993. Oak plantation establishment using mechanical, burning, and herbicide treatments. Oak Regeneration: Serious problems, practical recommendations. Symposium Proceedings: 1992; Knoxville, Tennessee. Presented the by Center for Oak Studies. Gen. Tech. Rep. SE-84. Asheville, NC: USDA, FS, Southeastern Forest Experiment Station: 264-289..

POPE, P.E. 1993. A historical perspective of planting and seeding oaks: Progress, problems, and status. P. 224–240. in Proc. of Oak regeneration: Serious problems, practical solutions. Loftis, D. and C.E. McGee (eds.). US For. Serv. Gen. Tech. Rep. SE-84. REICH, P.B., M.D.

SMITH, D.W. 1993. Oak regeneration: The scope of the problem. P. 40–52 in Proc. of Oak regeneration: Serious problems, practical recommendations. Loftis, D.L., and C.E. McGee (eds.). US For. Serv. Gen. Tech. Rep. SE-48. 319 p.

STROEMPL, G. 1985. Grading northern red oak planting stock. *Tree Planters' Notes* 36(1):15–18.

VON ALTHEN, F.W. 1990. *Hardwood planting on abandoned farmland in southern Ontario. Forestry Canada, Ontario Region, Great Lakes Forestry Centre, Sault Ste. Marie, ON, Canada. 77 p.*

AND THEY'RE OFF! – FOURTH CYCLE OF LOBLOLLY PINE BREEDING HAS BEGUN IN THE NCSU COOPERATIVE TREE IMPROVEMENT PROGRAM

Steve McKeand[1], Josh Steiger, Jadie Andrews, David Barker, Ross Whetten, Tori Brooks, and Fikret Isik

[1]NC State University Cooperative Tree Improvement Program, Department of Forestry and Environmental Resources, NC State University, Raleigh, NC

Members and staff of the North Carolina State University Cooperative Tree Improvement Program commenced the fourth-cycle breeding program in 2012. A Differential Evolution (DE) algorithm used in animal breeding programs was implemented to evolve an optimal solution (i.e., selection of mates). The objective was to increase genetic gain for the financial benefit of members while maintaining long-term genetic diversity so that gain can continue for multiple generations. Pedigree analysis tools were used to provide insights for our population management options. Our pedigree analysis shows that there is minimal inbreeding in any of the third-cycle populations. The numbers of selections being mated to date for the fourth cycle are 397 (759 crosses) in the large Coastal population, 281 (475 crosses) in the Piedmont, and 209 (304 crosses) in the Northern population. The Cooperative is using an alpha cyclic incomplete block design and rolling front progeny test approach to field test large numbers of trees. Such a strategy will allow better connection between progeny tests across years but also reduces the testing effort. The Cooperative aims to finish crossing for the fourth breeding cycle by 2015. We are also developing strategies to incorporate DNA markers for genomic selection. A high-throughput genotyping platform called genotyping by sequencing has been explored. These approaches will serve to increase genetic gains by reducing the time and effort required for progeny testing. Historical weather records and provenance growth data are also being analyzed to determine the universal response functions of different seed sources and genotypes for adaptive traits such as cold hardiness, heat tolerance, and drought stress, for incorporation into deployment strategies based on scenarios of future climate alternatives.

GENETIC IMPROVEMENT FOR ECOSYSTEM RESTORATION

C. Dana Nelson[1], Kurt Johnsen, Craig Echt, Barbara Crane, Chis Oswalt

[1]USDA Forest Service, Saucier, MS

University-Industry-State Cooperative Tree Improvement has been highly successful in the southern United States. For nearly 60 years, three Cooperative programs have led the way in developing and deploying genetically improved planting stocks for loblolly (*Pinus taeda*) and slash pines (*P. elliottii*). However, much lower levels of success have been achieved for species of lesser economic importance such as longleaf (*P. palustris*) and shortleaf pines (*P. echinata*) and the many southern hardwoods. The result is that many important forest tree species are in need of sustained genetic enhancement for both short-term silvicultural and long-term conservation purposes. To address this need, we are pursuing the concept of participatory plant breeding for application in forest trees. The basic concept includes three types of forest landowner as participants covering the main functions in tree breeding: mother tree selection, progeny testing, and seed production. A species' program would be organized through a web portal with a back-end database containing tree, test planting and orchard data. The program's goal is to provide landowners with an opportunity to actively participate in range-wide genetic improvement and gene conservation. In addition, all landowners would benefit from the low-cost availability of well-bred planting stock for optimal performance in a changing climate. Such improved materials could be planted in current breeding zones or used to improve adaptation potential via assisted migration.

PINEMAP: COOPERATIVE FORESTRY RESEARCH, EXTENSION AND EDUCATION FOR A CHANGING WORLD

Tim Martin[1], Tom Byram, Gary Peter, Ross Whetten, Martha Monroe

[1]School of Forest Resources and Conservation, University of Florida, Gainesville, FL

For over 50 years, university-corporate-governmental tree improvement cooperatives and the US Forest Service have conducted extensive genetic analyses and breeding with loblolly and slash pine. Large breeding populations have been established and evaluated in extensive regionwide replicated field tests. Using recurrent selection for growth, stem form, and disease resistance, 3rd generation loblolly and slash pine seedlings yield ~30% more wood compared with unimproved material, and essentially all of the ~1 billion southern pine seedlings planted annually have been through at least one generation of genetic improvement. The NIFA-funded Pine Integrated Network: Education, Mitigation and Adaptation Project (PINEMAP) will build on this existing infrastructure and tech transfer network to develop and disseminate knowledge needed to enable southern pine breeders and land managers produce and deploy germplasm with enhanced climate change mitigation and adaptation traits. The genetics and breeding efforts in PINEMAP are focused in two areas. First, we will create a tool to inform future deployment decisions with projected climate change scenarios taking into account uncertainty and risk introduced by performance instability. To accomplish this goal, available growth, survival, genetic and environmental data from our participants' and cooperators' provenance, family, and clone trials will be used to parameterize uniform response functions. We will integrate response functions with geospatially specific climatic predictions in a dynamic model to estimate the relative productivity and adaptation of genetic material to specific climatic conditions analogous to that developed for Douglas fir. This analysis will be conducted by all three breeding cooperatives and climatologists to provide guidance for seed deployment. Second, we will investigate the genetic basis of important mitigation and adaptation traits in loblolly pine by conducting linkage and association mapping to identify alleles that can be screened in breeding populations, helping to accelerate improvement of productivity and adaptive traits. Furthermore, the project provides an unprecedented opportunity to integrate genetics and breeding research with efforts in growth and yield, ecophysiology, economics, policy, and lifecycle analysis to refine future breeding objectives, and to produce enhanced, resilient forest management systems for changing climate. Research will be disseminated to stakeholders through a two-pronged extension program incorporating the land grant extension network coordinated by the Southern Regional Extension Forester, and the well-established corporate genetics and breeding research cooperative network. An innovative education program provides material and training for the next generation of scientists and informed citizens.

IMPROVING FOREST PLANTATIONS THROUGH BIOTECHNOLOGY: CAN A SYSTEMS-LEVEL UNDERSTANDING OF TREE BIOLOGY BE TRANSLATED TO MAJOR GAINS IN TREE IMPROVEMENT VIA TRANSGENIC TECHNOLOGY?

Amy M. Brunner

Department of Forest Resources and Environmental Conservation, Virginia Tech University, Blacksburg, VA

Next-generation sequencing, genomics technologies, and biological network reconstruction and integration are enabling systems-level understandings of organisms. At the same time, new plant transgenic methods are continuing to be developed that enable more precise manipulation of individual genes, as well as the ability to introduce a large number of transgenes. By revealing various types of molecular interactions that underlie interrelated traits such as higher wood yield, altered biomass composition, and resilience to biotic and abiotic stress, networks have the potential to accurately identify targets for manipulating multiple or single traits. However, plant networks are highly complex, and their accuracy depends on the underlying experimental plant biology. To what extent are networks identified in one plant species translatable to another species? How many different genotypes, environmental conditions, tissues, time points and developmental stages need to be studied to accurately predict targets? Do many genes need to be manipulated in order to alter complex traits? Although there are not single, straightforward answers to these questions, I will use specific examples to discuss some of the important issues.

PHYTOPHTORA CINNAMOMI THE "STEALTH" KILLER

Joseph James

Clemson University, Clemson, SC

- PHYTOPHTHORA is not a fungus, but an oomycete related to red and brown algae.
- There are probably 250 to 450 species worldwide.
- Cinnamomi is clearly the most virulent of the known species.
- C. Crenata, Mollisima, Sequinii, and Henryi evolved with Cinnamomi in Southeast Asia and are largely resistant.
- P. cinnamomi and perhaps several other species were most likely introduced to North America with experimental potted plants shipped to plantation owners around Charleston, SC circa 1780 to 1800.
- Wherever P.C. was spread it completely wiped out the pure American chestnut; this event went completely undetected by the scientific world until the 1940's.
- Through the hybridization process trace amounts of resistance derived from the original Chinese and Japanese trees have been found to persist in the various backcross generations. (James and Jeffers)
- By the use of random screening of large numbers of hybrid seedlings from different families, a few trees have been shown to survive P. cinnamomi.
- Early data shows that by breeding these survivors with each other the progeny's survival rate increases by a factor of 4 to 8 fold over their parent's generation. Herein lies the hope for the future.

Though difficult to prove, P. cinnamomi probably evolved in Southeast Asia. The area around Charleston, SC seems a quite logical point of entry; especially when one considers the extensive plantation culture with its accompanying interest in gardens, and with the introduction of exotic tropical plants to be developed for agricultural profit. These plants would have been potted if they were to survive the long ocean voyage. Any number of other exotic microorganisms including other Phytophthoras would have gained entrance also.

This large scale screening is being done on Chestnut Return Farm in Oconee Co. South Carolina. The work is entirely based on the premise that genes for resistance to P. cinnamomi would have been derived from the original Chinese trees. All the Chinese genes, including those for phytophthora resistance, would have been carried in progressively diluted form. Each successive backcross generation would statistically have an average reduction of Chinese genes of 50%. The F1 cross= 50% Chinese genes; B1= 25%; B2= 12.5%; B3= 6.25% Chinese genes. All survivors are not created equal. Some thrive while others just "get by". The difference is probably a reflection of the different resistance alleles a given hybrid seedling might carry. How many different alleles contribute to "complete" resistance is currently unknown. It is TACF's goal to eliminate most of the undesirable Chinese alleles while maintaining the genes for

resistance. When dealing with P. cinnamomi we have one strong factor in our favor, DEATH. Only those few seedlings that have, by chance, still retained sufficient genes for resistance from their Chinese ancestor will live **. These surviving trees will be grown in an open-pollinated orchard and will breed only with other B3F3 survivors. The seedlings in each generation will be inoculated directly with P. cinnamomi and trees with too many non-resistant genes will be eliminated. This natural culling process will concentrate the resistance alleles eventually toward 100% frequency and reduce the non-resistant alleles toward 0%.

We have now entered our third year of B3F3 screening. The results obtained by direct seeding in the field appear to closely mimic the results obtained from our standard tub tests. But there was glaring difference that I observed between Rows 3 and 4 from the 2011 planting. That first year of direct seeding of B3F3's in the field was supposed to result in the usual 70% or so die-off of seedlings in the first summer. That did not happen and I became worried that the existing level of field inoculum was too low. So, I decided to inoculate Row 4 but leave Row 3 uninoculated to gauge any differences in health and growth rates. The seedling count in Row 3 dropped from 103 to 80 living trees by April of 2012. The remaining trees in Row 3 continued to grow exceedingly well. 26 of 80 reached between 7.5 and 9 ft. in height by 15 mos. from the time of sprouting. Their individual biomass, I would estimate, was at least 4X greater than the best trees in Row 4. Could it be that we are missing a portion of the complete genetic complement for resistance?

CUGI: A RESOURCE FOR GENOMICS AND BIOINFORMATICS

Christopher Saski

Clemson University Genomics Inst., Clemson, SC

Genetics, genomics, and bioinformatics are entering a new technology phase. The explosive trend in DNA sequencing technologies is transforming the way scientists collect and measure an individual's genetic background (plant or animal) and gene dynamics, while bioinformatics and super-computing are merging to facilitate parallel sample computation and interpretation at unprecedented speeds. Organismal sequences are being collected at remarkable rates, but the resulting assemblies are typically highly fragmented and unordered. The Clemson University Genomics Institute provides a rich source of techniques and applications for genome refinement and enablement, and several examples will be discussed with applications to the simple diploid genome (*Theobroma* cacao) in search of pod color traits to the complex tetraploid genome of *Gossypium hirsutum*.

THE PINE REFERENCE GENOME SEQUENCE AND APPLIED TREE BREEDING

N.C. Wheeler[1] and R. Whetten

[1] Molecular Tree Breeding Services, LLC, Centralia, WA

Introduction

The PineRefSeq project was funded to produce a high-quality reference genome sequence for loblolly pine and related species. An early draft (v 0.6) of the loblolly pine genome was released at the project website in June, 2012. This draft and two subsequent improvements have been widely accessed by the scientific community. A complete reference sequence (v 1.0) should be released very shortly. Recently, draft genome sequences for white and Norway spruces were published. Long considered a task so daunting it might never be achieved, the development of next generation sequencing (NGS) technologies has opened the door to deciphering even these leviathan conifer genomes. In our talk we will discuss the continuous evolution of the state-of-the-art in genome sequencing and the adaptive approaches of a team of researchers funded by the USDA NIFA AFRI program ($14.625 million dollars over five years). It is anticipated that the reference sequences produced by the PineRefSeq project and others will dramatically change the landscape of forest genetics and pave the way for greater application of genomic resources in applied tree breeding.

The reference genome sequence

A reference genome sequence is that which results from *de novo* sequencing and assembly of a haploid complement of an organism's genome. It is the initial sequence to which all subsequent sequences are ultimately compared and therefore it must be as complete as possible given fiscal and technical constraints. The challenges to assembling the billions of bases of a conifer genome in the proper order are monumental. The reference genome leads to the identification of all or most of the genes in an organism, and reveals features of genome structure such as the amount and order of repetitive elements, the nature of regulatory elements, and so forth. Re-sequencing, or sequencing of other individuals of the same species, is vastly less time consuming and costly once a reference genome exists. Re-sequencing reveals the amount and distribution of genetic variation (mutations) within a genome on an individual or population basis.

While whole genome sequencing has become rather commonplace, and is recognized as the gold standard of genetic resource development in biological science, its history is really quite short. This is largely a function of the remarkable advancements in sequencing technology that have occurred over the last 15 or so years. The first major genome to be sequenced was the human genome. Though plans for a "Human Genome Project", or HGP, were taking shape throughout the late 1980s, the project itself did not kick off until 1990 when Congress allocated funds. Work on the publicly funded HGP was ultimately carried out by labs in 18 countries, but the bulk of the work was conducted in the USA, initially under the guidance of James Watson, and later by that of Francis Collins. A draft genome sequence was completed in 2000 and the project concluded in 2003, two years ahead of schedule, under budget, and with accomplishments far exceeding goals. The cost of sequencing declined over the course of the project from roughly

$10 per base in 1990 to around $0.09 per base at its conclusion. Today, one can obtain nearly 100,000 bases of sequence per penny. The completion of the HGP draft sequence was announced simultaneously with that of a privately funded human genome sequence project that was initiated only a few years before the announcement by Craig Venter at Celera Genomics. Since then, over 1000 individual human genome sequences have been completed and are publicly available (The Thousand Genomes Project Commission). As the quote above implies, the HGP opened the floodgates to genome sequencing of all manner of organisms.

Challenges

There are a number of challenges to obtaining a reference genome sequence for a conifer, not the least of which is the size of their genomes. Genome size varies considerably among conifer species, ranging from 6 billion to over 30 billion base pairs (Figure 1). Before next-generation sequences became available, it was estimated it would take nearly 30 years to sequence a conifer.
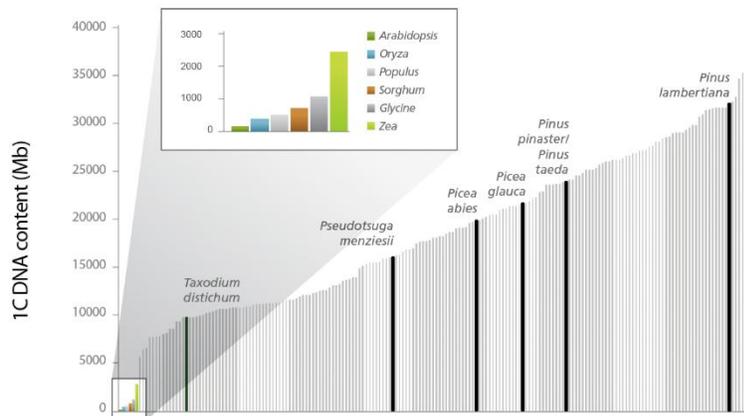

Figure 1 Range of genome sizes, in Mb (millions of bases), for an array of conifers and model plants. The image is modified from Daniel Peterson, Mississippi State University

Today, technically, it can be done in a matter of months. But size is not the only hurdle. Conifer genomes generally possess large gene families (duplicated and divergent copies of a gene), and abundant pseudo-genes. Beyond the duplicated gene (and pseudo-gene) content, it appears that the vast majority of the remaining conifer genome is composed of moderately or highly repetitive DNA of unknown or poorly understood function. Correct assembly of the genomic puzzle with so many identical or nearly identical pieces requires a more thorough and sophisticated sequencing and assembly effort than anyone has ever attempted.

Finally, we should note that most conifer species, and individual trees, retain an enormous reservoir of genetic diversity. Studies have shown that single nucleotide polymorphisms occur once every 50 to 100 bases throughout sampled areas of the conifer genome. Consequently, trying to sequence a diploid individual with all that variation can confound the sequence assembly function. Clearly the task of building a reference sequence for a conifer, or any other large genome organism for that matter, is a significant challenge. As scientists evaluate methods to overcome these challenges, an adaptive approach is being used. In the next slide we will describe a number of techniques that are being evaluated in our attempt to create reference genomes for three conifer species: loblolly pine, sugar pine and Douglas-fir.

Strategy for building a reference genome sequence

There is no single recipe or established strategy for sequencing large and complex genomes. Approaches for doing so are continually evolving and improving, and different organisms may require different approaches. In the project we will describe, an adaptive approach that embraces current and developing best sequencing technologies and assembly strategies will be used, carefully testing methods and techniques to ensure optimal efficiency is eventually approximated. The path chosen will be guided by approaches that will simplify assembly of the genome. These approaches can be generally described as 1) the use of complementary sequencing strategies designed to simplify the process through use of actual or functionally haploid genomes and 2) conducting assembly in iterative steps, beginning with reduced size of individual assemblies, and leading to a meta-assembly.

The process begins with a deliberate selection of an individual tree for which the genome will be sequenced and proceeds through sequencing, assembly, and annotation. These processes are facilitated by the use of large-insert and jumping libraries, genetic mapping, and transcriptome sequencing. The entire process is reliant on database creation, management, and access. Though we discuss these steps as if they were a linear flow of activities, in reality, all activities are conducted more or less simultaneously and iteratively.

Proportionally speaking, most sequence will be obtained from the whole genome DNA obtained from the haploid megagametophytic tissue of a single seed. Enough sequence will be generated from this source to represent the presumed genome size 40 to 60 fold, or 40X to 60X. As an example, consider the loblolly pine genome. It is approximately 24 Gb in size, or 24 billion base pairs, arrayed among 12 chromosomes. A 40X coverage means that 960 billion base pairs of sequence would be generated. The second major source of sequence comes from the creation and sequencing of pooled **fosmid** clone libraries, to a depth of about 5X. A fosmid clone is a unique bacteriophage lambda particle that contains a large piece of DNA from the target tree genome inserted into its own, circular DNA. The large, single-stranded inserts can be selected for size, and in this case, are typically around 37,000 to 40,000 (Kb) bases in size. Pools of fosmid clones are combined, each pool containing between 1000 and 4000 clones. A pool of 4000 fosmids would therefore contain about 160 Mb of sequence, or about 7/10ths of one percent of the total genome. Since few if any of the clones are likely to have the same fragment of target DNA as any other clone in the pool, the total sequence from that pool is effectively haploid in nature, even though it was derived from diploid tissue (needles) to begin with. 150 such pools would represent about 1X coverage of the genome. Finally, a series of jumping or joining libraries will be created from both the fosmid and whole genome DNA sources. The purpose of the jumping library is to connect or pull together sequence **contigs** into **scaffolds**. The jumping library sequences represent a very important element in assembling the reference sequence. These diverse libraries will collectively be sequenced to a depth of about 5X to 10X. The next few slides will look at each of these sources in greater detail.

The transcriptome

A complementary element of building a reference genome sequence is the characterization of the organism's **transcriptome**. The transcriptome is the entire set of RNA transcripts in the cell, tissue, or organ from which the RNA was collected. It is the product of all the genes that are

being expressed at that time and place. Since the transcriptome is tissue and time specific, any given attempt to sample it will surely under-represent the total complement of functional genes in the genome. It is simply a snapshot in time of what genes are being expressed, and how they are being expressed. Consequently, it is desirable to sample transcripts (mRNA) from many tissues, collected under different environmental conditions, and at different times in the development of the plant if a "complete" characterization of the plant's transcriptome is desired. In the conifer reference genome project, two dozen or more RNA/cDNA libraries have been used to characterize the transcriptome of each selected genotype or individual. In many cases, libraries are collected from plants that have been subjected to experimental treatments or stresses. cDNA, or complementary DNA, is the product of reverse transcription of mRNA. The cDNA's have only DNA sequence that is used to code for a gene product (does not contain intron sequences for instance). Collectively, cDNA libraries produce an array of EST's or Expressed Sequence Tags.

The transcriptome is substantially more complex than the genome. While the DNA content of an individual is virtually constant throughout every cell, the transcript of a gene may vary considerably. Transcripts may be modified, alternately spliced, edited, and degraded on the way to being translated into protein. The transcriptome can help us understand how cells differentiate and respond to changes in their environment.

While the most direct way to identify a gene is to document the transcription of a fragment of the genome, such as is done with the sequencing of ESTs, protein coding sequences may also be identified by a process known as ab initio gene discovery using software that recognizes features common to protein coding transcripts. This is done by analyzing the genome sequence directly. Generally, both approaches are used, though for the latter all putative genes must be confirmed by a second line of evidence before they may be elevated to gene status.

Use of the reference genome sequence in applied tree breeding

The primary goal of applied tree breeding is to produce genetically improved planting stock while maintain sufficient genetic diversity to manage risk. Tree breeders seldom know which genes they are selecting for or the biological mechanisms they are influencing. Genomic resources offer the promise of providing such insights. Today, the primary tools of the tree breeder is the genetic test. Phenotypic data from tests are, by-in-large, analyzed using BLUP, or best linear unbiased predictor methods, which rely heavily on kinship relationships between trees in the breeding program. While breeders have long been fascinated by the prospects of employing genomic resources to make tree improvement faster, less expensive and more efficient, attempts to do so have generally come up short, until now.

Development of the pine reference genome sequence will provide many genomic resources, the most important of which in the near future being genetic markers. A virtually unlimited number of markers will be readily available and could find immediate use in two applications: 1) the improvement of kinship matrices in BLUP analyses, and 2) the modeling of genetic merit using genomic selection.

In the long run, the identification of virtually all of the genes in the genome, an understanding of their function, and how those genes are regulated, should enable future advances in marker assisted selection strategies that could transcend and complement current breeding practices.

Resources

Wheeler, N., and Neale, D.B. Reference Genome Sequencing [Online Learning Module]. Pine Reference Sequence. eXtension Foundation. Available at: http://www.extension.org/pages/67931 (verified April 29, 2013)

One of 17 learning modules with a focus on genomics in tree breeding, this module expands on material presented in this talk/abstract with a voice-over Camtasia video presentation. http://www.extension.org/pages/60370/conifer-genomics-learning-modules

GENES TO PHENOTYPE, FUNCTION TO APPLICATIONS: NEAR-TERM POTENTIAL OF LARGE-SCALE GENOMICS

Jerry Tuskan

Oak Ridge National Laboratory, Oak Ridge, TN

The availability of information related to genetic diversity among individuals within natural populations, breeding populations and pedigrees is rapidly becoming affordably abundant. Utilizing such information to related genetic variation to phenotypic variation requires adequate computational infrastructure, reliable phenotypic data from relevant environmental settings and a defined economic target. The application of large-scale genomics approaches and genomic selection to forest tree species improvement comes with many benefits and costs. Although the cost of generating useful data is declining, there is still a substantial investment required to generate the data, including the cost of creating a high-quality reference genome. Moreover, the undomesticated nature of most forest tree species requires the generation of a large marker library to cope with linkage disequilibrium. However if these obstacles can be overcome then the direct use of DNA markers [e.g., single nucleotide polymorphisms (SNPs)] as selectable markers is fairly straightforward. Examples of this approach using *Populus trichocarpa* will be presented for genes controlling cell wall related traits and sugar yield data.

HIGH VARIABILITY IN BIOMARKER GENE RESPONSES TO *SIREX NOCTILIO* VENOM IN FIELD-GROWN PINES

J. Michael Bordeaux[1]**,** David R. Coyle, Jeffrey F.D. Dean, and Kamal J.K. Gandhi

[1]Warnell School of Forestry and Natural Resources, University of Georgia, Athens, GA

Introduction

Sirex noctilio is an exotic forest pest in North America and is capable of causing significant economic damage in plantation and naturally-grown pines (*Pinus* spp.) (Borchert et al., 2006). Hazard maps for *S. noctilio* are essential to predicting high-risk areas for establishment, and should account for such factors as stand density, edaphic effects, and host preference. Patterns in *S. noctilio* woodwasp host preference for southeastern pine species have previously been documented; the woodwasp preferred Virginia (*P. virginiana* Mill.) and white (*P. strobus* L.) pines to four other southeastern U.S. *Pinus* species in bolt assays (Dinkins, 2011). Though useful, these data are not sufficient to estimate host tree susceptibility. A susceptibility assay for trees in the field would have utility for predictions of *S. noctilio* establishment.

Figure 1: Cross-sections of Monterey pine (*P. radiata*) needles exposed to fresh *S. noctilio* venom, 10X. Treated needles were fixed in 4% formaldehyde and sections were stained with toluidine blue. Needles were treated as follows: Panel A, 24 hrs water; B, 24 hrs fresh venom; C, 48 hrs water; D, 48 hrs fresh venom. The predominant cellular phenotype caused by venom treatment was collapse of phloem cells as described by Fong and Crowden (1973).

Early observations of *S. noctilio* attack on pines noted that needles showed a characteristic wilt phenotype ('flagging'), which on a cellular level was correlated with collapsed phloem cells (Fong and Crowden, 1973). We were able to reproduce this phenotype in the laboratory (Fig. 1). Previously, we demonstrated that laboratory-grown seedlings and cuttings from potted trees can be used to gauge tree susceptibility to *S. noctilio* venom by measuring gene expression changes in two biomarker genes (PR4 and TLP) (Bordeaux et al., 2012). Biomarker gene response was detectable well before phloem collapse and wilt in needles. The assay has shown utility for measuring gene responses in several species of pine exposed to *S. noctilio* venom.

The purpose of the present study was to assess whether this biomarker assay would be similarly useful for detecting responses in a variety of field-grown *Pinus* species. We hypothesized that changes in biomarker gene expression in response to *S. noctilio* venom would be similar in field-grown pines of various species compared with seedlings grown under controlled conditions. We further hypothesized that these responses could be correlated with susceptibility or resistance to *S. noctilio* attack in a species-specific manner. If successful, such an assay would enable us to more efficiently study insect preference and host tree responses to *S. noctilio* under field conditions, and should permit development of better predictive models and incursion risk maps prior to woodwasp establishment in southeastern U.S.

Materials and Methods
Gene Expression Assays

A biomarker assay of pine gene responses to *S. noctilio* venom using quantitative real-time polymerase chain reaction (qRT-PCR) was previously described for use in laboratory-grown loblolly (*P. taeda*) and Monterey (*P. radiata*) tissues (Bordeaux et al., 2012). The assay measures changes in transcription levels for two defense-response genes in pine (PR4, pathogenesis-related protein; TLP, thaumatin-like protein) in comparison to several housekeeping (control) genes. For this study, actin (ACT1, ACT2), ubiquitin-conjugating factor (UBCF), and glyceraldehyde phosphate dehydrogenase (GAPDH) were used as control genes.

It has been widely recognized that pine gene sequences are highly conserved between species and as a consequence oligonucleotide primers developed for one species are often directly transferrable to other pine species (Barbara et al., 2007; Krutovsky et al., 2007; Lesser et al., 2012). To test whether the primer pairs for the biomarker and control genes listed above would amplify genes from species to be tested, needles were collected from field-grown loblolly, longleaf (*P. palustris* Mill.), Scots (*P. sylvestris* L.), shortleaf (*P. echinata* Mill.), table mountain (*Pinus pungens* Lamb.), Virginia, and white pines. One to three needle fascicle bundles from a mature tree yielded sufficient RNA to perform reverse-transcriptase PCR (RT-PCR) assays in replicate. Needle sampling was minimally invasive and deemed unlikely to affect gene expression in needles elsewhere in the tree. RT-PCR amplification produced products (amplimers) using RNA from each test species as template, and all products were the same size (Fig. 2), which
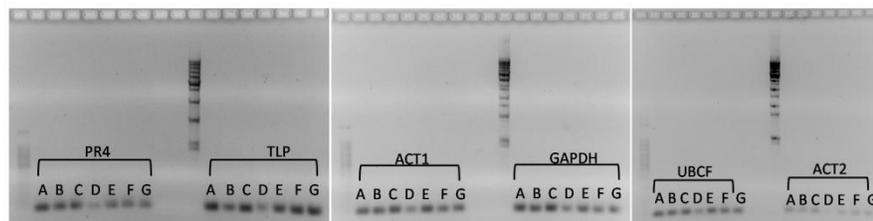
Figure 2: RT-PCR products amplified from seven species of *Pinus* using primer pairs based on *P. taeda* genes. Template: cDNA prepared from RNA isolated from needles incubated in water (control conditions). Initial concentrations of template were not standardized, which accounts for variations in spot density. Amplification ran for 30 cycles of conditions described in (Bordeaux et al., 2012). Lanes on far left and in the middle contain 100bp and 1kbp standards, respectively. Amplified genes are noted above the braces. Pine species were as follows: lane A, *P. sylvestris*; B, *P. strobus*; C, *P. pungens*; D, *P. virginiana*; E, *P. echinata*; F, *P. taeda* (positive control); G, *P. palustris*.

strongly suggested acceptable conservation of sequence between genes within these species of *Pinus* (Fig. 2).

Site selection and sampling plan

Trees were sampled from a common garden (The Pinetum at Thompson Mills Forest) near Braselton, Georgia (34°07'22.82"N, 83°47'48.95"W). The Pinetum contains examples of all conifer species native to Georgia. Sampling took place in February 2012 with a complete replicate collected four weeks later (March 2012). Six pine species native to Georgia -- loblolly, longleaf, shortleaf, table mountain, Virginia, and white – as well as a European species that is a natural host to *S. noctilio* (Scots pine) were sampled for this study. Samples were taken from six trees of each species (N=6), except for table mountain and Scots pines (N=3). Four samples (fascicle bundles) were taken from each sampled tree, two of which were used for venom treatment, and two for water treatment (control). After exposure to venom or water for 24 h, needles were flash-frozen in liquid nitrogen, and RNA was extracted as previously described (Lorenz et al., 2010; Bordeaux et al., 2012). Using protocols from the same studies, cDNA was synthesized from RNA and qRT-PCR was performed.

Results

Relative expression data for the biomarkers in venom- versus water-treated needles showed high variability between trees within species as well as between species (for example, between *P. strobus* vs. *P. palustris*). There was also high variability between the two biomarkers with respect to species response (Fig. 3A, PR4; Fig. 3B, TLP). Presenting the data in terms of fold-change (Fig. 3C, 3D) was useful for identifying general trends in the responses of some species, but did not provide for a reliable estimate of biomarker gene expression responses within a given species due to the high variability of response within species (see for example *P. sylvestris* and *P. palustris*). Variations between biomarkers in different species were especially pronounced (Fig. 3C, 3D).
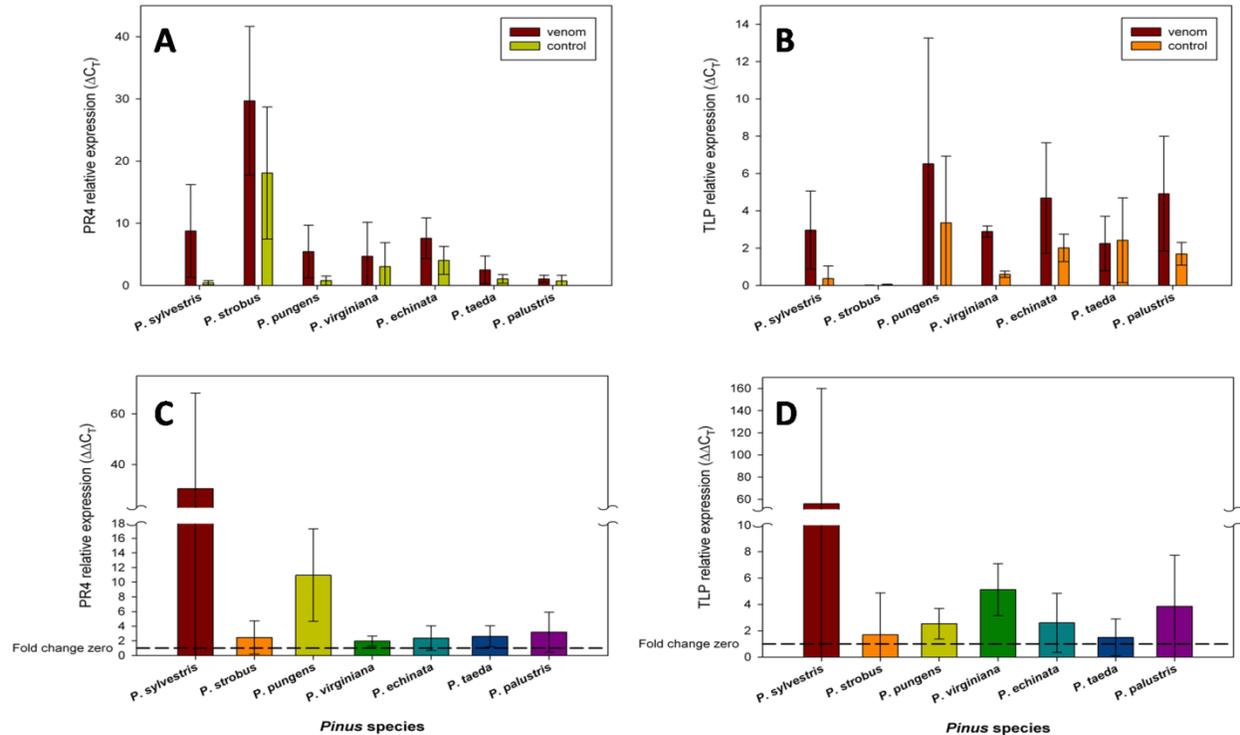
Figure 3: **Expression levels of biomarker genes in pine needles treated with *S. noctilio* venom. Needles from the same tree were treated either with an aqueous solution of *S. noctilio* venom or water, and gene expression levels were quantified using qRT-PCR. Panels A and B show relative expression for both venom-treated and water control needles. Panels C and D use fold change (ratio of treatment response to control response) to display the same data. In these figures the dashed line represents a ratio of one, signifying no change in relative expression. Panels A, C, PR4 expression; panels B, D, TLP expression. Note differences between y-axis scales in all panels.**

Comparing biomarker gene responses in two southeastern U.S. pine species to Scots pine, a natural host of *S. noctilio*, in the North American species the two biomarkers showed a similar magnitude of response, but did not agree in direction. In *P. palustris*, for example, PR4 expression changed very little in response to venom, whereas TLP was strongly up-regulated by *S. noctilio* venom exposure (Fig. 4). These results were not consistent with previous results using seedlings grown under controlled conditions, in which case expression was up-regulated for both biomarkers following venom exposure. Comparisons of biomarker expression changes in needles collected on different dates (Fig. 4A, 4B versus Fig. 4C, 4D) were somewhat more consistent, but any attempt to generalize from these data was confounded by high variability within species and between trials.
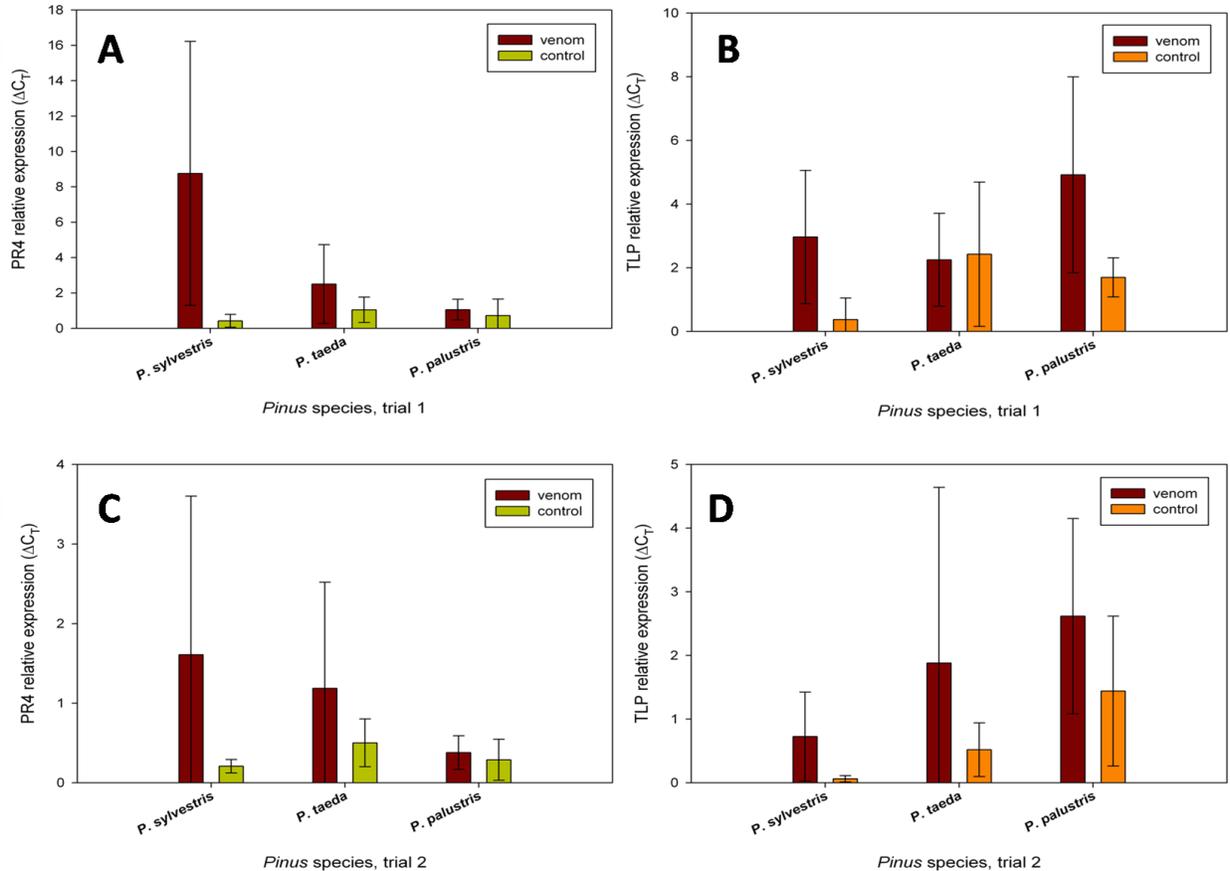
Figure 4: Apparent biomarker gene expression responses to *S. noctilio* venom in needles collected on different dates. Results from the first collection date (February 2012) appear in panels A and B, while results from the second collection date (March 2012) appear in panels C and D. Data are shown only for the three species (Scots, loblolly, and longleaf pines) that yielded fully-replicated data at both time points, and the data shown in panels A and B are a subset of the data in Fig. 3A, 3B. Trees sampled were N=5 in Trial 1, and N=6 in Trial 2 for *P. taeda* and *P. palustris*, respectively, and N=3 for *P. sylvestris* for both trials. Panels A and C show PR4 expression relative to a water control, while panels B and D show relative TLP expression. Note differences between y-axis scales in all panels.

One observation of note from these experiments was that up-regulation of the biomarker genes was consistently stronger in Scots pine species compared to either of the southeastern native pines (Fig. 5). Scots pine assays were particularly variable, which might be related to the small sample size (N=3 trees). While it was not possible to generalize broadly from these data, the magnitude of difference in biomarker expression (especially for TLP) in Scots pine relative to loblolly or longleaf pine was profound. Our previous studies showed that elevated levels of biomarker gene expressions was strongly correlated with the degree of wilt symptoms in needles (Bordeaux et al., 2012).
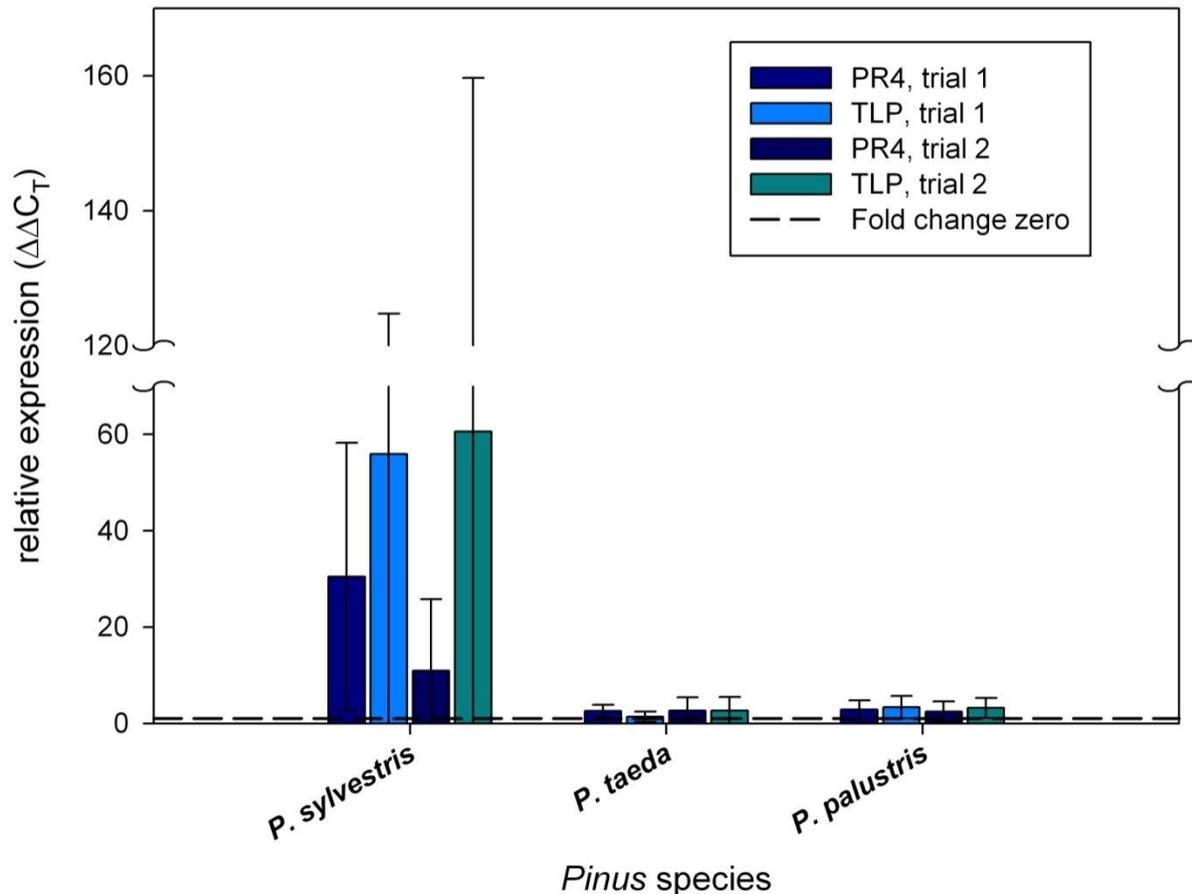
Figure 5: Fold-change in expression of biomarker genes in three pine species responding to _S. noctilio_ venom. The dashed line represents a ratio of one, signifying no fold-`change difference in relative expression. Trees sampled were N=5 in Trial 1, and N=6 in Trial 2 for _P. taeda_ and _P. palustris_, respectively, and N=3 for _P. sylvestris_ for both trials.

Discussion

      As sessile organisms that must cope with highly variable environmental conditions, plants monitor their growth conditions closely and respond quickly to changes by varying the expression levels of many different genes (Atkinson and Urwin, 2012). Thus, it is not surprising that gene expression patterns observed when plants are grown under controlled conditions in a laboratory differ significantly from the expression patterns in field grown plants. Among the more responsive genes in this regard are those involved with plant defense responses (Richards et al., 2012). Such variations in gene expression are problematic for studies within species and even within clonal or isogenic lines, not to mention between the highly heterozygous genotypes that are typical of pine populations or between species. Inherent variability in expression of some genes may be dampened in such situations by using trees grown in a common garden, but various microclimatic conditions, including differential lighting, soil chemistry and drainage, and water supply, will still lead to variations between samples. Despite these issues, this study

clearly demonstrated the utility of the PCR primer pairs developed on the basis of sequence information from loblolly pine for detecting and quantifying cognate transcripts in a wide variety of other pine species. Also, while the high degree of variability in gene expression from sample to sample indicate that these biomarker genes would be unreliable for detecting and assessing the degree of *S. noctilio* attack under field conditions, general trends across all experiments indicate that woodwasp venom tends to lead to increased expression of these genes to varying extents in the species tested.

Discoordinate expression of the two biomarker genes, PR4 and TLP, was not seen previously in venom-treated loblolly or Monterey pines grown under laboratory conditions, but was seen in several instances in this study. Thus, we observed that expression of the two biomarker genes differed not only in magnitude of relative expression (Fig. 3C, D) but in direction, as well (Fig. 4). Clearly field-grown trees offer a special challenge for the selection of useful biomarker genes, and experiments to identify potential biomarkers useful under these conditions will likely need to sample venom-treated field-grown trees.

One additional important observation from this study was that Scots pine, a European species that co-evolved with *S. noctilio*, consistently demonstrated stronger induction of biomarker gene expression in response to venom compared to all of the North American species we tested. Strong up-regulation of defense genes correlates with increased resistance in many plant-pest systems; however, it will take significantly more effort to establish whether or not this is the case for the *Sirex-Pinus* pathosystem. The degree of biomarker induction seen here indicates that Scots pine recognizes *S. noctilio* venom quickly and responds strongly. Further work with this species pairing could identify breeding targets for efforts to develop pines that are more resistant to this pest.

References

Atkinson, N.J., Urwin, P.E., 2012. The interaction of plant biotic and abiotic stresses: from genes to the field. J. Exp. Bot. 63, 3523-3543.

Barbara, T., Palma-Silva, C., Paggi, G.M., Bered, F., Fay, M.F., Lexer, C., 2007. Cross-species transfer of nuclear microsatellite markers: potential and limitations. Mol. Ecol. 16, 3759-3767.

Borchert, D., Fowler, G., Jackson, L. Organism pest risk analysis: risk to the conterminous United States associated with the woodwasp, *Sirex noctilio* Fabricius, and the symbiotic fungus, *Amylostereum areolatum* (Fries: Fries) Boidin. USDA-APHIS-PPQ-EDP, 2006.

Bordeaux, J.M., Lorenz, W.W., Dean, J.F.D., 2012. Biomarker genes highlight intraspecific and interspecific variations in the responses of *Pinus taeda* L. and *Pinus radiata* D. Don to *Sirex noctilio* F. acid gland secretions. Tree Physiol. 32, 1302-1312.

Dinkins, J., 2011. *Sirex noctilio* host choice and no-choice bioassays: woodwasp preferences for southeastern US pines. MS Thesis, University of Georgia, Athens, GA.

Fong, L.K., Crowden, R.K., 1973. Physiological effects of mucus from wood wasp, *Sirex noctilio* F., on foliage of *Pinus radiata* D. Don. Aust. J. Biol. Sci. 26, 365-378.

Krutovsky, K.V., Elsik, C.G., Matvienko, M., Kozik, A., Neale, D.B., 2007. Conserved ortholog sets in forest trees. Tree Genet. Genomes 3, 61-70.

Lesser, M.R., Parchman, T.L., Buerkle, C.A., 2012. Cross-species transferability of SSR loci developed from transciptome sequencing in lodgepole pine. Mol. Ecol. Resour. 12, 448-455.

Lorenz, W.W., Yu, Y.-S., Dean, J.F.D., 2010. An improved method of RNA isolation from loblolly pine (*P. taeda* L.) and other conifer species. J. Vis. Exp., e1751.

Richards, C.L., Rosas, U., Banta, J., Bhambhra, N., Purugganan, M.D., 2012. Genome-wide patterns of *Arabidopsis* gene expression in nature. PLoS Genet. 8, e1002662.

A MEDIUM THROUGHPUT GREENHOUSE PHENOTYPING ASSAY OF *POPULUS* SPP. FOR SEPTORIA CANKER RESISTANCE

Kelsey L. Dunnell[1], Achala Nepal, Jared M. LeBoldus

[1] North Dakota State University

Poplar trees are increasingly important for fiber production and as a potential feedstock for bioenergy. Septoria leaf spot and stem canker, two common diseases of hybrid poplar caused by the fungus, Septoria musiva, have limited the sustainability of commercial poplar plantations. In the north central region of the United States stem cankers have the greatest impact on hybrid poplar production as cankers weaken stems, increasing the risk of wind breakage. The best strategy for minimizing the impact of S. musiva is to plant resistant genotypes. Screening for disease resistance is challenging in terms of time and space requirements. A study was conducted to develop an increased throughput assay for greenhouse phenotyping of Septoria canker resistance in Populus spp. and their hybrids. To induce canker development a spore suspension spray of 1 x 10^6 conidia per ml was applied to the stem of each tree and incubated for 48 h in black plastic bags (incubation chambers). In the first trial the variation among incubation chambers was tested by placing 25 genotypes of hybrid poplar in each of 10 incubation chambers. The results indicated no significant differences among incubation chambers ($P = 0.1509$) whereas significant differences among genotypes ($P < 0.0001$) were detected. In the second trial we reduced the number of incubation chambers per genotype ($n = 5$) and increased the number of genotypes ($n = 59$) and were still able to detect significant differences among genotypes ($P < 0.0001$). In the third trial we increased the number of genotypes a second time ($n = 92$) and were able to detect significant differences between genotypes ($P = 0.0012$). The ability to screen large numbers of host genotypes for resistance to Septoria canker will facilitate QTL and association mapping of resistance loci in hybrid poplar.

DIVERSITY WITHIN A POPULATION OF *PHYTOPHTHORA CINNAMOMI* FROM ORNAMENTAL CROPS IN SOUTH CAROLINA

Simon Schreier[1] and Steven N. Jeffers

[1]School of AFES Clemson University, Clemson, SC

Phytophthora cinnamomi is a devastating pathogen that can infect over 900 hosts, including many urban and forest trees. It is the most common species of *Phytophthora* isolated from woody ornamental crops in South Carolina, but little is known about variability among isolates of *P. cinnamomi* that attack these plants. Therefore, 142 isolates of *P. cinnamomi* recovered from samples submitted to the Clemson University Plant Problem Clinic between 1996 and 2011 were characterized for growth rate, mycelium growth habit, mefenoxam sensitivity, and mating type. Average growth on PARPH-V8 selective medium was 60 mm in 72 h at 25Â°C in the dark. Mycelium growth habit on PARPH-V8 was classified as aerial, sparse, dwarf, or appressed; 85% of isolates had aerial mycelium. All isolates were sensitive to the fungicide mefenoxam at 100 ppm. The population was composed of 129 A2 and 13 A1 isolates with six A1 isolates recovered from camellia. DNA was extracted and the ITS region was sequenced. ITS had low diversity; only two genotypes were different from the majority of the population. One genotype consisted of an isolate of *P. cinnamomi* var. parvispora, and the other genotype included four morphologically diverse isolates. Consequently, there was high genetic uniformity in the ITS region for this population. Four other loci were sequenced for a subset of 61 representative isolates. Cluster analysis of the genotypes at each locus revealed five distinct groups. Dwarf isolates were genetically and morphologically distinct from the majority of isolates, which clustered in one group. The isolate of *P. cinnamomi* var. parvispora also was genetically and morphologically distinct from other isolates. Two other groups of isolates were genetically distinct but were not morphologically distinct based on the characters we evaluated. Host-pathogen relationships for this population were compared to reports in the literature, and 33 new host associations were found.

PROGRESS TOWARDS BETTER DNA MARKER SETS FOR SOUTHERN PINES

Sedley Josserand

USDA Forest Service, Saucier, MS

Microsatellite DNA (SSR) markers are appropriate for small to mid-scale studies to establish parentage and sibships, clone identification, gene conservation, biogeographic history, or forensic provenance. An ideal set of SSR markers can identify every unique tree genotype without error on the first pass of laboratory analysis, preferably in fully automated fashion. We are not there yet, but are getting closer. Over the past few years at the Southern Institute of Forest Genetics, we have developed various sets of SSR markers to analyze of populations of loblolly, slash, shortleaf, and longleaf pines. We also have SSR sets for DNA fingerprinting of specific loblolly clones, like 7-76 and 20-1010. Although we strived to develop a handful of robust and unambiguously informative markers from thousands of candidates, the more we work with them, the more we become aware of their shortcomings. Truly good markers are hard to find.
This has led us to try a new approach: use the billions of bases of DNA sequence available from the Pine Reference Sequence Project to find special classes of SSR sequences, be very selective during marker evaluations, and develop a new generation of SSR markers for southern pines. In a proof-of-concept study, we used very stringent criteria to search a portion of the loblolly pine genome sequence for perfect, unique SSRs with 4, 5 or 6 bp motif lengths. This approach was in part inspired by new standards in DNA forensics to use only tetranucleotide SSRs (4 bp motifs), which largely avoid allele calling problems encountered with markers of shorter motif length. We have screened 147 of these new markers in loblolly, shortleaf, longleaf and Table Mountain pines. Progress and prospects will be reported.

SCALING UP HYBRID SWEETGUM SOMATIC SEEDLING PRODUCTION

Siran Lu[1] and Scott Merkle

[1]University of Georgia, Athens, GA

Fast-growing hybrid southern hardwood trees should make excellent material for woody biomass production in the Southeastern U.S., if elite clones can be identified and efficiently propagated. We have enhanced the potential of sweetgum (*Liquidambar styraciflua*) as a biomass species by generating hybrids between the tree and its Chinese relative, *Liquidambar formosana*, and propagating the most promising clones via somatic embryogenesis. Some of the hybrid clones have already demonstrated superior biomass productivity compared to elite *L. styraciflua* trees. However, production of somatic seedlings from these clones remains labor-intensive. Bioreactors, specifically temporary immersion designs, such as the RITAÂ®, have been applied to improve in vitro propagation of a number of woody species. We tested RITAs for their potential to improve the production efficiency of high-quality hybrid sweetgum somatic seedlings. Somatic embryos of a single hybrid sweetgum clone were inoculated either onto semi-solid germination medium in plastic Petri plates (control) or into RITAs containing liquid germination medium at three inoculation densities (50, 100 or 200 embryos/RITA). RITAs were operated to provide immersion in liquid germination medium for 1 min every 12 hours. After 45 days, the germination rate for the control treatment was 81.3%, while the germination rates for the 50 embryos/RITA, 100 embryos/RITA and 200 embryos/RITA treatments were 85.3%, 87.7% and 88.3%, respectively. Embryos germinated in the RITAs had longer roots than those germinated on plates after 45 days. Selected somatic seedlings from all treatments were potted and grown in a hardening-off chamber. The survival rate of somatic seedlings germinated in the RITAs was almost double the survival rate of somatic seedlings from the control treatment, and the RITA-derived plantlets were also larger and more vigorous. Following acclimatization to greenhouse conditions, somatic seedlings from all treatments continued growth, with 100% survival.

SOMATIC EMBRYOGENESIS FOR ASH CONSERVATION AND RESTORATION

Scott Merkle[1], Jinfeng Zhang, Dan Li

[1]Warnell School of Forestry and Natural Resources, University of Georgia, Athens, GA

All North American ash (*Fraxinus*) species are under threat of extirpation from their native ranges by the emerald ash borer (EAB; *Agrilus planipennis*), an exotic wood-boring beetle that has already destroyed millions of ash trees in 15 U.S. states and Canada. Conventional breeding approaches to generate EAB-resistant ash trees could be enhanced and/or complimented by the availability of a system for in vitro propagation of the best EAB-resistant material, which could be used for both propagation of conventionally bred material and for testing candidate genes that might confer resistance or tolerance to EAB. We conducted a preliminary experiment to initiate embryogenic cultures from seeds of green ash (*F. pennsylvanica*). Seeds with embryos at various stages of development were collected from three local Athens, GA green ash trees and cultured on two different basal media with different combinations of plant growth regulators (PGRs). A very low percentage of seed explants at an early stage of development from all three source trees produced proembryogenic masses (PEMs) when cultured on a modified Woody Plant Medium with 2,4-dichlorophenoxyacetic acid and benzyladenine. Transfer of PEMs to PGR-free medium resulted in high-frequency production of somatic embryos. We believe that the apparent similarity of the ash embryogenic cultures to those of other hardwood species we have cultured gives them the potential to be scaled up via suspension culture and perhaps bioreactor culture for mass propagation and gene transfer purposes. The ability to produce embryogenic cultures also gives us the ability to conserve ash germplasm indefinitely by cryostoring the embryogenic cultures. We believe that eventually somatic embryogenesis will prove to be an invaluable tool to aid with reforestation efforts for these valuable tree species.

ESTABLISHMENT OF FUSARIUM OXYSPORUM-ARABIDOPSIS PATHOSYSTEM

Stefan Nakollari[1], Li Guo, Li-Jun Ma, Andy Berg

[1]Department of Biochemistry and Molecular Biology, University of Massachusetts, Amherst

Fusarium oxysporum is a fungal pathogen that causes vascular wilt diseases on a broad range of plants including field and plantation crops worldwide. *F. oxysporum* infection is economically important and can cause severe losses to host plants. Being a soil-borne pathogen, *F. oxysporum* is difficult to control and disease management currently relies on soil sterilization and the application of resistant cultivars. To make more effective disease control strategies, understanding of both pathogen virulence and host defense mechanisms is critically important. Using genetic and genomic resources of the host and pathogen, we are studying the interaction of *F. oxysporum* and model plant Arabidopsis thaliana in our lab. A pathosystem using Arabidopsis and *F. oxysporum* (Strain Fo5176) was established in a controlled environment. Fo 5176 causes necrosis and wilting on Arabidopsis Col-0 plants within one week post inoculation. The fungal colonization progress in plant root tissues was also visualized by glycoside-based root staining assay. RNA sequencing will be used to study the time-croused transcriptomes of both host and pathogen during different infection stages ranging from 0 to 5 days post inoculation. Differential gene expression analysis will shed light on the molecular basis of host defense and pathogenicity and the transcriptional regulation networks responsible for disease development.

EFFECTS OF TIMBER HARVESTING ON WATER RESOURCES IN THE SANTEE WATERSHED IN SOUTH CAROLINA

Gang Shao[1], Indrajeet Chaubey, Laurent Ahiablame, Guofan Shao

[1]Department of Forestry and Nature Resources Purdue University, West Lafayette, IN

The forest resources in South Carolina provide both economic and environmental benefits including manufacturing industry, bioenergy production, wildlife habitat protection and greenhouse gas consumption. Timber harvesting and forest management practices may have potential effects on water and soil resources, and these impacts vary geographically based on regional soil types and climatic zones. The watershed scale is appropriate for evaluating effects on water resources. Then, the specific management practices can be implement to protect natural resources against potential harmful consequences. In this study, we employed hydrologic models to estimate the stream flow, and pollutants loads such as inorganic nitrate and solid sediment of Santee River, and then analyzed the relationship between the stream pollutants and the timber volume harvested within this watershed. The results showed the timber harvesting had no effect on nutrient loads such as inorganic nitrate, but increased the following years solid sediment level.

GENOMIC RESOURCE DEVELOPMENT FROM LOW COVERAGE WHOLE GENOME SEQUENCING OF TEN HARDWOOD TREE SPECIES

Margaret Staton[1], Teodora Best, Tim McCleary , Sudhir Khodwekar, Sandra Owusu, Tao Xu, Yi Xu, Tara Jennings, Brian Knauss, Richard Cronn, Mark Coggeshall, Oliver Gailing, Haiying Liang, Jeanne Romero-Severson, Scott Schlarbaum, Nicholas Wheeler, John Carlson

[1]Clemson University, Clemson, SC

Low coverage whole genome sequencing is a viable and inexpensive technique to produce an initial set of genomic resources for under-researched species. For this project ten hardwood tree species were sequenced on a single lane of Illumina HiSeq: black cherry (*Prunus serotina*), black gum (*Nyssa sylvatica*), black walnut (*Juglans nigra*), green ash (*Fraxinus pennsylvanica*), honeylocust (*Gleditsia triacanthos*), red bay (*Persea borbonia*), sugar maple (*Acer saccharinum*), sweetgum (*Liquidambar styraciflua*), white ash (*Fraxinus americana*), and white oak (*Quercus alba*). The species chosen have little or no previous genomic data, are under bioitic and abiotic stress, and/or have significant economic impact in the United States. The amount of sequence recovered for each species was variable, and genome coverage ranged from .4X to 4.7X. Unique microsatellite loci were identified for all ten species and primers designed to enable mapping and diversity studies. A set of 96 SSR primer pairs for eight of the ten species were tested for amplification in a minimum of 6 individuals with varying levels of success. The genomic sequence data has been further analyzed to characterize the conserved and novel repeat sequences in each genome as well as overall sequence similarity to reference plant genomes. All sequence data and analysis will be made publicly available on the Hardwoods Genomics Website (hardwoodgenomics.org).

THE FREQUENCY OF LOBLOLLY— SHORTLEAF HYBRIDS IN SHORTLEAF PINE SEED ORCHARDS AND NURSERIES

John F. Stewart[1], Rodney E. Will, C. Dana Nelson, Barbara Crane

[1]Oklahoma State University, Stillwater, OK

Recent research has shown that hybridization and introgression between shortleaf pine *(Pinus echinata)* and loblolly pine (*P. taeda*) in natural stands has increased dramatically from 1950 to present, and shortleaf pine may be at risk of extinction through introgression. Artificial regeneration of shortleaf pine is an important component of shortleaf pine restoration and forest management, so we are investigating the frequency of hybrids in state and federal seed orchards and in nursery seedlings using simple sequence repeat (SSR) molecular markers. Preliminary results have shown that the rates of hybridization in the seed orchards (5% to 29%) and nurseries (10% to 20%) is higher than the rate observed in trees grown from seeds collected in the 1950s (4%) but is lower than the rate observed in natural regeneration collected 5 to 10 years ago (47%). Our results will be used to suggest management strategies for seed orchard managers and forest managers.

IDENTIFYING GENETIC VARIATION IN SITE ADAPTABILITY IN LOBLOLLY PINE

Laura Townsend[1] and Ross Whetten

[1]Tree Improvement Program, NC State University Dept. of Forestry and Natural Resources, Raleigh, NC

Pine plantations are an important economic commodity, and loblolly pine is the leading southern pine species produced in the U.S. The projected increase in severe weather events due to climate change has potential to harm plantation stands and impact the forest industry. Loblolly pine has a very diverse gene pool and is adapted to various environments, and is thus a good candidate to breed for adaption to climate change. An ideal way to accomplish this is to identify associations of individual genetic marker loci with growth or quality characteristics using climate factors as covariates. To accomplish this, a cost effective, easily implemented, and high-throughput genotyping method is needed. To identify the appropriate genetic markers, a set of phloem tissue samples will be collected in 2 different sites from the plantation selection seed source study (PSSSS). The PSSSS consists of 140 pollen mix families planted in 20 test locations representing the entirety of the loblolly pine range. Phenotypic data from measurements at ages 4 and 8 years are available to be used in genetic marker analysis. The DNA from the phloem samples will be extracted using cost effective buffers adapted from the Canadian Center for DNA Coding protocol. Genotyping-by-sequencing (GBS) will be employed using methylation-sensitive enzymes to enrich gene regions. A preliminary experiment was conducted to test the recovery of marker fragment sequences from DNA of a single loblolly pine parent tree and a set of 90 haploid megagametophytes from seeds of that parent. About 15% of single-end sequence tags show the expected 1:1 ratio of presence and absence in the haploid DNA samples and map to a single contig in the v 0.6 draft assembly of the pine genome sequence. These promising results will be extended with further processing of samples and improved software analysis.

CHARACTERIZATION OF REDBAY (*PERSEA BORBONIA*) GSSR MARKERS

Yi Xu[1], Tao Xu, Margaret Staton, Oliver Bukles, Scott E. Schlarbaum, Richard Cronn, John E. Carlson, Haiying Liang

[1]Department of Genetics and Biochemistry, Clemson University, Clemson SC

Through Illumina HiSeq paired end sequencing, 20,046 genomic microstallite (gSSR) markers with PCR primers have been identified for redbay [*Persea borbonia (L.) Spreng*.]. This resource is the first molecular markers developed for the species, which is now being threatened by laurel wilt, a new, nonnative disease that is causing widespread mortality in redbay populations indigenous to the coastal regions of South Carolina, Georgia and Florida. These markers, when fully characterized, will be valuable in linkage map construction, molecular characterization of germplasm collections, and analysis of genetic diversity in redbay. To validate the effectiveness of these gSSR markers, 94 markers, chosen randomly, were evaluated in a group of 25 unrelated redbay trees from eastern South Carolina, most of which were located at least five miles apart. PCR amplification success rate was 92.8%, indicating high quality of the gSSR markers. Polymorphism values for these loci are being investigated with DNA fragment analysis by automated capillary electrophoresis.

LIRIODENDRON EST-SSR MARKER DEVELOPMENT AND GENETIC CONSTITUTION OF A LIRIODENDRON BREEDING ORCHARD

Xinfu Zhang[1], Alanna Carlson, Margaret Staton, Scott E. Schlarbaum, Jeanne Romero-Severson, John E. Carlson, Haiying Liang

[1]Department of Genetics and Biochemistry, Clemson University, Clemson SC

Liriodendron tulipifera L., commonly known as yellow-poplar, is a member of the Magnoliaceae family. It is a fast-growing hardwood tree species with great ecological and economic value. Liriodendron occupies an important phylogenetic position as a basal angiosperm and has been used in studies of the the evolution of flowering plants. Genomic resources, such as EST databases and BAC libraries, have been developed for this species. In this project, we mined available EST databases for putative polymorphic sequence repeat (SSR) markers with the goal of developing 190 informative EST SSRs. These markers will be used to construct the first framework genetic linkage map of *L. tulipifera* with 380 full-sib seedlings. Such a linkage map is essential for future molecular breeding and quantitative trait locus (QTL) mapping, and as a framework for sequencing the Liriodendron genome in the future. Forty high-quality, single-locus markers have been selected to evaluate the genetic constitution of a *L. tulipifera* breeding orchard in Knoxville, TN. The orchard, established in 1966 as part of a tree breeding program in the University of Tennessee, contains approximately 100 grafted clones, representing 34 genotypes. These 34 genotypes will provide a first look at the genetic diversity and allele richness among selections of this unique native species.

GENETIC MAPPING OF RESISTANCE TO ROOT ROT DISEASE (*PHYTOPHTORA CINNAMOMI*) IN CHESTNUT (*CASTANEA* SPP.)

T. Zhebentyayeva[1], B. Olukolu , M. Staton, S. Jeffers, J. James, P. Sisco, F. Hebard, L Georgi, C. D. Nelson and A.G. Abbott

[1]Department of Genetics & Biochemistry, Clemson University, Clemson, SC

A soil-born oomycete *Phytophthora cinnamomi* (Pc) causing root rot and the ascomycete fungus *Cryphonectria parasitica* (Cp) causing chestnut blight are two major pathogens of the American chestnut *Castaneae dentata*. In the Southeastern forests, root rot disease has an especially severe impact on chestnut stands because of the favorable climatic and soil conditions for the *Phytophthor*a life cycle. Thus, introduction of the resistance to both Pc and Cp is crucial for restoration of American chestnut in the Southeast. To address this need, a collaborative network among the American Chestnut Foundation (TACF), Clemson University (Plant Pathology) and private enterprise The Chestnut Return Farm was established for screening Cp-resistant hybrid material from the TACF breeding program for resistance to Pc. Utilizing the backcross families derived from crosses of American chestnuts with two Chinese chestnut cultivars, Mahogany and Nanking, we initiated genetic mapping and marker development for genomic region(s) underlying Pc resistance in Chinese chestnut. In pilot experiments with a limited number of progeny issued from crosses of AdairKY1 × GL158 (Nanking background) and KY115 × AD98 (Mahogany background), resistance to Pc was mapped to linkage group E (LG_E). To verify and potentially refine these results, we increased the progeny size in the KY115 × AD98 cross and incorporated three new Nanking–derived families NK1, NK2 and NK3 into our mapping efforts. Based on Pc resistance screening in 2012, 333 individuals were available for local map construction and QTL analysis. Genotyping of these individuals with 22 SSR markers spanning Chinese chestnut LG_E is currently under way. Together, these materials and these analyses should help resolve the location of the QTLs on LG_E and test their co-location between two important sources of resistance to Pc in chestnut. Results of the data analysis will be reported and discussed in this presentation.

SELECTION OF HIGH-YIELDING SHRUB WILLOW GENOTYPES WITH IMPROVED BIOMASS CHARACTERISTICS FOR CONVERSION TO BIOFUELS

Michelle J. Serapiglia[1], Fred E. Gouker, Kimberly D. Cameron, J. Foster Hart, Faride Unda, Shawn D. Mansfield, Lawrence B. Smart

[1]Department of Horticulture, Cornell University, New York State Agricultural Experiment Station, Geneva, NY

Genetic improvement of shrub willow (*Salix*), a perennial energy crop suited for temperate climates, has led to the development of new cultivars with improved biomass yield, pest and disease resistance, and biomass composition for bioenergy applications (Serapiglia et al., 2009; Serapiglia et al., 2012). Cultivar development, resulting from controlled hybridizations, exploits the high levels of genetic diversity among natural populations of many willow species. Current research efforts continue to focus on improving willow biomass traits important to the renewable fuels industry, including production yield and compositional quality. Species hybridization leading to heterosis has been exploited by many breeders to improve growth and vigor in several tree species, although little is known about the genetic basis for this phenomenon (Li and Wu, 1996; Kopp et al., 2002).

The objective of this study was to evaluate progeny from crosses performed in 2001, 2002, and 2005 and to identify traits associated with heterosis and variation in biomass compositional quality. The genus *Salix* consists of approximately 300 species with a wide range in ploidy levels, including diploids, tetraploids, hexaploids, and even higher levels of ploidy in some cases. Many of the progeny evaluated in this study were triploids, which are known to generally display improved vigor relative to their diploid and tetraploid parents (Zsuffa et al., 1984). The top performing genotypes in this trial, representing advanced pedigrees compared to those in previous trials, were mostly triploid in nature and outperformed current production cultivars.

MATERIALS AND METHODS

A total of 76 genotypes were hand planted in May 2008 as rooted plants started from 10-cm cuttings in double row spacing at a density of 14,350 plants ha^{-1}. Each genotype was planted in 24-plant plots with six plants per row. The trial was a randomized complete block design with a total of three blocks. During the establishment year, a combination of hand-weeding and spot herbicide (glyphosate) application was used for weed management. In December 2008, at the end of the first growing season, the trial was coppiced. Prior to budbreak in the following spring, pre-emergence herbicide (oxyfluorfen) and post-emergence herbicide (clopyralid) were applied to control weeds. In addition, nitrogen fertilizer was applied at a rate of 50 kg ha^{-1}. Growth traits, wood density, and cell wall compositional traits were measured following each growing season (2009, 2010, and 2011). After the third post-coppice season, the above ground biomass was harvested in December 2011. Biomass composition analysis was performed on three-year old biomass using standard wet chemical methods and by a high-resolution thermogravimetric analysis method developed for low-cost phenotyping of shrub willow biomass (Serapiglia et al., 2009). Variation among key traits and their impacts on biomass production were analyzed.

RESULTS AND DISCUSSION

Many of the progeny examined in this trial displayed heterosis for yield compared to their parents. Among the 76 genotypes within this study, triploids produced the greatest yields. The top 50th percentile within the trial consisted of mainly triploids and tetraploids, while the diploids and pentaploids were lower yielding genotypes (Fig. 1). The highest yielding genotype in the trial was an interspecific hybrid triploid, (*S. koriyanagi* × *S. purpurea*) × *S. miyabeana,* producing a final yield of 17.4 oven dry Mg ha^{-1} yr^{-1}. Many of the triploid progeny in this trial were from crosses between diploid species, such as *S. purpurea*, and the tetraploid species, *S. miyabeana*. Consequently, most of these triploid genotypes have reduced reproductive

Figure 1 - Harvested biomass at 3-years post-coppice by ploidy of genotype.

fertility (Kopp, 2000), which reduces concerns about potential invasiveness. Current studies are underway to improve our understanding of the molecular basis for heterosis in these highly heterozygous willow triploids. However, due to the high heterozygosity in willow parental genotypes, models for heterosis based on crop systems with highly inbred and homozygous parental lines need to be reconsidered. A clearer understanding of this phenomenon and ability to predict the performance of progeny prior to making a cross will not only improve breeding efforts in willow, but in many other bioenergy crops and tree species.

Figure 2 – Changes in biomass composition across all three years by ploidy. A) % Hemicellulose, B) % Cellulose, C) % Lignin, D) % Ash.

The mean yield of the top five performing new genotypes in this trial was 16 oven dry Mg ha^{-1} yr^{-1}, while the mean yield of the top five current commercial cultivars was 14 oven dry Mg ha^{-1} yr^{-1}. This represents an improvement of 12.5 % in yield over current commercial cultivars on this single site. Larger scale yield and evaluation trials with a selection of the top genotypes have been planted in NY, PA, and WV to further test their performance prior to final selection, scale-up, and commercial deployment.

Biomass composition was significantly different across the three-year growth cycle (Fig. 2). Differences in biomass composition among genotypes with different ploidies were also observed. Cellulose content was greater in the second year compared with the first and third years of growth. The opposite pattern was apparent for lignin. There was a significant negative correlation between lignin content and yield over all three years (data not shown). In addition, the triploid and tetraploid genotypes displayed the lowest lignin content regardless of year. Further studies are required to determine if lignin content in the biomass has a negative impact on yield. Through controlled breeding, high-yielding triploid willow cultivars were produced with relatively low lignin content, which has been shown to improve enzymatic saccharification, potentially improving the quality of these cultivars as feedstocks for biochemical conversion to liquid biofuels (Studer et al., 2011).

ACKNOWLEDGEMENTS

REFERENCES

Kopp RF (2000) Genetic improvement of *Salix* using traditional breeding and AFLP fingerprinting Ph.D. Thesis, State University of New York College of Environmental Science and Forestry

Kopp RF, Smart LB, Maynard C, Tuskan G, Abrahamson LP (2002) Predicting within-family variability in juvenile height growth of *Salix* based upon similarity among parental AFLP fingerprints. Theoretical and Applied Genetics **105:** 106-112

Li B, Wu R (1996) Genetic causes of heterosis in juvenile aspen: A quantitative comparison across intra- and inter-specific hybrids. Theoretical and Applied Genetics **93:** 380-391

Serapiglia M, Cameron K, Stipanovic A, Abrahamson L, Volk T, Smart L (2012) Yield and woody biomass traits of novel shrub willow hybrids at two contrasting sites. BioEnergy Research**:** 1-14

Serapiglia MJ, Cameron KD, Stipanovic AJ, Smart LB (2009) Analysis of biomass composition using high-resolution thermogravimetric analysis and percent bark content as tools for the selection of shrub willow bioenergy crop varieties. Bioenergy Research **2:** 1-9

Studer MH, DeMartini JD, Davis MF, Sykes RW, Davison B, Keller M, Tuskan GA, Wyman CE (2011) Lignin content in natural *Populus* variants affects sugar release. Proceedings of the National Academy of Sciences, USA **108:** 6300-6305

Zsuffa L, Mosseler A, Raj Y (1984) Prospects for interspecific hybridization in willow for biomass production. *In* K Perttu, ed, Ecology and Management of Forest Biomass Production Systems, Vol 15. Swed. Univ. Agric. Sci., pp 261-281

List of participants

Name	Email	Address	
Albert Abbott	aalbert@clemson.edu	Dept. of Genetics and Biochemistry	Clemson, SC 29634
Joshua Adams	adamsj@uamont.edu	PO Box 3468	Monticello, AR 71656
Greg Albert	gralbert@ncsu.edu	1019 Biltmore Hall	Raleigh, NC 27695-8002
Jadie Andrews	jlandre2@ncsu.edu	1019 Biltmore Hall	Raleigh, NC 27695-8002
Benjamin Bartlett	bdbart@gmail.com	23332 Success Rd	Saucier, MS 39574
Onesphore Bitoki	ones.bitoki@dof.virginia.gov	2109 Bromby Street	Henrico, VA 23231
John Bordeaux	john.bordeaux@gmail.com	100 McAlpin Dr.	Winterville, GA 30683-1400
Gwendolyn Boyd	gboyd@alcorn.edu	1000 ASU Drive 703	Lorman, MS 39096
Amy Brunner	abrunner@vt.edu	448 Latham Hall	Blacksburg, VA 24061
Richard Bryant	TenMileWood@gmail.com	2100 Faceville-Attapulgus Rd	Bainbridge, GA 39819
Harold Burkhart	burkhart@vt.edu	319 Cheatham Hall	Blacksburg, VA 24061
Tom Byram	t-byram@tamu.edu	2585 TAMU	College Station, TX 77843-2585
Alanna Carlson	alannac@g.clemson.edu	130 McGinty Court	Clemson, SC 29634
Melissa Carvalho	melissapisaroglo@ufl.edu	747 SE 2 Place Unit 2	Gainesville, FL 32601
Anthony Cascio	tcascio@resourcemgt.com	31 Inverness Center Parkway	Birmingham, AL 35242
Vikram Chhatre	crypticlineage@gmail.com	Dept. of Ecosystem Science & Management	College Station, TX 77843
Thomas Conwell	tconwell@westervelt.com	P.O. Box 48999	Tuscaloosa, AL 35404
Barb Crane	barbaracrane@fs.fed.us	1720 Peachtree RD NW	Atlanta, GA 30309
William Cumbie	wpcumbi@arborgen.com	2011 Broadbank Ct.	Ridgeville, SC 29472
Michael Cunningham	mwcunni@arborgen.com	PO Box 180438	Tallahassee, FL 32318
Kelsey Dunnell	Kelsey.Dunnell@ndsu.edu	2002 35th Street Circle S	Moorhead, MN 56560
Craig Echt	cecht@fs.fed.us	23332 Success Rd	Saucier, MS 39574
Alfredo Farjat	aefarjat@ncsu.edu	1019 Biltmore Hall	Raleigh, NC 27695-8002
Graham Ford	gaford@ncsu.edu	1019 Biltmore Hall	Raleigh, NC 27695-8002
Salvador Gezan	sgezan@ufl.edu	363 Newins-Ziegler Hall	Gainesville, FL 32611
Andrea Gilich	agilich@fs.fed.us	23332 Success Rd	Saucier, MS 39574
Li Guo	lguo1@biochem.umass.edu	270 Stockbridge Rd	Amherst, MA 1003
Heidi Harrington	heidiharrington@bellsouth.net	1125 Oak Brook Way	Atlanta, GA 30319
Christine Holtz	christine.t.holtz@gmail.com	148 Wakefield trace	Athens, GA 30605
Dudley Huber	dudley@quantitative-genetics.com	957 Easy Street	Toccoa, GA 30577
Allan Humphrey	humphrey@uamont.edu	110 University Ct	Monticello, AR 71656

Name	Email	Address	
Joe James	s4e4j4@bellsouth.net	260 Steve Nix Road	Seneca, SC 29672
Steven Jeffers	sjffrs@clemson.edu	School of AFES	Clemson, SC 29634
Kurt Johnsen	kjohnsen@fs.fed.us	3041 E. Cornwallis Rd	Research Triangle Park, NC 27709
Henry Kodama	kodama@forestry.state.sc.us	PO Box 21707	Columbia, SC 29221
Jared LeBoldus	jared.leboldus@ndsu.edu	NDSu Dept 7660	Fargo, ND 58101
Ted Leininger	tleininger@fs.fed.us	432 Stoneville Road	Stoneville, MS 38776
Haiying Liang	hliang@clemson.edu	130 McGinty Court	Clemson, SC 29634
Siran lu	lusiran@gmail.com	107 college station Rd.	Athens, GA 30605
Mohammed Majid	majidbr@yahoo.com	1042 Agronomy Rd	College Station, TX 39574
Tim Martin	tamartin@ufl.edu	PO Box 110410	Gainesville, FL 32611-0410
Steve McKeand	tori_batista@ncsu.edu	1019 Biltmore Hall	Raleigh, NC 27695-8002
Scott Merkle	smerkle@uga.edu	School of Forestry and Natural Resources	Athens, GA 30602
Daniel Morrow	daniel.morrow@weyerhaeuser.com	P.O. Box 1060	Hot Springs, AR 71902
Lazarus Mramba	lmramba@ufl.edu	358 Newins Ziegler Hall	Gainesville, FL 32611-0410
Nicholas Muir	nmuir@interforestry.com	1265 GA Hwy 133 North	Moultrie, GA 31768
Nicholas Mustoe	nickamustoe@gmail.com	120 McKnight Drive	Monticello, AR 71655
Campbell Nairn	nairn@uga.edu	Warnell School of Forestry and Natural Resources	Athens, GA 30602
Stefan Nakollari	snakolla@gmail.com	310 MAY ST	Worcester, MA 1602
Dana Nelson	dananelson@fs.fed.us	23332 Success Rd	Saucier, MS 39574
Ronald Overton	roverton@fs.fed.us	Purdue University	West Lafayette, IN 47907
Gary Peter	gfpeter@ufl.edu	PO Box 110410	Gainesville, FL 32611
Greg Powell	glpowell@ufl.edu	PO Box 110410	Gainesville, FL 32611
Tania Quesada	tquesada@ufl.edu	352 Newins-Ziegler Hall	Gainesville, FL 32611
Earl Raley	fraley@tfs.tamu.edu	2585 TAMU	College Station, TX 77843-2585
Alejandro Riveros Walker	ariverosw@ufl.edu	368 Maguire Village Apt. 5	Gainesville, FL 32603
Christopher Rosier	clrosie@arborgen.com	13 Ballastone Ct	Savannah, GA 31410
Christopher Saski	saski@clemson.edu	310 BRC	Clemson, SC 29634
Simon Schreier	Simonsch.says@gmail.com	811 ISSAQUEENA TRL	Central, SC 29630``
Michelle Serapiglia	mjs534@cornell.edu	630 West North St	Geneva, NY 14456
GANG SHAO	gshao@purdue.edu	2101 Cumberland Ave Apt 4207	West Lafayette, IN 47906
Shakuntala Sharma	sharma@uamont.edu	1413 HWY 425 South	Monticello, AR 71655
Joshua Sherrill	josh.sherrill@rayonier.com	PO Box 819	Yulee, FL 32041
Jesse Spitzer	jespitze@ncsu.edu	1019 Biltmore Hall	Raleigh, NC 27695-

Name	Email	Address	
			8002
Margaret Staton	staton2@clemson.edu	304C BRC	Clemson, SC 29634
Josh Steiger	josh_steiger@ncsu.edu	1019 Biltmore Hall	Raleigh, NC 27695-8002
George Surritte	gsurritte@westervelt.com	1400 Jack Warner Parkway, NE	Tuscaloosa, AL 35404
Bijay Tamang	bxtaman@arborgen.com	PO Box 180438	Tallahassee, FL 32318
Zhenkun Tian	zhenkut@clemson.edu	130 McGinty Court	Clemson, SC 29634
Laura Townsend	laura.townsend.a@gmail.com	3412 Monsieur Court	Raleigh, NC 27610
CJ Tsai	cjtsai@uga.edu	120 Green Street	Athens, GA 30606
James Tule	jtule@hnrg.com	23194 Hwy 111	DeRidder, LA 70634
Nicholas Wheeler	nickwheeler@scattercreek.com	21040 Flumerfelt Rd SE	Centralia, WA 98531
Ross Whetten	ross_whetten@ncsu.edu	Dept Forestry & Environmental Resources	Raleigh, NC 27695-8008
Rodney Will	rodney.will@okstate.edu	008C Agricultural Hall	Stillwater, OK 74078
Yi Xu	yix@g.clemson.edu	104 Biosystems Research Complex	Clemson, SC 29634
Jianxing Zhang	zhangjx04@gmail.com	327 University Village APT 3	Gainesville, FL 32603-2052
Xinfu Zhang	xinfuz@clemson.edu	104 Regency Drive, apt48	Central, SC 29630
Tetyana Zhebentyayeva	tzhebe@clemson.edu	P & A Center, Room 154, 130 McGinty Court	Clemson, SC 296340001