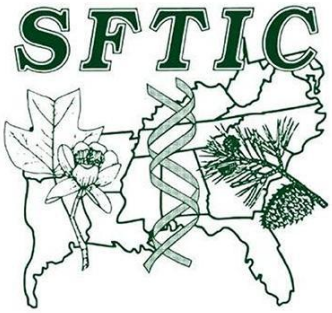

35th Southern Forest Tree Improvement Conference

Genetics and Improvement of Forest Health and Productivity

June 3-6, 2019

Lexington, Kentucky, USA

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Southern Forest Tree Improvement Committee

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ABOUT the SFTIC COMMITTEE (SFTIComm) and the 35TH SFTIC CONFERENCE (SFTIC)

The Southern Forest Tree Improvement Committee (SFTIComm) is the de facto professional organization of tree improvement specialists throughout the southeastern United States. SFTIComm has organized and coordinated tree improvement efforts and has sponsored many forest genetics studies including the first quantification of geographic variation in the southern pines. The Southern Forest Tree Improvement Conference (SFTIC) has met biennially since 1951 to exchange information and to discuss future needs. The Forest Health Research and Education Center (FHC, www.foresthealthcenter.org), the Department of Forestry and Natural Resources and Forestry Extension at the University of Kentucky, in cooperation with SFTIC, hosted the 35th biennial meeting of Southern Forest Tree Improvement Conference in Lexington, Kentucky at the Downtown Hilton Hotel on June 3-6, 2019. A meeting summary of the conference is in press at Tree Genetics and Genomes.

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Information on the SFTIC Proceedings

The abstracts in this document have been lightly reviewed by the SFTIC proceedings editors and may be cited as the Proceedings of the 35th Southern Forest Tree Improvement Conference.

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At any subsequent time until the next SFTIC, abstract authors may submit an amendment to their abstract consisting of an extended abstract, a full paper, or a reference to a publication that extends the work of their SFTIC abstract. The amendments will be added as new page numbers to the Proceedings in the order they are received and reviewed and will reference the page number of the original abstract. At the time of the next SFTIC, the Proceedings will be closed and the current edition will be considered the final edition.

PLENARY SPEAKER ABSTRACTS

BRINGING BACK THE AMERICAN CHESTNUT: ADVANCES IN GENETIC MODIFICATION, NAVIGATING FEDERAL REGULATION

Vernon Coffey[1], William Powell[1]

[1]American Chestnut Research and Restoration Project, State University of New York College of Environmental Science and Forestry (SUNY-ESF), Syracuse, NY

The American chestnut, once a major component of Eastern forests, was decimated by an invasive fungal pathogen introduced over a century ago. Researchers at SUNY-ESF have developed the 'Darling' transgenic American chestnut by incorporating into its genome an oxalate oxidase (OxO) gene from wheat. OxO is a common defense gene found in many plant species. American chestnut trees expressing the OxO enzyme have enhanced tolerance to chestnut blight, similar to or greater than that of Chinese chestnut controls. Several ecological and risk-assessment studies have been completed without finding significant differences between the transgenic and non-transgenic trees. The American Chestnut Research and Restoration Project at SUNY-ESF, in collaboration with the American Chestnut Foundation, are undertaking the lengthy process of applying for federal non-regulated status. This would allow the unrestricted planting and breeding of these trees with the long-term goal of restoring this iconic species to its native habitat throughout Eastern forests.

GENETIC REGULATION OF BRANCH ORIENTATION AND GRAVITROPIC POTENTIAL IN TREES

Chris Dardick[1]

[1] USDA Agricultural Research Service, Appalachian Fruit Research Station, Kearneysville, WV

Tree architecture is intimately tied to environmental signals, most importantly light. Light impacts branch growth angles and, in turn, branch orientation influences the light interception by both the tree and through competitive shading of neighbors. We've identified a number of genetic factors that control branch angles. Among these are genes in the IGT gene family, which consists of TILLER ANGLE CONTROL1 (TAC1) and LAZY1. Reduction of TAC1 gene expression leads narrow, upright branch angles, whereas reduction of LAZY1 leads to non-vertical angles. Here we describe how IGT genes contribute to light-induced changes in branch angle in trees and other plants. Collectively the data suggest that IGT genes influence branch angles by integrating light and photosynthetic signals to modulate gravitropic potential.

NOTES FROM THE MOUNTAIN TOP – WHERE WE GO FROM HERE IS UP TO US

Steve McKeand[1]

[1]NC State University Cooperative Tree Improvement Program, Raleigh, NC

Tree breeding programs are a challenge for landowners, foresters, citizens, and politicians to understand and to fund. Tree improvement is relatively easy to justify when there is short-term economic benefit. For long-term benefits that might include sustaining/enhancement of forest health or ecosystem services such as carbon sequestration, support for tree improvement is often a challenge. Long-term management of our natural resources is not a high priority for many citizens, so tree improvement like most forest management issues is not a high priority. How we raise the profile of our work is up to us, but success breeds success. As a community, we must highlight our successes and the benefits that we bring. Again, this is relatively straightforward for programs that yield short-term financial returns. For threatened or endangered species or sustaining long-term forest health under changing climates, success stories are more of a challenge. While politicking is not normally in most tree breeding job descriptions, gaining political support for our work will be key for our success.

THE CHINESE CHESTNUT GENOME: A REFERENCE FOR SPECIES RESTORATION

Margaret E Staton[1], Charles Addo-Quaye[2,3], Nathaniel Cannon[2,4], Tetyana Zhebentyayeva[2], Matthew Huff[1], Shenghua Fan[5], Emily Bellis[6], Nurul Islam-Faridi[7], Jiali Yu[1], Nathan Henry[1], Daniela I. Drautz-Moses[8], Rooksana E. Noorai[9], Stephen Ficklin[10], Chris Saski[11], Mihir Mandal[12,13], Tyler K Wagner[2], Nicole Zembower[2], Catherine Bodénès[14], Jason Holliday[12], Jared Westbrook[15], Jesse Lasky[6], Laura L Georgi[15], Frederick V Hebard[15], C. Dana Nelson[5,16], Stephan C Schuster[8], **Albert G Abbott**[2,5], John E Carlson[2]

[1]University of Tennessee, Institute of Agriculture, Knoxville, TN; [2]Schatz Center for Tree Molecular Genetics, Pennsylvania State University, University Park, PA; [3]Division of Natural Sciences and Mathematics, Lewis-Clark State College, Lewiston, ID; [4]Department of Biology, Southern Utah University, Cedar City, UT; [5]Forest Health Research and Education Center, University of Kentucky, Lexington, KY; [6]Department of Biology, Pennsylvania State University, University Park, PA; [7]USDA Forest Service, Southern Research Station, College Station, TX; [8]Singapore Centre for Environmental Life Sciences Engineering, Nanyang Technological University, Singapore 639798; [9]Clemson University Genomics and Bioinformatics Facility, Clemson, SC; [10]Department of Horticulture, Washington State University, Pullman, WA; [11]Department of Plant and Environmental Sciences, Clemson University, Clemson SC; [12]Virginia Polytechnic University, Blacksburg, VA; [13]Department of Biology, Claflin University, Orangeburg, SC; [14]UMR Biodiversité Gènes et Communautés, French National Institute for Agricultural Research (INRA), 69 route d'Arcachon, 33612 CESTAS Cedex – France; [15]The American Chestnut Foundation, Meadowview, VA; [16]USDA Forest Service, Southern Research Station, Saucier, MS

The American chestnut (*C. dentata*) is one of the most well-known and studied examples of near total mortality of a forest tree across its native range due to exotic diseases. It is one of the few species with large-scale genomics-enabled breeding programs, which rely on introgression of resistance genes from Asian chestnut species. To support the efforts to restore American chestnut, we assembled a chromosome-scale reference genome from the Chinese chestnut (*C. mollissima*) cultivar 'Vanuxem'. Comparative genomics with peach (*Prunus persica*) and oak (*Quercus robur*) reveal largely conserved genome organization, including across key quantitative trait loci (QTLs), but also significant expansion and contraction of particular gene families. Resequencing of 5 *C. dentata* and 5 *C. mollissima* genotypes enabled analysis of signatures of selection, providing insights into the evolution of resistance to chestnut blight (*Cryphonectria parasitica*) and new candidate resistance genes. With this genomic resource as well as additional upcoming resources, chestnut is becoming a promising platform for forest tree genotype-to-phenotype research and for leveraging genomics in species restoration.

CONIFER GENOMES AND IMPLICATIONS FOR PINE GENETICS AND IMPROVEMENT

Jill Wegrzyn[1], Sumaira Zaman[1,] Alyssa Ferreira[1], Madison Caballero[1], Ross Whetten[2]

[1]Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs, CT; [2]Department of Forestry and Environmental Resources, North Carolina State University, Raleigh, NC

The emergence of improved sequencing technologies, coupled with decreasing costs, inspired innovative assembly methods for large and complex genomes, such as the conifer megagenomes, that range from 16 to 40 Gbp in size. Although these megagenomes are increasing in contiguity, accurate genome annotations remain challenging. Questions surrounding genome evolution are answered by interrogating the genome and its associated annotation. The accuracy of these products impacts estimates of genome duplication, gene family expansion/contraction, and functional assessments. Applications related to genomic selection, classification of hybrids, and pangenome approaches also require robust annotations. Among conifer genome assemblies, the gene space annotations are complicated by the presence of repetitive elements, large gene families, numerous pseudogenes, and long introns. Existing annotation packages are challenged to differentiate among these features and provide high quality results. Recent efforts have focused on improving strategies for the annotation of several gymnosperms, including five conifer species. We examine the impact of using assembled transcriptomic evidence (full length transcript and protein sequences) versus RNA read alignments to train *ab initio* gene predictors to annotate these genomes. These approaches are evaluated with assays for accessible chromatin, such as ATAC-Seq, which can improve the detection of true gene models, and distinguish prevalent pseudogenes. The final loblolly pine genome annotation improves on both the estimated completeness and structural metrics of the proposed gene models. A total of 51,200 genes were annotated with a novel pipeline integrating RNA-Seq and protein alignments with Braker2 and two in-house developed pieces of software, EnTAP and gFACs. The ATAC-Seq data assisted in filtering of the mono-exonic genes which are frequently inflated in conifer genomes. This approach was benchmarked against previous annotations as well as those resulting from standard standalone pipelines (MAKER and Braker2). The most recent release of the loblolly pine genome annotation can be retrieved from the TreeGenes database.

THE EVOLVING EFFORT TO RESTORE THE AMERICAN CHESTNUT

Jared W. Westbrook[1], Jason A. Holliday[2]

[1]The American Chestnut Foundation, Asheville NC, [2]Virginia Tech, Department of Forest Resources and Environmental Conservation, Blacksburg, VA

For 30 years, The American Chestnut Foundation (TACF) has pursued backcross breeding to introgress blight tolerance from Chinese chestnut into hybrids that have the timber-type form of American chestnut. The underlying assumptions of the backcross program are 1) blight tolerance is controlled by Chinese chestnut alleles that segregate at two to three loci and 2) that progeny that inherited those alleles can be reliably selected in each backcross generation. To test these hypotheses and to perform genomic selection in backcross seed orchards, we genotyped ~1,200 American chestnut BC_3F_2 trees. We developed genomic selection models for blight tolerance by genotyping age 10 + trees phenotyped for traits indicative of long-term blight tolerance or whose progeny had been evaluated for blight tolerance. In addition, reference panels of Chinese chestnut and American chestnut were genotyped estimate hybrid index for the BC_3F_2 trees. We found that blight tolerance phenotypes of individual BC_3F_2 trees and their progeny were weakly heritable (h^2 ~ 0.1 to 0.3) and that there is a tradeoff between blight tolerance and the proportion of the genome inherited from American chestnut. On average, selected BC_3F_2 trees inherited 84% of their genome from American chestnut and had blight tolerance that was intermediate between F_1 hybrids and pure American chestnut. Results suggest that blight tolerance is controlled by more loci than previously assumed. TACF is pursuing multiple alternative routes to ensure restoration success. We are advancing additional backcross lines through fewer backcross generations to find a balance between blight tolerance and American chestnut characteristics. In addition, we plan to outcross transgenic blight-tolerant American chestnuts developed by SUNY-ESF to a diverse collection of wild American chestnut trees. Third, we are pursuing genomic research to identify variants that underlie blight tolerance in Chinese chestnut and to enable additional transgenic and gene editing approaches to developing blight-tolerant populations.

GENERAL SESSION SPEAKER ABSTRACTS

BREEDING FOR WEEVIL RESISTANCE IN NORWAY SPRUCE: BALANCING ATTACK, GROWTH AND WOOD QUALITY USING MULTI-TRAIT GENOMIC SELECTION

Patrick R.N. Lenz[1,2], Simon Nadeau[1], Marie-Josée Mottet[3], Martin Perron[3], Nathalie Isabel[2,4], Jean Beaulieu[2], Jean Bousquet[2]

[1]Natural Resources Canada, Canadian Wood Fibre Centre, Québec, Québec G1V 4C7, Canada; [2]Institute of Integrative Biology and Systems, and Centre for Forest Research, Université Laval, Québec, Québec G1V 0A6, Canada; [3]Gouvernement du Québec, Direction de la recherche forestière, Québec, Québec G1P 3W8, Canada; [4]Natural Resources Canada, Laurentian Forestry Centre, Québec, Québec G1V 4C7, Canada

Increasing pressure of pest and disease on forest plantations is becoming a growing issue in the context of climate change. Breeding approaches using genomics may offer efficient and flexible tools to face this pressure. Norway spruce (*Picea abies* (L.) Karst.) has been introduced to North America more than a century ago and is valued for its superior growth compared with native spruces. Initial selection efforts were focussing on growth and hardiness traits. In Canada, Norway spruce plantation can get heavily attacked by the white pine weevil (*Pissodes strobi* Peck). In the present study, we targeted genetic improvement of Norway spruce resistance to the native white pine weevil. Single and multi-trait genomic selection (GS) models and selection indices were developed considering the relationships between weevil resistance, intrinsic wood quality, and growth traits. Moderate to high heritability was detected for average wood density, acoustic velocity as a proxy for mechanical wood stiffness and weevil resistance. Weevil resistance was genetically positively correlated with tree height, height to diameter ratio, and acoustic velocity. The accuracy of the different GS models tested (GBLUP, Threshold GBLUP, Bayesian Ridge Regression, BayesCπ) was high and did not differ among those methods. Multi-trait models performed similarly than single trait models when all trees were phenotyped. However, with an increasing proportion of missing values, e.g. when weevil attack survey could not be extended to all trees, weevil resistance was more accurately predicted by integrating genetically correlated traits into multi-trait GS models. A genomic selection index that corresponded to the breeders' priorities achieved near maximum gains for weevil resistance, acoustic velocity, and height growth, but a small decrease for DBH. The results of this study indicate that it is possible to breed for high quality, weevil resistant Norway spruce reforestation stock with high accuracy achieved from single-trait or multi-trait genomic selection.

SELECTION AND BREEDING FOR FROST AND *FUSARIUM CIRCINATUM* TOLERANCE IN SAPPI'S *PINUS PATULA* X *PINUS TECUNUMANII* HYBRID PROGRAMME

André Nel[1], Fanele Mabasa[2], Sithembhile Malinga[2], Lebogang Mphahlele[1], Arnulf Kanzler[1], Hannél Ham[2]

[1]Sappi Research, Howick, South Africa; [2] Department of Forestry, University of Stellenbosch, Stellenbosch, South Africa; South Africa

The Pitch Canker Fungus (PCF) disease, caused by *Fusarium circinatum*, has caused high levels of mortality of the primary commercial species *Pinus patula* in South African forestry nurseries and has also caused poor post-planting survival. Artificial inoculation experiments have indicated low levels of resistance with species such as *P. patula* and *P. radiata*, and higher levels of tolerance with some species and hybrid combinations. The *P. patula x P. tecunumanii* hybrid has largely replaced *P. patula* as the commercial species of choice in South Africa. This hybrid is, however, less cold tolerant than *P. patula* and therefor limits the planting of the hybrid in frost-prone areas. Results are presented on field growth and adaptability, disease tolerance and cold tolerance screening using hybrid families from a large factorial mating design between *P. patula* and *P. tecunumanii* Low and High Elevation parents. Viable seed was put through vegetative propagation via rooted cuttings, and hybrid families were tested as a family mix representing the genetic diversity available for each hybrid family, and hybrid status was confirmed with DNA fingerprinting. Results of the screening have shown a wide range of tolerance for both *F. circinatum* as well as cold tolerance, while a substantial increase in growth has been realized with the hybrid. Wood property studies of older hybrid material have also shown that the wood is highly desirable for both sawn timber and Kraft pulp. General and specific hybridizing abilities (GHA and SHA) were also calculated to identify superior parents for future commercial hybrid families.

EXPLORING THE POTENTIAL FOR USE OF RNA INTERFERENCE FOR EMERALD ASH BORER MANAGEMENT

Lynne K. Rieske-Kinney[1]

[1]University of Kentucky Department of Entomology, Lexington, KY

The emerald ash borer (EAB), *Agrilus planipennis*, is an exotic, invasive tree pest that has caused the death of hundreds of millions of urban and forested ash in North America. Adult EAB is responsible for only minor feeding damage, but larval feeding and tunneling beneath the bark disrupts water and nutrient transport, and tree death is rapid. All North American *Fraxinus* are susceptible, and EAB has more recently been reported on other Oleaceous hosts. Therefore, development of efficient and target-specific products for EAB management is essential. RNA interference (RNAi) technology is emerging as a next generation pest control method. Double-stranded RNA (dsRNA) molecules activate the RNAi pathway, which is a natural antiviral defense mechanism that disrupts normal protein synthesis. We've shown that RNAi can silence genes in EAB and cause rapid and extensive mortality. In order to move this technology to the deployment stage, its specificity to EAB must be demonstrated, and practical delivery methods must be developed. We are currently evaluating specificity by assessing potential non-target effects, and also evaluating methods of delivery.

INTEGRATED GENOMIC AND GENETIC APPROACH FOR DISCOVERY OF CANDIDATE GENES ASSOCIATED WITH RESISTANCE TO *PHYTOPHTHORA CINNAMOMI* IN CHESTNUT

Tetyana N. Zhebentyayeva[1], Steven N. Jeffers[2], Paul H. Sisco[3], Margaret E. Staton[4], Rooksana E. Noorai[5], Emily Bellis[6], Jesse Lasky[6], C. Dana Nelson[7], Albert G. Abbott[7], John E. Carlson[1]

[1]Schatz Center for Tree Molecular Genetics, Pennsylvania State University, University Park, PA; [2]Department of Plant and Environmental Sciences, Clemson University, Clemson, SC; [3]The American Chestnut Foundation, Asheville, NC; [4]Department of Entomology and Plant Pathology, University of Tennessee, Knoxville, TN; [5]Genomics and Bioinformatics Facility, Clemson University, Clemson, SC; [6]Department of Biology, Pennsylvania State University, University Park, PA; [7]Forest Health Research and Education Center, Southern Research Station, USDA Forest Service, Lexington, KY

Soilborne oomycete *Phytophthora cinnamomi* is one of the most devastating plant pathogens that occurs on all continents of the world except Antarctica and affects several thousand species. Genus *Castanea* is well suited to studying genetics of resistance *Phytophthora cinnamomi* due to availability of resistant Asian and susceptible American and European chestnut species with weak reproductive barriers. We generated and phenotyped for severity of root rot symptoms more than 1,800 individuals derived from interspecific crosses between resistant Chinese chestnut, *Castanea mollissima* ('Mahogany' and 'Nanking' resistance donors), and susceptible American chestnut, *C. dentata* (multiple parents). Saturated genetic linkage maps were constructed with sequence-based markers and used for QTL mapping. Altogether 17 QTLs were detected, and three most consistent QTL intervals were found on the top (qPcE.1), in the middle (qPcE.2) and at the lower end (qPcE.3) of the LG_E. Taking advantage of *C. mollissima* (Vanuxem) v 3.2 genome assembly we re-sequenced five Chinese and five American chestnut accessions and searched for signature of natural selection potentially present in resistant Chinese chestnut genomes coexisting with *P. cinnamomi* in the East Asia. To facilitate candidate gene discovery within QTL intervals, we also initiated a functional genomics study by analyzing gene expression profiles in chestnut roots interacting with *P. cinnamomi* zoospores. In total, 49 regions under selective sweep were detected, and 34 of them were located within QTL intervals on LG_E. Using assembled transcriptome data, we determined if genes within LG_E QTLs were expressed in chestnut roots infected with *Pc* zoospores. As indicated by transcript annotations, genes under selective sweep encode proteins primarily involved in the phenylpropanoid pathway, cell-wall formation, transmembrane transport and signal transduction, and production of reactive oxygen species. Groups of genes either potentially involved in host response to *Pc* or confer plant innate immunity —i.e., glucan endo-1,3-beta-D-glucosidase, patatin-like phospholipase, G-type lectin S-receptor-like serine/threonine-protein kinases, and cysteine-rich receptor-like kinases—were placed on the short list of genes for functional characterization in transgenic American chestnut.

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PERFORMANCE OF SWEETGUM VARIETIES ON UPLAND SITES IN NORTH LOUISIANA

Joshua P. Adams[1], Robert Hane[1], Michael Blazier[2], Curtis Vanderschaaf[1], Eric McConnell[1]

[1]School of Agricultural Sciences and Forestry, Louisiana Tech University, Ruston, LA; [2]School of Renewable Natural Resources, LSU Agricultural Center, Homer, LA 71040

Hybrid sweetgum (*Liquidambar styraciflua x formosana*) has shown remarkable growth rates through the first few years of growth relative to native sweetgum (*L. styraciflua)*. In 2015, alongside a genotype trial at two sites in North Louisiana- Homer, LA (LSU Hill Farm Research Station) and Ruston, LA (Louisiana Tech University)- five sweetgum genotypes were also planted in a herbicide trial. Genotypes consisted of four elite commercially-available hybrid clones, a fifth additional clone not commercially-available, and two superior native sweetgum full-sibling families. Families were planted in eight-tree strip-plots within a randomly assigned herbicide main plot treatment. After two years of monitoring the effect of herbicide treatments, half of the trees in each subplot were cut down and allowed to resprout. First-year results indicate successful coppicing of the hybrid, and growth potential of these hybrid sweetgum will be reported after the first year of growth.

EXTENSIVE CLONAL VARIATION IN DROUGHT-INDUCED GENE EXPRESSION CHANGES IN LOBLOLLY PINE

Jingjia Li[1], Jason West[2], Carol Loopstra[2], **Claudio Casola[3]**

[1]Department of Ecosystem Science and Management, Texas A&M University, College Station, TX

In the next few decades, drought intensity is expected to dramatically increase in multiple areas along the range of the loblolly pine (*Pinus taeda* L.). Identifying and propagating loblolly varieties with high drought tolerance will be instrumental to maintain or increase productivity through large-scale replanting efforts. While phenotyping and breeding efforts have long recognized and tested varieties with increased resistance to aridity, determining the genetic basis of drought tolerance represents a more direct way to select loblolly varieties capable of sustaining a prolonged water deficit. Previous studies relying on population genomic or microarray-based gene expression data have begun to unveil the genetic underpinning of drought tolerance in loblolly pine. However, these analyses were based on a limited set of loblolly genes. Here, we applied for the first time an RNA-seq transcriptomic approach to specifically investigate the genetic response of two loblolly clones that were previously shown to exhibit either low or high drought tolerance. We analyzed 54,826 loblolly transcripts from 24 root samples and found significant variation in expression patterns between control and treatment (drought-simulated conditions) in both clones. More than 3,800 genes significantly changed their expression level in roots of water deficit stressed vs. control ramets. The two clones shared only 6-13% of upregulated and 10-11% downregulated differentially expressed (DE) genes. Although genes from some families known to be involved in drought responses, including dehydrins and LEAs, showed differential expression between control and treatment ramets in both clones, we found many gene families whose expression was affected only in one of the two clones. Notably, most DE genes were downregulated in the drought-sensitive clone and upregulated in the drought-tolerant clone. Our results unveiled thousands of genes involved in drought tolerance in loblolly and underscore the importance of investigating the genetic mechanisms of stress resistance across multiple varieties.

DNA FINGERPRINTING RESULTS FOR THE FOREST SERVICE LONGLEAF PINE AND SHORTLEAF PINE SEED ORCHARDS AND SEED BANK

Barbara Crane[1], Valerie Hipkins[2], Sedley Josserand[3], Craig Echt[3]

[1]USDA Forest Service Southern Region, Atlanta, GA; [2]National Forest System Genetics Lab, USDA Forest Service, Placerville, CA; [3]USDA Forest Service, Southern Research Station, Southern Institute of Forest Genetics, Saucier, MS

USDA Forest Service National Forest System (NFS) in the Southern Region provides oversight for the management of approximately 800,000 acres of longleaf pine (*Pinus palustris*) and 1,440,000 acres of shortleaf pine (*Pinus echinata* Mill.). About 97% of the longleaf pine ecosystem and 53% of the shortleaf pine ecosystem have been lost over the past century. Consequently, longleaf and shortleaf pines are priority species targeted for increased restoration on the thirteen national forests in the Southern Region (R8). Quality seed is needed to support successful artificial regeneration of these species. And the genetic integrity of a species is important to ensure adaptation, survival and resilience of future forests. Recently, the question has arisen about potential increased hybridization between pine species. Currently, longleaf x loblolly pine hybrids (*Pinus sondereggeri*) and shortleaf x loblolly pine hybrids are known to occur in the general forests, but at a very low rate of 5% or less. Climate variability can trigger extreme fluctuations in temperatures, which could influence flower receptivity and pollen flight windows between species, potentially resulting in increased inter-species hybridization. Hybridization may degrade the inherent genetic adaptive traits of a species and present challenges to successful restoration. To assess the genetic purity of R8's germplasm, the National Forest System Genetics program chose to DNA fingerprint longleaf and shortleaf pine families and seed bank samples from the regional seed orchards. Simple sequence repeats (SSRs) were the DNA markers used to fingerprint the orchard trees and seed bank samples. Three markers were developed from GenBank chloroplast DNA sequences that together identify species-specific profiles (haplotypes) among longleaf, shortleaf and loblolly pines. Because chloroplast DNA is only inherited through the pollen in pines, loblolly pine chloroplast DNA was the differential indicator for detecting hybrids in the samples. Approximately 250 longleaf pine clones and 619 shortleaf pine clones were tested. Seed samples, spanning 1981 – 2017 were also tested. Source locations for the clones and seed represented Alabama, Arkansas, Florida, Georgia, Kentucky, Louisiana, Mississippi, Missouri, North Carolina, South Carolina, Tennessee, Texas and Virginia.

IMPLEMENTATION OF GENOMIC PREDICTION IN A LOBLOLLY PINE BREEDING POPULATION

W. Patrick Cumbie[1], Dudley A. Huber[2], Salvador Gezan[3], Victor Steel[1], Michael Cunningham[1]

[1]ArborGen, Inc, Ridgeville, SC, [2]Quantitative Genetics Services, LLC, Toccoa, GA,
[3]School of Forest Resources and Conservation, University of Florida, Gainesville, FL

The application of genomic based breeding and selection is not a one size fits all proposition. As ArborGen builds genomic information and resources we are exploring different approaches to incorporate genomic tools to accelerate the breeding and selection of superior genotypes of loblolly pine. A population of 1850 clonally replicated varieties was genotyped with 50,000 single nucleotide polymorphisms (SNPs) for genomic analysis. The Bayes Cpi model appeared to be successful in identifying significantly associated SNPs for all traits in this population (Table 1). The number of significantly associated SNPs ranged from 63 for fusiform rust (with 3 large effect SNPs) to 327 SNPs for volume (with no large effect SNPs). There were 58 SNPs in common between volume, height, and DBH suggesting some SNPs may have biological meaning. Modeling efforts to incorporate genomics for trait improvement show promise for parental and varietal selection but pedigrees must be represented in the training population. The prediction model worked well in this population with a correlation of 0.83 (Figure 1). A sub-sampling exercise in which 30 individuals were randomly removed from the population yielded correlations for true vs. predicted ranging from 0.63 to 0.97. The application of this technology could significantly reduce the testing and selection timeline for forest trees and improve selection intensity be pre-screening of test seedlings to remove the predicted poor performers and allow field testing of trees with a higher probability of desirable phenotypes.

Table 1. Significant SNPs and true vs. predicted correlations for each trait from Bayes Cpi modeling in the Coastal varietal population.

Trait	Significant SNPs	Correlation True vs. Pred.
Height	201	0.81
DBH	103	0.76
Volume	327	0.83
Rust	63	0.84
Straight	147	0.89
Forking	300	0.90

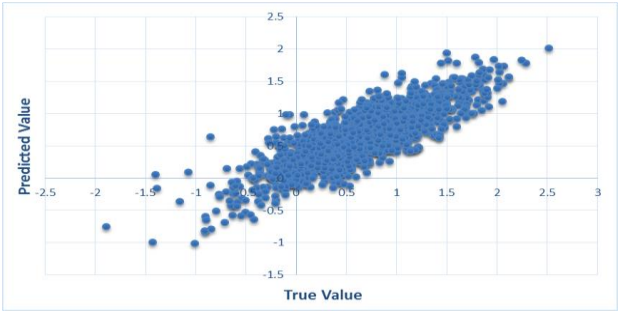

Figure 1. True vs. predicted values (r = 0.83) for age 6 volume in the Coastal varietal population. A total of 1523 samples out of the 1850 were used in the training model.

GENETIC IMPROVEMENT IN WHITE OAK (*QUERCUS ALBA*): CONNECTIONS TO FOREST HEALTH, CLIMATE CHANGE, AND FOREST PRODUCT INDUSTRIES

Laura E. DeWald[1], Ellen V. Crocker[1,2], Abe Nielsen[3], Rachel Thunder[1,2], Albert Abbott[2], C. Dana Nelson[2,4]

[1]Department of Forestry and Natural Resources, University of Kentucky, Lexington, KY; [2]Forest Health Research and Education Center, Lexington, KY; [3]Kentucky Division of Forestry, Frankfort, KY; [4]USDA Forest Service Southern Research Station, Lexington, KY

White oak influences ecosystem processes throughout eastern US forests and is thus critical to forest health. The species is also highly valued for forest product industries (e.g., cooperage industry). The long-term sustainability of white oak is uncertain and there are few long-term improvement programs for the species. To address these problems, we are developing a white oak genetic improvement program in the Forest Health Research and Education Center (University of Kentucky) focused on growth, yield, and health related to improving forest health and forest products. Our program will complement other white oak improvement programs to provide a range-wide effort. The improved white oak will be grown in managed, natural forests, thus genetic structure and adaptation to local and future climate conditions must be conserved. Therefore, program development is occurring concomitant with genomic, silvicultural and health research. Field identification of superior white oaks is particularly challenging given the large portion of private ownership in eastern forests, and thus a variety of partners and methods including TreeSnap are being used. Additionally, little genetic information exists to guide tree improvement in terms of how white oak responds to stressors, or how adaptive and non-adaptive traits such as wood organoleptic characteristics vary genetically. Limited studies indicate that despite extensive gene flow, species coherence is strong, there is high within-population genetic variation, and high phenotypic plasticity. Genotypes are correlated with environmental variation, population density, and site productivity. Some functional genes critical to environmental variation have been characterized and can be used to predict risk of non-adaptedness. Molecular information can maximize genetic gain per time by rapidly improving output quality of seed orchards. The superior trees being field selected serve as in-situ genetic reserves and they provide material for ex-situ seed orchards that can be used to uncover genetic variation patterns in traits of interest.

IDENTIFICATION OF CANDIDATE INTERACTING RESISTANCE AND AVIRULENCE GENES IN THE FUSIFORM RUST PATHOSYSTEM

Daniel Ence[1], Katherine E. Smith[2], Amanda L. Pendleton[3], Thomas L. Kubisiak[4], Claire L. Anderson[5], Asaf Salamov[6], Andrea Aerts[6], Robert W. Riley[6], Alicia Clum[6], Erika A. Lindquist[6], Nicolas Feau[7], Robin Paul[8], Braham Dhillon[9], Michael C. Campbell[10], Zev Kronenberg[11], Igor Grigoriev[6], Mark Yandell[12], Richard C. Hamelin[7], Matias Kirst[1], Leandro Neves[13], Jill Wegrzyn[14], C. Dana Nelson[15], John M. Davis[16]

[1]School of Forest Resources and Conservation, University of Florida, Gainesville, FL; [2]USDA Forest Service, Southern Research Station, Gainesville, FL; [3]Purdue University, West Lafayette, IN, USA 47907; [4]retired Southern Institute of Forest Genetics, USDA Forest Service, Southern Research Station, Saucier, MS; [5]Research School of Biology, Australian National University, Canberra, ACT 0200, AU; [6]Joint Genome Institute, US DOE, Walnut Creek, CA; [7]Department of Forest and Conservation Sciences, University of British Columbia, Vancouver, BC, CA; [8]Indiana University School of Medicine, Bloomington, IN; [9]Department of Plant Pathology, University of Arkansas, Fayetteville, AR; [10]10X Genomics, Pleasanton, CA; [11]Pacific Biosciences, Menlo Park, CA; [12]Department of Human Genetics, School of Medicine, University of Utah, Salt Lake City, UT; [13]Rapid Genomics, Gainesville, FL; [14]Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs, CT; [15]Forest Health Research and Education Center, USDA Forest Service, Southern Research Station, Lexington, KY; [16]IFAS, University of Florida, Gainesville, FL

Fusiform rust is a disease incited by the fungus *Cronartium quercuum f.sp. fusiforme* (*Cqf*) on southern pines (where it causes galls on stems and branches) and on oaks (where it causes minimal leaf damage). Fusiform rust is a major disease threat to the timber industry in the US. Rust galls cause yield losses that exceed US$100M/year. A high priority for breeders and forest managers is to identify candidate resistance genes in loblolly pine (*Pinus taeda* L.; *P. taeda*) and avirulence genes in *Cqf*. However, identifying the specific loci that regulate phenotypic traits in conifers is a major undertaking because of their very large genomes. During the process of annotating the genome of *P. taeda*, an expressed sequence tag (EST) was identified that contains a single nucleotide polymorphism (SNP) mapping to Fusiform rust resistance locus 1 (*Fr1*), which interacts with the *Cqf* gene, Avirulence locus 1 (*Avr1*). This EST aligns to a transcript from RNA-sequencing data and a TIR-NB-LRR protein, thus identifying it as a candidate *Fr1* gene. Here we present the results of work mapping *Fr1* in the *Pinus taeda* genome and *Avr1* in the *Cronartium quercuum* genome. We conducted bulk segregant analysis of next-generation sequence data from both host and pathogen. In pine, half-sibling progeny from a resistant mother were phenotyped as either resistant or susceptible to *Cqf*. These progeny were sequenced with a custom sequence-capture method targeting a genomic region linked to resistance by prior work. In *Cqf*, analysis of whole-genome sequence of rust grown on resistant or susceptible seedlings identified a 200kbp region containing several likely effector proteins. By identifying candidates for an interacting avirulence and resistance gene pair in this conifer-rust pathosystem, we will discover markers that will guide breeding and deployment of resistant pine.

QTL MAPPING BLIGHT RESISTANCE IN CHINESE X AMERICAN CHESTNUT HYBRID FAMILIES

Shenghua Fan[1], Laura L. Georgi[2], Frederick V. Hebard[3], Tatyana Zhenbentyayeva[4], John E. Carlson[4], Albert G. Abbott[1,4], C. Dana Nelson[1,5]

[1] Forest Health Research and Education Center, University of Kentucky, Lexington, KY; [2] Forest Health Research and Education Center, Meadowview, VA; [3] The American Chestnut Foundation, Meadowview, VA; [4] Schatz Center for Tree Molecular Genetics, Pennsylvania State University, University Park, PA; [5] Southern Institute of Forest Genetics, USDA Forest Service, Southern Research Station, Saucier, MS

Chestnut blight (caused by *Cryphonectria parasitica*, *Cp*) and Phytophthora root rot (caused by *Phytophthora cinnomomi*, *Pc*) have nearly extirpated American chestnut throughout its native range in eastern North America. We have mapped QTLs for resistance to both pathogens using several control-pollinated hybrid families (American and Chinese chestnuts) scored in artificial inoculation experiments. Results for *Pc* resistance will be discussed in a separate presentation. For *Cp* we implemented a genome wide association (GWAS) analysis using multiple backcross families and one F_2 family that were derived from three unrelated Chinese chestnut donor trees. Adding to the complexity of the genetic materials, the inoculation experiments were run in different years, such that years were confounded, but with standard protocols and the same two pathogen isolates across years. Standardizing resistance phenotypic data for each isolate and each inoculation experiment enabled an across-families GWAS. Results from the GWAS identified several resistance-associated QTLs. All but one of these QTL (on LG_B) were isolate specific. Thus it appears that *Cp* resistance is relatively complex due in part to important interactions with pathogen isolates. We recommend that breeding programs consider this apparent pathogenic variability in their screening and deployment activities.

PINE-BREED: OPTIMAL MATE SELECTION IN *PINUS TAEDA*

Khushi Goda[1], Fikret Isik[2]

[1]NC State University Cooperative Tree Improvement Program, Genetics Program, North Carolina State University, Raleigh, NC; [2]NC State University Cooperative Tree Improvement Program, North Carolina State University, Department of Forestry and Environmental Resources, Raleigh, NC

Loblolly pine (*Pinus taeda*) is the most important tree crop in the US, planted over 25 million acres in the south. The Tree Improvement Program at North Carolina State University manages the genetic improvement of Loblolly pine. Loblolly pine has a high genetic load and suffers greatly from inbreeding depression. It is a challenge to balance two important but contrasting goals of capturing as much genetic gain as possible and managing short- and long- term inbreeding. While methods and algorithms for breeding of several other types of trees have been improving, an efficient algorithm suited to this species remains elusive. Developing an algorithm to design mating that optimizes genetic gain whilst putting constraints on relatedness is imperative for loblolly pine breeding. Towards this goal, we have adopted mate selection algorithms commonly used in animal breeding. Pine-breed is an optimization algorithm developed that can utilize pedigree-based relationships to create optimal mating list for breeding. Modified second-order cone programming and differential evolution algorithms have been applied to create mating lists that can be realized to give maximum return of genetic gain in future progeny while minimizing the increase in average co-ancestry in the population. The completion of this study will see the development of a suite of software that is able to not only utilize genetic relationships from pedigree but also utilize genomic relationships derived from SNP markers. The framework and methods adapted for loblolly pine breeding have relevance to breeding of other monoecies species as well.

EFFECTS OF POLLINATION BAGS ON FLOWER DEVELOPMENT AND COLD DAMAGE IN A LOBLOLLY PINE SEED ORCHARD

Austin Heine[1], Fikret Isik[1], Steve McKeand[1], Trevor Walker[1]

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

The NCSU Cooperative Tree Improvement Program and its members installed the third round of prototype pollination bags for the ongoing PBS Pollination Bag Study in spring 2017. The installation included an open-pollinated control, a kraft paper pollination bag with support wire, the previously tested PBS-A bag, and two new prototypes from PBS International (bags A2 and I2). At the NC Forest Service seed orchard in Goldsboro, NC bags were installed on March 6 just prior to female strobili opening. Temperature recorders were installed into all bags and one open-pollinated branch per tree. Eight days after bagging and just prior to the arrival of a significant cold front, flower development was checked to see if variation existed in flower stage. Bag type PBS-I2 had a significantly higher proportion of flowers with female strobili that had not yet opened compared with all other treatments which were further developed. Two days later there was an extreme cold event (low of -6.5°C/20°F) for two nights. The flower survival at time of bag removal was assessed on April 5, 2017. The bag main effect was highly significant ($Pr<0.0001$) for flower survival. Female strobili that were bagged using PBS-I2 were approximately twice as likely to survive to bag removal than strobili bagged using the kraft paper bag with support wire. Open-pollinated strobili were approximately three times more likely to survive than flowers bagged using the kraft paper bag with support wire. Bag type PBS-I2 slowed the development of flowers compared to the other bag types, which was likely the reason for the higher survival of flowers in bag type PBS-I2 at time of bag removal. Heat sums were calculated from the temperature data, but these did not completely explain the reduced flower development in PBS-I2. Open-pollinated strobili were superior for flower survival when compared to all bag types.

A BRIEF HISTORY OF SOME SHORTLEAF PINE PROGENY TESTS IN THE OUACHITA AND OZARK NATIONAL FORESTS

Shaik M. Hossain[1], Don C. Bragg[2], Virginia L. McDaniel[3], Barbara Crane[4]

[1]USDA Forest Service, Southern Research Station, ORISE Program, Monticello, AR; [1]USDA Forest Service, Southern Research Station, Monticello, AR; [3]USDA Forest Service, Southern Research Station, Hot Springs, AR; [3]USDA Forest Service, Southern Region, Atlanta, GA

The considerable economic value and recent rapid and range-wide decline of shortleaf pine (*Pinus echinata*) have increased interest in this species for tree improvement work. The USDA Forest Service has studied the genetics and improvement of shortleaf pine since at least the 1950s. For instance, the cooperative Southwide Southern Pine Seed Source Study included shortleaf amongst other major southern pines in a comparison of the performance of different geographic sources. In the 1960s, a formal tree improvement effort used breeding material from 50 superior trees from 12 geographic sources, including three from Arkansas and Oklahoma. This effort led to the establishment of five shortleaf pine first generation seed orchards in the 1970s in which full-sib families were developed. Intended to help support the Forest Service's silvicultural program at that time, scores of shortleaf pine progeny tests were installed between 1978 and 1990 on national forests using seedlings from these families. As a part of this effort, 84 shortleaf progeny tests were established based on 33 full-sib families grown in the Mt. Ida Seed Orchard using three local geographic seed source regions. Following analysis of early results, selections were made from these progeny tests to establish second generation seed orchards in the 1980s. However, a shift in agency management priorities led to most of these plantings being abandoned in the 1990s. Recent interest in large-scale planting of shortleaf pine has renewed investigations into the Ouachita and Ozark progeny tests that remain in good condition. We hope that these investigations can help guide the Forest Service towards more informed management decisions about the quality of available shortleaf pine families for today's conservation needs and future tree improvement programs.

SNP DISCOVERY IN TROPICAL AND SUBTROPICAL PINES USING REDUCED REPRESENTATION SEQUENCING METHODS

Colin Jackson[1], Nanette Christie[2], Madison Caballero[3], Melissa Reynolds[2], Christopher Marais[2], Erik A. Visser[2], Sanushka Naidoo[2], Gary R. Hodge[1], Ross Whetten[1], Fikret Isik[1], Juan J. Acosta[1], Jill L. Wegrzyn[3], Alexander A. Myburg[2]

[1]Department of Forestry and Environmental Resources, North Carolina State University, Raleigh, NC; [2]Department of Biochemistry, Genetics and Microbiology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa; [3]Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs, CT

This study performs SNP discovery and characterization using RNA-seq and targeted sequencing for use in developing a high throughput genotyping assay for tropical and subtropical pine species. Targeted sequencing was performed on six species of pine: *Pinus patula, Pinus tecunumanii, Pinus oocarpa, Pinus greggii*, *Pinus caribaea* and *Pinus maximinoi*. Sequence data was generated from a custom set of 40K capture probes (RAPiD Genomics Gainesville, FL) of which 30K were designed from single copy locations in v2.01 of the *Pinus taeda* genome assembly and 10K were designed from the P. *tecunumannii* and P. *patula* transcriptome assemblies. A total of 81 pooled samples were sequenced among the six species. The 81 pools represented between 4-8 trees from a single provenance and covered the natural ranges of the species in Mexico and Central America. Target sequencing generated between 3.1 and 7.7 million reads per pool with coverage of 20-30X across capture regions. Approximately1.1 million SNPs were detected in at least two of the 81 provenances, of which 403K are shared among most species. RNA-seq data was generated for the species mentioned above minus P. *caribaea*. Pooled RNA was isolated from shoot tissue of between 8-16 seedlings from two or more families per species. Paired end sequencing generated between 29.4 and 67.7 million raw reads per pool. Reads were trimmed and mapped to each species' respective transcriptome assemblies. SNP detection yielded between 426K and 1.3M SNPs per species. SNP probe design resulted in 1.8 million candidate probes designed between species and across platforms. The probes generated from each dataset were further assessed for unique vs. repetitive mapping against the v2.01 P. *taeda* genome assembly and similarity across species. Assessment of RNA-seq derived probes showed a large proportion of probes being unique to a given species with few being shared between. Approximately 53% of probes mapped to a unique location in the reference assembly. Target capture derived probes showed a larger proportion of probes being shared across species with greater than 80% of probes mapping to a unique location. From these 1.8 million probes, 323K RNA-seq and 121K target capture derived probes were selected for assessment on a screening array. Currently, 480 samples have been submitted for genotyping.

GENETIC ANALYSIS OF REGIONAL MULTI-ENVIRONMENT TRIALS OF *PINUS TAEDA* REVEALS FAMILY LEVEL PATTERNS OF GEOTYPE-BY-ENVIRONMENT INTERACTION

Edwin Lauer[1], Andrew Simms[2], Fikret Isik[3]

[1]NC State University Cooperative Tree Improvement Program, Raleigh NC;
[2]University of Florida Cooperative Forest Genetics Research Program, Gainesville, FL;
[3]Department of Forestry and Environmental Resources, NC State University
Cooperative Tree Improvement Program, Raleigh NC

Loblolly pine (*Pinus taeda* L.) is the predominant plantation timber species in the southeast United States, covering more than 80m hectares of plantation forests from east Texas to southern Virginia. This range encompasses a wide array of environmental conditions, such as a gradient of minimum winter temperatures, climatological differences between coastal and continental regions, and a large variety of different soil types. For this reason, growers of loblolly pine have environmental adaptability as one of their primary concerns when choosing genotypes to deploy in their production area. In order to understand the genetic potential of loblolly pine genotypes in breeding programs, genetic tests are conducted in multiple environments representing, as accurately as possible, the range of potential environments in which they will be deployed. In this report, genetic analysis was conducted on a multi-environment trial dataset with 324 half-sib families planted in five test series across thirty-eight locations ranging from southeast Mississippi to northeastern North Carolina. These test locations span a range of latitude from 35.5N to 29.7N, and a range of mean minimum winter temperatures from to -11.5C to -5.1C. Each series represented a group of tests established with the same genetic entries, but tests in different series were highly unbalanced. Out of (329 x 38)=12502 possible genotype-by-environment combinations, 2640 were present in the data. To account for this imbalance, a hierarchical analytical approach was undertaken. Single test series were analyzed independently using factor analytic mixed models in order to estimate genetic correlations and variance components, followed by a combined analysis in which the parameters estimated from the first step were held fixed. In the combined analysis, the type B genetic correlation between test sites in different series was fixed at a global value estimated as the average of the among-site correlations from the first step. Traits measured included tree height, volume, and stem form. The average type B genetic correlation for height, volume, and stem form were 0.73, 0.64, and 0.78 respectively, indicating GxE was more important for volume than for height or stem form. In the combined analysis, the inclusion of a nested parent-by-site mean minimum temperature interaction effect significantly improved model fit. These results contribute to a more granular understanding of the magnitude of genotype-by-environment interaction in loblolly pine, as well as the relationship between geographic origin and genotype performance over a wide environmental range.

RESTORING GREEN ASH (*FRAXINUS PENNSYLVANICA*): BREEDING FOR RESISTANCE TO THE EMERALD ASH BORER (*AGRILUS PLANIPENNIS)*

Jennifer L. Koch[1], David W. Carey[1], **Mary E. Mason[2]**, Therese M. Poland[3], Kathleen S. Knight[1], Jeanne Romero-Severson[4], Charles Tubesing[5], Roger Gettig[5]

[1]Northern Research Station, USDA Forest Service, Delaware, OH; [2]Department of Evolution, Ecology and Organismal Biology, The Ohio State University, Columbus, OH; [3]Northern Research Station, USDA Forest Service, East Lansing, MI; [6]Department of Biological Sciences, University of Notre Dame, Notre Dame, IN; [5]The Holden Arboretum, Kirtland, OH

The invasion of emerald ash borer (*Agrilus planipennis*) threatens the survival of green ash (*Fraxinus pennsylvanica*) in the United States. Green ash is extensively used for soil conservation, rural water management, urban green spaces, and is a common component mixed hardwood forest, especially riparian and swamp hardwoods forest. Initial selection of green ash is from long term monitoring plots in EAB infested natural forests where surviving, or "lingering" ash trees, are those trees with DBH greater than 10cm and healthy canopies for at least two years after all other ash trees have died. Lingering ash are grafted and ramets are tested in EAB egg bioassay experiments, which have confirmed that these trees possess an increased level of resistance due to multiple types of host defense responses. Lingering ash phenotypes include increased mortality (defense killing) of early instar larvae, development of larvae having significantly lower weights, and reduced adult feeding preference of foliage. Replicated field test are also being installed for the first 42 green lingering ash to be accessioned and complete bioassay. Field test will allow identification of additional phenotypes potentially related to adult EAB preference and correlation of bioassay and field results. Lingering ash that perform well in bioassays are being used as parents to produce control cross families to directly investigate inheritance of EAB resistance. To date we have produced and are testing 16 full-sibling families utilizing 8 female trees and 15 male trees bred in a systematic cross design. Additional families will be added as select parent trees begin to flower. Bioassay evaluation of seedling progeny from 7 lingering x lingering families demonstrate variation both within and between families. Between 15 to 40% of lingering x lingering progeny had a more effective defensive response to EAB than either parent suggesting a polygenic or quantitative model of inheritance. Progeny (including re-sprouted bioassay seedlings) are also being evaluated in field trials being planted over the next 2-5 years. Polycross seed orchards will be installed using the best lingering ash parents based upon parental and progeny phenotypes. In addition, progeny test plantings may be rouged by selecting both the best families and individuals within families to produce seedling orchards if results suggest genetic gains will be sufficient.

COMBINING BREEDING, SOMATIC EMBRYOGENESIS AND CRYOSTORAGE TO CREATE EMERALD ASH BORER-RESISTANT ASH VARIETALS

Scott A. Merkle[1], Jennifer L. Koch[2], A. Ryan Tull[1], David W. Carey[2], Paul M. Montello[1], Brittany F. Barnes[1], Logan T. House[1], Kira R. Eidle[1], Daniel A. Herms[3], Kamal J.K. Gandhi[1]

[1]Warnell School of Forestry and Natural Resources, University of Georgia, Athens, GA; [2]USDA Forest Service, Northern Research Station, Delaware, OH; [3]Davey Tree Expert Company, Kent, OH

Emerald ash borer (*Agrilus planipennis*; EAB) has devastated populations of ash trees in at least 20 U.S. states and Canada over the past few decades. To date, control measures have had minimal impact on halting the infestation. However, there is evidence of genetic resistance or tolerance to EAB in natural populations of white ash (*Fraxinus americana*) and green ash (*Fraxinus pennsylvanica*) trees, as demonstrated by the continued survival of scattered trees for more than five years following infestations that killed over 99% of the trees. These "lingering" or "surviving" ash individuals may form the basis for reforestation programs in EAB-impacted areas, if these genotypes or the best of their progeny can be clonally mass-propagated. Over the past five years, we have initiated embryogenic cultures by culturing immature zygotic embryos from open-pollinated (OP) seeds collected from several surviving white ash and green ash trees in Michigan, Minnesota, Pennsylvania, Virginia and North Carolina. In addition, in 2018, we initiated cultures from crosses made between lingering green ash parents by USDA Forest Service personnel in Delaware, Ohio. Somatic embryos were produced by growing cultures in liquid suspension, followed by fractionation and plating on semisolid medium. Somatic embryo germination and conversion were enhanced by a combination of pre-germination cold treatment and addition of gibberellic acid to the germination medium. Ash somatic seedlings derived from OP explants grew rapidly following transfer to potting mix and 42 somatic seedlings representing 10 ash clones were acclimatized, grown in the greenhouse and planted in a preliminary field test, along with EAB-resistant (*Fraxinus mandshurica*) and EAB-susceptible control seedlings. Somatic seedling production is underway from two cultures that originated from seeds derived from a cross between lingering ash parents. An ex vitro germination protocol to accelerate plantlet production and a cryostorage protocol for the ash embryogenic cultures are under development.

NEW SEED COLLECTION ZONES FOR THE EASTERN UNITED STATES

Carolyn C. Pike[1], Barbara Crane[2], Paul Berrang[3]

[1]State and Private Forestry, Eastern Region, USDA Forest Service, West Lafayette, IN; [2]Southern Region, USDA Forest Service, Atlanta, GA; [3]Eastern Region, USDA Forest Service, Milwaukee, WI

The USDA Forest Service was assigned the task of developing seed collection zones (herein referred to as seed zones) for the Eastern and Southern Regions of the US, in collaboration with States, NGOs, nursery and seed industry representatives. Various seed zone guidelines exist, but either are not standardized, or are limited to state boundaries. Standardized seed zones are needed to define the origin of seed across Federal, State, and Private land ownerships. We created new seed zones by combining Plant Hardiness Zones (PHZ), with Ecological Provinces (EP). EP define broad landscape-level characteristics including elevation, soils, and other features that may impact the genetic structure of plant populations. In the eastern US, counties are generally of small stature and were utilized as the minimum administrative area to define a seed zone. A first draft was created by snapping PHZ's and EP's to county lines, and then these layers were overlaid. Seed collection zones were defined as areas with common PHZ and EP. Some resulting seed zones were revised by the project committee to reduce heterogeneity on the map. Both maps, the original and revised, are freely available online at http://www.easternseedzones.com/. In addition, a downloadable list of counties, and the seed zone to which they are assigned, will be posted to this website. The over-arching goal of this project is to develop a standardized system to define seed origin for all taxa that can be used in accordance with existing seed transfer guidelines and seed transfer tools.

MOLECULAR GENETICS AND THE EFFORT TO CONSERVE SOUTHERN HEMLOCKS DECIMATED BY HEMLOCK WOOLLY ADELGID

Kevin M. Potter[1], Robert M. Jetton[2], Lia Campbell[1], John M. Hastings[1], Sedley A. Josserand[3], Valerie D. Hipkins[4], C. Dana Nelson[5], William S. Dvorak[2]

[1]Department of Forestry and Environmental Resources, North Carolina State University, Research Triangle Park, NC; [2]Camcore, Department of Forestry and Environmental Resources, North Carolina State University, Raleigh, NC; [3] Biological Technician, Southern Research Station, USDA, Forest Service, Saucier, MS, USA, 39574; [4]Pacific Southwest Research Station, USDA Forest Service, Placerville, CA; [5]Forest Health Research and Education Center, Southern Research Station, USDA Forest Service, Lexington, KY

An exotic insect is decimating populations of the two hemlock species native to the eastern United States, eastern hemlock (*Tsuga canadensis*), a widespread and ecologically important tree species, and Carolina hemlock (*T. caroliniana*), a rare species endemic to the Southern Appalachian Mountains. The insect pest is hemlock woolly adelgid (*Adelges tsugae*), which was brought to the eastern United States from southern Japan in the early to mid-twentieth century. Both hemlock species have been the focus of long-term cooperative efforts between the United States Forest Service and the Camcore conservation cooperative at North Carolina State University to conserve representative seed collections. To help guide these *ex situ* gene conservation efforts, we employed rangewide microsatellite molecular marker studies of both species to identify locations of high and low genetic variation, evaluate genetic variation in peripheral disjunct and core range populations, and assess regional patterns in genetic diversity to better understand their phylogeographic history. The results for eastern hemlock demonstrated widespread inbreeding and found that peripheral disjunct populations are less genetically diverse than main-range populations, but that some are highly genetically differentiated or contain unique alleles. Bayesian clustering analyses suggested that three or four Pleistocene glacial refuges may have existed in the Southeastern United States, with a main postglacial movement from one of those refuges into the North. The results for Carolina hemlock discovered that its populations are extremely inbred and surprisingly highly differentiated from each other, with most populations containing at least one unique allele. This level of differentiation, most likely the result of very low interpopulation gene flow, is unprecedented for a North American conifer. These findings have been employed in analyses to prioritize populations for additional conservation seed collections from both eastern hemlock and Carolina hemlock.

PRIORITIZING THE CONSERVATION NEEDS OF U.S. TREE SPECIES: EVALUATING VULNERABILITY TO FOREST PESTS

Kevin M. Potter[1], Maria E. Escanferla[2], Robert M. Jetton[3], Barbara S. Crane[4]

[1]Department of Forestry and Environmental Resources, North Carolina State University, Research Triangle Park, NC; [2]Department of Forestry and Environmental Resources, North Carolina State University, Raleigh, NC; [3]Camcore, Department of Forestry and Environmental Resources, North Carolina State University, Raleigh, NC; [4]Southern Region, National Forest System, USDA Forest Service, Atlanta, GA

Insect and disease infestations pose the most serious threat to several North American forest tree species. Scientists and managers from throughout the United States Forest Service have cooperated to develop a conservation priority-setting framework for forest tree species at risk from insects and disease and other threats. The Project CAPTURE (Conservation Assessment and Prioritization of Forest Trees Under Risk of Extirpation) framework is data-driven and guided by expert opinion, allowing the quantitative grouping of species into vulnerability classes that may require different management and conservation strategies. We applied this framework to categorize and prioritize 419 native North American tree species for conservation, monitoring, and management, based on trait data and insect and disease threat data for each host tree species. The categorization is based on vulnerability factors relating to each tree species' (1) insect and disease threat severity, (2) sensitivity to insect and disease infestation, and (3) capacity to adapt to insect and disease infestation. We used K-means clustering to group species into 11 classes based on these three vulnerability dimensions. The three most vulnerable classes encompassed 15 species which require the most immediate conservation intervention. Two additional classes face less severe insect and disease threats and may be good candidates for resistance breeding efforts. Other groups had traits associated with high sensitivity and/or low adaptive capacity to potential future insect and disease threats, suggesting that these species need close monitoring. This assessment tool should be valuable for decision-makers determining which species and populations to target for monitoring efforts and for pro-active gene conservation and management activities. Several Southern tree species were among those identified as those needing immediate conservation intervention, including Florida torreya (*Torreya taxifolia*), Allegheny chinquapin (*Castanea pumila*), pumpkin ash (*Fraxinus profunda*), Carolina ash (*F. caroliniana*), redbay (*Persea borbonia*), Carolina hemlock (*Tsuga caroliniana*), eastern hemlock (*T. canadensis*), and butternut (*Juglans cinerea*). Additionally, the Project CAPTURE framework was used to help identify a short list of imperiled tree species as potential targets for Forest.Health, **a focused, high intensity intervention U.S.-Canadian effort that could apply biotechnology applications such as** genome sequencing and bioinformatics, population genotyping for breeding, and transformation of native genotypes with resistance genes**.**

ASSESSING LONG-TERM IMPLICATIONS FOR DISEASE RESISTANCE SCREENING IN PINES: GROWTH RESPONSE AND VIRULENCE OF THE PITCH CANKER *FUSARIUM CIRCINATUM* IN A CHANGING CLIMATE

Tania Quesada[1], Sunny Lucas[2], Katherine Smith[3], Kathleen McKeever[2], Gary Peter[4], Jason Smith[1]

[1]School of Forest Resources and Conservation, University of Florida, Gainesville, FL; [2]USDA Forest Service, Resistance Screening Center, Asheville, NC; [3]USDA Forest Service, Southern Research Station, Gainesville, FL; [4]School of Forest Resources and Conservation, Genetics Institute, University of Florida, Gainesville, FL

The fungus that causes pitch canker disease, *Fusarium circinatum* has caused recent outbreaks on southeastern U.S. pine plantations. This has resulted in significant economic losses to the timber industry. The intensification of these outbreaks is unknown but may be related to changes in climate. As our climate continues to warm, it is important to anticipate the effects of this disease under future environmental conditions and to develop strategies for obtaining trees resistant to current and future variants of *F. circinatum*. We tested fifteen *F. circinatum* isolates collected from Florida and Georgia and evaluated their growth response in culture at 25, 28, and 31°C. We also evaluated the sporulation and pathogenicity of a subset of these isolates on loblolly (*Pinus taeda*) and slash pine (*Pinus elliottii*) open-pollinated families at the USDA Forest Service Resistance Screening Center (RSC). Our results showed overall slower mycelium growth at 31°C, although a small number of isolates showed no significant growth between 25°C and 28°C. These isolates could potentially be better adapted to warmer climate conditions and need further assessment. Furthermore, some of the newly-collected isolates showed higher virulence than those routinely used at the RSC for screening. The RSC is a public institution created to screen seedlings of pine and other tree species for genetically-controlled tolerance to different diseases, including pitch canker and provides services to numerous private and public institutions for over 40 years. As a result of this study, the RSC is now using these new *F. circinatum* isolates in their operational screening program. We are now working together to develop a long-term plan to include material from the entire geographic range of loblolly and slash pine and to periodically renew the pathogen collection used in the RSC's screening tests.

COMPARING AND VALIDATING ASSOCIATION MAPPING FOR DISEASE RESISTANCE IN TWO LOBLOLLY PINE POPULATIONS USING INCREASED MARKER COVERAGE

Tania Quesada[1], Gary F. Peter[2]

[1]School of Forest Resources and Conservation University of Florida, Gainesville, FL;[2]School of Forest Resources and Conservation, Genetics Institute, University of Florida, Gainesville, FL

Association genetics is a powerful approach to identify markers in genes related to traits of interest. Association genetic analyses were used loblolly pine to identify single nucleotide polymorphisms (SNPs) significantly associated with fusiform rust and pitch canker resistance in two populations: an association population composed of over 400 largely unrelated individuals (ADEPT2 population), and a population with known structure composed of 71 full-sib families derived from a circular diallel mating design with 41 parents (CCLONES - Comparing Clonal Lines on Experimental Sites). We conducted association tests between over two million polymorphic SNPs in the ADEPT 2 population and over 67,000 SNPs in the CCLONES population to identify candidate genes for pitch canker and fusiform rust resistance. Analyses were done using PLINK, an open-source toolset for whole-genome association and population-based analyses. For pitch canker resistance phenotypic data, obtained by measuring lesion length, we detected eight significant SNPs in the ADEPT 2 population at 90% confidence using PLINK. This number was similar to previously published data obtained the platform BAMD. However, when used in the CCLONES population, which has known structure, we obtained 1,695 significant SNPs at 90% confidence. For fusiform rust, the number of significant SNPs varied between two and 499 at 90% confidence, depending on whether gall score (presence/absence - a binary trait) or gall length (a quantitative trait) were used as phenotypic data. Gall score showed more differences in the number of significant SNPs obtained using different platforms (PLINK and BAMD). However, no overlap was observed between the SNPs significant for pitch canker or rust across the CCLONES and ADEPT2 populations; however, whether these significant SNPs are in linkage disequilibrium remains to be determined. Future steps involve the annotation of such significant SNPs and validation across platforms.

BREEDING AND SELECTION OF FAST-GROWTH HARDWOOD SPECIES IN THE SOUTH

Randall J. Rousseau[1]

[1]Department of Forestry, College of Forest Resources, Mississippi State, MS

Efforts in fast-growth hardwood species in the South reached its height from the early 1960's to the end of the 1980's. In particular the U.S. Forest Service program in eastern cottonwood not only led this effort but supplied numerous companies along the Lower Mississippi River with selected genotypes. Additionally, this effort was able to share eastern cottonwood clones on a world-wide basis resulting in many of these genotypes serving as the basis in hybridization programs that are still production today. In the South, only a few industrial eastern cottonwood programs, such as Westvaco and Crown Vantage continued *Populus* improvement effort due to the exceptional production from their breeding and selection programs. However, by the turn of the century and the shift from vertically integrated companies to TIMOs and REITs halted most of the research and development programs. Unfortunately much of the improved fast-growth hardwood material was loss due a variety of reasons including insufficient funds available for preservation or only limited number of genotypes transferred to either academia or other entities such as ArborGen. The current interest in lignocellulosic production has focused on *Populus* for the production of renewable jet fuel and co-products such as carbon fiber. Efforts in breeding of selected 1st and 2nd-Generation selections of eastern cottonwood was restarted along with a greater emphasis on clonal testing of a variety of *Populus* hybrid taxa. Like *Populus*, *Salix* is also being examined for biomass production but on poorly drained agricultural sites that are unsuitable for *Populus* production. The one species that had seemingly been forgotten was American sycamore. This loss of interest was due to severe disease problems exhibited in a numerous plantations along the Atlantic and Gulf coastal areas. The possibility of using this species in the bioenergy field resulted from a small control-pollinated test in 1997 that provided the insight into the viability of breeding resistant genotypes.

EVALUATING SLASH PINE SEED SOURCES FOR USE IN RESTORATION OF A BARRIER ISLAND

Ronald C. Schmidtling[1], C. Dana Nelson[1,2]

[1]USDA Forest Service, Southern Institute of Forest Genetics, Saucier MS; [2]Forest Health Research and Education Center, USDA Forest Service, Southern Research Station, Lexington, KY

Many barrier islands in the northern Gulf of Mexico were severely damaged by Hurricane Katrina in 2005. Now that restoration of the islands is being considered, questions arose about suitable seed sources for replanting of the native slash pine (*Pinus elliottii)*. Would seedlings obtained from local nurseries be well enough adapted to the pure-sand soil and the occasional inundation with salt water, or should seed be collected from barrier islands? We installed a small test planting on Deer Island, a near-shore barrier island off of Biloxi, Mississippi that was severely damaged. The western end recovered well with successful natural regeneration of Slash Pine and Live Oak (*Quercus virginiana)*. Our experimental planting to evaluate seed source differences was installed on the nearly treeless eastern end. Seedlings from half-sib families of 12 Deer Island mother trees and 38 north Harrison County mother trees (30 miles north of the Coast) were planted in January 2017. Survival in the fall of 2017 averaged 46%, with little differences among sources. A storm in summer of 2018 resulted in flooding of the planting with brackish water. Subsequent survival in early 2019 averaged 27% in the Deer Island source and 32% in the north Harrison source. Height measured in early 2019 averaged 7.8 ft. for the Deer Island source vs 8.0 ft. for the north Harrison source, a non-significant difference. Differences among families within sources were highly significant for height, however. At this early point in the study, it does not appear that collecting seed from island sources will be necessary. However, it does appear that selection of families for growth on the islands could be productive.

CORRESPONDENCE BETWEEN BREEDING VALUES OF THE SAME LOBLOLLY PINE GENOTYPES FROM CLONAL TRIALS AND HALF-SIB SEEDLING PROGENY TESTS

Nasir Shalizi[1], Fikret Isik[1], Salvador Gezan[2], Steve McKeand[1]

[1]Cooperative Tree Improvement Program, Department of Forestry and Environmental Resources, North Carolina State University, Raleigh, NC; [2]School of Forest Resources and Conservation, University of Florida, Gainesville, FL

In loblolly pine genetic trials, we expect that if seedlings are cloned, selection within families would be more efficient than selecting seedling progeny. In this study, 65 loblolly pine clones from 32 crosses were tested at four locations in the southern US. About seven copies of each clone were tested at each site. In order to compare the breeding values of 65 clones based on clonal tests with seedling progeny, about 40 wind-pollinated seedlings (half-sib progeny) of each the same 65 clones were later tested at four different location. For both the clonal trials and the progeny trials, tree height, diameter at breast height, and the incidence of fusiform rust disease caused by *Cronartium quercuum* f. sp. *fusiforme* were assessed at age four years. Individual-tree models were fit to partition observed phenotypic variance into genetic and environment effects. Breeding values of the same 65 genotypes were estimated both from the clonal trials and from half-sib progeny tests. Clones and their half-sib progeny did not differ for mean height and volume growth. However, fusiform rust disease differed between clones and their half-sib seedlings. On average half-sib seedlings had 23% more fusiform rust disease incidence than clones. For growth traits, the additive genetic variance based on cloned genetic tests was about 2.5 times greater than based on their half-sib progeny test. For fusiform rust incidence, the additive genetic variance was even greater (~6 times) for cloned tests compared to their half-sib progeny tests. Family-mean heritability estimates were 0.88, 0.82 and 0.95 for height, volume and fusiform rust incidence, respectively. Clone-mean heritability estimates were slightly higher than family mean heritability estimates. Correlations between breeding values of 65 genotypes obtained from clonal trials and seedling half-sib progeny were about 0.59, suggesting that the correspondence between breeding values from clonal tests is moderate for both growth and fusiform rust disease incidence in loblolly pine.

DEVELOPING HOST RESISTANCE TO THE HEMLOCK WOOLLY ADELGID

Ben C. Smith[1], Fred P. Hain[2]

[1]Forest Restoration Alliance, Department of Entomology and Plant Pathology, North Carolina State University, Waynesville, NC; [2]Forest Restoration Alliance, Department of Entomology and Plant Pathology, North Carolina State University, Raleigh, NC

The hemlock woolly adelgid (HWA; *Adelges tsugae*), an invasive exotic insect, has caused widespread decline and mortality in the two hemlock species native to the eastern US, eastern hemlock (*Tsuga canadensis*) and Carolina hemlock (*T. caroliniana*). Eastern hemlock, broadly distributed from northern Georgia northward into Canada, was ecologically important as a foundation species, and economically important in regions as a nursery crop, enjoying widespread use as a landscape and ornamental tree due to its shade-tolerance, ability to hedge, and availability of numerous cultivars. The Forest Restoration Alliance (FRA) seeks to produce hemlocks resistant to or tolerant of HWA that will be suitable for both species restoration and ornamental uses. FRA strategies include identification of resistant and tolerant genotypes through screening from our native hemlocks for inclusion in a resistance breeding program, and creation of interspecific hybrids between native and HWA-resistant or tolerant exotic hemlocks, including Chinese (*T. chinensis*), northern Japanese (*T. diversifolia*), and southern Japanese (*T. sieboldii*) hemlocks. We are currently testing 26 genotypes from native populations of eastern and Carolina hemlock with potential for resistance using artificial infestation with HWA, with infested and non-infested treatments. Stem cuttings from each of the genotypes were clonally propagated by rooting in 2015, and artificially infested using a rain-down technique (Jun 2017) and through direct attachment of infested branches (April 2019). We have not yet observed statistically significant differences among clones or between potentially resistant and putatively susceptible genotypes for adelgid density. The only significant differences observed for plant growth and health have been among clones, and not between infested and not-infested treatments, and are due to propagation differences, as some clones respond better to rooting than others. The lack of significant differences due to adelgid infestation is very likely due to our difficulty so far in attaining dense adelgid infestations that persist over time. We are continuing to search for native resistance using reports from natural resources professionals and citizen scientists, utilizing the TreeSnap app that was developed for Android and iPhone mobile devices. Working with the U.S. Forest Service Southern Institute of Forest Genetics, which has developed five species-specific chloroplast DNA SSR markers, we have verified 85 hybrid genotypes produced by our program between Carolina hemlock maternal parents and Chinese and southern Japanese paternal parents. To date, we have not been able to produce any verified hybrids between eastern hemlock and any of the three Asian hemlock species above.

SLASH PINE GROWTH AND YIELD MODEL CALIBRATED TO INCORPORATE GENETICS IN PLANTATION OF SOUTHEAST UNITED STATES

Priscila A. Someda Dias[1]; Salvador A. Gezan[1]; Gary F. Peter[1], Timothy A. Martin[1]

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

Slash pine (*Pinus elliottii* Engelm.) has an important economical, social and ecological role in the southeast United States. The increased use of genetic improved material, together with more intensive silvicultural practices in slash pine stands, expands the demand for suitable growth and yield (G&Y) models to help predict conditions about forests and to plan future silvicultural managements. With the aim of bridging the gap between slash pine growth and yield model, and their need to consider silviculture and genetic specific modules, this study tests and recalibrates an existing slash pine model that considers silviculture information and updates the G&Y model to incorporate genetic specific modules of dominant height, mortality and basal area. The calibration stage begins by using existing long-term plot data from unimproved and improved genetic plots measures from ages 5 to 21 years (average 9 years). The new model was calibrated for the unimproved plots and validated with the improved plots. At a later stage, the calibrated model was modified to consider as input the genetic entry, by developing flexible genetic specific modules, such as dominant-height and mortality. Here, long-term plot-level data from different genetic entries is correlated in the simulations against its estimated breeding values, generating a system including silvicultural and genetics. This system of equations is converted into an application to predict and project G&Y of stands, with silviculture information, accepting tree measurements or stand-level data input. Our tool also includes an option to perform simulations by projections starting at the desired age from known initial stand conditions. The open source code is based on R programming with a flexible modular structure and an interactive web application build using Shiny. This tool constitutes a framework that can be extensively modified to easily construct G&Y models for other tree species with an array of dynamic web interfaces.

POSTER SESSION ABSTRACTS

POSTER SESSION ABSTRACTS (continued)

CAMCORE AND THE FOREST SERVICE NATIONAL FOREST SYSTEM: A TEN-YEAR PARTNERSHIP IN TREE CONSERVATION

Barbara Crane[1], Andy Whittier[2], Robert Jetton[2], Kevin Potter[3]

[1]USDA Forest Service Southern Region National Forest System, Atlanta, GA; [2]Camcore, Department of Forestry and Environmental Resources, North Carolina State University, Raleigh, NC, [3]Department of Forestry and Environmental Resources, North Carolina State University, Research Triangle Park, NC

Climate variabilities, increasing pest and pathogen infestations and continued land-use changes will increase the likelihood that forest trees could experience population-level extirpation or species-level extinction. Based on the 2010 Forest Tree Genetic Risk Assessment System (FORGRAS) framework, genetic risk-related attributes for 131 Southern Appalachian tree species were used to assess and rank the predisposition of forest tree species to genetic degradation. The end product was a list of trees ranked from most to least endangered or threatened to aforementioned threats. The FORGRAS framework is used by the Southern Region (R8) National Forest System (NFS) Genetics program as a tool to plan and guide tree conservation efforts. Using the list produced by FORGRAS, NFS began tree conservation collections in 2010 for some of the most highly endangered tree species in the Southern Appalachian national forests. The goal was and is to preserve forest tree genetic diversity and safeguard existing adaptedness within species. Because of limited resources, NFS enlisted Camcore as a partner to assist with gene conservation efforts. Camcore had already been working with the Forest Service Forest Health Unit in collecting Eastern and Carolina Hemlocks. Camcore's reputation for doing worldwide tree conservation work for over 35 years is renowned, thus affording the NFS an exemplary partner. To date, this partnership has focused on range wide collections of Table Mountain pine, Atlantic white-cedar, red spruce, Balsam and Fraser firs, Ash (Texas, Carolina, Pumpkin, Blue), and continuing with the hemlocks. Funding for these projects is provided by both the Washington Office Forest Health Unit as well as R8 Forest Management and Timber Unit. The seed collected is distributed for multi-purpose goals: 1/ to Camcore for research, 2/ to ARS Genetics Resources Preservation Center for long-term preservation storage and 3/ to NFS for establishing conservation banks at the seed orchards and for operational use in restoration of these species.

TREESNAP: A CITIZEN SCIENCE TOOL TO HELP OUR FORESTS

Ellen Crocker[1], Bradford Condon[2], Abdullah Almsaeed[2], Albert G. Abbott[1], C. Dana Nelson[1,3], Margaret E. Staton[2]

[1]Forest Health Research and Education Center, Department of Forestry and Natural Resources, University of Kentucky, Lexington, KY; [2]University of Tennessee, Institute of Agriculture, Knoxville, TN; [3]USDA Forest Service, Southern Research Station, Lexington, KY

Invasive diseases and pests threaten the health of forests worldwide. We created a mobile app available for iOS and Android, TreeSnap, which connects interested citizens with tree breeding and research programs to help fight these threats through both awareness and research. In the past, restoration tree breeding programs each had their own portals and requirements for submitting potential trees for inclusion in breeding programs. TreeSnap provides a unified submission gateway for citizen scientists to submit information to scientists that will meet their individual requirements. TreeSnap prompts users to take photos of trees and answer questions specified by each tree breeding programs. This information, along with GPS coordinates, is sent to the TreeSnap web database, where scientists can access it, curate, and follow up with users. Our goal is that scientists will gain data on trees to use in research programs while the public will become more engaged in and informed about forest health. Since its release in 2017, TreeSnap has attracted over 2,389 users and been used to record information about over 3,000 trees. Trees currently featured on TreeSnap with partnering research programs include American chestnut, American elm, ash, eastern larch, Florida torreya, hemlock, Pacific madrone, tanoak, and white oak.

GENETIC CONTROLS OF GROWTH TRAITS IN CHERRYBARK OAK (*QUERCUS PAGODA*) ESTIMATED WITH POST-HOC EXPERIMENTAL DESIGN ADJUSTMENTS IN ABLUP

Chen Ding[1], Benjamin Bartlett[1], Tom Byram[1], Yu-hui Weng[2], Fred Raley[1]

[1]Western Gulf Forest Tree Improvement Program, Texas AM Forest Service, College Station, TX;
[2]Arthur Temple College of Forestry and Agriculture, Stephen F. Austin State University, Nacogdoches, TX

Optimal experimental design of genetic trials provides accurate estimates of quantitative genetic parameters for tree breeders and forest managers to select elite reforestation and urban forests. Traditional randomized block design could not justify the within block environmental variances in the level that are efficient for the genetic parameter (e.g., heritability) estimation. In this study, we used a restoration and plantation species *Quercus pagoda* as an example to adjust the row-column factors in field progeny tests to improve the selection of the breeding program. Although previous genetic trials have not employed the incomplete block design, our post-hoc adjustment method could utilize the existing design information and reduce the environmental residual noise that leads to an increased estimation of genetic control.

GENETIC ANALYSES OF THE LAUREL WILT PATHOGEN, *RAFFAELEA LAURICOLA*, IN ASIA PROVIDE CLUES TO THE SOURCE OF THE CLONE CAUSING THE CURRENT USA EPIDEMIC

Tyler J. Dreaden[1], Marc A. Hughes[2], Randy C. Ploetz[3], Adam Black[4], Jason A. Smith[5]

[1]USDA Forest Service, Southern Research Station, Forest Health Research and Education Center, Lexington, KY; [2]College of Tropical Agriculture and Human Resources, University of Hawaii at Manoa, Hilo, HI; [3]Tropical Research and Education Center, University of Florida, Homestead, FL; [4]Peckerwood Garden Conservation Foundation, Hempstead, TX;[5]School of Forest Resources and Conservation, University of Florida, Gainesville, FL

Laurel wilt is caused by the fungus *Raffaelea lauricola* T.C. Harr., Fraedrich and Aghayeva, a nutritional symbiont of its vector, *Xyleborus glabratus* Eichhoff, the redbay ambrosia beetle. Both are native to Asia but were found in Georgia in the early 2000s and have since spread to much of the southeastern United States killing >300 million trees in the Lauraceae plant family. Our goals were to elucidate the genetic structure of populations of the pathogen, *R. lauricola*, examine its reproductive strategy, and investigate the number of times the pathogen was introduced to the USA. A panel of 12 simple sequence repeat (SSR) markers identified 15 multilocus genotypes (MLGs) among 59 isolates from the USA (34 isolates), Myanmar (18), Taiwan (6) and Japan (1). Limited diversity in the USA isolates and the presence of one MAT idiotype (mating type locus) indicated that *R. lauricola* was probably introduced into the USA a single time. Only three closely related MLGs were detected in the USA, the most prevalent of which (30 of 34 isolates) was also found in Taiwan. MLG diversity was far greater in Asia than the USA with isolates from Myanmar being distinct from those from Japan, Taiwan and the USA. Although both MAT idiotypes were present in Myanmar and Taiwan, only the population from Taiwan had the genetic structure of a sexually reproducing population. The present results suggest that a Taiwanese origin is possible for the population of *R. lauricola* in the USA, although more work is needed. The results highlight the need to prevent the introduction of additional genotypes and the second mating type into the USA because this could allow the pathogen to rapidly overcome the resistance that is being developed. The pathogen population should be monitored so that new genotypes can be identified and incorporated into resistance screening trials.

CYTO-MOLECULAR CHARACTERIZATION OF CHINESE CHESTNUT LINKAGE GROUP-SPECIFIC CHROMOSOMES

Nurul Islam-Faridi[1,2], Tatyana Zhebentyayeva[3], Laura L. Georgi[4], Margaret E. Staton[5], Frederick V. Hebard[4], Paul H. Sisco[6], John E. Carlson[3], Albert G. Abbott[2,8], **C. Dana Nelson[7,8]**

[1] USDA Forest Service, Southern Research Station, Southern Institute of Forest Genetics, Forest Tree Molecular Cytogenetics Laboratory, College Station, TX; [2] Department of Ecosystem Science and Management, Texas A&M University, College Station, TX; [3] Schatz Center for Tree Molecular Genetics, Pennsylvania State University, University Park, PA; [4] Meadowview Research Farms, The American Chestnut Foundation, Meadowview, VA; [5] Entomology and Plant Pathology Department, Institute of Agriculture, University of Tennessee, Knoxville, TN; [6] The American Chestnut Foundation, Asheville, NC; [7] USDA Forest Service, Southern Research Station, Southern Institute of Forest Genetics, Harrison Experimental Forest, Saucier, MS; [8] Forest Health Research and Education Center, Department of Forestry, University of Kentucky, Lexington, KY

Recent advances in molecular cytogenetics have made it possible to unequivocally identify individual chromosomes and to visualize the location of specific genes and/or molecular markers (of specific traits). We used molecular cytogenetics to validate the genome assembly using genetically mapped BAC clones. One to four BACs from each of the upper and lower end of each of the 12 linkage groups (LGs) were used in fluorescent in situ hybridization (FISH), to delineate the centromeric positions and identify the short and long arms of each LG-specific chromosome. In addition, ribosomal rDNA (45S and 5S rDNA) probes were used to identify their locations and LG-specific chromosomes. The chestnut chromosomes were found to be metacentric and sub-metacentric and individual LGs were assigned to each chromosome. The centromeric positions enabled to designate six of the 12 Chinese chestnut LG specific chromosomes (LG_A, LG_B, LG_C, LG_F, LG_G and LG_I) as metacentric and/or near metacentric, four (LG_E, LG_H, LG_J and LG_K) as near sub-metacentric and two (LG_D and LG_L) as clearly sub-metacentric chromosomes. The origination (zero cM) of each LG map was found to be associated with the short arm of nine LG specific chromosomes and the long arm of three chromosomes (LG_C, LG_G and LG_L). Since LG_C and LG_G re metacentric, we are only recommending that LG_L be corrected. The major 45S rDNA locus, was assigned to LG_H chromosome, and that part LG_H is missing from the linkage map. The 5S rDNA locus was found to be localized interstitially in the short arm of LG_E chromosome. The cytological positions (i.e., orientations) of all but three of the 54 BAC clones three were found to be concordant with their expected linkage map positions.

CHARACTERIZATION OF LIGNIN IN BOURBON BARRELS

Jarrad Gollihue[1], Justin K. Mobley [2], Tonya Morgan [3], Mark Crocker [3], Harlan Wheatley [5], Victoria G. Pook[1,6], John Ralph[4], Seth DeBolt[1,6]

Department of Horticulture, University of Kentucky, Lexington, KY; [2]Department of Chemistry, University of Kentucky, Lexington, KY; [3]Center for Applied Energy Research, University of Kentucky, Lexington, KY;1 [4] Biochemistry Department, University of Wisconsin-Madison, Madison, WI, 53706 [5] Sazerac Buffalo Trace Distillery, Frankfort, KY; James B Beam Institute for Kentucky Spirits, University of Kentucky, Lexington, KY

Kentucky Straight Bourbon whiskeys are aged for at least 2 years in newly charred barrels made from American White Oak (*Quercus alba*). Bourbon production both the grain mash that is fermented and then distilled and in the staves of the barrel than contain lignin is the source of many of these phenolic compounds, which have desirable aromas and flavors. In this study, we investigated the staves of Bourbon barrels to find how lignin content and composition is altered by whiskey maturation and how variation in distillate could interact with oak lignin in alter extractable lignin moieties from the barrel. We found that the C layer (the layer of the stave in the interior of the barrel which has been charred) had a higher proportion of lignin than the outer layers of the stave and was increased further in staves from barrels that had been used to age Bourbon. Lignin is a complex biopolymer with many connections motifs we found difference in linkages the lignin linkages present in the C layer were also different from the outer layers of the barrel but were similar across barrels. The same pattern was observed in the aromatic compounds were present at higher levels in the C layers of each barrel. The results presented here show that this charring may have dramatic effects on the lignin in the staves, which could have a strong influence on the flavor of the spirit aged in the barrel.

LOBLOLLY PINE INTER-PROVENANCE HYBRIDS AND FUSIFORM RUST

Stephen Goodfellow[1]

[1]College of Forest Resources, Mississippi State University, MS

Fusiform rust *(Cronartium quercum f. sp. fusiforme)* is a noxious pathogen that severely impacts loblolly pine *(Pinus taeda)* tree health and stand productivity in the Southeast. Southern pine tree improvement programs incorporate breeding strategies that focus on the development of fusiform rust-resistant planting stocks. Many of these selections are inherently resistant to fusiform rust, however tree form, volume production and adaptability may not be optimal. A solution is to incorporate loblolly pine inter-provenance hybrids as planting stocks that express strong family heritability for rust-resistance, site adaptability and desirable growth attributes. In this experiment, six progeny tests were established in 2017 across the natural range of loblolly pine. The field trials were laid out in randomized complete blocks design with border trees adjacent to the perimeter. Each block is replicated six times and within each block there are six representations resulting in thirty-six observations of each family selection. Tested selections include; full-sib loblolly pine inter-provenance hybrids, within provenance full-sib loblolly pine crosses, and loblolly pine open-pollenated selections from the piedmont, coastal, and western gulf provenances. After 3-years of growth, the field trials will be measured to determine if there is a positive correlation between inter-provenance hybrids performance regarding fusiform rust-resistance, stem form, and growth attributes across a wide geographic region.

DEVELOPMENT AND OPTIMIZATION OF A CRYOSTORAGE PROTOCOL FOR EMBRYOGENIC ASH CULTURES

Logan T. House[1], Cristian R. Montes[1], Paul M. Montello[1], A. Ryan Tull[1], **Scott A. Merkle**[1]

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

Efforts to conserve ash germplasm and eventually restore ash species to their native ranges and as landscape trees following their devastation by emerald ash borer (*Agrilus planipennis*; EAB) could be greatly aided if ash genotypes could be safely archived for later propagation and out-planting. One approach for storing forest tree germplasm indefinitely is cryopreservation. Embryogenic cultures of several forest tree species have been shown to be highly amenable to cryostorage and recovery. The goal of this study was to develop and optimize cryostorage and recovery protocols for white ash (*Fraxinus americana*) embryogenic cultures for use in ash germplasm conservation and species restoration. Three experiments were conducted testing different cryoprotection agents and concentrations, which included sorbitol, sucrose, glucose, polyethylene glycol and DMSO, using six different ash embryogenic culture lines. Following pre-treatment, all cultures were stored in a liquid nitrogen cryofreezer for at least 48 h before removing them for thawing and recovery. Regrowth of cryostored cultures was scored visually 3-5 weeks following their removal from cryostorage. Experiment 1 and its replication, Experiment 2, which tested three different DMSO concentrations, indicated that 10% DMSO provided higher recovery rates than 5% or 15% DMSO across the six tested genotypes, although the only statistically significant difference was between 10% and 5% DMSO in Experiment 2. Recovery varied widely among genotypes, with some genotypes re-growing with multiple DMSO concentrations, and some failing to regrow regardless of DMSO concentration. The failure of some genotypes to regrow following cryostorage prompted us to test different cryoprotection treatments in Experiment 3, employing sucrose or glucose and PEG as osmotica in place of sorbitol. Results from this experiment indicated that, compared to sorbitol, neither sucrose/PEG nor the glucose/PEG treatment improved regrowth of the cultures following cryostorage, and that the major determinant of potential for regrowth remained the genotype of the culture line.

THE HARDWOOD GENOMICS PROJECT: AN ONLINE DATABASE FOR TREE GENETIC AND GENOMIC DATA

Matthew Huff[1], Abdullah Almasaeed[1], Bradford Condon[1], Ming Chen[1], Amanda Devine[1], Casey Richards[1], Raymond Senu[1], Patrick Sisler[1], Jill Wegrzyn[2], Doreen Main[3], Stephen P. Ficklin[3], Albert G. Abbott[4,5], John E. Carlson[5], Margaret E. Staton[1]

[1]Department of Entomology and Plant Pathology, University of Tennessee, Knoxville, TN; [2]Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs, CT; [3]Department of Horticulture, Washington State University, Pullman, WA; [4] Forest Health Research and Education Center, University of Kentucky, Lexington, KY; [5]Schatz Center for Tree Molecular Genetics, Pennsylvania State University, State College, PA

With the vast increase in genetic and genomic data for woody tree species, it is necessary for this data to be easily accessible to scientists. However, much of this data is not available publicly, or only raw or unannotated versions of the data are available. The Hardwood Genomics Website (HWG) is dedicated to housing and annotating tree genomic datasets, including ecologically relevant and threatened forest tree species. Our site houses genomes and transcriptomes of trees unavailable on any other platform and also provides searchable functional annotation for genes and transcripts, including plant protein databases, protein domains, KEGG pathways, and gene ontologies identified through BLAST and InterProScan; we further characterize gene sequences by housing gene expression data from high throughput RNASeq experiments. HWG houses simple sequence repeats (SSRs) and their primers from genome and gene sequences, either obtained from published data or developed by our team, where they are available for use as genetic markers. We have integrated a number of tools for researchers to easily access our data, including a powerful search engine, BLAST sequence similarity searching, and JBrowse for genome browsing. As a recent example, we have incorporated Galaxy software to run bioinformatics analysis workflows, allowing users to map DNA or RNA reads to a reference, perform differential expression analysis, call variants in reads, and others. Furthermore, this system allows users to use data available on HWG, as well as data uploaded from their computer, as input in a workflow. As the scope of forest tree breeding programs and genetic programs grows, we are also building support for high throughput genotyping and phenotyping data. As Hardwood Genomics grows, we welcome new data submissions, suggestions, and partnerships to continue development. HWG is supported by NSF Awards #1443040 and #1444573.

QUANTITATIVE GENETICS OF HYBRID POPULATIONS OF *EUCALYPTUS NITENS* X *EUCALYPTUS GLOBULUS*: GENETIC PARAMETERS AND IMPLICATIONS FOR BREEDING STRATEGIES

Luis Ibarra[1,2], Juan José Acosta[1], Claudio Balocchi[2], Christian De Veer[2], Gary Hodge[1]

[1]Camcore, Department of Forestry and Environmental Resources, North Carolina State University, Raleigh, NC; [2]Division of Genetic Improvement, Bioforest Arauco, Coronel, Chile

Interspecific hybridization in forestry is used to produce genotypes with intermediate characteristics between two parents. Often, for particular traits, hybrids can exhibit superiority over an intermediate phenotype (mid-parent heterosis) or may perform better than either parent (high-parent heterosis). In Chile, forest plantations are established mainly with *Pinus radiata*, *Eucalyptus nitens* and *E. globulus.* Interspecific hybrids between *E. nitens* x *E. globulus* were developed by Arauco Bioforest with the goal of capturing specific traits from each parental species: growth rate and cold resistance from *E. nitens* and wood properties from *E. globulus*. Field tests of *E. nitens* x *E. globulus* were distributed in two geographic zones: Arauco (12 tests) and Valdivia (15 tests). Arauco zone is located in the central part of the country and is the best area for growing E. *globulus* due to absence of frost events. Valdivia zone is in the south of the country, being the best area for *E. nitens*, with a high precipitation and some frost events. The hybrid population is composed of clones from 28 full-sib families, being the result of crossing 12 *E. nitens* females and 8 *E. globulus* males. Progeny from each of these families were vegetatively propagated and tested on each growth zone, with a total of 1214 clones developed. The main objectives of this research are 1) to compare if hybrids perform better than the pure species in both geographic zones, 2) to develop and test statistical models to estimate genetic parameters for hybrid breeding populations for growth and wood properties. Broad-sense heritability values for volume were moderate (Valdivia: 0.51, Arauco: 0.54). Results indicate that *E. nitens* parents have a stronger effect on hybrid growth performance than *E. globulus* parents. Additionally, no Genotype-Environment interaction was found within each geographic zone (r_{Bg} 0.91 in Valdivia, 0.92 in Arauco).

CANDIDATE GENES INVOLVED IN LEAF DEVELOPMENT IN *LIRIODENDRON CHINENSE*

Jikai Ma[1], Huogen Li[1]

[1]College of Forestry, Nanjing Forestry University, Nanjing, China

Leaves play an essential role in plant photosynthesis and transpiration and represent enormous diversity that exhibits adaptation to the environment. *Liriodendron chinense* is a common ornamental tree in China, and its leaf is termed “robe tree” since it resembles a Chinese robe of the Qing dynasty. *L. chinense* is very popular and is regarded as an excellent species to research leaf shape development. Stereoscopic and scanning electronic microscopy were employed to determine leaf shape development periods. To find candidate genes controlling leaf shape development, we evaluated different stages of leaf development transcriptome and their different expressed genes. The candidate genes were then expressed in model plants. The results showed that the leaf bud was 1-2 cm and there were always 4-8 tender leaves within a bud. The leaf primordium was about 50-100㎛ and was the initial tissue of the leaf development. It then developed into a "hook-like" tissue and finally expanded to a complete leaf. Accordingly, the leaf morphological development process of *L. chinense* could be divided into three stages: leaf primordium occurrence, leaf morphology establishment, and leaf extension. In addition, the identification of candidate genes demonstrated some transcription factors prominently resulted in leaf change. Also, a few genes mediating plant hormone also affected individual height and flower timing. In summary, we attempt to provide an insight into leaf shape development mechanisms that can serve as a reference for the breeding of ornamental traits in *Liriodendron chinense*.

CHROMATIN ACCESSIBILITY IN THE LOBLOLLY PINE GENOME

Lilian P. Matallana[1], Sumaira Zaman[2], Jill Wegrzyn[3], Ross W. Whetten[1]

[1]Department of Forestry and Environmental Resources, North Carolina State University, Raleigh NC; [2]Department of Computer Science, University of Connecticut, Storrs CT; [3]Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs CT

The nucleus of a diploid loblolly pine cell is less than 15 microns in diameter, but contains about 14 meters of double-stranded DNA. Packing that DNA into the cell nucleus requires efficient organization of DNA into chromatin, a protein-DNA complex with higher-order structure. Some regions of chromatin are highly condensed and relatively inaccessible to nuclear factors, while other regions are less condensed and more accessible. Results from several species indicate that both regulatory and transcribed DNA sequences are enriched in regions of accessible chromatin, and that DNA sequence variation in regions of accessible chromatin can be associated with observed phenotypic variation. We conducted two experiments to explore chromatin structure in the loblolly pine genome, using nuclei isolated from the same tissue type. One experiment used Assay for Transposase-Accessible Chromatin by Sequencing on six replicate samples, while a second experiment used digestion with micrococcal nuclease followed by sequencing on a single sample of isolated nuclei. Comparing and contrasting the results of these experiments shows that the transposase-based method seems to be more tightly focused on a smaller proportion of the entire genome, while the micrococcal-nuclease based method yields a broader distribution of sequence reads across a larger proportion of the genome. Regions with coverage above the 99th percentile within each experiment were combined and used to search for transcribed regions of the pine genome that do not contain predicted gene models in the current version of the genome annotation. The recovered candidate regions were examined for the presence of conserved protein coding domains and putative single-copy orthologs of genes described in other Embryophyta. The results of these analyses suggest that chromatin accessibility can add value to genome annotation efforts, improving the ability to discriminate between functional genes and processed pseudogenes in conifer genomes.

USDA FOREST SERVICE RESISTANCE SCREENING CENTER: UPDATES AND NEW MANAGEMENT

Katie McKeever[1]

[1]USDA Forest Service, Resistance Screening Center, Asheville, NC

The Resistance Screening Center (RSC) was established in 1973 primarily to evaluate seedlots of slash and loblolly pines for resistance to fusiform rust (*Cronartium quercuum* f.sp. *fusiforme*) and pitch canker (*Fusarium circinatum*). Although these services remain the primary foci of the RSC; additional services that have been performed at the center include screening flowering dogwood for resistance to anthracnose (*Discula destructiva*), testing chestnut for resistance to both blight (*Cryphonectria parasitica*) and root rot (*Phytophthora cinnamomi*), and screening seed for presence of *Fusarium*. In January of 2019, the RSC welcomed Katie McKeever, Plant Pathologist, as the new manager to join the two veteran Biological Science Technicians that staff the center. The 2019 season promises to be busy with both traditional and research-based pine screening requests as well as the expansion of the cooperative *Phytophthora* project with The American Chestnut Foundation. The RSC's current goals include solidifying existing partnerships and forging new relationships with potential collaborators to continue to offer services that promote the advancement of forest resources.

A POPULATION GENETICS STUDY OF THREE FLORIDA PERSEA SPECIES EFFECTED BY THE LAUREL WILT EPIDEMIC

Katherine Smith[1]; Craig Echt[2]; Sedley Josserand[2], Jason Smith[3]

[1]Southern Research Station, USDA Forest Service, Gainesville, FL; [2]Southern Institute of Forest Genetics, Southern Research Station, USDA Forest Service, Saucier, MS; [3]School of Forest Resources and Conservation, University of Florida, Gainesville, FL

Laurel wilt disease was introduced into the southeastern US in 2002 and since then has spread as far west as Texas. The causative fungus, *Raffaelea lauricola*, is a symbiont of the redbay ambrosia beetle, *Xyleborus glabratus*. The fungus has a host range that includes a variety of lauraceae family members including avocado, sassafrass and redbays. Ecologically important Florida *Persea* species effected by the disease include redbay (*Persea borbonia*), swampbay (*Persea palustris*) and silkbay (*Persea humilis*). Particularly devastating losses have occurred in populations of redbay and swampbay, an important component of tree islands in the Florida Everglades. At Florida sand scrub preservation sites, all three *Persea* species occur relatively near one another. Silkbay grows exclusively on sandhill, xeric sites, swampbay grows in wetlands and swamps and redbay grows on high spots along the edges of streams, swamps or hammocks. Differences between redbay and swampbay habitats can be subtle, making identification tricky at times and underscoring the need to choose replanting sites carefully. Twenty samples per species were collected from five Florida sandhill preservation sites. Genetic diversity of species and population differentiation between species and locations was determined using SSR (simple sequence repeat) markers, at twelve loci. The fact that *Persea* are outcrossing and generally diverse species was confirmed by this study. Genetic differentiation between species was highest between silkbay and swampbay and lowest between silkbay and redbay. Given the restricted range of silkbay and its genetic similarity to redbay, silkbay could be an ecotype of redbay. In general, a better understanding of the genetics within and between these closely related species will inform replanting of areas effected by laurel wilt disease.

PRELIMINARY ANALYSIS OF NORTHERN RED OAK PROGENY TRIALS AT HTIRC

James Warren[1], Carrie Pike[2], Jim McKenna[1], **Keith Woeste**[1], Mark Coggeshall[1]

[1]Northern Research Station, USDA Forest Service Hardwood Tree Improvement and Regeneration Center, West Lafayette, IN; [2]State and Private Forestry, USDA Forest Service, Eastern Region, West Lafayette, IN

Forest landowners in the Central Hardwood Forest Region value northern red oak (*Quercus rubra* L.) as a timber, wildlife, and restoration species. In addition, northern red oak grows well across a wide range of sites, which provides prospective tree planters with considerable flexibility. The Hardwood Tree Improvement and Regeneration Center (HTIRC) has a large collection of source-identified red oak families originating from collections made in the mid 1980's by the Indiana Department of Natural Resources (IDNR). By the mid 2000's, 21 progeny tests derived from these selected red oaks had been planted in Indiana and neighboring states. Here we describe the evaluation of HTIRC northern red oak progeny tests at least 10-years-old growing at three sites. These were measured or rated for height, dbh, volume, and stem quality. Tree performance was also compared to a check seedlot that represented average red oak in Indiana. Stem quality was evaluated as: presence of clear 12 ft log, number of branches greater than 1 inch on the bottom log (12 ft), presence/absence of sweep, overall branch angle (trees with strongly upswept limbs are downgraded). Because of pronounced differences in growth across the three sites, each test site was independently analyzed. Individual tree heritability estimates for volume ranged from 0.15 to 0.44. Heritability estimates for stem quality traits, including branch angle, sweep, and branchiness, were extremely high, so we expect selection for these traits will be highly effective. Improvement for volume and other traits was calculated relative to the checklot. Twenty trees with the greatest genetic potential were identified at each site. The average improvement of these 20 selections for volume ranged from 32-66% (depending on site), in comparison to the checklot. The trees selected for improved volume also showed 3 – 12% improvements in stem quality, depending upon which quality trait was examined.

WON'T YOU BE MY NEIGHBOR? COMPACT AND EFFICIENT EXPERIMENTAL DESIGNS TO ANALYZE COMPLEX INTERACTIONS

Keith Woeste[1], Mo Zhou[2], Michael Saunders[3], Jennifer Vandenbussche[4]

[1]Northern Research Station, USDA Forest Service Hardwood Tree Improvement and Regeneration Center, West Lafayette, IN; [2]Department of Forestry and Natural Resources, Purdue University, West Lafayette, IN; [3]Department of Forestry and Natural Resources, Purdue University, West Lafayette, IN; [4]Department of Mathematics, Kennesaw State University, Kennesaw, GA

Current monoculture breeding methods for hardwood trees result in selections likely to be ill-suited to the mixed-species plantations in which they will be planted. Dissonance between breeding methods and preferred deployment persists in part because there are no practical experimental designs that enable forest geneticists to estimate the importance of direct and indirect genetic effects of competitors during plantation development. *To address this need*, we have developed several practical designs for evaluating complex neighbor effects on the growth and quality of trees (or any organism). These designs efficiently and systematically place a target species (or each of several target species) at the center of and equidistant from six neighbors. The neighbors constitute a neighborhood that varies from monospecific to mixtures of species in all possible combinations. We will use three methods to assess neighborhood effects: spatial Durbin model, spatial causality model, and nonparametric neighborhood analysis. The former two will establish structured spatial models to test the impacts of neighboring plant biomass on target plant biomass, while the last one will classify target plant biomasses into groups by neighborhood characteristics This novel class of experimental designs aims to identify and measure community effects on phenotype, opening the door to the genetics of multi-species interactions. These designs are especially suited to precise forest phenotyping methods such as aerial or ground-based LiDAR.

TRANSCRIPTOMIC PROFILING REVEALS FLOWER DEVELOPMENT GENE INDUCTION DURING ENDODORMANCY TO ECODORMANCY TRANSITION IN APRICOT AND PEACH FLORAL BUDS.

Jiali Yu[1], Anna O. Conrad[2], Véronique Decroocq[3], Tetyana Zhebentyayeva[4], Chris Dardick[5], Zongrang Liu[5], Albert G. Abbott[2], Margaret E. Staton[1,6]

[1]UT-ORNL Genome Science and Technology, TN; [2]Forest Health Research and Education Center, Lexington, KY; [3]INRA-Bordeaux, France [4]Schatz Center for Tree Molecular Genetics, Pennsylvania State University, University Park, PA; [5]Appalachian Fruit Research Station, USDA Agriculture Research Service, Kearneysville, WV; [6]Department of Entomology and Plant Pathology, University of Tennessee, Knoxville, TN

Winter dormancy is a mechanism to protect buds on perennial trees from harsh conditions. Trees enter endodormancy, cease growth and start accumulating chilling hours at the beginning of winter. Once a set chilling requirement (CR) is fulfilled, the trees move into ecodormancy, a stage where trees remain dormant until the arrival of optimal conditions for growth. The transition between dormancy stages is strictly regulated by physiological factors and environmental cues. However, the genetic mechanisms underlying dormancy transition from endodormancy to ecodormancy are still unclear. Here we utilized the transcriptome profiles of apricot (*Prunus armeniaca*) and peach (*Prunus persica*) cultivars with different CRs (early blooming vs late blooming) to explore the genetic regulation of flower bud dormancy. Floral buds from grafted replicates of four apricot cultivars and four peach cultivars with different CRs at time points spanning from early winter to bud break were subjected to RNA-seq analysis. The transcriptome variances showed that the trees with different chill hours were moving through stages of dormancy at different rates. Thus, the samples were analyzed based on physiological stages instead of time points. Of 26,872 genes, 608 were consistently differentially expressed between endodormancy and ecodormancy in both species. Functional analysis reveals these genes are involved in lipid localization, pollen development, cell wall biosynthesis and stimuli response. 99 of them are within previously identified peach CR quantitative trait loci (QTL) regions, indicating that they are strong candidates for dormancy regulation. Co-expression analysis identified two clusters of genes, related to abiotic and biotic stress, flower and pollen development, lignin biosynthesis and lipid transportation, that were highly induced at ecodormancy in peach and apricot. Our analysis reveals that the induction of flower development genes may be an indicator of endodormancy to ecodormancy transition, providing new insights into bud activity at winter dormancy.