Proceedings

33rd Southern Forest Tree Improvement Conference

Using Tree Improvement to Grow New Products for a Changing World

Hosted by:

Arkansas Forest Resources Center; University of Arkansas Division of Agriculture

School of Forest and Natural Resources; University of Arkansas at Monticello

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Proceedings of the 33rd Southern Forest Tree Improvement Conference

Edited by

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Foreword

The 33rd Southern Forest Tree Improvement Conference (SFTIC), marking 64 years of biennial technical conferences, was held June 8-11, 2015 in Hot Springs, Arkansas. This marks the first time the conference has been in the state of Arkansas. Direction of the conference was coordinated by the Southern Forest Tree Improvement Committee and with co-hosts Arkansas Forest Resources Center; University of Arkansas Division of Agriculture School of Forest and Natural Resources; University of Arkansas at Monticello and Weyerhaeuser Inc.

The theme for this year's meeting was "Tree Improvement to Grow New Products for a Changing World". Across four days, foresters, tree breeders, scientists and practitioners met and listened to invited and volunteer presentations describing the many ways that forest genetics adds value to our region's and our nation's forests and how this field can be furthered and conserved.

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 Patrick Cumbie with his talk entitled "Marker-assisted selection for trait improvement in loblolly pine *(Pinus taeda L.)"* and Tim Albaugh with his talk entitled "Improving our understanding of growth differences of *Pinus taeda* in the United States and Brazil: A common garden experiment".

The **Bruce Zobel Award** is given for the best oral presentation by a student. The winner was Bridget Piculell from the University of Mississippi with her talk entitled "Correlations between traits in loblolly pine, including interactions with mycorrhizal fungi".

The **Belle Baruch Foundation Award** is given for the best poster. The first place winner was Changho Ahn from the University of Georgia with the poster "Application of somatic embryogenesis for mass propagation of hybrid hemlocks". Changho Ahn also won the second place prize with the poster "A clonal propagation and cryopreservation system for Atlantic white cedar (*Chamaecyparis thyoides*) via somatic embryogenesis". The third place poster was presented by Jessica Mitchell, also from the University of Georgia, entitled "Propagation of "lingering ash" genotypes via somatic embryogenesis for emerald ash borer resistance testing".

The 33rd SFTIC Planning and Scientific Committee:

The Planning Committee was chaired by Joshua Adams (Louisiana Tech University). Members included Victor Ford (Director and Professor of the Southwest Research and Extension Center, University of Arkansas Division of Agriculture) and Robert Purnell (Weyhaueser Company). Alexander Hoffman (University of Arkansas at Monticello [UAM]) served as the graduate representative and de facto representative of UAM after the departure of Joshua Adams from Arkansas to Louisiana Tech University.

The Executive Committee: Dana Nelson (chair), Joshua Adams (vice-chair), Greg Powel (past chair), and Fred Raley (secretary/treasurer)

Proceedings typesetting was conducted by Grace Zhang (Louisiana Tech University undergraduate student), editing was conducted by the planning committee and final editing was done by Joshua Adams.

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Conference Co-Hosts:

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In Memoriam

Dr. F. Thomas Ledig

Aug. 13, 1938-May 20, 2015

4 old-time Forest Geneticists taken in 2011 at UC Berkeley Faculty Christmas party. Left to right Bro Kinloch, Ron Schmidtling, Tom Ledig, Tom Conkle

Resident of Vallejo, CA. Dr. F. Thomas Ledig passed away on May 20, 2015 at 76 from metastatic melanoma. He was a world renowned research scientist in the fields of forest genetics and conservation biology. He could not have been loved more by his family and a host of wonderful friends from all over the world.

Tom was born in Dover, New Jersey. After earning his B.S. from Rutgers University and his M.S. and Ph.D. from North Carolina State University, he went on to become a full professor and member of the Board of Permanent Officers at Yale University. In 1979 Tom joined the U.S. Forest Service's Pacific Southwest Research Station as Director of the Institute of Forest Genetics in Berkeley and Placerville, California.

Tom traveled the world as a highly sought after expert on forest genetics and conservation, consulting with government and scientific institutions. His research took him to Australia, Belgium, Canada, France, Hungary, India, Italy, Kenya, Korea, Luxembourg, Mexico, Morocco, Portugal, Scotland, Spain, Sweden, Turkey, Vietnam, and throughout the United States. He was familiar through travel with forest conditions in many other countries.

Major recognition of his life's work includes the Society of American Foresters Barrington Moore Memorial Award for outstanding achievement in biological research; Fellowship in the American Association for the Advancement of Science; a special award from the Universidad Autonoma Agraria Antonio Narro, Saltillo, Mexico; and two North American Forest Commission awards. His service as secretary of the UN/FAO/North American Forest Commission's Forest Genetic Resources Working Group was one of the most rewarding experiences of his career. Yet, his most treasured honor was a 1997 letter, signed by former colleagues at Yale University that reads, in part, "We miss the joie de vivre that left when you left."

Tom retired in 2008 as Senior Scientist in the Pacific Southwest Research Station and continued as an adjunct professor at the University of California-Davis. He remained active in research. His most recent work involved genetic diversity in conifers, evaluating experiments he established in 1973-1974 on the effects of climate change on pitch pine; and working on a National Geographic Society grant to study Coulter pine in Baja, California. His career is represented in part by over 135 publications in genetics and physiology. To the end, he maintained his long-time interests in hiking the mountains and the Southwest desert, photography, SCUBA diving, and collecting fine and folk art.

Tom is surived by wife, Linda Marie Lux; three children from a former marriage: Colleen Stanton Cassidy, Sean Cormac Ledig, and Brendan Owen Ledig; his grandson, Nicholaus Vitaly Ledig; brother Alan Lloyd Ledig; and farther-in-law Raymond Clyde Lux.

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Plenary Session

THE ROLE OF PLANTING IN ECOSYSTEM RESTORATION—PROS AND CONS

Jim Guldin[1]

The role of planting in the restoration of southern pine ecosystems that are underrepresented on the landscape is simple to the point of being rudimentary--when a seed source of the desired species is absent from a stand, planting is the only way to re-establish the desired species in the stand being restored. The advantages are obvious, especially when application of planting is done in a manner that draws on existing knowledge of seed sources, nursery practice, site preparation, and planting methodology in a manner that resembles reforestation after clearcutting. Much headway is being made with longleaf pine restoration, for example, using this model. However, there are a number of issues associated with the use of planting that raise questions on public and private lands, including cost, quality of genetic stock, and the speed with which restored habitat can be obtained. As a result, a broader approach to ecological restoration efforts in southern pines will involve the use of planting in stands that are partially to fully stocked. That raises questions about silvicultural prescriptions for planting in stands with residual overstory and midstory stocking, and how those prescriptions might address questions of robustness of seed sources, genetic diversity, and seedling establishment and growth. An overview of these issues will be presented to stimulate discussion and develop innovative applications of planting methodology especially with respect to regional ecological restoration initiatives with longleaf pine and shortleaf pine.

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PINE: A SURVEY OF ITS VALUE ADDED PHYTOCHEMICALS

Julie Carrier[1]

Despite the fact that U.S. forests can absorb up to 14% of U.S. CO2 emissions, forest values is at constant risk because of the decline in housing, paper and paperboard markets. The 746 million acres of U.S. forests have the potential to supply more than 650 million m^3/year for industrial use, and southeast U.S. forests have the potential to supply 167 million m^3/year of pine. In addition of supplying wood for lumber, paper and pellet manufacturing, pine contains numerous phytochemicals that could be extracted prior to traditional uses. Dating back to the Great Depression, compounds leading to turpentine manufacturing were incorporated into southern US pulp and paper mills. As we continue to confront a changing environment of forest product needs, these alternative products may need to be assessed for tree improvement. Pine bark is a prime candidate for extraction of compounds with biological activity; work conducted in Scandinavia reported on bark extracted with ethanol, yielding phenolic compounds that displayed anti-inflammatory properties. Recently, Arkansas-grown pine needles were extracted by hydrodistillation, yielding essential oils (EO) containing, among others, α-pinene, β-pinene, D-limonene, terpineol, and (-) caryophyllene. It was determined that pine EO composition did not change as a function of sampling date. The sum of the percent content of α-pinene, β-pinene, D-limonene, terpineol and (-) caryophyllene, added together, in pine EO for April, May, June, July and September samples were 67.3, 67.7, 69.4, 67.5, and 75.5 %, respectively. Using the disk diffusion assay, the produced pine EO was tested against methicillin resistant strains of Staphylococcus aureus. Results showed that growth of *S. aureus* strain 13136, an intermediate methicillin resistant strain, was inhibited by pine EO. Pine EO was also tested for inhibition of Listeria monocytogenes, an important food borne pathogen, using the disk diffusion assay. Toxicity of pine EO was tested using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. Results showed that pine EO preparations containing up to 0.63% did not affect the viability of mammalian cells, which in this assay were Caco-2 human colon cancer cells.

The above-described results indicate that phytochemicals could be extracted from pine bark and needles, providing compounds that have multiple societal benefits. Successful identification of phytochemicals and ensuing use could prove to be a mode of adding value to traditional forestry operations.

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BIOENERGY MARKETS COME DOWN TO EARTH

Matthew Pelkki[1]

For 10 years forest scientists and engineers have pursued the hope of advanced cellulosic biofuels. Difficulties in scaling up these conversion processes has led to investigation of practical alternatives that can be implemented with incremental advances in biomass conversion. Wood pellets, both white and "torrified" offer immediate opportunities for reduction of carbon emissions and efficient bio energy production from woody cellulose supplies. The production of wood pellets and characteristics of woody biomass supplies that lend to successful pellet production will be presented.

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PROJECT CAPTURE: A NATIONAL PRIORITIZATION ASSESSMENT OF TREE SPECIES FOR TREE SPECIES FOR CONSERVATION, MANAGEMENT, AND

Kevin M. Potter[1], Barbara S. Crane[2], and Valerie D. Hipkins[3]

A variety of threats, most importantly climate change (Parmesan 2006) and insect and disease infestation (Logan et al. 2003; Dukes et al. 2009; Sturrock et al. 2011), will increase the likelihood that forest tree species could experience population-level extirpation or species-level extinction during the next century. Project CAPTURE (Conservation Assessment and Prioritization of Forest Trees Under Risk of Extirpation) is a cooperative effort across the three deputy areas of the USDA Forest Service to establish a framework for conservation priority-setting assessments of forest tree species across the entire United States.

Project CAPTURE uses extensive lists of life history trait data, as well as climate change and pest and pathogen threat information, to categorize and prioritize native tree species for conservation, monitoring, management and restoration. The geographic scope of the initial phase of the Project CAPTURE encompasses approximately 400 tree species that occur on all forested lands within the contiguous United States and Alaska. Differences in data availability require separate prioritization efforts for the native tree species of Hawai'i and other U.S. Pacific islands and for the native species of Puerto Rico and the U.S. Virgin Islands; these efforts are now under way.

The foundation of this overall effort is a flexible framework that rates species based on risk factors relating to (1) *intrinsic attributes*, such as population structure, fecundity and seed dispersal ability; (2) *external threats* to genetic integrity; and (3) *conservation factors*, including evolutionary distinctiveness and regional responsibility. The Project CAPTURE framework allows for the quantitative grouping of species into vulnerability classes that may require different management and conservation strategies for maintaining the adaptive genetic variation of the species contained within each class.

Project CAPTURE builds on previous regional National Forest System efforts to assess the vulnerability of forest tree species to climate change and other threats (Aubry et al. 2011; Potter and Crane 2010; Devine et al. 2012). It aims to address three overarching principles necessary to enhance forest resilience and resistance on forested lands of the United States in the face of climate change: (1) Genetically diverse and adapted seed and planting stock will provide the foundation for healthy forests and ecosystems in the future; (2) Gene conservation is key to preserving vulnerable species and populations for the future; and (3) Establishing and maintaining partnerships will be more important than ever (Erickson et al. 2012).

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In the face of multiple threats and uncertainty, an important forest management goal will be to safeguard existing adaptedness within tree species and create conducive conditions for future evolution, with a focus on the conservation of variability in adaptive traits (Myking 2002). Our understanding of relevant adaptive traits is incomplete or non-existent for many tree species, however. Several researchers have therefore proposed using ecological and life-history traits to evaluate species' genetic resources and predisposition to threats including climate change (Sjostrom and Gross 2006; Myking 2002; Aitken et al. 2008).

Maintaining genetic variation across multiple species will require tailoring conservation, management, monitoring and restoration efforts to species with similar vulnerabilities. Specifically, priority-setting approaches will become increasingly important when conditions are rapidly changing and needs are greater than the available capacity to respond (Millar et al. 2007). It has become increasingly clear that triage, for example, may be necessary to prioritize forest tree species and populations for conservation (St Clair and Howe 2011). Such priority-setting can include the assessment of extinction risk, but also can integrate a wide variety of other information, such as economic and ecological importance; the probability of success; and the availability of funds (Gauthier et al. 2010; St Clair and Howe 2011).

The results of priority-setting assessments can be applied for restoration and conservation planning, for the evaluation of species' genetic resources, and for the early detection of vulnerability (Devine et al. 2012; Aubry et al. 2011). Importantly, such assessments should be based on a scientifically defensible and transparent process. These assessments also must be ongoing and adaptive to account for new information (Carter et al. 2000; Millar et al. 2007; Coates and Atkins 2001).

In this context, 25 USDA Forest Service resource managers and scientists participated in a Project CAPTURE workshop in March 2014 workshop, at The Arbor Day Foundation's Lied Lodge and Conference Center, to work toward agreement on a scientifically defensible and transparent process to categorize and prioritize tree species for conservation, monitoring, management and restoration.

Consensus at the workshop was that the project focus should be to identify and categorize forest tree species on U.S. forested lands that are expected to be most vulnerable to genetic degradation in the face of multiple threats. Genetic degradation is defined as a significant reduction in the ability of a species to persist for the next century without the loss of important variation in adaptive traits. Maintaining variation in adaptive traits is an important forest management goal because it increases the likelihood (1) that individuals across the species will have adaptedness to greater range of environmental conditions, and (2) that the species (or a population within the species) will be able to continue undergoing the evolutionary process of adaptation via natural selection and thus to be able to persist in its habitat or habitats.

Conceptually (Foden et al. 2013; Booth 2013), the prioritization framework aims to assess the relationship between the severity of each of three distinct threats (Exposure to Pests and Pathogens, Expected Climate Change Pressure, and Lack of Structural Sustainability) and two intrinsic vulnerability dimensions (Sensitivity and Low Adaptive Capacity) associated with each of those threats.

Data for two external threats (Exposure to Pests and Pathogens and Expected Climate Change Pressure), 23 intrinsic vulnerability species traits, and six conservation factors have been collected for each of the species included in the assessment. Most of the data were available from a handful of publicly available sources.
The proposed categorization and prioritization process is hierarchical and data-driven, and consists of five steps:

Step 1: Decision of which species traits (such as drought tolerance, site affinities, and frequency of large seed crops) should be included in each of several broad species attributes (such as Rarity, Regeneration Capacity, Dispersal Ability, and Environmental Limitations) in the context of each of three major threats to tree species (Exposure to Pests and Pathogens, Expected Climate Change Pressure, and Lack of Structural Sustainability). Each species is given a relative score (on a scale of 0-100) for each broad attribute.

Step 2: Determination of which species attributes (such as Rarity and Regeneration Capacity) belong in each of the two intrinsic vulnerability dimensions (Sensitivity to Threats and Low Adaptive Capacity) associated with the three distinct external threats.

Step 3: For each threat (Exposure to Pests and Pathogens, Expected Climate Change Pressure, and Lack of Structural Sustainability), grouping of species into vulnerability classes based on vulnerability dimension and individual threat severity scores using a quantitative clustering approach. For each of the three threats, species are given a vulnerability rating.

Step 4: Determination of each species' overall vulnerability score. This can be done in one of two ways: (1) Selecting the highest of the vulnerability scores from the three threats. (2) Combining the vulnerability scores for the three threats in a manner that weights each threat differently based on the perceived immediacy and severity of the threat to forest trees.

Step 5: If appropriate, assignment of higher prioritization weights to species based on additional factors, such as NatureServe conservation status and the degree to which a species' range occurs within the boundaries of the United States ("regional responsibility").

The flexibility of the Project CAPTURE framework allows for its application at multiple scales and across any area for which the relevant data exist for the species of interest. Only by considering extinction as a synergistic process of external threats and intrinsic biological traits will it be possible to make predictions of risk that approximate reality for most species, and therefore to increase the likelihood that conservation efforts will be effective (Brook et al. 2008). The Project CAPTURE assessment tool should be valuable for scientists and managers attempting to determine which species and populations to target for monitoring efforts and for pro-active gene conservation and management activities.

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Stress and Disease

TREE GENES INITIATIVE-ACCELERATING STRESS ADAPTED TREE

Adam Costanza[1], Keith Woeste[2], and Susan McCord[1]

The health of many forests in North America is under threat now, and forests will be even more susceptible in the future because of a changing climate. Trees can naturally adapt to changing environments by slow migration - less than ½ a kilometer per year-but suitable habitats are predicted to shift at 10 times that rate. More extreme weather events are likely to increase the intensity of wildfires. Warmer winters allow pests, both native and invasive, to expand rapidly and devastate areas faster. We need an integrated approach to develop the next generation of trees that can better withstand current and future stressors. Several large research projects are working to understand how trees respond to climatic and biotic stressors at a genetic level. Unfortunately, the benefits from this research face obstacles that keep them from progressing to downstream users.

Too often good research gets lost along the path to operational use. Currently there are several large research projects working to understand how trees respond to the climate and biotic stressors at the genetic level. Connecting this research to downstream users will speed up the process of showing on-the-ground value for research money spent, and increase the chance of operationalizing research discoveries. The Tree Genes Initiative (TGI) brings experts together to identify obstacles, collaboration opportunities, and communicate unmet needs to organizations including those funding research, setting forest policy, and managing forest resources.
The TGI is producing its first Forest Tree Adaptation Obstacles and Recommendations Report. This, and future TGI reports, are developed to identify obstacles along the chain of events (value chain) from research, to growth, to use of improved trees, and make recommendations that could speed the use of trees better adapted to stress. There are three categories of obstacles identified by the TGI consortium this year: obstacles to innovation, outdated policies, and market disconnects. These topics are explored in some detail with examples and recommendations to overcome the obstacles.

The TGI includes all points along the research-stewardship chain. The TGI works for on-the-ground changes by conveying practical information in a timely manner to stakeholders. This is accomplished by identifying current efforts, needs, and obstacles to using stress adapted trees.

Upstream Research
- Funding agencies
- Forest biotechnologists
- Tree breeders

Midstream Growth
- Land owners

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- Forest growers
- Forest managers

Downstream Use

- Timber management companies
- Forest products companies
- Federal land management agencies

At its core the TGI is a consortium of organizations engaged in the research, growth, deployment, and management of stress-adapted trees. Consortium experts include forest biotechnologists, tree breeders, forest growers, and forest managers. The TGI fosters communication among organizations throughout the research-management chain, and to outside stakeholders.

NOT ALL CLONES ARE EQUAL: VARIATION IN DROUGHT RESPONSE AMONG THREE CLONES OF LOBLOLLY PINE

Miles W. Ingwers[1], Mary Anne McGuire[1], Ridwan A. Bhuiyan[1] and Robert O. Teskey[1]

Abstract-- To better understand loblolly pine (*Pinus taeda* L.) drought response we conducted an intraspecific study on three 2-year-old loblolly pine half-sibling clones. Three soil water treatments were imposed. Two were constant soil water treatments at either high (-0.3 MPa) or low (-1.5 MPa) water availability and the third was a wetting-drying treatment that alternated between three weeks of drought (-1.5 MPa) and one week at field capacity (-0.3 MPa). The experiment was replicated three times with 171 ramets per clone and treatment combination. Biomass growth was measured by harvesting a subset of plants every four weeks. In addition, pre-dawn needle water potential, morning water potential, photosynthesis, stomatal conductance, transpiration and instantaneous water use efficiency were measured throughout the experiment

Increases in the frequency and severity of drought necessitate a better understanding of loblolly pine drought response. We conducted a study on intraspecific variation of loblolly pine (*Pinus taeda*) drought response in which 171 plants of each of three closely related 2-year-old loblolly pine clones were grown in well-watered and simulated drought conditions. Three soil water treatments were imposed. Two were constant soil water treatments at either high (-0.3MPa) or low (-1.5 MPa) water availability and the third was a wetting-drying treatment that alternated between three weeks of drought (-1.5 MPa) and one week at field capacity (-0.3 MPa). Biomass growth was measured by harvesting a subset of plants every four weeks. In addition, pre-dawn needle water potential, afternoon water potential, photosynthesis, chlorophyll fluorescence, stomatal conductance, transpiration, instantaneous water use efficiency, and leaf anatomy were measured throughout the experiment.

There was a significant difference in biomass growth among clones with a clear genetic by environment (G x E) interaction (p=0.004). Specifically, clone 1 preformed the best under low water conditions but significantly worse under high water conditions. In contrast, clones 3 preformed the best under high water conditions and significantly worsted than the other two clones under low water conditions. We interpret the results to suggest a fundamental tradeoff in which a particular set of traits can maximize growth under low water conditions at the expense of growth at high water or vise versa. Carbon isotope discrimination (12C/13C) also showed clear intraspecific variation and a significant treatment response, with average delta 13C of -32.09, -31.64 and -32.32 for clones 1, 2, and 3, respectively under well-watered conditions ($P<0.001$), with a similar response in drought conditions, indicating variation in water use efficiency. Needle water potential, needle gas exchange and chlorophyll fluorescence measurements all showed strong treatment effects but little variation among clones. This study indicates that there is genetic variation, even among closely related clones, in

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response to drought. These results will help provide tools for developing greater drought resistance in loblolly pine.

REALIZED GAINS FOR RUST RESISTANCE IN SLASH PINE

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One relevant aspect to be executed in any breeding program is to have an evaluation of the realized gain obtained from selection of material for a specific trait. Most experiments that aim to select outstanding genotypes establish trials that are based in single-tree plots, which maximize the use of the area, and therefore, number of genotypes tested; and they are based in tree-level traits, such as tree height and DBH. However, operational deployment occurs in large blocks of genotypes of the same, or similar, genetic background with different levels of competition, and more importantly, they express stand-level traits, such as total volume and dominant height. Given this difference between selection and objective traits, it is critical in these programs to evaluate realized gains. For Slash pine, the CFGRP from the University of Florida has a program that concentrates in volume and rust resistance. In the current study we evaluated the genetic gains from rust using an array of experiments. Breeding values for rust incidence were calculated from a second breeding cycle of slash pine that established two polymix (PMX) single-tree plot trial series, and one series of full-sib block plots (FSBP). All three series had sites established in Florida and Georgia with 16 sites for the polimix series and 111 locations for the FSBP. We correlated the breeding values between these series which yielded correlations between PMX and FSBP greater than 0.954. In addition, within the FSBP, we compared improved vs unimproved genotypes to quantify realized gains, and we evaluate the reliability conditions for which, the common measure, R50 scores is a reliable indicator of rust resistance. We conclude that important agreements exist for the genetic rankings between the selection and objective traits for rust resistance in Slash Pine.

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CONIFER TERPENES: ENGINEERING AN ANCIENT PLANT DEFENSE PATHWAY FOR RENEWABLE CHEMICALS AND BIOFUELS

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Conifer terpenes were the first “industrial” chemical and are feedstocks for the largest nonfood based hydrocarbon industry today. Terpenes are an important class of defense molecules in plants. Conifers evolved specialized duct systems that synthesize and store oleoresin composed of monoterpenes and diterpenoids. Sapwood typically contains 3-5% of terpenes which protect the stem against boring insects and their associated fungi. We are characterizing the genetics of resin duct formation and terpene synthesis in loblolly pine with the goal of increasing the content in the stem for increased resistance to southern pine beetles and as a better source of chemical feedstocks for renewable chemicals and biofuels. I will describe our progress towards metabolic engineering of the conserved terpene precursor pathway and regulators of resin duct development.

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Seed Source

SEED SOURCE INFLUENCES ON HEIGHT-DIAMETER RELATIONSHIPS IN LOBLOLLY PINE PLANTATIONS

Trevor D. Walker[1], Steve McKeand[1], Fikret Isik[1] and Bronson Bullock[2]

Height-diameter relationships describe the mean tree height across the range of diameter at breast height and are influenced by the environmental and genetic factors involved in stand development. Tree breeders are interested in the genetic influences on the height-diameter relationship because variation in height-diameter curves reflects differences in stem allometry and resource allocation. Timberland managers routinely use regional height-diameter models in growth and yield systems that account for stand age, density, and site productivity but do not explicitly include genetic information (Burkhart and Tome 2012, p. 281), which has created the need to assess the amount of variation that exists among families. Previous work with loblolly pine data has indicated that for a given site, family and seed source affect the level of the height-diameter curve without altering the basic shape of the curve (i.e. an anamorphic effect) (Buford and Burkhart 1987, Lynch et al. 2010). Furthermore, the level of the curve was found to be strongly related to the dominant height, suggesting that family and seed source can be adequately accounted for by adjusting site index inputs in regional height-diameter models. However, more recent research has found differences in curve shape (i.e. polymorphic effects) among loblolly pine clones (Sabatia and Burkhart 2013, Egbäck et al. 2015). The objectives of this study were to test for a genotype by environment interaction on the height-diameter relationship and determine if family effects could be effectively explained by seed source and dominant height.

Materials and Methods

This analysis utilized age eight measurements from the Plantation Selection Seed Source Study, which is comprised of more than 140 pollen-mix families of first generation loblolly pine from seven seed sources (Chamblee 2011). Data were available for 18 locations that were planted across the southeastern US using single-tree plots and 24 blocks per site. The height-diameter relationship was investigated using an allometric equation of the form

$$\ln(H) = \alpha + \beta D^{-1} \qquad (1)$$

where H is the total tree height for diameter at breast height D and $\ln()$ is the natural logarithm. The intercept, α, is the asymptote parameter and dictates the level of the curve. The slope, β, isthe shape parameter and describes how rapidly the asymptote is approached. A mixed-effects form of Equation 1 was used to assess the effects of family, location, and their interaction as

$$\ln(H_{ijk}) = (\alpha_0 + a_{1i} + a_{2j} + a_{3ij}) + (\beta_0 + b_{1i} + b_{2j} + b_{3ij}){D_{ijk}}^{-1} + \varepsilon_{ijk} \qquad (2)$$

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where a_{1i}, a_{2j}, and a_{3ij} are random effects for location i, family j, and their interaction, respectively, corresponding to the asymptote parameter and similarly for b_{1i}, b_{2j}, and b_{3ij} for the shape parameter. The formulation of Equation 2 is such that the fixed effects parameters α_0 and β_0 represent the population average values analogous to α and β from Equation 1. The ε_{ijk} is the random stochastic error due to tree k with $\varepsilon_{ijk} \sim N(0, \sigma_\varepsilon{}^2)$. Random effects were assumed to follow a normal distribution with mean zero with independence among subjects. The variance-covariance matrix of the random effects allowed correlations between the level and shape parameters for a given factor. Models were fit with restricted maximum likelihood. Likelihood ratio tests (LRT) were used to compare reduced forms of Equation 2 to assess the significance of family and interaction effects. Seed source was added as a fixed effect into the asymptote and shape parameters using dummy variables. A forward selection procedure was used to identify the combination of seed source regions that were most important. The adequacy of utilizing site index to account for genetic variation was assessed by adding dominant height as a fixed effect into the asymptote and shape parameters of Equation 2 and using likelihood ratio tests to determine if the random effects for family and family by location interaction could be removed without significantly weakening model fit.

Results and Discussion

The LRTs indicated significant family and location effects were present in both the level and shape of the height-diameter curve. These results suggest that pollen-mix families vary in curve shape, whereas previous studies have found that open-pollinated half-sibs did not (Buford and Burkhart 1987) and clones did (Sabatia and Burkhart 2013, Egbäck et al. 2015). The model was ill-conditioned when including the family by location interaction in both the asymptote and shape parameters, indicating that the interaction effect can be explained by either the level or shape of the curve (Pinheiro and Bates 2000, p. 156). Including dominant height in the model eliminated the need for the interaction effect, suggesting that the phenomena that influence genotype by environment interaction for height are the same as detected by the height-diameter model. The reader is referred to Chamblee (2011) for a thorough discussion on the family by location interaction effect on height observed in this data set.

Including seed source in the asymptote parameter produced a significantly better model based on the AIC. Including seed source in the shape parameter did not produce a better fit than the model with seed source in the asymptote alone. The model from the forward selection procedure that produced the best AIC had dummy variables in the asymptote for Coastal North and South Carolina combined and for Virginia. The Coastal North and South Carolina families corresponded to height-diameter curves with higher asymptotes, whereas the Virginia families had a lower asymptote (Figure 1a). Based on the AIC, it was not necessary to differentiate between Lower Gulf, Upper Gulf, Coastal Georgia-Florida, and Piedmont regions.

When including dominant height in the model, the LRT indicated the family random effect for the asymptote could be removed without reducing model fit; however, removing the family effects for shape significantly impaired model fit. The estimated shape effects remaining after including dominant height are presented in Figure 1b, which illustrates the variation that cannot be explained by simply adjusting site index inputs to account for genetics. Although, a practical observation from Figure 1b is the unexplained variation among families decreases with increasing diameter (and thus individual tree value). Estimates of the potential bias in stand volume estimates when excluding genetic effects from height-diameter models are presented in Egbäck et al. (2015).

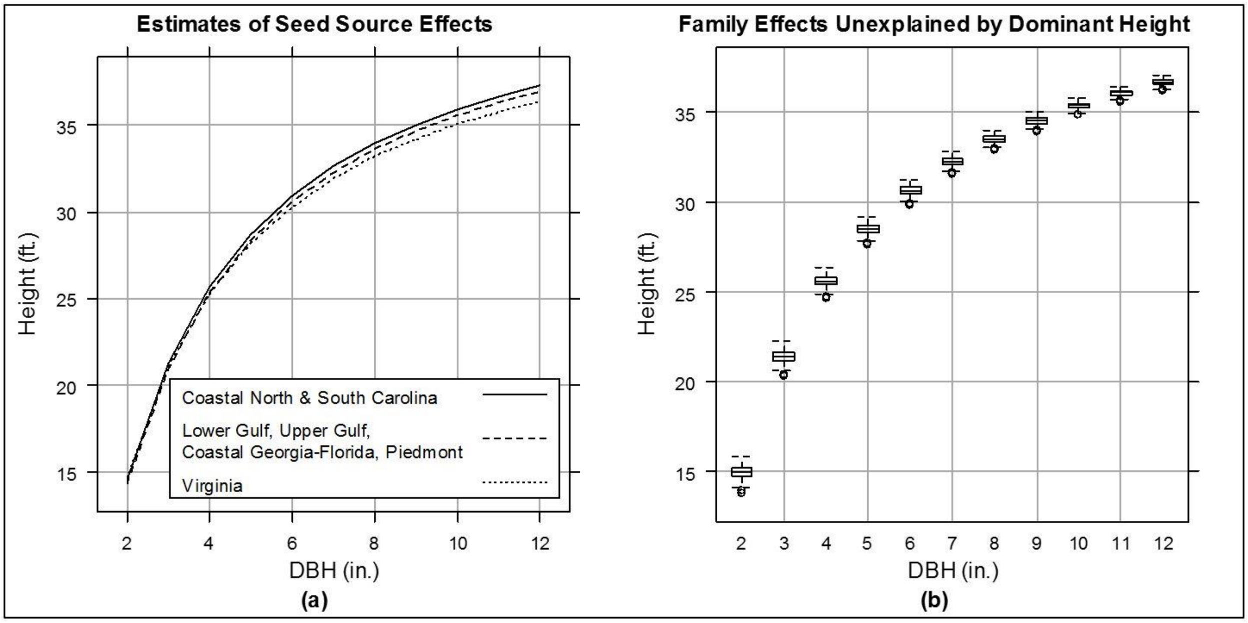

Figure 1. Height-diameter models with (a) estimated fixed effects for important seed sources and (b) boxplots of the spread of estimated random effects for family after accounting for dominant height.

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BAYESIAN SPATIAL MODELING OF LOBLOLLY PINE SEED SOURCE MOVEMENT

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

The establishment and analysis of provenance tests for investigating the genetic variation among forest trees has a long tradition in forestry. Such tests are generally intended to identify superior seed sources for planting at specific locations. The trials are usually replicated experiments established with seed from parent trees collected from different geographical regions within the species distribution and grown at several locations. The geographic location of the sites where the seed parents were collected and the location of the sites where the progeny trees were grown provide meaningful information for assessing the response of genotypes to environmental change. Furthermore, the climatic differences between the seed source and test site locations can be used to make predictions about the relative performance of provenances under different environmental conditions.

We developed a spatial Bayesian approach for modeling the expected height of trees from seed sources using as predictors climate variables associated with the location of origin and the planting site, and the growth potential of the planting site. The proposed modeling technique introduces a separable covariance structure that provides flexible means to describe and estimate effects associated with the origin and planting site locations. The model is developed and tested using the Plantation Selection Seed Source Study, a large replicated seed source test of loblolly pine (*Pinus taeda* L.) with test sites distributed throughout the southeastern United States. Application examples are presented and discussed using projections of future climate scenarios. The statistical model can be used as a quantitative tool for seed deployment aimed to identify the location of superior performing seed sources that could be suitable for a specific planting site under a specific climate scenario.

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DEPLOYMENT AND PROCUREMENT OF LOBLOLLY PINE (*PINUS TAEDA* L.) SEED SOURCES GUIDED BY THE APPLICATION OF CATEGORICAL UNIVERSAL RESPONSE FUNCTION (CURF)

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Loblolly pine (*Pinus taeda* L.) is a commercially and ecologically important forest tree species dominant in the southeastern USA. Throughout its history it has likely experienced recurrent climate changes (Millar, 1993). The most recent period of glaciation that ended about 15 thousand years ago was thought to have limited the natural range of loblolly pine to two southern refugia (Wells *et al.*, 1991; Schmidtling, 2003). Subsequent climate warming allowed for the gradual northward expansion of the species, likely causing adaptational lag between evolutionary adaptations expressed as growth rate vs. local climate conditions. This mismatch was quantified by Schmidtling who showed that the optimal height growth could be achieved when seed sources are moved northward to zones experiencing minimum winter temperatures cooler by about 2-3°C (5°F) (Schmidtling, 1994). These recommendations formed the basis for loblolly pine seed movement guidelines in the southeastern USA. Today foresters essentially assist selected families migrate northward to optimize the match of growth adaptations to local climate, exploiting the growth potential of the families. Survival is an additional factor, therefore maladaptation to such environmental factors as e.g. cold or drought could lead to increased mortality, and consequently compromised timber yield. To better understand these relationships, and to investigate factors that may be potentially limiting to species expansion to the west and north, we developed Categorical Universal Response Function (CURF) (Koralewski *et al.*, 2015). CURF models the timber yield vs. climate relationship, providing support for loblolly pine assisted migration decisions. However, much within-family and within-provenance variability has been observed for growth traits, which limits the utility of performance models based solely on climate for predicting growth.

Research is ongoing to identify approaches to best match evolutionary adaptations with local environment. Some of the notable advances include transfer functions (e.g., Matyas, 1994; Rehfeldt *et al.*, 1999), population response functions (e.g., Rehfeldt *et al.*, 1999; Wang *et al.*, 2006), and the universal response function (URF) (Wang *et al.*, 2010) which combines the former two methods. The major difference between the URF and CURF is that CURF implements multinomial logistic regression and thus operates on a categorical level. In brief, CURF follows a preliminary step in which the response variable is categorized according to a predefined scheme. Next, probabilities are calculated that a performance of a given family will fall within each of the predefined categories on a given planting site.

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We applied CURF to 15-year loblolly pine planted tree volume as a response to mean minimum temperature of the coldest month and growing season precipitation. We also considered the annual variation of these two metrics, as this may help explain range limiting factors in the west and north where the species is not bound by geographical barriers. The individual tree measurements originated from the Geographic Seed Source Study (GSSS) established and maintained by the members of Western Gulf Forest Tree Improvement Program (WGFTIP).
Estimated climate conditions at the provenance sites over a 30-year period using PRISM data (PRISM Climate Group; Daly *et al.*, 2008) were considered as surrogates for long term effects shaping evolutionary adaptations. The weather conditions during the 15-year progeny testing period corresponded to the actual environmental impact upon the tested families. Therefore, the weather at the test site could be seen as a shift in climate from the local conditions at the site of origin to which the families are presumably adapted. Furthermore, this effect could be seen as a surrogate for a potential future climate change.

The model generally confirmed the guidelines proposed by Schmidtling (1994). South to north movement was well supported, with some local variability that could probably be attributed to precipitation and variation in the two climate variables. All, or almost all (depending on the evaluation criterion) selected interactions among climate variables were significant. Although interactions did not cause major differences when compared to the model without interactions, their effect was more evident for sites near the edge of the loblolly pine distribution range. Since all explanatory variables are solely climate-related, and thus the method operates in a climate space, extrapolation beyond the spatial boundaries of the studied region is possible. Such exercises, however, must be verified with practical trials as growing conditions in the areas to which the model is extrapolated may be influenced by factors other than climate, e.g. soil type or water table level. This is especially important when focusing on areas along the edge of the distribution range where climate conditions are generally harsher. For example, in practice, seed movement in east Texas generally involves selection of better quality soils to facilitate survival. The model did not support the provenances located along the northern edge of the distribution, such as those in southern Oklahoma and southern Arkansas, as good candidate procurement sources. However, due to exposure to harsher and more variable continental climate these sources have likely developed essential survival adaptations, and may be valuable for novel areas beyond loblolly pine range located in the north – e.g., in Tennessee or Kentucky. For the same reason, they may be considered in wide crosses with fast growing seed sources (e.g. Atlantic coastal).

The model performed well and is consistent with current understanding of the risk-benefit balance in loblolly pine seed movement in the Western Gulf area. As the model is purely climate-based, it does not account for other factors that may impact species performance, including soil type, elevation, aspect, or silvicultural practices. Moreover, when considering future climate projections, one should be aware of climate models' sensitivity to long-term assumptions, and thus uncertainties that may result from both stochasticity of natural phenomena and unknown direction of human actions. As the ultimate procurement and deployment decisions are made by practitioners, the categorical output and probabilistic nature of CURF make it a good candidate approach to consider for Decision Support Systems.

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ANALYSIS OF WOOD PROPERTIES IN COASTAL AND PIEDMONT POLLEN MIX TESTS

Andrew Sims[1], Graham Ford[1], Austin Heine[1], Fikret Isik[1], and Steve McKeand[1]

Wood density and bending strength are among the most important properties related to the value of wood products. Traditional sampling procedures for these traits require time-consuming and expensive lab work, both of which are major impediments to incorporating these traits into tree breeding programs. Fortunately, there are now efficient and rapid procedures to sample these wood properties. The Fakopp TreeSonic measures acoustic stress-wave velocity, which has a strong genetic correlation with wood bending strength. The IML Resistograph is a micro-drilling tool that measures drilling resistance across the cross-sectional profile of a tree and is strongly correlated with wood specific gravity. Use of these non-destructive techniques have been promising for reducing the time and cost of selecting genotypes with superior wood properties.

The first phase of the study was to validate the efficiency of the new Resistograph version IML Resi PD400 series for correlating with volumetric specific gravity. Additionally, acoustic stress-wave velocity from Fakkop TreeSonic tool was used as an estimate of modulus of elasticity. Here, we found that genetic correlation between Resistograph drilling amplitude and specific gravity was high (r_G = 0.90). This phase represented two test sites from the Coastal Pollen Mix 1 series, inculding 70 half-sibling families and two checklots. The second phase of the study extended these measurement and analysis protocols to a wider range of Coastal and Piedmont families, representing ten sites in 5 different series. We found moderate to high heritabilities for wood property traits with family mean heritabilities ranging from h_fm^2 = 0.44 to 0.76 for stress-wave velocity and h_fm^2 = 0.50 to 0.78 for relative amplitude. These findings indicate that the Cooperative can use these rapid screening methods to effectively characterize families for wood properties. To date, we have used these rapid screening tools to estimate breeding values for the surrogates of wood density and bending strength for 280 Coastal families and 74 Piedmont families.

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Conservation

GENETIC RESOURCE CONSERVATION OF TABLE MOUNTAIN PINE IN CENTRAL AND SOUTHERN APPALACHIAN MOUNTAINS

Robert Jetton[1], Barbara Crane[2], Andrew Whittier[1], and Bill Dvorak[1]

Table Mountain pine (*Pinus pungens* Lambert) was historically a widespread pine species native to the central and southern Appalachian Mountains, but its current natural distribution has been reduced to less than 12,000 ha in recent decades. Reasons for this decline include wildfire suppression programs of the early 20th century, southern pine beetle outbreaks, and recent climate fluctuations. Part of the effort to mitigate this decline is a 5-year, cooperative, genetic-resource conservation effort being conducted by Camcore (International Tree Breeding and Conservation, North Carolina State University) and the U.S. Department of Agriculture (USDA), Forest Service, Southern Region National Forest System. The goal of the project was to target seed collections from up to 300 mother trees in 30 populations distributed across the natural range of the species. During five field seasons, cones were collected from a total of 262 mother trees in 38 populations distributed across the geographic range of the species. Collections represent 5 of 8 ecoregions, 5 of 6 plant hardiness zones, and all 4 seed zones occupied by the species. A total of 390,530 seeds were collected for conservation that have been distributed to the USDA Agriculture Research Service-National Center for Genetic Resources Preservation for long-term storage (55,828 seeds), the USDA Forest Service Ashe Nursery Facility for seed orchard and reforestation activities (193,395 seeds), and the Camcore Seed Bank for research and field plantings (135,361 seeds). Collectively, the seed stored at these three facilities represent the largest genetic resource of Table Mountain pine that exists outside of natural stands. Ongoing research on Table Mountain pine population genetics using microsatellite markers will compare the genetic diversity in natural stands to that of the seed sample to evaluate the effectiveness of this genetic resource conservation effort in capturing a representative sample of the species. A summary of this project is available in Jetton et al. 2015. *Tree Planters' Notes* 58(1): 42-52.

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SHORTLEAF PINE GENETIC RESOURCE-SUPPORTING RESTORATION IN THE SOUTHERN REGION

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The USDA Forest Service National Forest System (NFS) in the Southern Region actively supports shortleaf pine (*Pinus echinata* MILL) restoration. Shortleaf pine has the largest range of the four major southern pines. It is a long lived species, genetically diverse, and successfully managed using fire. NFS's reforestation activities include planting several million shortleaf seedlings annually. State and private nurseries grow both bare root and container stock for the Forest Service, using our seed orchard seed. Forest Inventory and Analysis has estimated that the shortleaf pine ecosystem has declined by 50% of the original range noted in 1915. Hence shortleaf pine is the second most important species targeted for restoration by the Forest Service.

The NFS Genetic Resources Management Program (GRMP) actively manages 1200 acres of shortleaf pine seed orchards in North Carolina, Mississippi, Louisiana and Arkansas. This is 70% of all known shortleaf pine seed orchard resources in the south. The orchards contain varying amounts of 1st and 2nd generation material. Cones are harvested on 5-7 year intervals. The GRMP seed bank has 900 pounds viable shortleaf pine seed in storage, 2-25 years old, representing 9 southern seed zones. The GRMP maintains 155 shortleaf progeny tests, which will provide scion material for orchard expansion, backup seed production areas and opportunities for climate change research.

The GRMP is involved in several projects and partnerships, with the purpose of supporting shortleaf pine restoration. The GRMP has sent samples of each of the seed zones to ARS's National Center for Genetic Resources Preservation facility (Ft. Collins, CO) for long term storage and research. The GRMP is working with the Eastern Environmental Threat Assessment Center (EFETAC) to develop new planting zones to prepare for climate change challenges. The GRMP and North Carolina Forest Service have a MOA in place, for the purpose of sharing shortleaf pine improved genetic material. Exchange of scion material between the two agencies helps to replenish seed orchards, increase genetic diversity, and provide a secondary site for some of the material. In support of the Shortleaf Initiative, the GRMP is circulating a Shortleaf Pine Seed and Orchard Resources Survey throughout the south. Data collated includes information about the state of shortleaf seed orchards, e.g. ownership, acreage, age, seed zones, generations, cone crop cycles, and seed inventory. This information will help identify seed zone gaps. The GRMP, in collaboration with Southern Research Station, is helping to fund Oklahoma State University to study shortleaf X loblolly hybrids, and identify any in our shortleaf seed orchards. Forest Service seed orchards typically manage four or more pine species in each orchard, therefore all trees are being DNA fingerprinted to ensure species purity. The work is being done at the NFS National Genetics Lab (NFGEL, Placerville, CA).

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FREQUENT FIRE AND THE REDUCTION OF LOBLOLLY PINE X SHORTLEAF PINE INTROGRESSION

John F. Stewart[1], Rodney E. Will[1], Kevin M. Robertson[1], and C. Dana Nelson[2]

A recent study showed that the hybridization rate between shortleaf pine (*Pinus echinata*) and loblolly pine (*P. taeda*) has increased during the last 60 years, creating a cause for concern about the future of shortleaf pine in particular. Whereas shortleaf pine seedlings have strong basal crook as a fire adaptation, loblolly pine seedlings do not, and hybrids have intermediate crooks that do not appear to provide adequate protection from fire for their dormant buds. Thus, regular fire could select against hybrids in shortleaf pine habitat. The Tall Timbers Research Station (TTRS) conducts biennial burns in open canopy pine and grass savanna but also keeps closed canopy woodland adjacent to the burned sites. We used 25 microsatellite markers to estimate the hybrid character of 184 saplings and 111 overstory trees from 4 paired burned and unburned sites at the TTRS. We found that the overstory was an undifferentiated mix of loblolly pine, shortleaf pine, and hybrids and that there was no difference in the frequencies among those samples (P = 0.36). Along the edges of unburned areas, all three classifications appeared in frequencies similar to the overstory population. However, we sampled no loblolly pine seedlings and only one shortleaf pine x loblolly pine seedling in the regularly burned sites, and the hybrid frequencies of the burned and unburned sites were significantly different (P = 0.006) Thus, introgression of shortleaf pine by loblolly pine may be controlled by regular controlled burning of open sites. These results are the first to show that frequent fire can prevent introgression in two co-occurring native species, preserving the genetic integrity of at least one of those species. These results were also recently published in the Journal of Conservation Genetics, and we present them to an audience different from that journal.

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Tree Improvement

PREDICTION OF GENETIC MERIT IN A CLONAL POPULATION OF LOBLOLLY PINE

Alfredo Farjat[1], Fikret Isik[1], and Steve McKeand[1]

Cloned progeny testing provides more reliable predictions of individual-tree breeding values than does seedling progeny testing. In this study, we analyzed a large cloned progeny test established by the NC State University Tree Improvement Program. The main objective of the study was to identify superior genotypes for breeding. From each of 51 full-sib families of loblolly pine (*Pinus taeda* L.), an average of 46 progeny were cloned using rooted cutting techniques. A total of 2362 clones were tested in seven test sites across the southeastern United States using a incomplete block row-column design. Growth measurements (height and diameter at breast height), stem straightness, and forking were assessed at tree age 4 years; only the results for height are presented here.

Linear mixed models were used for the statistical analysis. Predictions of genetic merit of clones for tree height were obtained using various G and R covariance structures that account for heterogeneity and genotype by environment interactions. Models were compared through fit statistics (Akaike Information Criterion, Bayesian Information Criterion, Log Likelihood). Best linear unbiased predictions of clones were compared using rank correlations, Pearson's correlations, and overall standard error of predictions. To estimate the aggregate genetic merit of clones from the multi-environmental trial analysis, weights for each site were obtained from separate analyses of individual trials.

Among the fitted models for the genetic analysis of height, factor analytic models G structure combined with a separable autoregressive structure of first order for the residuals at each site were superior based on the Akaike information criterion. However, the model was computationally demanding and required good starting values to converge. On the other hand, factor analytic G structure combined with block diagonal covariance R structure for the residuals was robust for modeling genotype by environment interactions. The model was parsimonious, informative, and could be fit without running into convergence problems since the computational burden of the mixed model analysis is greatly reduced.

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OPTIMAL FIELD DESIGNS AND ANALYSIS OF SPATIALLY AND GENETICALLY CORRELATED DATA USING MIXED MODELS

Lazarus Mramba[1] and Salvador Gezan[1]

The existence of varied levels of environmental heterogeneity and genetic relatedness among genotypes in plant breeding is a major challenge in the design and analysis of field trials. The standard traditional design methods assume independent residual errors, ignore genetic relationships and often treat both blocking and genetic effects as fixed, which often results in suboptimal designs, which are biased and present misleading findings in addition to poorly estimating the genetic values. This study investigates the dynamics of varying levels of heritabilities, spatial and genetic correlations using both A- and D-Optimality criteria to generate optimal field designs. An algorithm to optimize the design of a randomized complete block experiment is presented.

The results from this study indicated that under the D-Optimality criteria, the percentage improvement between the original and the final optimal designs was consistently higher with increasing heritability and spatial correlations for all the genetic scenarios (full-sibs, half-sibs, and independent genotypes). The percentage improvement with respect to the A-Optimality criteria varied with the type of pedigree structure, levels of heritability and spatial correlations. A strong Spearman's correlation between the D- and A- Optimality criteria of about 0.97 was obtained for both the original and final designs, implying that a design with small/large traces (from the A-Optimality criteria) is likely to have small/large determinants values (from the D-Optimality criteria).

Complete datasets with response variables, field patches (surfaces) and nuggets (measurement errors) for both non-optimal and "near optimal" designs were generated and analyzed for prediction of random effects and heritability estimates. Very high Pearson's correlation coefficients were observed ranging from 0.84 to 0.99 between the predicted and the true genetic effects. The optimal designs provided more accurate, consistent and precise estimates of heritabilities and breeding values with smaller standard errors compared to the initial non-optimal designs. In conclusion, the results indicate that simultaneous consideration for both genetic and environmental conditions is indispensable for generation and analysis of optimal field designs.

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UNDERSTANDING GENOTYPE-BY-ENVIRONMENT INTERACTION ON LOBLOLLY PINE CLONAL TRIALS

Melissa Carvalho[1], Josh Sherry[1] and Salvador Gezan[1]

The success of genetic improvement program is to provide genotypes with guaranteed superior performance in terms of productivity and or quality across environmental conditions. To achieve this goal it is necessary to have a good understanding of the phenotype, which is a result of the combination between genotype's performance and environmental. The present study evaluates a series of six loblolly pine (*Pinus taeda* L.) clonal trials established on the southeast U.S. that were measured for total stem volume and survival. The main objective of this study is to explore GxE interaction for this clonal population by fitting a range of linear mixed models. The dataset came from a series of genetic trials established by the FBRC from the University of Florida (CCLONES). This study contains 61 full-sib loblolly pine families planted in single-tree plots as an incomplete block design with eight full replicates assigned to a silvicultural treatment (operational or intensive management). The variables evaluated were total stem volume and survival.

Initially, a single-site analysis was fitted for each of the two silvicultural treatments, the objective was to evaluate if there are statistical differences among treatments, in terms of genetic control of the traits of interest. Also one- and two-stage analyses were performed to obtain the information required for an MET analysis, in both cases, the silvicultural treatment effects were ignored. For the two-stage MET analysis several models of lesser complexity were fitted. The first stage consists on performing single-site analyses where all the design features of each trial are considered. For the second stage, the predicted means are used as response to fit a simpler weighted linear mixed model of the following form based on the implicit model.

The results of this study indicate that it is expected to obtain very similar rankings for genotypes selected under the two silvicultural levels evaluated (operational and intensive) in this trial series. The results of the explicit model analysis provided with an interesting Type B genetic correlations of 0.627 and 0.566 for VOL and SURV, respectively. For the implicit model, the results for VOL show values ranging from 0.412 to 0.802 (average of 0.568).

The one- and two-sage analyses provided very similar correlations, rankings and breeding values; however, the two-stage analysis resulted in increment in the genetic correlations. This increment ranged from 0.62% to 36.91% (average increment of 14.46%). Factor analytic analysis is appropriate when there are a large number of trials with high genotype-by-environment interaction. Biplots analysis summarized the genotype-by-environment successfully and confirmed the positive correlation between all the environments for volume found earlier.

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Genomics

MARKER-ASSISTED SELECTION FOR TRAIT IMPROVEMENT IN LOBLOLLY PINE (*PINUS TAEDA* L.)

W. Patrick Cumbie[1], Dudley A. Huber[2], Victor C. Steel[1], William Rottmann[1], Les Pearson[1] and Michael Cunningham[1]

A set of 900 clonally replicated loblolly pine varieties (*Pinus taeda*, L.) from 35 full-sib families were assessed for growth, stem form, and disease resistance after 6 growing seasons. Using a set of more than 5000 SNPs we identified 3340 SNPs that were informative in this population and were subsequently used in genetic analyses. SNP markers were used for the estimation of genomic relationships and significant SNP associations were identified for height growth, volume, rust resistance and stem straightness. Resistance to fusiform rust infection caused by Cronartium quercuum (Berk) Miyabe ex Shirai f.sp. fusiforme was heritable (H^2=0.44) and highly significant SNP associations were identified using a Bayes CPi analysis. The five most significant (5) SNP loci accounted for 45% of the total variance for fusiform rust infection. Current results and potential applications will be discussed.

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DRAFT GENOME SEQUENCE OF MYCOSPHAERELLA DEARNESSII FROM TWO ISOLATES INFECTING LONGLEAF PINE

B. D. Bartlett[12], J. H. Roberds[1], K. C. Showmaker[2], D. G. Peterson[2], and C. D. Nelson[13]

Longleaf pine (*Pinus palustris* Mill.) once occupied over 30 million hectares in the southeastern US, but in recent times has been drastically reduced to an area consisting of 1.2 million hectares. Currently, a restoration effort is underway to increase longleaf pine habitat to a total of 3.2 million hectares. *Mycosphaerella dearnessii* is a fungal pathogen of longleaf pine that could hinder restoration efforts. To better understand the pathogen and develop strategies to limit its damage, a draft genome sequence has been produced. To date, two isolates of the fungus has been sequenced and an assembly of 30Mbp genome sequence with an N50 of 19,120bp has been obtained from one of these. This represents 65% of the estimated genome size of 46Mbp from 12,317 contigs. Gene prediction software has yielded 17,633 protein coding genes. Comparative genomics will be employed to recognize the genes responsible for pathogenicity. Of particular interest is detection of effector protein genes, which are responsible for the fungus entering the plant cell. Knowledge of the pathogen's genome will lead to reduce incidence of the disease and provide a starting point to develop disease-resistant longleaf pine trees.

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BREEDING WITHOUT BREEDING: SCIENTIFIC JOURNEY TO REAL-LIFE APPLICATIONS

Milan Lstiburek[1] and Gary R. Hodge[2]

Breeding without Breeding (BwB), broadly defined, is the use of molecular marker technology to make selections and genetic gain in tree breeding programs with a greatly reduced effort in control crossing and progeny testing. In this presentation, we cover the development of BwB strategies and highlight some key features, including phenotypic preselection and pedigree reconstruction. Next, we discuss the latest development on the extension of the BwB system to uncover genetic information directly from commercial forest plantations. This approach could be particularly useful at the initiation of a tree improvement program to save the time and expense of traditional progeny trials. The details of a suggested approach will be described, which include the use of both a random and phenotypically selected population (to minimize genotyping costs), pedigree reconstruction, and the use of standard REML-BLUP methodology to predict breeding values and make selections. Quantitative genetic theory and computer simulation were used to determine expected genetic gains, and estimate optimum population sizes for pre-screening and genotyping. In summary, we think BwB is a competitive cost-efficient approach that has potential value to tree improvement programs worldwide.

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GENOMICS RESEARCH AND FUSIFORM RUST DISEASE

Katherine E. Smith[1][2], Daniel Ence[3], Zev Kronenberg[2], C. Dana Nelson[3][4], Mark Yandell[2] and John M. Davis[1]

Two of the major goals of our fusiform rust disease research are to identify resistance genes in pine selections and to monitor fungal virulence in the field as a tool to predict the best selections to plant. Advances in high-throughput sequencing and bioinformatics have provided new methods for achieving these goals. The 23.2 gigabase loblolly pine genome is approximately 232 times larger than the 100 megabase genome of *Cronartium quercuum*, the causative fungus, yet we have a genomic reference for both, as well as, RNA sequence data to support gene annotation. Ongoing research in the pine host includes: 1. Employing known SNP (single nucleotide polymorphism) data and specialized target selection with high-throughput sequencing to fine map the Fr1 locus; and 2. Identifying resistance genes expressed in elite families by leveraging the large depth of known RNA sequences to select resistance gene candidates. On the fungal pathogen side of the interaction, a genomic location for the Avr1 gene has been identified using previously obtained marker sequences and high-throughput re-sequencing techniques. Advances in the assembly of genomes using longer reads and improved software have made it feasible to sequence the Cqf genome using the Pacific Biosciences sequencing platform. These data are being used to improve the Cqf reference sequence and to more accurately pinpoint the Avr1 gene. Progeny of the fungal isolate P2 were sequenced in order to locate the Avr1 locus. Sequencing of P2 progeny resulting from inoculation on pine families segregating for resistance gene alleles corresponding to Avr2 and Avr3 can be used to locate these avirulence genes. This reinforces the importance of maintaining germplasm of relevant pine families and fungal isolates.

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Tree Improvement Part II

THE CROSSETT EXPERIMENTAL FOREST'S CONTRIBUTIONS TO SOUTHERN PINE IMPROVEMENT PROGRAMS

Don C. Bragg[1], Jess Riddle[2], Joshua Adams[3], and James M. Guldin[4]

Abstract--Long renowned for its contributions to silvicultural practices in naturally regenerated loblolly (*Pinus taeda*) and shortleaf (*Pinus echinata*) pine, the Crossett Experimental Forest (CEF) has also played an important, if much less well known, role in southern pine tree improvement. A decades-long program centered at Crossett started in 1951. Roland E. Schoenike was hired in 1952 to run the CEF's tree improvement program, then largely "exploratory" in nature. A combined soils and genetics laboratory was constructed on the CEF in 1954, and following Schoenike's departure in 1956, plant geneticist Hoy C. Grigsby was hired from the Mississippi Forestry Commission as the scientist in charge of the program. In the late 1960s, Grigsby installed part of a full- and half-sibling plus tree loblolly pine progeny test on the CEF compared to "woods-run" materials collected from the experimental forest. However, Grigsby would not see this study to completion; after the CEF was shut in 1974 he was transferred to Pineville, Louisiana. Although long closed, portions of the CEF program still offer current and future research opportunities. For example, some plus tree progeny tests remain, and at 46-49 years post-establishment, these tests have unique opportunities to reevaluate growth, bole quality, and other performance measures for known families, as well as the promise of new studies related to genetics, tree defense strategies, carbon allocation, and bole/crown dynamics.

INTRODUCTION

The Crossett Experimental Forest (CEF) was established in 1934 to help refine silvicultural practices in the naturally regenerated loblolly (*Pinus taeda*) and shortleaf (*Pinus echinata*) pine-dominated forests of the Upper West Gulf Coastal Plain (Reynolds 1980). Founding project leader Russell R. Reynolds had a knack for uneven-aged silviculture and quickly developed a successful research and demonstration program over most of the original 680 ha of the CEF (Reynolds 1959). Over the years, other researchers have continued this uneven-aged work while studying many other aspects of these pine forests, including even-aged silvicultural practices, regeneration ecology, competition control, growth and yield, and unmanaged stand dynamics. The CEF's research program has yielded hundreds of publications and many thousands of foresters, students, landowners, and policy makers have toured the facility. Much less well-known is the once very active forest genetics/tree improvement (FG/TI, which also included a significant nursery component) program on the CEF that operated for over 20

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years. When established, the CEF's FG/TI effort was a part of the USDA Forest Service's (USFS's) multi-pronged effort in this discipline, and its waning mirrored trends seen elsewhere in federal FG/TI (Wheeler et al. in press). Today, vestiges of the CEF program remain and offer the promise of new opportunities to learn from work established nearly 50 years ago. This paper provides a brief summary of the CEF FG/TI program, including some current and future plans for research based on the surviving plus tree progeny tests.

THE CEF FG/TI PROGRAM

Reynolds and Wakeley Set the Stage

As successful as the uneven-aged silviculture work on the CEF proved to be, Reynolds was criticized for not including even-aged management as one of his comparisons. Tree improvement pioneer and fellow USFS scientist Philip Wakeley was one of the most vocal critics, pulling no punches in his evaluation (Wakeley and Barnett 2011, p. 57):

> The [CEF] was organized in heyday of "selective cutting"…The Forest was deliberately and avowedly set up to "demonstrate" the virtues of such selective cutting…this unabashed move to "demonstrate" the worth of a current fad and particularly the failure to match many-aged management with the most obvious check, namely, even-aged management, seemed to me a regression…Despite what I consider its long run with only half its cylinders firing—and the poorer half at that!—there is no denying the immense impetus that the Crossett Experimental Forest, under Russ Reynolds' direction, has given both to the Station and to technical forestry throughout the South. The data it has yielded on many-aged management, even without the obvious check, are uniquely valuable.

In correspondence not reprinted in Wakeley and Barnett (2011), Reynolds strenuously objected to Wakeley's characterization of the CEF not having even-aged checks, citing a methods-of-cutting study on 43 ha installed between 1937 and 1942—a move promptly dismissed by Wakeley as inadequate.

Wakeley's criticism notwithstanding, Reynolds did not avoid even-aged research and demonstration—in addition to the aforementioned methods-of-cutting study, one of the first demonstrations on the CEF included an arboretum, and woods-run seeds were provided in 1935 for outplanting studies across the world (e.g., Wakeley 1951). By the early 1940s, new studies that incorporated even-aged, naturally regenerated pine forests began on the CEF. To do this, in March of 1942 the CEF was expanded (to 1,408 ha) with the addition of adjoining land leased from the Crossett Lumber Company (Harris 1945, Reynolds 1980).

The momentum for FG/TI in southern pines was irresistible. Wakeley and other like-minded individuals recognized the potential to improve upon southern pine growth and yield, insect and disease resistance, and wood quality. The greater degree of control under FG/TI was notably more promising than that from the preferential selection of trees during the harvest process or the reliance on poorly provenanced woods-run seedlings to establish plantations (e.g., Wakeley 1951, Haig 1951, Dorman 1955). In the early 1950s, the Southern Forest Experiment Station (SOFES) of the USFS greatly expanded its FG/TI program by establishing a number of formal research projects at experimental forests and in 1954 the SOFES opened the Southern Institute of Forest Genetics to coordinate these efforts.

Figure 1. One of the better performing longleaf (*Pinus palustris*) x slash (*Pinus elliottii*) pine hybrids produced at the CEF. These seedlings started height growth the first year (no apparent grass stage) and reached 2.74 m tall in four years. USFS photograph, circa early 1960s.

The Schoenike Years

According to Grigsby (1969), the CEF FG/TI program began in 1951—however, this early start was probably limited to the contribution of shortleaf pine seed and provenance plantings for the Southwide Seed Source Study (see Wells and Wakeley 1970). A more proper beginning of the CEF FG/TI program was in 1952, when Roland E. Schoenike was hired to lead this effort (USFS SOFES 1953, Anonymous 1963). Much of the CEF FG/TI work was done in collaboration with the Crossett Company (and eventually Georgia-Pacific), who provided research funding, plant materials, and field logistical support over the years (USFS SOFES 1953, Anonymous 1963). During these early years, CEF's program was exploratory in nature, including local pine seed collection, southern pine hybridization, nursery and propagation techniques, exotic species tests, and the beginnings of loblolly and shortleaf pine plus tree selection, testing, and improvement (USFS SOFES 1955, Wakeley 1955). This period also witnessed considerable investment in infrastructure on the CEF, including the construction of a combined soils and genetics laboratory building (completed in 1954 for $3,338.63; a greenhouse addition was built in 1958 for an additional $6,145.04), planting beds and associated equipment, and the hiring of support staff.

FG/TI work on the CEF was as prominently featured in most of the SOFES annual reports from this period as the better-known uneven-aged silvicultural research (e.g., USFS SOFES 1955, 1956, 1957, 1958). However, although useful for program establishment and other logistical purposes, the early CEF FG/TI studies were rarely published outside of station study plans and annual reports. To date, only two formal publications relating to Schoenike's work on the CEF have been found: both were brief (1 page) notes; one on weather factors leading to the demise of loblolly and shortleaf pine seed crops in 1955 (Schoenike 1955) and the other on using plastic tubes for the controlled pollination of pines (Schoenike 1956). The epic droughts and other weather-related challenges of the early- and mid-1950s also sorely tested many FG/TI projects across the region, and those on the CEF were no exception—for example, widespread drought-related mortality of progeny test seedlings at the CEF occurred in 1954 (USFS SOFES 1955). A later unpublished closing report (Nance 1978, p. 2) criticized many of the earliest CEF studies, calling them "…generally poorly designed, [of] limited objectives, and in general contribut[ing] little toward the long-term goals of the project." However, not all of these early studies were destined for obscurity—for instance, a comparison of woods-run loblolly pine seedlings from the Crossett area were planted across much of the region to compare with local-origin stock produced multiple publications (Sihvonen 1955, Grigsby 1955, 1975, USFS SOFES 1958). Pine hybridization was also an active part of the CEF program—Schoenike was credited by Wakeley as being one of the first (in 1954) persons to successfully produce Sonderegger pine (*Pinus* x *sondereggeri*) using controlled pollination (Wakeley and Barnett 2011). Hybrid crosses (Figure 1) between different species were sought to improve southern pine resistance to ice damage, insect and disease resistance, high growth rates, and good wood quality (Grigsby 1959).

The Grigsby Era

Schoenike left the CEF in 1956 to start a doctoral program in FG/TI at the University of Minnesota. Later that year, plant geneticist Hoy C. Grigsby was hired to replace Schoenike as the scientist in charge of the program. Grigsby was familiar with the CEF, having aided Schoenike in the establishment of the Arkansas loblolly pine seed source study across Mississippi when he was employed by the Mississippi Forestry Commission (Grigsby 1955). Grigsby was involved in many different aspects of FG/TI research, including some projects associated with nursery practices. An unpublished 1959 summary of the CEF's research program listed 29 active, numbered studies under the "Forest Genetics" category, all assigned to Grigsby (Table 1). While many of these used loblolly and shortleaf pine from local (Crossett area) sources, southern pines from other regions were often tested, as were exotic conifer species. Some of these studies originated with Schoenike, but Grigsby also established quite a number of projects himself. During his tenure, the CEF-based tree improvement and genetics research ranged from nursery practices and seed quality to experimentation with mutagens (both chemical- and radiation-based) and tests of "unique" parents (e.g., "bull" pines, figured wood).

The most prominent CEF FG/TI project related to plus tree selection, breeding, and progeny testing (Figure 2), most of which were summarized in an establishment and progress report written in the late 1960s (Grigsby 1969). These studies ranged considerably in their objectives, but one of the largest was installed between 1966 and 1969 and involved planting the progeny of controlled plus tree crosses in Compartment 3 of the CEF, as well as other blocks placed elsewhere (we will cover this project more in later).

One of the more unusual projects CEF scientists contributed to involved the irradiation of pine seed to see how different levels of exposure affected pine germination, seedling survival, and growth performance. Initiated by Schoenike and Wakeley in 1954, loblolly pine seed was exposed to different dosages of x-rays and then planted at both Crossett and the Harrison Experimental Forest in southern Mississippi. Grigsby contributed to the analysis and eventual publication of the results, which found decreasing survival of pine seed with increasing levels of radiation but few other significant influences (Snyder et al. 1961). In addition to its uniqueness, this work also highlighted some of the challenges the FG/TI had at the CEF. Because of space and site condition limitations, it was not unusual for some of the FG/TI research directed by CEF staff to be installed elsewhere.

Table 1. Genetics studies listed in unpublished 1959 summary of the CEF's research program, all of which were assigned to Hoy Grigsby.[1]

USFS study number	Study title	Notes
CR-11.01	Geographic seed source	Shortleaf pine seed sources for Southwide Seed Source Study
CR-11.02	Forest nursery	
CR-11.03	Plus tree selection	For "outstanding" loblolly pine in the Crossett area
CR-11.04	Branch habit selection	Loblolly pine
CR-11.05	Seed grading selection	Loblolly pine
CR-11.06	Nursery stock selection	
CR-11.07	Hybridization and controlled breeding	From pine hybrids produced at CEF, Gulfport, and Alexandria
CR-11.08	Exotics and other non-natives	Multiple conifer species
CR-11.09	Local test of Crossett loblolly pine seed	
CR-11.12	X-ray irradiation for cytogenetic effects	Loblolly pine
CR-11.13	Morphological seedling types	Loblolly pine
CR-11.14	Nursery stock test	From outside nurseries in southern Arkansas and northern Louisiana
CR-11.15	Shortleaf pine seed grading	Tested seed size
CR-11.16	Loblolly pine seed grading	Tested seed size
CR-11.17	Controlled breeding—1953	Seedlings produced from 1953 breeding program
CR-11.18	Controlled breeding—1954 breeding program	Seedlings produced from 1954
CR-11.19	Controlled breeding—1955 breeding program	Seedlings produced from 1955
CR-11.20	Controlled breeding—1956	Seedlings produced from 1956 breeding program
CR-11.21	Seed source study	Used loblolly pine seed from Ozan Lumber Company
CR-11.22	Missouri seed source study	Loblolly pine for southern Missouri
CR-11.23	Ultrasonic treatment for cytogenetic tests	Loblolly pine
CR-11.24	Gamma irradiation treatments for cytogenetic effects	Loblolly pine
CR-11.25	Phenotypic variation in open pollinated	Loblolly pine
CR-11.26	Colchicine experiments for cytogenetic effects	Loblolly pine
CR-11.27	"Bull" pine progeny tests	

CR-11.28	Exotic trials in southwest Arkansas	Various exotic conifers
CR-11.29	Loblolly pine seed source	At Prescott, AR (36 seed sources)
CR-11.30	Performance and progeny test for seed lots from Arkansas, Louisiana, and Mississippi	Loblolly pine
CR-11.32	Chemical effects on rooting	Loblolly pine cuttings

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[1] Document on file with the senior author.

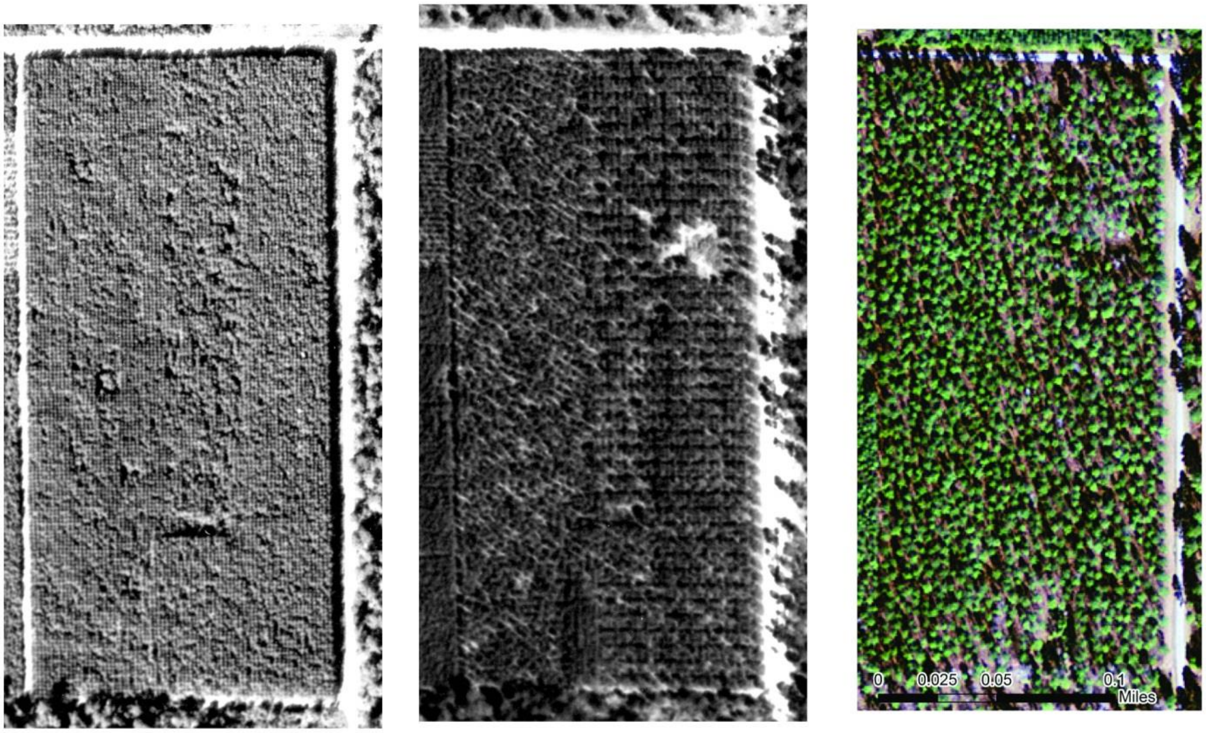
Schoenike and Grigsby established a number of their progeny tests and other studies on nearby industry lands, as well as on other experimental areas in Arkansas, Louisiana, Mississippi, and other locations across the southeastern US. For example, Grigsby installed some of the replicates of a study on the impacts of tip moth (*Rhyacionia* spp.) on loblolly, shortleaf, and hybrid pines at a site near Many, Louisiana, to complement the treatments installed on the CEF (USFS SOFES 1960).

The End of an Era

It is worthy of noting that Wakeley, even after his sharp critique of the selective cutting focus of the early years of the CEF, later saw fit to praise its FG/TI program: "The Crossett Project's contributions to forest tree improvement are not to be lightly brushed aside and will grow in value as the trees in its hybrid-, progeny-, and provenance-test plantations grow tall." (Wakeley and Barnett 2011, p. 57). Wakeley had penned this statement in 1964 during what probably could have been considered the zenith of the CEF tree improvement program. Obviously, at this time he could not have anticipated the closing of the CEF in a decade, followed by the termination of its FG/TI work.

Figure 2. Hoy Grigsby scaling one of the plus tree loblolly pines on the CEF. Trees with superior form, growth, and disease resistance formed the basis for many of the progeny tests conducted by the CEF staff during the 1950s and 1960s. USFS photograph, circa early 1960s.

The studies established in the mid- to late-1950s were closed by early 1969 (Grigsby 1969). The CEF was shuttered by the SOFES in 1974, with all remaining staff moved to other duty stations; Grigsby was reassigned to Pineville, Louisiana. Some of the FG/TI studies were continued for years afterwards by other US Forest Service scientists, Georgia-Pacific, the University of Arkansas-Monticello (UAM), and other collaborators. Plant geneticist Warren L. Nance assumed the responsibilities for any remaining CEF tree improvement studies in 1974 (Nance



1980 1985 2006

Figure 3. Aerial photographs of Compartment 3 on the CEF from 1980, 1985, and 2006, showing the unthinned (1980) and thinned (1985 and 2006) plus tree progeny tests. USFS photographs.

1978). After 1974, some of the outlying CEF-related plantings were measured by others—for instance, Professor Elwood Shade measured a number of the progeny tests established on the UAM POW Camp, and staff of Georgia-Pacific and other cooperators followed other outplantings during this period.

When the CEF reopened in 1979, its mission then focused on the development of low-cost silvicultural techniques for small forest landowners. Thinnings had been designed into the original study plans and were to have occurred after 10 growing seasons (Grigsby 1969). However, this would have meant thinnings were to have been done between 1976 and 1979, when the CEF was closed. An aerial photograph of the CEF from 1980 shows that Compartment 3 had not received any thinnings, however, the aerial photograph taken a few years later was obviously thinned (Figure 3). In late 1985, the plus tree progeny was thinned, followed by operational thins in 1996 and 2002.

CURRENT AND FUTURE DIRECTIONS

Although the formal CEF FG/TI program has long been closed, vestiges remain that present current and future research opportunities. For example, the plus tree progeny tests of 1966-1969 are largely intact, and at 46-49 years post-establishment offer unique opportunities to reevaluate growth, bole quality, and other performance measures for known families. However, we have found the records on this work fragmentary and hard to reconstruct, especially since the fate of the plus tree progeny tests on Compartment 3 became murkier following Nance's (1978) internal closing report. The temporary CEF closing and then years of shuffling files around the agency further disrupted the documentation of this plus tree study. Regrettably, very few of the principles in these studies remain to consult, as all have since retired or died—Reynolds passed away in 1986, Schoenike in 1988, and Grigsby in 2009. Furthermore, some of the original installments have been cut, burned, blown over, or even bulldozed—parts of the plus tree pine progeny tests were lost as recently as 2014.

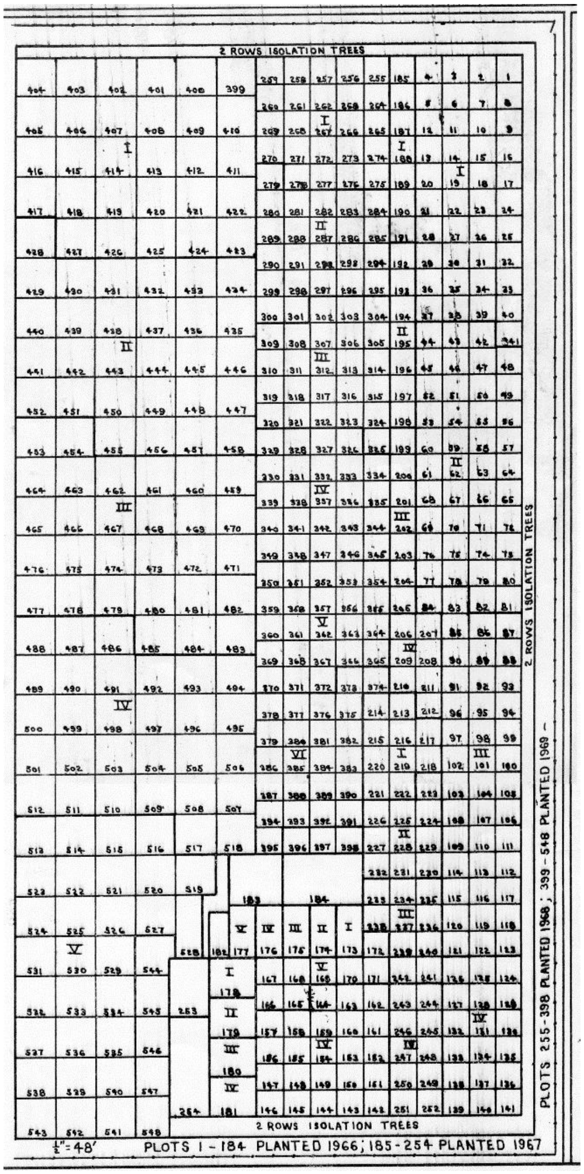


Figure 4. Plus tree deployment map for CEF Compartment 3 progeny tests, circa 1970. This map will help researchers reconstruct the family structure of this planted loblolly pine stand, allowing for new and expanded analysis of this now nearly 50-year-old progeny test.

These challenges notwithstanding, we are optimistic that interesting insights can be gained from the pieces that remain. For example, we recently started reestablishing the original layout of the plus tree pine progeny tests in Compartment 3. From our preliminary assessment, most of the treatment replicates from Grigsby's study survive. Our first step was to gather the remaining study documentation, in which the deployment maps were found (Figure 4). We then used GPS and a laser distance measuring device to produce a stem map that we could overlay a digitized representation of the deployment map to identify the family of each remaining pine. The process will need to be improved, as the horizontal accuracy of the stem map (currently about ± 2 m) is insufficient to definitely assign the correct family for specific trees. To date, we have found a number of the plot corners in Compartment 3 using a metal detector that are probably still in place (tags on wooden posts were used, and the posts have long since decayed away). If this approach works in Compartment 3, a number of other progeny tests remain at least partially intact in CEF Compartments 36 and 46, and some may survive at UAM's POW Camp.

Once the plus tree progeny tests are accurately mapped, we plan to recover the original data collected when these trees were young. This

information was not published when completed in the 1970s, and when combined with diameters and other measurements collected in 2014, we think some interesting lessons could still be learned. For instance, would the best performers indicated by early height growth trends measured in the 1970s still be the best performers decades later? What progeny have shown the best growth performance? Survival? Vulnerability to insects or disease?
We also believe that the plus tree pine progeny tests in Compartment 3 (and elsewhere) offer promise for future studies related to genetics, tree defense strategies, wood properties, reproductive potential, carbon allocation patterns, and bole/crown dynamics, amongst others. As an example, which progeny will do best under future climate scenarios? Having a large number of full- and half-sib families in a concentrated area under relatively similar environmental conditions and consistent management histories can provide a degree of experimental control rarely available.

CONCLUSIONS

With the prominence of the CEF's research programs in naturally regenerated pine forests, few are aware of the substantial, decades-long FG/TI program once centered at Crossett. Undoubtedly, part of this is because the lead investigators of this program did not publish much of their research, especially in the peer-reviewed literature. Using several online search engines (e.g., Google Scholar, Digitop Navigator, ProQuest Natural Science Collection, EBSCOHost Environment Complete) and some forestry publications and reports (e.g., Dorman 1976), only 20 papers were found that had been published by Schoenike, Grigsby, Grano, and their other collaborators on CEF-related tree improvement work (including nursery practices, genetics, etc.) between 1955 and 1978. Many of these papers were in the "gray" literature (e.g., conference proceedings, Forest Service publications) and are hard to access. However, we should have enough information to add considerably to our knowledge of southern pine silviculture using the surviving examples and the past data of the CEF's FG/TI program.

ACKNOWLEDGMENTS

We would like to recognize the efforts of many people over the years to establish and maintain the CEF FG/TI program, including Russ Reynolds, Phil Wakeley, Roland Schoenike, Charles Grano, Hoy Grigsby, and all the other support staff who shepherded the projects over time. Regrettably, most of these people are now deceased, so we can't thank them in person.

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CORRELATIONS BETWEEN TRAITS IN LOBLOLLY PINE,INCLUDING INTERACTIONS WITH MYCORRHIZAL FUNGI

Bridget J. Piculell[1], C. Dana Nelson[2], James Roberds[1], and Jason D. Hoeksema[1]

Genetic correlations between traits may influence how species respond to both natural and artificial selection. Loblolly pine (*Pinus taeda*) is important both as an economic crop and as a wide-ranging dominant species throughout the southeastern United States. It is subject to natural selection from both biotic and abiotic sources, as well as artificial selection for favorable economic traits (such as disease resistance). Mycorrhizal fungi are common symbionts of most plants, including pines, deriving mineral nutrients from the soil and transferring them to the host, while the host provides carbohydrates to the fungi. Mycorrhizal fungi have also been shown to affect essential host traits such as drought and disease tolerance. Loblolly pine interacts with a diverse guild of ectomycorrhizal fungi, with host plants capable of simultaneous interaction with several fungal partners, each of which may play an important role in nutrient acquisition and soil health. We investigated the underlying genetic structure of multiple traits in loblolly pine. Specifically, we measured traits of 140 loblolly genotypes (each clonally triplicated) in a pedigree population to determine i) the narrow-sense heritability of traits, and ii) the degree to which these traits are genetically correlated with one another and with single-nucleotide polymorphisms (SNPs) in loblolly candidate genes.

In this field study, we found significant narrow-sense heritability for both above- and below-ground pine traits, including susceptibility to the disease-causing fusiform rust (*Cronartium fusiforme*), resistance to attack by tip moth (*Rhyacionia* sp.), compatibility in forming mycorrhizae with particular fungal species, and overall mycorrhizal fungal diversity. We also found significant additive genetic correlations between pairs of these traits, and associations between several of these traits and SNPs located in loblolly candidate genes. These results shed light on the underlying genetic structure of coevolving traits and how it may affect the evolution and adaptation of species. In addition, understanding the genomic architecture of how important adaptive traits are related to one another is an essential component of successful breeding and management strategies. Breeding practices that focus on one or a few traits, while neglecting other important traits may be inadvertently selecting for suboptimal genotypes, especially if variation in local planting site conditions are not considered.

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TRENDS IN GERMPLASM DEPLOYMENT OF LOBLOLLY PINE IN THE SOUTHERN US

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Deployment practices have changed dramatically for southern pine as breeding programs have intensified and better germplasm has become available. As part of the Pine Integrated Network: Education, Mitigation, and Adaptation project (PINEMAP) sponsored by a USDA National Institute of Food and Agriculture grant, we surveyed seedling vendors who are members of the three pine tree improvement cooperatives in the South to quantify how many seedlings of various types are being planted and determine any evidence of risks associated with planting relatively more genetically homogeneous seedlots such as specific full-sibling families or clonal varieties.

In the 2011, 2012, and 2013 planting seasons, 31 cooperative members grew an average of 843.5 million tree seedlings per year. This is 37% fewer than the 1.35 billion seedlings grown yearly during the previous survey period of 2000-2002. Of these 843.5 million seedlings, 87.1% (734.6 MM) were loblolly pine, 6.1% (51.1 MM) slash pine, 5.7% (48.5 MM) longleaf, and 1.1% other conifers and hardwoods.

In the last 10 years, the most dramatic change for loblolly pine is that 95% of plantations are now being established as genetically more homogeneous stands to capture greater gains in yield and value from improved genetics. In the previous survey, 59% of loblolly stands were planted with open-pollinated (OP) families and the rest with mixtures of seedlings from different mother trees. Today, the vast majority of stands (85%) are planted with OP families. About 8% of stands are planted with full-sib families and about 2% with clones. Landowners can now choose families that best match their management goals.

To determine risks associated with establishing plantations with relatively more homogeneous germplasm, we asked respondents if they were aware of any unexpected environmental or pest problems (e.g. diseases, insects, cold, or storm damage) encountered with family block plantings. One of the 33 respondents experienced freeze damage with one southern coastal family planted in northern environments that were colder than recommended, and one family consistently showed about 10% lower survival compared to others. No respondents were aware of any outright plantation failures due to the use of family blocks.

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USDA'S ROADMAP FOR PLANT BREEDING

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Plant breeding is critical for sustainable plant production for security, health, and quality of life. Contemporary attention to plant breeding is increasing for a number of reasons, including the importance of plant breeding for adapting to future circumstances; the development of a range of new enabling tools for plant breeding; and the value of plant breeding across scales and contexts, including both organic and conventional agriculture. In response, in 2013, USDA's Chief Scientist held a public Listening Session on Plant Breeding. Subsequently, USDA has developed a Plant Breeding Roadmap to align its forward vision for plant breeding with USDA's strategic goals. Both documents are now posted on the web page of the Office of the Chief Scientist (OCS) at http://www.usda.gov/wps/portal/usda/usdahome?navid=OCS. Stakeholder comments to USDA included the value of USDA's work in genetic resources-- including conservation, characterization, distribution, and pre-breeding; the importance of the recruitment and education of future plant breeders in active breeding programs; and the value of the federal-state partnership and associated grower-groups partnerships. Additional needs expressed for USDA's work in plant breeding included public-sector plant varieties (cultivars) when /as needed for meeting national goals; translational work in the incorporation of biological research results in new breeding methods, tools and approaches, to address ever-more complex goals; and extramural funding programs both appropriate and adequate for long-term health of the robust federal/state model for public plant breeding in the United States. Some of the issues raised by stakeholders are broader than any single entity, including recruitment of young people; optimal understanding and use of intellectual property rights and tech transfer mechanisms; and fostering optimal public/private investment balance to ensure that all critical needs are addressed. This talk will invite questions and discussion regarding the USDA Plant Breeding Roadmap and possible next steps.

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Hardwoods

APPLICATION OF HYBRID BREEDING AND SOMATIC EMBRYOGENESIS TO DEVELOP SWEETGUM VARIETIES FOR THE BIOENERGY AND PULP AND PAPER INDUSTRIES

Scott A. Merkle[1] and Michael Cunningham[2]

The application of hybrid breeding to forest trees has resulted in some very useful and productive genotypes for some forest crops. Notable successes include hybrid poplars (e.g. *Populus trichocarpa* x *Populus deltoides*), hybrid Eucalyptus (e.g. *E. urograndis*), and hybrid pines (e.g. *Pinus rigida* x *Pinus taeda*). However, hybrid breeding has not been a widely used tool in U.S. plantation forestry in the southeastern U.S. Similarly, somatic embryogenesis, which at one time appeared to have great potential for forestry applications, in particular for clonal propagation of elite southern pine genotypes, has yet to attain a significant percentage of southern pine annual regeneration. Combining these two technologies, however, creates a very powerful approach that appears to have created its first product with real economic potential in the southeastern U.S.—in a hardwood tree, hybrid sweetgum. The timing of the appearance of this new hardwood feedstock coincides with an unusual convergence of pine and hardwood pulpwood prices in the region, where prices for hardwood have traditionally trailed those for pine. Trends indicate that this change is due to either rising demand for hardwood, decreasing supply of hardwood or both. In any event, the case for purpose-grown hardwoods in the southeast is becoming stronger, in particular in areas where a reliable supply of hardwood fiber is needed year-round, since purpose-grown trees have the ability to stabilize wood costs and provide predictable price caps for mills. Sweetgum (*Liquidambar styraciflua*) plantations, first established in the southern U.S. in the 1960s, experienced some limitations that have largely been overcome with the development of improved planting stock (Wright and Cunningham 2008). Even more dramatic improvements in performance may be possible by hybridizing *L. styraciflua* with, *L. formosana*, which is native to China and Formosa. Even though the two species have been separated by continental drift for 10 million years they are still inter-fertile. Santamour (1972) reported the first interspecific *Liquidambar* hybrids.

To generate hybrid sweetgum varieties, in 1999, pollen was collected from three mature *L. formosana* trees growing in a U.S. Forest Service demonstration planting near Saucier, MS and used by International Paper Co. breeders to conduct controlled pollinations with three *L. styraciflua* selections from the N.C. State Hardwood Program at IP's Southlands Experiment Forest near Bainbridge, GA, for a total of nine crosses. Immature fruit were collected from the *L. styraciflua* mother trees in June and July and shipped to the Merkle lab at UGA, where the immature seeds were used for culture initiation. Briefly, immature fruits were surface-disinfested using a sequence that included Clorox, then the fruits were dissected and immature seeds removed, nicked with a scalpel blade and cultured on a semi-solid induction-maintenance medium (IMM) that included 2 mg/L 2,4-D (Vendrame et al. 2001). A second round of hybrid

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breeding and culturing was conducted in 2005. Seed explants were transferred to fresh medium after one month.

Approximately 2% of the cultured seeds produced proembryogenic masses (PEMs) within 2 months following culture initiation. PEMs could be maintained by monthly transfer to fresh medium and grew more rapidly once inoculated into liquid IMM to produce embryogenic suspension cultures. Suspension cultures were size-fractionated on stainless steel sieves, and a selected size fraction between 38 µm and 140 µm collected on filter paper using a Büchner funnel produced up to 6000 synchronously developing somatic embryos per 0.5 g of PEMs within 2 months following plating on semi-solid basal medium, which was the same as IMM but lacking 2,4-D (Fig. 1A; Dai et al. 2004). Eighty to 90 percent conversion of the somatic embryos to somatic seedlings could be obtained following an 8-week pre-germination cold treatment at 10º C (Merkle et al. 2010). Recently, further improvements in PEM production were obtained using air-lift-bioreactors and early growth of somatic seedlings was accelerated using RITA® temporary-immersion bioreactors (Lu and Merkle, in preparation). Several hundred hybrid sweetgum somatic seedlings, representing multiple varieties, were produced, transferred to potting mix and planted in field tests and demonstration plantings by IP and ArborGen collaborators over the past 10 years. These trees can be identified as hybrids by their leaf shape, which is intermediate between the five-lobed *L. styraciflua* leaf and the three-lobed *L. formosana* leaf. In addition, embryogenic cultures and trees derived from them were verified to be hybrids using RAPDs (Vendrame et al. 2001).

The hybrid somatic seedlings produced displayed a range of growth rates and habits in field tests on multiple sites. On the oldest planting, established in 2002 on IP property near Aiken, SC, a small number of the varieties showed faster growth rates than elite native sweetgum genotypes, as well as significantly higher wood specific gravity. For example, one variety in the test had a DBH of almost 11 in and a height of 69 ft at age 12, with a wood specific gravity of almost 0.55, for an estimated whole tree MAI of over 12 green tons/acre/yr, compared to an average DBH of 7.3 in, height of 59 ft and specific gravity of 0.45 for native American sweetgum seedlings. This variety and two others displaying similar performance were selected by ArborGen for commercial production. Other hybrid varieties showed potential for use as landscape trees and ornamentals, with dwarf phenotypes and striking fall color. The second round of breeding and culturing in 2005 resulted in the production of another eight hybrid varieties, which were planted in field tests at four locations in 2008. One of the tested varieties showed superior growth rates at all four sites and so was selected as a fourth commercial variety. Copies of the hybrid cultures had been cryostored following the protocol of Vendrame et al. (2001) with the intention of thawing and scaling up production from those cultures that produced the best trees, based on the results of the field tests. However, the desired varieties could not be successfully re-grown following recovery from cryostorage. Instead, a previously published method for initiating embryogenic cultures from sweetgum inflorescence tissues (Merkle and Battle 2000) was used to start new embryogenic cultures from the top hybrid varieties growing in the Aiken, SC field test, which had begun to produce flowers. Plantlets regenerated from these cultures were used to establish hedges for scaled-up production of rooted cuttings (Fig. 1B). In the past year, approximately 200,000 trees, representing four elite hybrid varieties, were produced by ArborGen and planted by landowners in four states, with the goal of providing a ready source of hardwood fiber for pulp mills in their areas. If the hybrid varieties continue to perform well, they may provide a new fiber crop for pulp and paper as well as biomass energy applications.

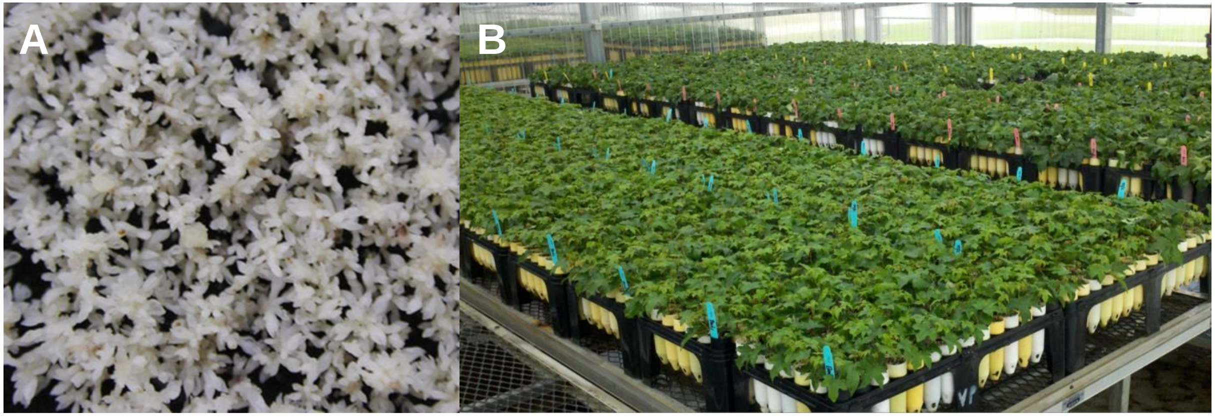

Fig. 1. Hybrid sweetgum varietal production. **A.** Embryogenic cultures produced thousands of hybrid sweetgum somatic embryos. **B.** Rooted cutting production in ArborGen greenhouse.

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EVALUATION OF EUCALYPTUS VARIETIES FOR COMMERCIAL APPLICATIONS IN THE SOUTHEASTERN UNITED STATES

Bijay Tamang[1], Victor Steel[1] and Mike Cunningham[1]

Eucalypts are preferred hardwood species for short rotation woody crop systems because of their faster growth and multiple end uses. They are also adapted to various edaphic conditions due to their diverse genetics. Selected *Eucalypts* have been recommended for commercial planting in the southeastern United States based on growth performance and tolerance to freezes. South Florida has received more attention lately due to the subtropical climate where the trees grow throughout the year maximizing productivity. Current commercial *Eucalyptus* plantings in south Florida are limited to *Eucalyptus grandis* and a hybrid (*E. grandis x urophylla*). Well managed *Eucalyptus* hybrid has productivity up to 32 green tons/acre/year at 7-year rotation. ArborGen has been testing 66 new *Eucalyptus* varieties in south Florida. The best Varietal had 19% and 36% height and diameter at breast height (DBH) gain, respectively, over the current commercial hybrid in a single tree test at age 1.5 years. The same Varietal had 22% and 27% height and DBH gain, respectively, in a block test at the same age. Three more block tests were planted in 2013. Combined best linear unbiased predictor (BLUP) analysis was done using the standardized height and the result suggested that the best Varietal had at least 27% height gain over the current commercial hybrid. Five new hybrid *Eucalyptus* Varietals were introduced for commercial applications in south Florida. Factors such as growth performance, resistance to blue gum chalcid (*Leptocybe invasa*) and non-native species planting permit requirement in the state were considered during selection.

Keywords: biomass, genetic improvement, hybrid eucalyptus, short rotation woody crops

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SWEETGUM: AN IDEAL FEEDSTOCK FOR A BIOCHEMICAL REFINERY PLATFORM IN THE SOUTHERN USA

Angele Djioleu[1], Elizabeth M. Martin[1], Matthew Pelkki[2], D. Julie Carrier[1]

Liquidambar styraciflua L., commonly called American sweetgum, is a natural occurring understory hardwood in US southern pine forests. As sweetgum is widely found in southern pine forests, it competes with pine for critical natural resources, It is estimated that the management of southern pine forest in view of eliminating competition can cost up to $150 millions per year to the timber and lumber industry. Therefore, as opposed to be a nuisance, sweetgum can be harvested and used as a feedstock for the production of fuels and chemicals in a biochemical refinery.

Wood from mature sweetgum trees, grown in Drew County Arkansas, was determined to contain 42% glucan and 20% xylan. These complex carbohydrates were converted into fermentable sugars, using dilute acid pretreatment and enzymatic hydrolysis. Results showed that this two-step hydrolysis process yielded 82% and 86% of available xylose and glucose, respectively. Overall, 72% of total fermentable sugars were successfully extracted from sweetgum wood. With the objective of increasing biorefinery revenues, hydrolysis of carbohydrates was also investigated in sweetgum bark. Although it was determined that 93% of xylose contained in sweetgum bark could be recovered, hydrolysis of glucan bark was more difficult. However, it was observed that bark contained extractable phytochemicals that displayed interesting biological activities. Using disc diffusion assays, sweetgum bark hot water extracts inhibited growth of Staphylococcus aureus and Listeria monocytogenes, with zones of inhibition up to 17 mm and 11 mm, respectively. Additionally, at a solid concentration of 16 g/L, the sweetgum hot water extract completely inhibited copper-induced oxidation of human low-density lipoproteins.

The above results indicate that sweetgum wood and bark could be good candidates as biochemical refinery feedstock, where wood would provide sugars that could be fermented into ethanol or other biobased compounds, while its bark could be a source for value-added compounds.

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Tree Improvement Part III

IMPROVING OUR UNDERSTANDING OF GROWTH DIFFERENCES OF PINUS TAEDA IN THE UNITED STATES AND BRAZIL:A COMMON GARDEN EXPERIMENT

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Evidence suggests that loblolly pine (*Pinus taeda* L.) grows much better in South America than it does in its native range in the southeastern United States. Reports of typical annual growth in Uruguay, Argentina and Southern Brazil range from 300 to 500 ft^3 ac^{-1} yr^{-1} while in the US comparable rates are less than 200 ft^3 ac^{-1} yr^{-1} (Cubbage et al. 2007). Interestingly, carrying capacity of exotic loblolly pine plantations (~275 to 400 ft^2 ac^{-1}) also exceeds that of native plantations (~200 ft^2 ac^{-1}). Our understanding of how intensive silvicultural practices including site selection, resource management, stocking and elite genetic material including clones influence potential productivity for loblolly pine has increased tremendously in the past 50 years. However, questions still remain as to why the growth and carrying capacity differences occur. We established three sites (Virginia: outside native range but in the southeast US, North Carolina: native range, and Brazil: southern hemisphere), where the same clones, mass control pollinated and open pollinated families were planted at three stocking levels (250, 500, 750 stems ac^{-1}) and two levels of resource availability (operational, and intensive for maximum growth). This study will allow us to examine growth differences in the US and Brazil without the confounding effects of different genetics. Trees grew up to two times faster in the southern hemisphere across all treatments. Varietal response to silviculture varied across site, and clone by silviculture interactions were observed. Across site, individual tree stem volume growth was linearly related to crown width, crown foliage mass and fine root length. Photosynthetic measurements indicate relatively minor differences across variety and site which do not explain the observed growth differences to date. The trees at all sites are currently about the same size (although the Brazil trees are two years younger) which will allow comparison of ecophysiological processes when all sites are at the same developmental stage. Consequently intensive measurement campaigns are planned for all sites in the coming year.

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BIOMASS AND ADAPTIVE RESPONSES OF FOUR PINUS SPECIES (P.STROBUS, P.RESINOSA, P.BANKSIANA, P.RIGIDA) TO A CO_2 X MOISTURE STESS FACTORIAL

John E. Major[1], Alex Mosseler[1], John Malcolm[1], and Moira Campbell[1]

Biomass, nutrient, and chlorophyll responses were quantified for 4 species of Pines: White pine (*Pinus strobus*), red pine (*P. resinosa*), Jack pine (*P. banksiana*), and Pitch pine (*P. rigida*) grown under CO2 x moisture stress factorial experiment after 3 years of treatment. The CO2 treatments were ambient and 2x elevated CO2 and the moisture stress treatments included irrigated and drought. The experiment was also grown with 4 spruce species; however, we will only present results comparing genus and within the pine species. Results often showed significant genus x CO2 interaction effect due to magnitude effects which resulted in pine having a better positive response than spruce. The genus x moisture stress interactions was often not significant. Height growth had a small CO2 effect, while biomass had a much larger CO2 effects. White pine often had the greatest nutrient concentrations while pitch pine had the lowest nutrient concentrations. There was more photosynthetic down regulation with spruces than pines (chloroplast, and carboxylation). It would appear that pines will do better in an elevated CO2 world than spruces.

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COMPARISON OF POLLINATION BAGS FOR MASS CONTROL CROSS SEEDS IN LOBLOLLY PINE

Austin Heine[1], Graham Ford[1], J.B. Jett[1], and Steve McKeand[1]

Over the past 10 years, deployment of full-sib families has gained prominence relative to traditional improved loblolly pine (*Pinus taeda* L.) seedling stock, such as open-pollinated families or seed orchard mixes. To produce control cross seed, a pollination bag must be used to isolate female strobili from outside pollen contamination, and a single, known pollen is applied at time of maximum female strobili receptivity. In the spring of 2014, the members and staff of the NCSU Cooperative Tree Improvement Program designed and installed a study to compare four pollination bag prototypes. Bags from PBS International were compared to the industry-standard Lawson pollination bag with and without a support wire. Open pollinated flowers were also added as control treatments to this study. The main objective of this study is to compare seed yields and seed efficiencies of cones produced from these bags to determine the optimal bag for maximizing production efficiency of control cross loblolly pine seed.

To quantify utility of each bag, total seed and seed efficiency per bag will be calculated after cone harvest in fall 2015. At this point in the study, we have assessments of female strobili survival at time of bag removal and following June drop. Based on these preliminary results, there was a highly significant difference between two of the PBS prototype bags and the industry-standard Lawson bags. PBS bags constructed of more rigid material and the Lawson bags with a support wire had the greatest survival of female strobili in June. If the preliminary conelet survival results hold true for seed yield and seed efficiency per bag, these better bags could produce about 25% more seed over the Lawson bags with no support wires. This 25% increase in seed production could also have a large economic impact on the mass production of control cross seeds.

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TOWARDS A FOREST HEALTH PARADIGM BASED ON HOST GENETICS AND PARTICIPATORY BREEDING

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Invasive pests and pathogens cause extensive damage to native forest trees and the ecosystems that depend on them and, given the continuing increase in globalization and mean global temperature, introductions of destructive invasive species are predicted to increase. To address these issues, the newly formed Forest Health Research and Education Center (FHC) at the University of Kentucky is focused on developing host resistance in forest trees and understanding the broader impacts of forest health issues on society. As is well known, the longstanding issues related to working on the genetics of forest trees presents a large challenge to breeding host resistance. However, they are no excuse to opt out and depend on shorter-term, less reliable or less environmentally friendly options. Instead, we argue that the long-term, proactive development of genetic, genomic and biotech resources in foundational forest tree species is critical to the implementation of host resistance when and if pest and pathogen problems arise. In addition, to develop these resources and implement these breeding programs over range-wide spatial- and multi-generational time-scales, participatory research networks will be required. We will discuss these concepts in relation to the ongoing work on various forest tree species by the FHC.

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Hardwood Genome

GENOME ORGANIZATION AND CYTO-MOLECULAR MAP OF CHESTNUT

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INTRODUCTION

The American chestnut (*Castanea dentata*, $2n = 2x = 24$), once a foundational forest species over 800,000 km^2 in eastern North America, was decimated by chestnut blight caused by an introduced fungal pathogen, *Cryphonectria parasitica*. The devastating disease was first reported in 1904 by Hermann Merkel, a forester at the New York Zoological Park (Murrill, 1906). Numerous measures were taken to control the pathogen, but these attempts failed as the disease spread rapidly, covering the entire species range by the late 1920s and killing nearly 4 billion trees by the 1950s (Hepting, 1974). Although now reduced to an understory species (Anagnostakis, 1987; Burnham, 1988), American chestnut persists through repeated, vigorous sprouting from its root collar, occasionally producing flowers and seeds before succumbing again to the blight (Paillet, 2002).

Chinese chestnut (*Castanea mollissima*), a species closely related to American chestnut, is relatively resistant to blight. Efforts are underway to transfer resistance from Chinese chestnut to American chestnut, including a backcross breeding program operated by the American Chestnut Foundation (TACF) (Hebard, 2006; *www.acf.org*) and a biotechnology-based program sponsored by the Forest Health Initiative (Thompson, 2012; Nelson et al., 2014; www.foresthealthinitiative.org). Recently an integrated genetic/physical map of Chinese chestnut was published (Kubisiak et al., 2013) and the species genome is being sequenced (Nelson et al., 2014); however, little cytogenetic data are available to confirm and correct these genomic resources. Fluorescence *in situ* hybridization (FISH) is an important cytogenetic technique for assigning and orienting genetic markers to specific chromosomes. Presently we are using as many as 16 genetically and physically mapped BAC clones, distributed across each linkage group, as FISH probes to develop a cyto-molecular map of chestnut.

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MATERIALS AND METHODS

Actively growing root tips were harvested from chestnut seedlings growing in potting soil and treated with an aqueous solution of α-monobromonaphthalene (0.8 % v/v) for 1.5 h at room temperature in the dark to accumulate metaphase stage cells, then fixed in 4:1 (95% ethanol : glacial acetic acid). Fixed root tips were treated with cell wall degrading enzymes in 0.01 M citrate buffer and the chromosome spreads were prepared as described previously (Sakaanokho and Islam-Faridi, 2013).

The 18S-28S rDNA of maize (Zimmer et al., 1988), 5S rDNA of sugar beet (Schmidt et al., 1994) and various BAC clones from Chinese chestnut (Fang et al., 2013) were used as probes. The BAC clones were selected from BAC contigs associated with genetically mapped markers near the ends of each linkage group (Kubisiak et al., 2013). In addition the BAC clones were selected to avoid repetitive regions of the genome, using information available from the physical mapping project (Fang et al., 2013). Probe DNAs were labeled with biotin-16-dUTP (Biotin Nick Translation Mix, Roche, USA) and/or digoxigenin-11-dUTP (Dig Nick Translation Mix, Roche, USA) following the manufacturer's instructions.

Fluorescent *in situ* hybridization (FISH) was performed as described previously (Islam-Faridi et al. 2009). Probe hybridization sites were detected with Cy3-conjugated streptavidin (Jackson ImmunoResearch Laboratories, USA) for biotin labeled probes and FITC-conjugated anti-digoxygenin (Roche, USA) for digoxigenin labeled probes. The FISH preparations were mounted with Vectashield containing DAPI (Vector Laboratories, USA) to prevent photo-bleaching the fluorochromes. Digital images were recorded using an epi-fluorescence microscope (AxioImager M2, Carl Zeiss, Germany) with suitable filter sets (Chroma Technology, USA) and a Cool Cube high performance CCD camera, and processed with ISIS V5.1 (MetaSystem Inc., USA) and Adobe Photoshop CS v8 (Adobe System, USA).

RESULTS AND DISCUSSION

The chestnut genome size is 780 Mb/1C, composed of 12 pairs of metacentric and near meta-centric chromosomes. We were able to obtain chromosome spreads that were free of cell walls, nuclear membranes and cytoplasmic debris, allowing for efficient probe hybridization.

The 18S-28S and 5S rDNA probes showed two 18S-28S rDNA (one major and the other minor) and one 5S rDNA sites. These three rDNA loci are located on three different homologous pairs of chromosomes in both Chinese chestnut and American chestnut. The positioning of ribosomal genes in Chinese chestnut reported here is in agreement with that in American chestnut reported previously (Islam-Faridi et al., 2009).

For FISH with BAC clones as probes, we used two BACs (one labeled with biotin and the other with digoxigenin) from either end of each linkage group to identify the corresponding chromosome and their physical positions and orientations within the chromosome. We followed those experiments by using four BAC clones (two from either end of a linkage group) to confirm that all four BACs hybridize to a specific chromosome. To date we have not observed any discrepancies between integrated physical/genetic map and cytogenetic data, i.e., BAC clones from an individual linkage group hybridized to a specific chromosome, and the physical positions of the BACs in chromosome spreads were relatively similar to their respective genetic map positions.

In a FISH experiment with four BAC clones (BB134N22, 1.3 cM; BB171MO4, 6.3 cM; BD176N08, 50.2 cM; and BB055C18, 57.9 cM) from linkage group H, hybridization signals identified a homologous pair of satellited chromosomes, suggesting that this linkage group contains the major 18S-28S rDNA locus. In another FISH experiment, two BAC clones from opposite ends of linkage group H (BB171M04, 6.3 cM and BB055C18, 57.9 cM) and 18S-28S rDNA were used as probes (Fig. 1). The results showed that the 18S-28S rDNA probe hybridized to the nucleolus organizer region (NOR) of the satellited chromosome, confirming that the major rDNA locus is located on linkage group H.

We are now in the process of locating as many as 16 genetically and physically mapped BAC clones to each linkage group to form the basis of a cyto-molecular map for chestnut. Such a map should help improve the understanding of the chestnut genome by serving as a reference karyotype for comparative genomic studies that will facilitate gene discovery and mapping, interspecies breeding and genetic engineering.

CONCLUSION

The major 18S-28S rDNA locus in chestnut is located on linkage group H. Chestnut BAC clones can be selected based on genetic and physical map positions and physical mapping characteristics that facilitate FISH. Using mapped BACs as FISH probes allows the integration of the genetic and physical maps with the karyotype and the production of a cyto-molecular map.

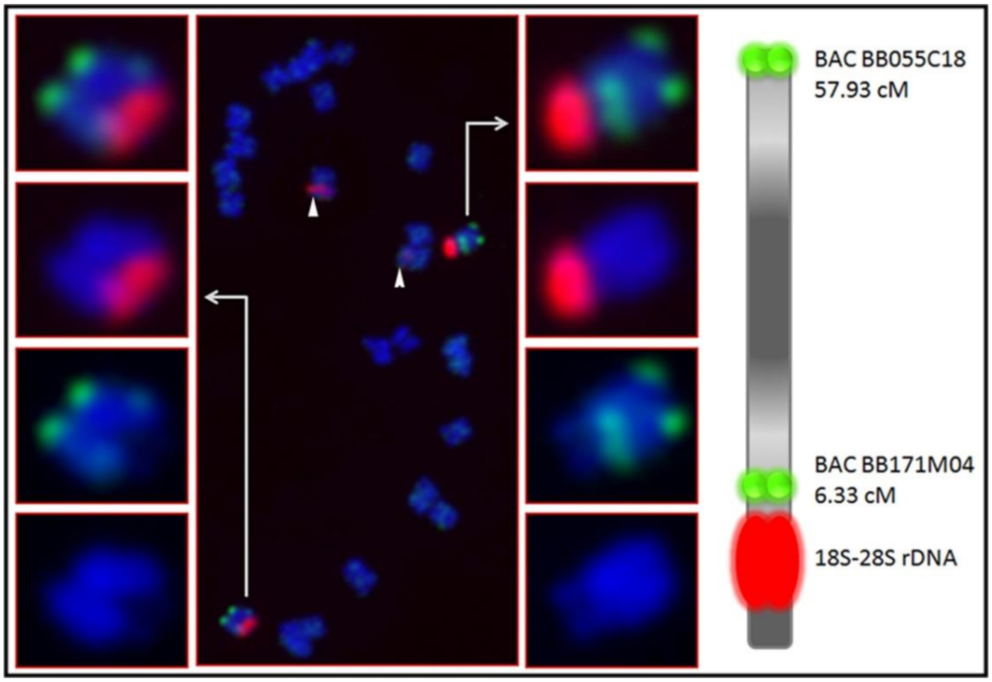

Figure 1. Fluorescent in situ hybridization of Chinese chestnut using BAC BB171M04 (green signal), BAC BB055C18 (green signal) and 18S-28S rDNA (red signals) as probes. The arrow heads show the minor 18S-28S rDNA signals. A schematic diagram of the satellited chromosome (i.e., linkage group H) is presented on the right hand side.

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MAPPING OF RESISTANCE TO PHYTOPHTORA CINNAMOMI (PC) IN INTERSPECIFIC AMERICAN/CHINESE AND AMERICAN/JAPANESE CHESTNUT POPULATIONS

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Root rot disease (*Phytophthora cinnamomi*) and chestnut blight (*Cryphonectria parasitica*) are the two most destructive diseases affecting American chestnut *Castanea dentata* (Marsh.) Borkh. Although American chestnut appears not to have any significant resistance to these pathogens, Chinese chestnut, (*C. mollissima)* and Japanese chestnut (*C. crenata)* do have natural resistance. The ability to cross these species with American chestnut provides an avenue to introgress the resistance from these Eastern Asian species into American chestnut. To support introgressive breeding of resistance into American chestnut from these sources, extensive genomic and genetic resources (EST database, physical and transcriptome-based genetic maps) were developed. Three QTLs for resistance to chestnut blight disease (CBD) were detected and delineated on a saturated Chinese chestnut genetic map composed of transcriptome-derived markers. Using a strategy based on sequence-informative markers and a comparative genomics approach with peach and other fully sequenced plant genomes, candidate genes were identified within these genetically marked QTL intervals. Currently these are being tested in transgenic chestnut trees.

For decades, progress in genetic analysis of resistance to *Phytophthora cinnamomi* (*Pc*) the causal pathogen for Phytophthora Root Rot (PRR) was hampered by the lack of a reliable phenotyping system that has been established only recently. Parental Chinese chestnut genotypes transferring *Pc* resistance to later generation Chinese/American hybrid chestnut progenies were selected and verified in small-size progeny tests. An initial genome-wide linkage map was constructed for the BC_1F_1 cross carrying resistance from the Chinese chestnut tree 'Nanking'. In this cross, a QTL was detected and mapped to linkage group E (LG-E). To obtain a more comprehensive understanding of the genetics of *Pc* resistance, we developed 7 additional interspecific hybrid crosses, 6 American/Chinese and 1 American/Japanese. These hybrid families in BC_1F_1, BC_3F_1 and BC_4F_1 configurations represent two Chinese ('Nanking' and 'Mahogany') and one Japanese ('Morrow Mountain') sources of *Pc* resistance. We phenotyped this hybrid material over multiple years and confirmed a QTL signal on LG-E by constructing local LG-E maps composed of transferable EST-SSRs markers from the reference Chinese

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chestnut genetic map. To further saturate these maps with markers and to search for other potential QTL signals, we are exploiting a single-family QTL mapping approach using genome-wide genotyping by sequencing (GBS) for a total of 1576 individuals from crosses phenotyped in 2011-2014. Our current progress in the genetics of *Pc*-resistance in chestnut is reported and discussed in this presentation.

Plenary Session Part II

TREE IMPROVEMENT IN THE SOUTH: THE STATE OF THE COOPERATIVE TREE IMPROVEMENT PROGRAMS

Tom D. Byram[1], S.E. McKeand[2], and G.F. Peter[3]

Tree improvement cooperatives focused on the southern pines have recently weathered significant changes in the structure of the forest industry, product demands, and land ownership patterns. The cooperatives have done this by streamlining their breeding programs and by broadening their appeal to include a variety of land owner objectives. In addition to the economic incentives that should continue to support investment in forest productivity, emerging issues such as increasingly unpredictable weather and the increased potential for sudden outbreaks of pests and pathogens that may disrupt forest health, make the continued support of forest genetics/tree improvement crucially important.

The demise of the vertically integrated forest industry and the emergence of forest land as an important investment vehicle have had both negative and positive consequences. The resulting fragmentation and volatility in land ownership has made it increasingly difficult to operate tree improvement programs and seed orchards targeted to specific landbases. On the other hand, the need to realize a financial return on land ownership has put a premium on maximizing growth rates to reduce the production cost of stumpage. Encouragingly, the market place is beginning to recognize the value of genetics in with faster growing seedlings demanding concomitantly higher prices and making it possible to monetize access to the performance databases. Creativity, however, will continue to be needed to provide sustained support for the traditional mainline breeding and testing programs. The authors discuss the changes experienced by the cooperatives in recent decades, give brief status updates of their current programs, and speculate as to how the tree improvement programs of the future will capture the value added by genetics and return it to those doing the breeding and testing.

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

Gary Peter[1], Tim Martin[1], R. Wynne, Heather Dinon, Corey Davis, Ryan Boyles and + 50 Co-PIs

The USDA NIFA-funded Pine Integrated Network: Education, Mitigation and Adaptation Project (PINEMAP) is building on the existing university-industry-agency cooperative research infrastructure and tech transfer networks 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. Integrated research aims to 1) increase carbon sequestration by southern pine forests, 2) foster a more robust and resilient forest-based economy in the southeast, 3) enhance connections between corporate and noncorporate landowners and climate experts and the capacity for regional interdisciplinary collaboration, 4) support public policies that promote sustainable management of planted pine under future climate scenarios, and 5) educate students and decision makers on the importance of climate, forest ecosystems, and forest management. To achieve these outcomes, PINEMAP researchers are conducting region wide analyses of carbon, nitrogen and growth dynamics in planted loblolly pine stands to determine probable changes in forest productivity and carbon sequestration with two future climate change scenarios. An important focus is a coordinated spatially explicit modeling program that is evaluating potential impacts of predicted changes in climate on net primary productivity, stand growth, timber supply and economics. A second important focus is development of the PINEMAP decision support system (DSS). The DSS is a map centric set of tools aimed to help professional foresters incorporate predicted changes in climate into their management decisions. One tool in the DSS is the seed deployment tool. 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 research cooperative network.

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Concluding Speaker

Barry Goldfarb

Tree improvement in a changing environment—Reflections of a pseudogeneticist

POSTER SESSION

A CLONAL PROPAGATION AND CRYOPRESERVATION SYSTEM FOR ATHANTIC WHITE CEDAR (*CHAMAECYPARIS THYOIDES*) VIA SOMATIC EMBRYOGENESIS

Changho Ahn[1], Ryan Tull[1], Paul M. Montello[1], and Scott A. Merkle[1]

Atlantic white cedar (AWC; *Chamaecyparis thyoides*) populations in the eastern U.S. have suffered dramatic declines due to over-harvesting, fire suppression, hydrologic alteration, and conversion of coastal bogs to agriculture and development. A clonal mass propagation system for the species could aid greatly with germplasm conservation and restoration efforts. With the goal of generating embryogenic cultures for the species, developing AWC seeds were collected periodically from the end of June to the end of July, 2014. Seeds were dissected and megagametophyte explants containing pre-cotyledonary embryos were cultured on a modified EM medium with 2,4-dichlorophenoxyacetic acid and 6-benzylaminopurine. While only a few explants produced embryogenic callus, we believe this was due to the fact that filled seed percentages were very low. Embryogenic tissues have been maintained and proliferated for more than 9 months on semi-solid medium. For long-term germplasm storage, we cryopreserved the embryogenic tissues using a standard protocol, and embryogenic tissues re-grew following 3 months of frozen storage. Basal medium type, abscisic acid (ABA) and activated charcoal were tested at different levels for their effects on somatic embryo development and maturation. EM medium with 2 g/L AC produced the highest number of mature embryos. Cotyledonary stage somatic embryos produced from these experiments and transferred to a modified EM germination medium without plant growth regulators germinated and have continued growth. We are also testing the effect of light quality on AWC somatic embryo germination and conversion, comparing the effects of LEDs of different wavelengths to fluorescent lights. This is first report describing an *in vitro* propagation and cryopreservation system for Atlantic white cedar via somatic embryogenesis.

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APPLICATION OF SOMATIC EMBROGENESIS FOR MASS PROPAGATION OF HYBRID HEMLOCKS

Changho Ahn[1], Lisheng Kong[1,2], Paul M. Montello[1], Ryan Tull[1] and Scott A. Merkle[1]

The eastern North American hemlock species, eastern hemlock (*Tsuga canadensis*) and Carolina hemlock (*Tsuga caroliniana*) were important components of eastern forest ecosystems, but over the past few decades, they have been devastated by hemlock woolly adelgid (HWA). Interest in hybridizing these hemlocks with Asian hemlock species, Chinese hemlock (*T. chinensis*), Northern Japanese hemlock (*T. diversifolia*), and Southern Japanese hemlock (*T. sieboldii*), has recently increased, due to the Asian species' resistance to HWA. Breeders have successfully crossed Carolina hemlock with each of the Asian species, but testing and eventual scaled-up production of promising hybrids could be greatly enhanced by combining hybrid breeding efforts with *in vitro* propagation and cryopreservation. In this study, embryogenic cultures of hybrid hemlocks were initiated by culturing immature zygotic embryos derived from interspecific crosses on a modified Litvay medium containing 2,4-dichlorophenoxyacetic acid and 6-benzylaminopurine. Abscisic acid (ABA) and activated carbon were tested at different levels in embryo development medium for their effects on somatic embryo development and maturation, and we found that 56 µM ABA and 2 g/L activated charcoal produced the highest numbers of cotyledonary-stage somatic embryos. Cotyledonary somatic embryos were given a pre-germination desiccation using a slow-drying process and then used in experiments to test the effect of light quality on somatic embryo germination and conversion, which employed LEDs of different wavelengths in comparison to the fluorescent light control. Preliminary results indicated that hemlock somatic embryos incubated under fluorescent light developed red hypocotyls, while hypocotyls of those incubated under red light supplied by LEDs remained green. Different light qualities also differentially affected other aspects of hemlock somatic embryo germination. We also demonstrated the potential to conserve germplasm of these hybrids via cryopreservation of the embryogenic cultures. This is first report describing an *in vitro* propagation and cryopreservation system for hybrid hemlocks via somatic embryogenesis.

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ALTERNATIVE APPROACHES TO BREEDING VALUE PREDICTIONS WITHIN FAMILY

Adam Festa[1] and Ross Whetten[1]

The goal of this study is to increase the selection intensity within loblolly pine breeding programs, by assessing the relationship between unique patterns of family gene expression and parental breeding values (BV). We hypothesize that selection intensity can be increased in pine breeding programs under two conditions -first, that there are genetic differences among families in gene regulatory networks, and second, that those differences are correlated with family mean performance in field tests of progeny. Currently, selections for advanced generations of loblolly pine are made on the basis of family mean phenotype, where phenotypically superior individuals are selected from top-performing families, and progeny tested to screen for those trees that have the best BVs. However, there is little confidence that phenotypically superior selections from a progeny test will carry forward the traits intended from the family, because many traits of interest to breeders have low individual-tree heritability.

In order to estimate the BV of a tree, a BLUP (Best linear unbiased predictor) analysis is conducted where phenotype and pedigree data are utilized to help define the genetic covariance among a set of families from a mating design. It is reasonable to suspect that variation in family mean phenotypes can be partially accounted for by differences in gene structure, and partially by gene regulation patterns. Sequencing DNA copies of messenger RNA is a means to collect information on both types of differences. Using covariance structures based on variation in gene structure or gene regulation in a BLUP analysis instead of, or in addition to, the standard numerator relationship matrix, may provide a higher prediction accuracy of BV's.

To test this hypothesis, we have chosen 43 different parents, from a wide geographic distribution, with pre-existing progeny phenotype data available from field tests across multiple sites. Seeds (open-pollinated or pollen-mix in 37 cases, controlled-cross in 6 cases) from each of these parents were grown in a greenhouse, and seedlings were harvested at 3 months for RNA extraction and sequencing, which is still underway. The RNA expression results from these families will be used to create covariance matrices reflecting shared genetic variation in coding sequences on one hand, and shared variation in gene regulatory networks on the other. Cross-validation of BLUP models using these covariance matrices, as well as a standard numerator relationship matrix, will be used to test the hypothesis that phenotypic variation can be accurately modeled by covariance of these classes of genetic variation. This analysis should provide insight into the value of using RNA expression patterns as another screening effort in selecting individuals as parents for future breeding populations.

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APPLICATION OF LASER CAPTURE MICRO-DISSECTION (LCM) MICROSCOPY IN FOREST TREE GENETICS

M.D. Islam[1], Claudia Casola[1], Nurul Faridi[1,2], Tom D. Byram[1,3] , C. Dana Nelson[4,5]

Laser Capture Microdissection (LCM) has been widely used in molecular biology since the early 1990s to study individual cells (Emmert-Buck et al., 1996). Until recently, LCM has not been widely applied to plants due to the complex plant cell wall structures (Kehr et al., 2001). However, LCM can be a very efficient tool in plants as well as animals for precisely harvesting targeted tissues, cells or chromosomes. When applied to loblolly pine (Pinus taeda. L), a very important and widely distributed forest tree in the southern United States, it may be possible to reduce genome complexity for further analysis such as genome sequencing. Loblolly pine has a haploid genome size of 23 Gbp (Neale et al, 2014) with a chromosome number of 2n = 2x = 24 (Islam-Faridi, 2007). Specific loblolly pine chromosomes (or portions of chromosomes) have been successfully captured with LCM and have yielded large amounts of DNA following PCR using whole genome amplification (WGA) methods. Fifteen copies of a targeted chromosome (~15 picograms of DNA) yielded 7 Âµg of DNA from PCR amplification (Islam et al, 2014 unpublished). DNA from such WGA can potentially be used for downstream analysis such as making chromosome-specific probes for fluorescence in situ hybridization (FISH) or for chromosome-by-chromosome genome sequencing. LCM promises to be a very powerful tool for studying conifer genomes. We will provide results and discussion of our recent experiments with LCM and WGA of pine chromosomes.

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PHYLOGENY OF MAJOR SOUTHERN PINES (SUBSECTION *AUSTRALES*, GENUS *PINUS*, FAMILY PINACEAE)

Tomasz E. Koralewski[1], Mariana Mateos[2], and Konstantin V. Krutovsky[1,3,4,5]

Southern pines are an evolutionarily young and closely related group. Their natural habitat stretches across southeastern states of the USA. Four of these species have been considered of major economic importance: shortleaf (*Pinus echinata* Mill.), slash (*P. elliottii* Engelm.), longleaf (*P. palustris* Mill.) and loblolly (*P. taeda* L.) pine. They are largely sympatric although recent glacial periods could have forced them into isolated refugia. Hybridization observed among these species in both natural and controlled environments further suggests tight links among them. The short time since speciation, sympatry, and demographic processes are likely primary causes of difficulties with systematic classification within this group in studies published to date. We investigated the phylogeny of the four southern pines using data from nuclear molecular markers. We utilized various phylogeny reconstruction techniques, including maximum likelihood and Bayesian methods. Despite a representative data sample and modern analytical approaches, our results reflected the difficulties in reaching classification consensus evident in past studies. However, we also observed a tendency for certain alternative topologies being supported by groups of genes that differed in their putative functionality.

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REINVIGORATING OLEORESIN COLLECTION IN THE SOUTHEASTERN USA: EVALUATION OF STAND MANAGEMENT AND TREE CHARACTERISTICS WITH BOREHOLE TAPPING

Jennifer Lauture[1] and Gary F. Peter[1]

The borehole tapping method was used to extract oleoresin from the xylem in slash pine (*Pinus elliottii*) in North Florida. This closed collection system allows for the recovery of higher quality resin, which can be used for a variety of commercial products as well as a natural liquid biofuel. Conifers produce oleoresin naturally as a biochemical defense against plant pests, such as boring bark beetles. The objectives of this project are to develop cost effective methods to collect oleoresin in North Florida and assess the feasibility and impact of expanding collection of pine terpenes for renewable chemicals and biofuel production on a large-scale. Treatments were applied manually using a gas powered drill as well as with an automated drilling machine mounted on a tractor designed to drill 3 connecting boreholes at the base of the tree. Oleoresin yields were compared from North Florida slash pine plantations aged 11, 15, and 22 years collected in the summer and fall. Oleoresin yields increased with stand age and DBH, as well as in stands managed for pine straw raking. Methyl jasmonate stimulated higher rates of oleoresin production compared to all other chemical stimulants.

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DEVELOPMENT OF A WHITE OAK SOMATIC EMBRYOOGENESIS SYSTEM TO AID PRODUCTION OF ELITE WHITE OAK GENOTYPES

Scott A. Merkle[1], Jessica Mitchell[1], Paul Montello[1], Ryan Tull[1], Albert G. Abbott[2] and C. Dana Nelson[3]

White oak (*Quercus alba*) is one of the most valuable North American oaks for a range of products, including furniture, flooring, and perhaps most critically, barrels for aging fine bourbon. The world-wide rise in demand for U.S. bourbon over the past decade has resulted in a concomitant rise in demand for white oak cooperage, which has in turn resulted in a shortage of high-quality white oak wood. A combined breeding and clonal propagation system for production of elite white oak genotypes could make a real long-term contribution to resolving this shortage. However, oaks are notorious for their long life cycles (i.e. time from seedling to flower production) and for their recalcitrance to clonal propagation by such methods as rooted cuttings or tissue culture. A robust somatic embryogenesis system for the species could help resolve both issues by providing a route for the gene transfer needed to accomplish rapid cycle breeding using early flowering genes, and by supplying the means to clonally propagate elite white oak genotypes once they are identified. In a preliminary experiment, developing acorns were collected from two white oak source trees periodically throughout July 2014 and dissected to obtain immature seeds, which were cultured on a modified woody plant medium (WPM) supplemented with either 2 or 4 mg/L 2,4-D. Within two months, multiple explants from both trees produced either clusters of repetitive somatic embryos or proembryogenic masses (PEMs). Once established, cultures were transferred to fresh medium every 3 weeks. Cultures showing the most rapid growth were inoculated into liquid medium and grown on a gyratory shaker at 90 rpm, where they formed suspension cultures. Suspension cultures were size-fractionated on stainless steel sieves and the selected size fraction was cultured on basal WPM for somatic embryo production. Relatively synchronous populations of somatic embryos were produced from six of the culture lines, representing both source trees. As the embryos matured, those with the best form were picked and moved to fresh basal WPM for further enlargement, prior to being given different pre-germination treatments, which tested the effects of pre-germination cold treatment and activated charcoal (AC) on germination. Cold storage at 8º C for 8 weeks are resulted in germination of multiple somatic embryos from different culture lines following transfer to a lighted incubator in GA-7 vessels (Magenta Corp) containing basal WPM with or without 0.5 g/L AC. Several embryos germinated (i.e. produced taproots) on medium with or without AC. Some embryos on medium with AC produced both roots and shoots with expanding leaves.

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PROPAGATION OF “LINGERING ASH” GENOTYPES VIA SOMATIC EMBRYOGENESIS FOR EMERALD ASH BORER RESISTANCE TESTING

Jessica Mitchell[1] and Scott A. Merkle[1]

Ash (*Fraxinus* sp.) is an economically and ecologically significant genus of hardwood forest tree. Unfortunately, North America's native ash resource is currently threatened by the rapid spread of emerald ash borer (EAB; *Agrilus planipennis*), an exotic wood-boring beetle, which is causing widespread damage and mortality of ash trees in North America. Our research employs germplasm gathered from green ash *(Fraxinus pennsylvanica*) and white ash (*Fraxinus americana*) trees to optimize a somatic embryogenesis *in vitro* propagation system, ultimately, in order to generate EAB-resistant planting stock to aid ash restoration efforts. Building upon preliminary green ash somatic embryogenesis work (Li et al. 2014), our work focuses on producing embryogenic cultures and somatic seedlings of “lingering” white ash. “Lingering” ash trees are healthy trees which are intermingled with dead ash trees where EAB infestation has caused almost complete mortality of mature ash trees in a particular area. The fact that these lingering ash trees have survived infestation may indicate genetically-based resistance or tolerance to EAB. By cloning lingering ash trees through somatic embryogenesis, it may be possible to propagate trees that are naturally resistant to EAB, eliminating the need to use gene transfer techniques to produce transgenic trees that carry resistance to EAB. A culture initiation experiment using seed and zygotic embryo explants from lingering ash parents in Michigan showed that seed collection date and explant type (naked zygotic embryo versus whole seed) significantly affected embryogenesis induction, while, 2,4-D concentration (2 or 4 mg/L) and explant length did not, as long as the zygotic embryo was over 4.5 mm long. Induction ranged as high as 37.5% for some genotypes and dates. White ash embryogenic cultures produced somatic embryos that were used in subsequent germination experiments, which tested the effects of activated charcoal and gibberellic acid (GA3) on germination. The treatment combining 0.5 g/L activated charcoal and 10 mg/ L GA3 produced the most vigorous somatic seedlings in the shortest time. Several of these somatic seedlings have survived transfer to *ex vitro* conditions and are growing in the greenhouse. By successfully inducing embryogenesis from explants gathered from lingering ash trees, our results show a promising step toward clonal propagation of potentially EAB-resistant trees. Once optimized, this system could eventually be scaled-up for mass clonal propagation of EAB-resistant planting stock to aid forest restoration in areas affected by EAB.

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DIFFERENTIAL GROWTH RESPONSE OF FUSARIUM CIRCINATUM ISOLATES FROM SOUTHEAST UNITED STATES TO INCREASED TEMPERATURES: IMPLICATIONS FOR A CHANGING CLIMATE

Tania Quesada[1] and Jason Smith[1]

Pitch canker is one of the major diseases in pines, affecting multiple species in forests and commercial plantations worldwide. This disease is incited by the necrotrophic fungus *Fusarium circinatum* and causes resinous lesions in stems and branches. Combinations of environmental conditions, especially high temperature and humidity, can trigger major outbreaks, but the pathogen may also remain latent as an endophyte without causing symptoms. In the context of increased global temperatures and altered precipitation regimes, future climate in the region may favor pitch canker outbreaks; however, current mitigation strategies rely mainly on breeding and selection of resistant host material, which takes many years to achieve. In an effort to foresee a potential disease scenario under elevated temperature conditions, a pilot study was implemented to determine growth patterns of *F. circinatum* isolates at 25, 28, and 31 Â°C. Preliminary growth data on cultured isolates collected from north and central Florida showed significant changes between cultures at 25 and 31°C, suggesting latitude-associated differential response to temperature stress. Additional fungal isolates from other regions will be evaluated and pathogenicity tests on slash pine using selected isolates are also projected. The results of this study, along with our current knowledge on species-wide host susceptibility to pitch canker, would help implement an ideal platform to evaluate pathogen, host and environment, and assess the effects of this disease in a changing climate. Studying the effects of a climate change on pitch canker disease would also allow a better understanding of other host-pathogen systems and develop adequate prediction models to help implement more efficient mitigation efforts.

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ANALYSIS OF WOOD PROPERTIES IN COASTAL AND PIEDMONT POLLEN MIX TESTS

Andrew Sims[1], Graham Ford[2], Austin Heine[1], Fikret Isik[1], and Steve McKeand[1]

Wood density and bending strength are among the most important properties related to the value of wood products. Traditional sampling procedures for these traits require time-consuming and expensive lab work, both of which are major impediments to incorporating these traits into tree breeding programs. Fortunately, there are now efficient and rapid procedures to sample these wood properties. The Fakopp TreeSonic measures acoustic stress-wave velocity, which has a strong genetic correlation with wood bending strength. The IML Resistograph is a micro-drilling tool that measures drilling resistance across the cross-sectional profile of a tree and is strongly correlated with wood specific gravity. Use of these non-destructive techniques have been promising for reducing the time and cost of selecting genotypes with superior wood properties.

The first phase of the study was to validate the efficiency of the new Resistograph version IML Resi PD400 series for correlating with volumetric specific gravity. Additionally, acoustic stress-wave velocity from Fakkop TreeSonic tool was used as an estimate of modulus of elasticity. Here, we found that genetic correlation between Resistograph drilling amplitude and specific gravity was high (r_G = 0.90). This phase represented two test sites from the Coastal Pollen Mix 1 series, inculding 70 half-sibling families and two checklots. The second phase of the study extended these measurement and analysis protocols to a wider range of Coastal and Piedmont families, representing ten sites in 5 different series. We found moderate to high heritabilities for wood property traits with family mean heritabilities ranging from h_fm^2 = 0.44 to 0.76 for stress-wave velocity and h_fm^2 = 0.50 to 0.78 for relative amplitude. These findings indicate that the Cooperative can use these rapid screening methods to effectively characterize families for wood properties. To date, we have used these rapid screening tools to estimate breeding values for the surrogates of wood density and bending strength for 280 Coastal families and 74 Piedmont families.

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SOFTWARE PACKAGE TO SOLVING COMPLEX SEED ORCHARD LAYOUTS

Jan Stejskal[1], Katerina Chaloupkova[1], Milan Lstiburek[1]

Lstiburek and El-Kassaby (2009) suggested the use of a quadratic assignment problem as a global heuristics to solving complex seed orchard layouts and named the resulting scheme as the Minimum-Inbreeding (MI) seed orchard layout. While the original theoretical concept was demonstrated using a small 10x10 orchard block, the same authors later expanded the MI scheme to large-scale operational sizes (Lstiburek et al., 2015). Here we describe the formation of distance and flow matrices using the actual software package, which is composed of two parts: (1) improved version of the computer program OPTIQAP (extension of the algorithm by Miscevicius 2005), and (2) a series of routines developed in R system. All advantages of the original MI scheme have been retained in the extended version. These may include, either alone or in combination: unequal clonal sizes, related genetic entries, and preferential allocation. We believe that this package is useful to many operational tree improvement programs.

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SINGLE GENOTYPE ISOLATES OF THE FUSIFORM RUST FUNGUS: PROCESS AND PROGRESS

CS Burdine[1], KE Smith[12], BD Bartlett[1], JM Davis[2] and CD Nelson[1]

Valuable timber is degraded and destroyed every year in loblolly pine (*Pinus taeda*) and slash pine (*P. elliottii* var. *elliottii*) plantations across the southern United States by fusiform rust (caused by *Cronartium quercuum* fsp. *fusiforme*, *Cqf*). Research indicates that a gene-for-gene system determines the host-pathogen interaction and subsequent infection and disease development. Additional research has indicated that multiple interacting gene pairs are responsible for the interactions between host and pathogen genotypes. Furthermore it has been shown that genes in the fungal pathogen can defeat (i.e., be virulent to) corresponding resistance genes in the pine host. Thus when these genes are present in high frequency in the pathogen population, host resistance can be overcome in the field. Knowledge of these interactions and outcomes, combined with awareness of which fungal genotypes are present, are critical in determining the pine families that should be used to reforest specific areas. At the Harrison Experimental Forest (Saucier, MS) an inventory of *Cqf* genotypes, informative for specific host-pathogen interactions, are being increased and stored for ongoing and future research. In our system, juvenile northern red oaks (*Quercus rubra*) are inoculated with a specific isolate of *Cqf* and kept in optimal conditions for spore development inside environmental growth chambers. Once uredinial pustules begin to develop, urediniospores from a single pustule are then transferred on to another juvenile oak and allowed to develop. The harvested spores from these re-infections are kept separate for each so called single-uredenial-pustule line (SUP). DNA is isolated from each SUP and analyzed with specific DNA markers (i.e., simple sequence repeats, SSRs) to verify the SUP's identity (same genotype as starting genotype) and purity (a single genotype). The SUPs developed and verified in this manner have been used in a variety of research projects and continue to be useful for current and future work in fusiform rust research.

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SALT TOLERANCE OF BARRIER ISLAND SLASH PINE

Ron Schmidtling[1], Vikram Chhatre[1], and C. Dana Nelson[12]

Hurricane storm surges in coastal areas often flood barrier islands and mainland beaches with salt water causing extensive mortality of forest trees. Hurricane Katrina in 2005 was especially lethal to slash pine (*Pinus elliottii* var *elliottii*) on the barrier islands in the northern Gulf of Mexico. Recently, restoration of slash pine on some of the islands has been proposed. The question was raised about planting stock. Would nursery-run seedlings from the mainland survive the next storm? Little is known about salt tolerance of slash pine from the islands compared to mainland populations. We collected seed from individual slash pine trees from three populations: 1. Mainland, mostly Harrison County in south Mississippi, 2. Deer Island, just offshore from Biloxi MS, and 3. Cat Island, a barrier island 10 miles offshore from Long Beach, Mississippi. We also included two families of loblolly pine (*P. taeda*) as it is common in south Mississippi but does not occur on the islands. Seed from the half-sib families were germinated and grown for 9 weeks in containers prior to treatments in this experiment. There were four treatments:

1) 3.5% salt water concentration (approximating sea water), 15 minutes total submersion
2) 3.5% salt water concentration, 15 minutes root submersion only
3) 1.75% salt water concentration, 15 minutes total submersion
4) 1.75% salt water concentration, 15 minutes root submersion only

Treatments 1, 2, and 3 caused total mortality by 10 weeks. Treatment 4 caused around 60% mortality by 13 weeks, but showed differentiation among seed sources and half-sib families. The order of survival of sources after 13 weeks was: 1.Cat Island, 2. Deer Island, 3. Mainland, and 4. Loblolly pine. This preliminary trial showed the existence of differences in salt tolerance among and within seed sources of slash pine.

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OPTIMAL MANAGEMENT AND PRODUCTIVITY OF EUCALYPTUS ON FORMER PHOSPHATE MINED AND CITRUS LANDS IN CENTRAL AND SOUTHERN FLORIDA: INFLUENCE OF GENETICS AND SPACING

Kyle W. Fabbro[1] and Donald L. Rockwood[2]

Eucalyptus short rotation woody crops (SRWC) with superior genotypes are promising in central and south Florida due to their fast growth, freeze resilience, coppicing ability, and site tolerance. Four *Eucalyptus grandis* cultivars, E.nergy™ G1, G2, G3, and/or G5, were established in 2009 at varying planting densities on a reclaimed clay settling area (CSA) in phosphate mined land in central Florida and a bedded former citrus site in southern Florida. Planting densities were 1025, 2050, and 3416 trees/acre on the CSA, and 581, 869, 1162, 1452, and 1742 trees/acre on the citrus site. Modified land expectation values (LEV) for coppicing species are reported for G2, G3, and/or G5 SRWCs on CSAs and citrus land. Optimal coppice stage and cycle lengths to the nearest 1/10th year were estimated for each cultivar × spacing × land scenario, assuming a range of coppice yields, cultural treatments (weed control and fertilization), plantation establishment and maintenance costs, stumpage prices, and real discount rates of 6, 8, and 10%. For example, at a 10% discount rate, stumpage price of $14 green/ton, costs of $250, 50, 974, 55, 90, and 10/acre for land preparation, bedding, planting, pre– and post–establishment weeding, fertilization, and annual management, respectively, and expected coppice yields, the LEV of CSAs under G3 at 1025 trees/acre was $561/acre or an equal annual equivalent of ~$56/acre/year. Currently, *Eucalyptus* is primarily harvested for landscape mulch, but markets are likely to expand into bioenergy and pulpwood applications.

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INVESTIGATING THE GENETIC BASIS OF VARIATION IN RESISTANCE TO CHESTNUT BLIGHT AMANG CHINESE CHESTNUTS AND INTERSPECIFIC HYBRIDS USING WHOLE-GENOME RESEQUENCING

Nicholas R. LaBonte[1] and Keith E. Woeste[2]

Chinese chestnut (*Castanea mollissima* Blume) is the main donor of disease resistance in the breeding program being undertaken by the American Chestnut Foundation (ACF) to restore chestnut forests to the eastern United States. The use of only a few *C. mollissima* resistance donor trees could be a liability for the restoration program if blight resistance in Chinese chestnut is maintained by diverse sets of alleles at blight resistance loci, as is the case in many plant/disease systems. To determine whether Chinese chestnut possesses diverse resistance alleles or a restricted set of "best" resistance alleles, we are using the Chinese chestnut v1.1 draft genome and three blight QTL scaffold sequences (hardwoodgenomics.org) to assemble individual genomes for 24 Chinese chestnuts and interspecific hybrids of variable blight resistance. Currently 14 trees have been sequenced using the Illumina HiSeqplatform, with approximate genome depth between 10 x and 25x, and preliminary analysis has revealed some polymorphisms (SNPs) that appear to segregate strongly between resistant and susceptible trees.

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NITROGEN RECYCLING: A PERENNIAL PROBLEM

Benjamin A. Babst[1], Fei Gao[1], Mike Schueller[2], Fei He[3] and Sergei Maslov[2,3]

Since nitrogen (N) is often the most limiting nutrient for plant growth in terrestrial ecosystems, efficient use of N by trees is crucial to environmental and economic sustainability of bioenergy and forestry. Although perennial plants, such as trees, may recycle a large proportion of N by remobilizing N from senescing leaves, few of the transporters involved in N remobilization from leaves have been identified. We are developing a combination of computational and experimental phenotype screening approaches to identify genes, especially transporters, involved in nitrogen transport throughout the plant. Here, as a medium-throughput phenotype screen, we administered radioactive $^{13}NH_3$ tracer as a gas to leaves, which was incorporated into biological molecules, and we measured export of assimilated ^{13}N from leaves. The high specific activity of ^{13}N allowed administration of tracer well below the concentrations of NH_3 normally found in plant tissues. Using Arabidopsis thaliana for demonstration purposes, coexpression network analysis was used to winnow the list of candidate transporter-encoding genes from more than 1000 to fewer than 50. In the radiotracer screen, several mutants of these genes exhibited reduced ^{13}N export from leaves, indicating that several of those candidate genes, whose functions were previously unknown, are necessary for normal export of N from leaves. We are currently working to apply this approach to genetically tractable trees, such as poplars, where N recycling is especially important in the context of seasonal growth and dormancy. Knowledge of the genes and systems underlying N recycling may present novel opportunities for breeding programs to maintain or improve N use efficiency in trees.

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OBSERVATIONS OF EARLY SURVIVAL OF LOBLOLLY PINE: ARE WE GAINING SERVIVAL ADVANTAGES WITH IMPROVED GENETICS

Valerie West[1], B. Landis Herrin[1], and Jason Cromer[1]

The availability and affordability of improved genetic level of Loblolly pine (*Pinus taeda* L.) planting stock has increased over the past decade. A series of trial locations was established in 2014 across Mississippi in an effort to better understand the performance of the wide range of available seedling stock on non-industrial private forest (NIPF) landowner sites. For the purposes of this trial we compared two second generation open pollinated stock (OP) against three mass control pollinated (MCP) stock and three varietal stock. All seedlings were bareroot stock. Our observations of early survival indicate that MCP and varietal seedlings had greater initial survival than the OP stock types. The added survival gains further supports the benefits of utilizing improved genetic material stock types for private landowners.

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AN ANNOTATED CONSENSUS GENETIC MAP FOR *PINUS TAEDA* AND EXTENT OF LINKAGE DISEQUILIBRIUM IN THREE GENOTYPE-PHENTOYPE DISCOVERY POPULATIONS

Jared W. Westbrook[1], Vikram E. Chhatre[2], Le-Shin Wu[3], Srikar Chamala[456], Leandro Gomide Neves[7], Patricio Muñoz[8], Pedro J. Martínez-García[9], David B. Neale[9], Matias Kirst[1], Keithanne Mockaitis[10], C. Dana Nelson[211], Gary F. Peter[1], John M. Davis[1], and Craig S. Echt[2]

A consensus genetic map for loblolly pine was constructed by merging three previously published maps with a map from a pseudo-backcross between loblolly pine and slash pine. The consensus map positioned 3700 markers via genotyping of over 1200 individuals from four pedigrees. Functional predictions for nearly 3500 mapped EST markers were improved by alignment to full-length loblolly pine transcripts. Alignments to the loblolly pine genome mapped over 3000 scaffold sequences onto the 12 linkage groups. The consensus genetic map was used to compare the extent of genome-wide linkage disequilibrium in an association population of distantly related individuals (ADEPT2), a multiple-family pedigree used for genomic selection studies (CCLONES), and a full-sib quantitative trait locus mapping population (BC1). Weak linkage disequilibrium was observed in CCLONES and ADEPT2. Average squared correlations, R2, between genotypes at SNPs less than 1 cM apart was less than 0.05 in both populations, and R2 did not decay substantially with genetic distance. By contrast, strong and extended linkage disequilibrium was observed among BC1 full-sibs where average R2 decayed from 0.8 to less than 0.1 over 53 cM. The consensus map and analysis of linkage disequilibrium establish a foundation for comparative association and QTL mapping between genotype-phenotype discovery populations.

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FGMRI CZECH REPUBLIC – CONNECTION WITH THE COST ACTION FP1202

Josef Frýdl[1] and Jaroslav Dostál[1]

In relation to the international EU project COST Action FP1202 "Strengthening conservation: a key issue for adaptation of marginal/peripheral populations of forest trees to climate change in Europe (MaP-FGR)" the main objectives of the project COST CZ LD14116 „The Czech Republic participation in the implementation of essential measures aimed at verifying the adaptability of threatened forest tree species in connection with ongoing environmental changes in Europe through the use of results gained from selected field trials with *Abies* species tested under various environmental and site conditions“ having been solved by the Forestry and Game Management Research Institute (FGMRI), Jiloviste–Strnady, Czech Republic from IV. 2014 to X. 2016 are focused to the acquisition of new knowledge of genetically conditioned variability of ecologically and economically important of characteristics of the species of genus *Abies*. The project is also focused on the verification of the use of these species in the Czech Republic forest management at the time of ongoing environmental change. In the context of this research project, the results will be obtained from evaluation of three long-term research plots with progenies of selected *Abies* genus species. These research plots were established by FGMRI in earlier times in the Czech Republic on sites with different environmental and habitat conditions.

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