37th Southern Forest Tree Improvement Conference

Using State-of-the-Art Technologies to Enhance Forest Tree Adaptation for the Future Bioeconomy and Climate

Proceedings

37th SFTIC Conference Website

Proceedings of the 37th Southern Forest Tree Improvement Conference

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Foreword

The Southern Forest Tree Improvement Conference (SFTIC) has met biennially since 1951 to exchange information and to discuss future needs in the fields of forest genetics and tree improvement, focusing primarily on the southeastern United States. The 37th Southern Forest Tree Improvement Conference was held on June 20-23, 2023, under the auspices of the Southern Forest Tree Improvement Committee, and hosted by the US Department of Energy's Oak Ridge National Laboratory (ORNL) and the University of Tennessee, Knoxville, with the cooperation of the Schatz Colloquium for Tree Genetics Fund. The conference was held at the University of Tennessee Conference Center in Knoxville, TN, and was attended by seventy-four registered participants, representing forest industry, university forestry programs, and federal and state agencies with interests in tree genetics. The theme of the conference was "Using State-of-the-Art Technologies to Enhance Forest Tree Adaptation for the Future Bioeconomy and Climate."

The conference was preceded by a meeting of the North American Quantitative Forest Genetics Group, organized by Dr. Chen Ding (Auburn University) and a Tree Improvement 101 workshop, organized by Dr. Carolyn Pike (US Forest Service, Eastern Region). A Career Panel for students and postdocs was organized by David Barker (Rayonier). The conference opened with a plenary session, "American Chestnut Restoration Genetics, Silviculture and Ecology," with invited speakers Dr. Stacy Clark (US Forest Service, Southern Research Station), Dr. Douglass Jacobs (Purdue University) and Dr. Jason Holliday (Virginia Tech). A second plenary session, "Poplar Genomics to the Rescue - Bioenergy and Climate Mitigation," opened the second day of the conference, with invited speakers Dr. Melissa Cregger (ORNL), Dr. Wellington Muchero (ORNL) and Dr. Yumin Tao (Living Carbon). Volunteer presentations were made in sessions focusing on pine genetics and tree improvement, hardwood genetics and tree improvement, *Populus* genetics and tree genetics and improvement tools. One volunteer session provided updates on US Forest Service programs by Forest Service research leaders Dr. Keith Woeste (National Program Leader, Genetics Biodiversity and Conservation, Research and Development), Dr. Marcus Warwell (Regional Geneticist, Southern Region) and Dr. Arnaldo Ferreira (National Program Leader, Genetic Resources Management, National Forest System). This session also included a presentation by Dr. Dana Nelson (US Forest Service, Southern Research Station) on the history and future of SFTIC. This presentation formed the basis for organized group discussions on the future direction of SFTIC at a working lunch. The poster session included seventeen posters on a range of topics including forest health, tree molecular genetics and clonal propagation. The final session of the conference was a symposium sponsored by the Schatz Colloquium for Tree Genetics Fund, "Synching Forest Genetics with Climate Change." The three invited speakers for the Schatz Symposium were Dr. Matthew Huff (University of Tennessee, Knoxville), Dr. Vikram Chhatre (US Forest Service, Northern Research Station) and Dr. Laura Leites (Pennsylvania State University). The conference ended with an optional field trip to tour ORNL's Woody Biomass Research Site hosted by Dr. Wellington Muchero, Dr. Brian Davison, and Dr. Jerry Tuskan.

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

The **Tony Squillace Award** is given for the best oral presentation based on content, style, and use of visual aids. There was a tie this year and the co-winners were Austin Thomas (US Forest Service, Southern Research Station) and Graham Ford (IFCO Seedlings).

The **Bruce Zobel Award** is given for the best oral presentation by a student, and again, there was a tie for this award. The co-winners were Beant Kapoor and Meher Ony (both University of Tennessee, Knoxville).

The **van Buijtenen/Belle Baruch Foundation Best Student Poster Award** went to Aaron Onufrak (University of Tennessee, Knoxville). The second and third place posters in the competition, which won **van Buijtenen Poster Awards,** were presented to Julia Wolf (Holden Forests and Gardens) and Yao Tao (ORNL), respectively. A poster by Xavier Tacker (Oregon State University) received **Special Mention**. The 37th SFTIC Planning Committee would like to thank the award judges, Dr. Gary Peter (University of Florida) and Dr. Jason Holliday (Virginia Tech).

In 2021, the 36th SFTIC hosted a research support funding competition. This competition awarded five grants of $2000 each, provided by the Schatz Colloquium for Tree Genetics Fund, for graduate students, postdocs and junior faculty/early career government researchers to continue or enhance the research projects on which they made presentations at the 36th SFTIC. Two of the research support grant awardees presented updates on their research at the 37th SFTIC: Austin Thomas (North Carolina State University) and Matthew Huff (University of Tennessee, Knoxville).

The 37th SFTIC was a success thanks to the outstanding conference organizing efforts of Judy Potok (ORNL) and Carmen Jones (ORNL). Dr. Eva Levi (University of Georgia) produced the conference program and abstract booklet and Matt Huff (University of Tennessee, Knoxville) populated and edited the conference webpage.

SFTIC would like to offer special recognition and thanks to Dr. Jill Hamilton, Director of the Schatz Center for Tree Molecular Genetics at Pennsylvania State University, and Dr. Kimberly Bohn (Pennsylvania State University, Mont Alto) who arranged financial support for the Schatz Symposium.

The 37th SFTIC Planning Committee:
Meg Staton, University of Tennessee, Knoxville (Co-Chair)
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In Memoriam

James Hall Roberds

Nov. 28, 1938 – Jan. 28, 2023

SIFG in 2008. L-R: Ron Schmidtling, Dana Nelson,Yousry El-Kassaby (visiting from Canada), Tom Kubisiak (kneeling), Milan Lstibůrek (visiting from Czech Republic), Craig Echt, Jim Roberds

Jim Roberds, a true Gentleman and Scholar, departed from the ranks of forest geneticists January 28, 2023, while still active in research, at the age of 84. Jim was born in Estil, SC, just north of Savannah, on November 24, 1938. He graduated from Savannah High School in 1956, and from North Carolina State University in 1960 and 1967 with Bachelor's and Master's Degrees in forestry. While he was a graduate student at NC State, he formed a friendship with Gene Namkoong, who was on educational assignment there from the US Forest Service's Southern Institute of Forest Genetics (SIFG) in Gulfport, MS. In recognition of Gene's outstanding abilities in genetics, the Forest Service established a "Pioneering Genetics Project" with Gene as Project Leader, around 1970 in Raleigh, NC. Gene hired Jim for a scientist position in the project, thus began a long relationship between Jim Roberds, Gene Namkoong and the Forest Service.

Jim had many of his own research projects but was most productive when he was teamed up with Gene in theoretical research. The synergism between Gene and Jim surely contributed in some small way to Gene's being awarded the Wahlenburg Prize in 1994 (Jim was part of the retinue that traveled to Stockholm for the ceremony). After Gene retired, the Forest Service closed his Pioneering Project, with Jim moving to the SIFG, now at Saucier, MS on the Harrison Experimental Forest, around 1996. Jim retired officially in 2008, but continued working as Scientist Emeritus until he was felled by a massive stroke in early January 2023.

Jim was preceded in death by his wife Kay Hensley Roberds, an infant son, and his brother Max Roberds. He is survived by his sons: Mike and wife Lisa Woodruf Roberds of Apex, NC; Alan and wife Shellie Holerud Roberds of Savannah, GA; Sean and wife Clara Clonz Roberds of Herndon, VA; and grandchildren Anna and James Roberds of Apex, NC, Sydnie and Dylan Roberds of Savannah, GA., and Cole and Eli Roberds of Herndon, VA.

Ron Schmidtling
Scientist Emeritus, USDA Forest Service - Southern Institute of Forest Genetics
Saucier, MS

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Plenary Session 1:

American Chestnut Restoration Genetics, Silviculture and Ecology

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American Chestnut Restoration: Effects of Breeding on Blight Resistance and Field Performance in Tennessee, USA

Stacy L. Clark[1], Scott E. Schlarbaum[2], and Arnold M. Saxton[3]

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The American chestnut (*Castanea dentata*) was a keystone species in eastern North America providing important ecosystem services and economic benefits to rural communities, particularly in the southern Appalachian mountains. Chestnut blight caused by *Cryphonectria parasitica*, has drastically reduced American chestnut populations and altered tree species composition for the last 100 years. A field reintroduction trial using hybrid American chestnuts and parental species was established in 2010 in eastern Tennessee USA. The hybrids were produced using a backcross breeding approach that integrates blight resistance from Asian chestnut species into the American chestnut genome. Pedigreed bare-root (1–0) nursery seedlings (n=513) were planted to test effects of breeding, genetics, and seedling size class on field performance. Mortality was highest the first two years after planting, and Phytophthora root rot (PRR), caused by *Phytophthora cinnamomi*, was probably a significant contributor. Chinese chestnut (*C. mollissima*) had the highest eight-year survival (96 percent) compared to American chestnut (34 percent) and hybrid generations, including the most advanced generation, the BC_3F_3 (41 percent). Less advanced hybrid generations, such as the BC_1F_3 and BC_2F_3, had the tallest eight-year stem height. We identified superior and inferior BC_3F_3 families based on blight resistance rankings and growth. Seedling size class, identified at the time of lifting seedlings from the nursery just prior to planting, affected survival and growth of generations and genetic families differently. Nursery production and restoration plantings should consider genetic effects and interactions with seedling size class to better refine and improve planting outcomes. The breeding program was successful in integrating desired American chestnut growth traits into the hybrid genome, while transferring an intermediate level of resistance from the Chinese chestnut. Long-term viability of the BC_3F_3 generation seedlings in this experiment is questionable as trees increasingly succumb to chestnut blight over time.

Beyond Blight: Silviculture and Policy Considerations for American Chestnut Restoration

Douglass F. Jacobs[1]

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American chestnut (*Castanea dentata*) was functionally extirpated from eastern US forests by chestnut blight. As efforts to produce blight-resistant American chestnut germplasm advance, approaches to reintroduce chestnut throughout its former range are being developed. Over the past twenty years, I have collaborated with colleagues to better understand how reintroduced chestnut will perform across the landscape. We have parameterized chestnut ecophysiology, responses to silviculture, seed dispersal and establishment, and carbon storage / decay; and then used these findings to conduct modeling experiments. We have produced several key findings from this work. First, American chestnut is very fast growing, relatively long-lived, and resistant to decay. Nevertheless, aggressive restoration efforts are needed to ensure that chestnut will become a significant and stable component of the forest within a century under current or future conditions (i.e., pests, climate change). Additionally, despite its fast growing and long-lived nature, chestnut will produce only modest increases in carbon storage, mainly through slow decay of its wood. Second, analysis of non-structural carbohydrate (NSC) pools in different organs of American chestnut trees found that coarse roots were a remarkably important storage site, suggesting that chestnut may thrive under disturbance-based management. Third, the pathogen *Phytophthora cinnamomi*, which causes a root rot disease, generally reduced chestnut biomass on the landscape even when modeling using the highest levels of resistance to root rot infection that are incorporated into current breeding efforts. As root rot is expected to increase in virulence and migrate north in response to climate change, we suggest the need to further increase root rot resistance through biotechnology, as well as to target reintroduction to sites where root rot is not expected to be present well into the future. Finally, although remarkable progress has been made in developing blight-resistant chestnut, we suggest that successful reintroduction will ultimately depend on regulatory policy, informed by science and with public support.

Genomic Tools for American Chestnut Restoration

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The early 20th-century introduction of *Cryphonectria parasitica* in North America caused the functional extinction of American chestnut (*Castanea dentata*), resulting in profound ecological, economic, and societal losses. Two potential solutions—introgressive hybridization with resistant Asian Castanea species and genetic modification—are currently being explored to develop disease-resistant chestnut populations. The hybrid breeding program has produced families with moderate resistance, but the approach is based on a flawed three-gene resistance model. The low heritability of the trait has further complicated the selection process. Genetic modification, specifically the overexpression of an oxalate oxidase (OxO) transgene, produces strong resistance. However, multiple outcrossing generations are required to restore genetic diversity. To overcome these challenges, we have produced several population and functional genomic datasets. We first created a genomic prediction model using progeny-test data, which assisted in selecting the top 1% of backcross families. We also sequenced the whole-genomes of approximately 350 wild C. dentata stump sprouts to characterize neutral and adaptive patterns of variation, thus informing *ex situ* conservation of wild germplasm for breeding with transgenic lines. A subset of backcross families was also sequenced to gauge the breeding program's efficacy in capturing wild adaptive diversity. Finally, although OxO-based resistance is expected to be long-lasting, this is not guaranteed. Hence, we are in the process of identifying additional candidates for transgenic manipulation. This is being done by integrating RNA-seq of a blight canker development time series in resistant and susceptible species, a whole-genome comparative analysis of all Castanea species, and QTL mapping within the backcross breeding program. The outcomes from these diverse projects will underpin our long-term objective of developing disease-resistant, locally-adapted American chestnut populations, thereby aiding in the species' restoration.

Concurrent Session 1A:

Hardwood Genetics and Tree Improvement I

Red Oak Resilience to Winter Flooding in Bottomlands: Is There Potential for Tree Improvement?
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Forty-Year Survey of White Oak (*Quercus alba*) Common Garden Experiment Planted in South-Central Indiana: Insights Into Growth and Mortality of Seed Sources Across a Latitudinal Gradient
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– *Beant Kapoor, University of Tennessee*...pg. 8

Genomic Resources for Sustaining and Improving American White Oak
– *Margaret Staton, University of Tennessee*..pg. 9

Red Oak Resilience to Winter Flooding in Bottomlands: Is There Potential for Tree Improvement?

Benjamin A. Babst[1], Jonathan M. Kressuk[2], Tucker Collins[3], Emile S. Gardiner[4], and Mohammad Bataineh[1]

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Red oaks in bottomlands are experiencing tree die-off and poor regeneration, highlighting the need for better science-based management and tree improvement. One key knowledge gap is our understanding of root physiology in moderately flood tolerant oaks, like willow oak and Nuttall oak (*Quercus phellos*, *Q*. *texana*), as altered hydroperiods and unusual weather patterns are more frequently exposing bottomland ecosystems to flooding when trees are not fully dormant. We conducted a series of greenhouse and field studies to determine phenology of roots and the impacts of flooding during winter dormancy transitions on red oak seedlings. Root growth and respiration declined during winter, due to low soil temperature. Air temperature also influenced roots, probably through effects on photosynthesis, still active during winter in the semi-persistent leaves common to willow oak and Nuttall oak seedlings. Soil flooding that occurred during winter had minor impacts, such as halting root growth, but caused no seedling mortality. Seedlings that experienced flooding in warm soil had greater, but still limited, stress. Additionally, seasonal flooding beginning before dormancy or extending after release from dormancy can negatively impact seedling growth and survival. Furthermore, flood intolerant *Q*. *shumardii* had reduced growth during the spring and summer following exposure to dormant season flood. Our results indicate that roots could continue to grow during typical winter weather and soil temperatures in southern bottomlands, but at slow rates. Apparent stress of soil flooding on willow oak and Nuttall oak seedlings under these conditions was low. Still, our results indicate that seedlings require some underlying physiological mechanisms to tolerate or recover from winter flooding, because reduced root activity, which may contribute to stress avoidance during winter flooding, does not persist as air and soil temperatures fluctuate over winter. The implications for tree improvement, as well as challenges and opportunities, will be discussed.

Forty-Year Survey of White Oak (*Quercus alba*) Common Garden Experiment Planted in South-Central Indiana: Insights into Growth and Mortality of Seed Sources Across a Latitudinal Gradient

Austin Thomas[1] and C. Dana Nelson[1]

[1]USDA Forest Service, Southern Research Station, Forest Health Research and Education Center, Lexington, KY, USA; *austin.thomas@uky.edu

We surveyed a common garden experiment of white oak (*Quercus alba*) planted in 1983 in south-central Indiana at Starve Hollow State Recreation Area and synthesized the data collected on this study over the 40-year growing period. Seed sources planted at this location covered the latitudinal range of white oak, from as far south as northern Mississippi to as far north as southeastern Minnesota. Our survey reveals four important findings. (1) White oak performs exceptionally well in a plantation setting at this location, far exceeding site index and DBH growth curves previously observed in naturally regenerating stands of white oak in the central hardwoods region. (2) Southern seed sources do not experience excess mortality due to harsh freezing temperatures. Mississippi seed sources were the best-performing trees, with low mortality and superior growth and form. (3) Budburst and fall senescence did appear to vary among seed sources, but the differences in budburst were very small (approximately 4 days across all latitudes), while peak senescence varied by as much as 17 days. (4) Northern seed sources have very slow growth rates, becoming suppressed by year 20 (even after thinning at year 12) and experiencing mortality by year 40, presumably due to competition for light. Our findings have important implications for the management and conservation of white oak populations in the face of climate change. We suggest that white oak may be a prime candidate for assisted migration as southern seed sources appear well adapted for current conditions in the central hardwoods region.

Characterization of Structural Variation in a *Q. rubra* Three-Generation Pedigree

Beant Kapoor[1], Jerry Jenkins[2], Jeremy Shmutz[2], Mark Coggeshall[3], Jeanne Romero-Severson[4], John Carlson[5], and Margaret Staton[1]

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Northern red oak (*Quercus rubra* L.), an economically and ecologically important tree species of North America, is a member of the *Quercus* genus in the Fagaceae family. A chromosome-scale, haplotype-resolved genome of *Q. rubra* was generated by combining PacBio sequences, Illumina short reads and chromatin conformation capture (Hi-C) scaffolding. This resource represents the first reference genome from the red oak clade (section *Lobatae*). The *Q. rubra* assembly is 739 Mb, with 33,333 protein-coding genes and 12 chromosomes containing 95.27 percent of the genome's sequences. High overall collinearity along with intrachromosomal structural variation was observed when *Q. rubra* was compared to *Q. lobata* and *Q. mongolica* genomes. Based on orthologous gene family analysis with other oak and rosid tree species, the *Q. rubra* genome exhibited simultaneous expansion and contraction of gene families linked to the defense response. Out of the nine plant species examined, *Quercus rubra* had the highest number of CC-NBS-LRR and TIR-NBS-LRR resistance genes. The *Q. rubra* individual selected for the reference genome was a progeny of a two-generation pedigree. The genomes of the parents of the original cross and the two F_1 progeny parents of the reference tree were sequenced, scaffolded against the reference genome, and annotated. We characterized structural variants (SV) in this *Q. rubra* pedigree and produced a set of 117,854 high-quality SV events. We also found that most of these SVs (88.27%) called were less than 1 Kb in length which constitutes a novel genetic diversity resource in *Q. rubra* tree species. The high-quality red oak genomes generated are a crucial resource for the oak genomics community and will add to our understanding of *Quercus* genomics.

Genomic Resources for Sustaining and Improving American White Oak

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Quercus alba is a keystone species in eastern North American forests and provides valuable ecosystem services, including supporting immense biodiversity and sequestering carbon. Mature *Q. alba* trees are harvested for their lumber as well as specialty products including staves for bourbon barrels. Emerging concerns for white oak sustainability include reduced natural regeneration, increased harvest, new pests and pathogens, and climate change. To support the increasing genetic research and tree improvement programs in this species, we have produced a high-quality reference genome for *Q. alba*. It is haplotype resolved, with 796Mb from the primary haplotype and 793Mb from the secondary haplotype, and 97% of the genome anchored to 12 chromosomes for each. The genome was found to be highly heterozygous for both nucleotide and structural variation, reflecting the obligate outcrossing nature of the species. Resequencing *Q. alba* individuals from six different geographic locations and representatives from seven other common white oak species with sympatry to *Q. alba* has yielded a dataset of over 50 million SNPs to leverage for future genotype to phenotype studies. Comparison to the publicly available genomes from other oak species reveals a largely collinear structure at the chromosome scale but also significant expansion and contraction of specific gene families. We identified over 2,500 candidate R-genes, including 302 RLKs (receptor-like kinases), 308 RLPs (receptor-like proteins), 102 CC-NB-LRRs, and 19 TIR-NB-LRRs. As oaks have no recent whole genome duplication, we found that R-genes are primarily created through dispersed and proximal gene copying. This genome will provide the foundation for a white oak pangenome, an ambitious project that has the potential to accelerate oak research and provide new insights into the unique evolution of the *Quercus* species complex in North America.

Concurrent Session 1B:

Pine Genetics and Tree Improvement I

Analysis of Fusiform Rust Incidence and its Impact on Growth and Stem Quality
– *Patrick Cumbie, ArborGen*..........pg. 11

Field Evaluation of Fertilizer Effects on Pitch Canker Severity in Slash Pine Families
– *Tania Quesada, University of Florida*..........pg. 12

Managing Cold-Hardiness in a Tree Breeding Program While Balancing Gain and Diversity
– *Trevor Walker, NC State University*..........pg. 13

Genetic Variation in Growth and Survival Through 40 Years in a Southern Mississippi Longleaf Trial
– *Graham Ford, IFCO Seedlings*..........pg. 23

Rotation-aged Genetic Parameters for Shortleaf Pine (*Pinus echinata* Mill.) and Their Implications for Tree Improvement, Disturbance Response, and Species Restoration in a Changing Climate
– *Chen Ding, Texas A&M*..........pg. 24

[Note: this talk was not actually presented at this meeting but is included here as one of the winners of the 36th SFTIC Schatz Research Support Awards]

Analysis of Fusiform Rust Incidence and its Impact on Growth and Stem Quality

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The identification and selection of disease-resistant seedlings is critical to loblolly pine (*Pinus taeda* L.) reforestation programs. Fusiform rust (caused by *Cronartium quercuum* (Berk.) Miyabe ex Shirai f.sp. *fusiforme*) is a wide-spread disease across much of the southeastern United States and rust resistance is a primary trait for selection and deployment in high-hazard areas. Rust resistance has been incorporated into selection indexes along with growth and stem form traits, but the incidence level on any given site depends on many factors. In this study we will explore the relationships between field trial and greenhouse incidence, the gall location on the tree, and the impact that trial incidence level may have on selection index weights. A total of 50 trials planted in the Piedmont and Coastal regions of the southeastern United States were assessed at age 6 and ranged from 8% to 72% in rust incidence. An additional set of data from 25 greenhouse trials at the USDA Forest Service Resistance Screening Center (RSC) in Asheville, NC were also included which ranged in rust incidence from 15% to 77%.

Field Evaluation of Fertilizer Effects on Pitch Canker Severity in Slash Pine Families

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Pitch canker is an episodic disease of southern pines, caused by the fungus *Fusarium circinatum*. Commercial pine stands are routinely fertilized after planting and at age 5 to enhance biomass production; however, while pine breeding programs have led to increased growth efficiency in the past decades, fertilizer rates have not changed much. Reports of fertilizer applications exacerbating pitch canker severity have been mainly anecdotal and from controlled greenhouse experiments. We set up two field trials near the towns of Brooker and Hampton, Florida, located 8 miles apart. Each site consisted of three-year-old slash pines from “resistant” (Brooker) and “susceptible” (Hampton) seedlots set in a randomized complete block design with four replicates and three treatments: Control (no fertilizer), low (200 lbs/acre DAP), and high (200 lbs/ac DAP + 237 lbs/ac of urea) fertilizer rates, applied in July 2022. Each plot consisted of 28 trees, of which 10 were inoculated in April 2023 with a local isolated of *F. circinatum* obtained from the Hampton site. Trees were evaluated for symptom severity (branch browning and resin production) at 7 weeks post inoculation. Preliminary results show greater occurrence of severe browning (75%-100% brown needles) and high resin production (large amounts of resin dripping onto branches or ground below) in plots with high fertilizer rates, compared to low fertilizer and controls. This inoculation will be repeated in 2024 on an alternate subset of 10 trees within those same plots. In addition, two more field trials were selected this year near Fargo, Georgia, using the same design and fertilizer treatments as the Florida trials. Fertilizer treatments were applied in March 2023 and inoculations with a local “Fargo” isolate will occur in March 2024 and 2025. Information from these field experiments will help optimize fertilizer rates to current slash pine stands to minimize pitch canker severity.

Managing Cold Hardiness in a Tree Breeding Program while Balancing Gain and Diversity

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Abstract: In addition to the balance between gain and diversity, forest tree breeding programs must also ensure that adaptability is maintained when choosing the trees and crosses to breed. Maintaining adaptability can be a challenge when there are undesirable genetic correlations, such as for cold hardiness and growth rate in *Pinus taeda*. Breeding zones have traditionally been used to maintain adaptability, but rigid demarcations might result in a sub-optimal crossing plan when the adaptability variation is clinal. For example, a strict breeding zone strategy would exclude crosses among trees that may have similar provenance origins but are separated by the zone boundary, such as the upper coastal plain and lower piedmont. Further, provenance origins become difficult to define after a few cycles of breeding, especially when trees from wide crosses are selected for breeding. A better approach may be to permit crosses among all individuals but apply a negative weight against wide crosses in a mate selection algorithm. Using the NC State University *Pinus taeda* breeding program as an example, we demonstrate this approach and evaluate the gain, diversity, and adaptability consequences for different scenarios for managing cold hardiness. The scenarios were evaluated using the differential evolution algorithm as applied by MateSel, which has grown in popularity among livestock, aquaculture, and plant breeding programs. The flexible breeding zones scenario provided the most desirable solution but required close monitoring of the results. Further research should include simulations over multiple generations to evaluate the long-term consequences and identify rules-of-thumb for choosing the appropriate weighting values.

INTRODUCTION

Tree breeding programs often use breeding zones to maintain adaptability within a species. A breeding zone is the set of environments for which improved varieties are being developed, and corresponds to a particular geographic region (White et al. 2007). Each breeding zone essentially acts as a distinct population, so there is an upfront cost of maintaining many breeding zones. There is also an opportunity cost of reduced gain per unit time, as managing multiple zones distracts from the number of crosses, progeny tested, and selections that can be afforded in any particular zone. However, too few breeding zones risks the loss of adaptability in part of the deployment range.

Because the inclusion of non-adapted material in a breeding zone will reduce overall suitability, breeders are challenged to define how wide any single zone can be before incurring a substantial loss of local adaptability (Namkoong et al. 1988).

Provenance-progeny trials (also known as common garden experiments) are critical for defining breeding zones. By testing every seed source in a replicated experimental design on multiple sites across the range of deployment environments, these trials allow evaluation of the important adaptability traits within a species. These trials also allow evaluation of the genetic correlations between adaptability traits and the traits targeted for selection. For example, provenance trials for *Pinus taeda* indicated that cold hardiness is the primary trait affecting adaptability, and that genotypes with warmer-source origins tend to have faster growth (Schmidtling 2001; Farjat et al. 2017). Using provenance trials and climatic date, Schmidtling (1994) presented a seed transfer distance metric for *P. taeda* that is calculated as the difference between the mean minimum winter temperature (MWT) of the genetic source origin (e.g., where the parent tree was selected) and the MWT of the planting site. It was found that genetic sources from warmer climates often outperformed the local source for growth rate whenever the seed transfer distance was 5°F or less, yet transfer distances greater than 10°F incurred cold damage that offset the gains in growth. Following the observations from provenance trials and progeny tests, the NC State University Cooperative Tree Improvement Program organized three breeding zones that represented the Coastal, Piedmont, and Northern deployment regions of the southeastern United States in their third and fourth cycles (McKeand and Bridgwater 1998; Isik and McKeand 2019). The purpose of these breeding zones was to ensure that cold hardiness is maintained within the Piedmont and Northern zones, because there is a strong undesirable genetic correlation between cold hardiness and growth, which results in a temptation to use less cold hardy material when designing mating plans.

Strict adherence to breeding zones is simple and effective but has limitations (in addition to the upfront and opportunity costs of managing multiple populations). The rigid demarcations between zones do not have a biological justification for clinal traits like cold hardiness. The zones arbitrarily prevent crosses among parents with genetic origins near the border, even though they may be similar in terms of adaptability. Further, in practicality, breeding programs have a history of experimenting with “wide” crosses (matings between parents from different zones), and selections made from these crosses will not have a straightforward zone assignment. An example of such a selection with a wide pedigree is shown in Figure 1, where the founders (represented by the top row of circles) come from disparate climates. The figure also demonstrates how MWT is calculated for advanced-generation selections using mid-parent values. This approach assumes polygenic inheritance of cold hardiness, which is a reliable premise given our current understanding of forest trees (Howe et al. 2003; Wisniewski et al. 2018; De La Torre et al. 2021).

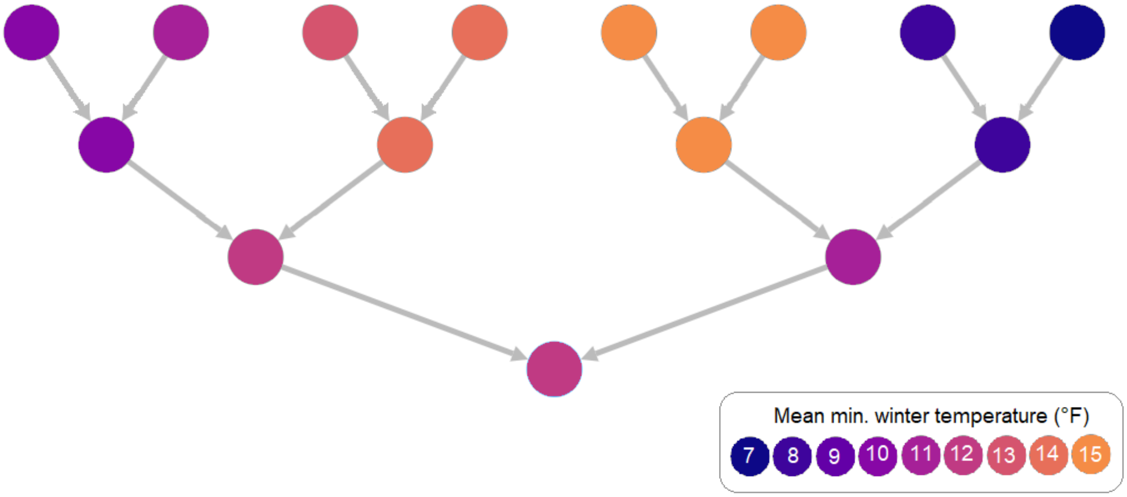

Figure 1. Illustration of the mean minimum winter temperature calculation for an advanced cycle selection with a particularly diverse pedigree of founders across the range of cold hardiness.

When developing a mating plan, one quickly encounters the "breeder's dilemma": the conflict between maximizing the short-term response to selection and maintaining diversity to avoid inbreeding and allow continued extraction of gain over the long-term (Lindgren and Mullin 1997). Modern breeding programs use mate selection algorithms (such as optimum contribution selection) to design their crossing plans to balance short term gain and preserve diversity (Mullin 2017; Yamashita et al. 2018; Isik and McKeand 2019). Computer programs are a necessity after the first two generations because pedigrees become too complex to design matings manually. The differential evolution algorithm is a popular method in livestock, aquaculture, and plant breeding (Kinghorn 2011). The objective function uses a weighting between 0 and 90 degrees that corresponds to the emphasis placed on short-term gain and diversity in the progeny population, with lower angles indicating a higher emphasis on short-term gain. The commercial software MateSel applies this algorithm to design mating plans while also incorporating many other features to accommodate the complex logistics of a breeding plan, such as biological limits on mating frequency per parent, accounting for crosses and selections already made but not yet reproductively mature, and logistical constraints on mating animals from different farms (Kinghorn and Kinghorn 2021). In this study, we investigate using the latter feature to introduce a concept of "flexible breeding zones", where the breeding population is managed as one, but a negative weighting is used to discourage making crosses among parents from different zones. We expect that managing the population as one will result in more short-term gain and adequate diversity compared to strict breeding zones, and we compare a few scenarios here to evaluate.

MATERIALS AND METHODS

Pine breeding population

The breeding population used in this study consisted of 369 selections made for the Cooperative Tree Improvement Program's 5th-Cycle breeding plan, which were topgrafted at the Arrowhead Breeding Center near Hawkinsville, Georgia, United States and in members' orchards across the southeastern United States. These selections were made from 4th-Cycle progeny tests planted across the southeastern United States (Figure 2 (a)). The progeny tests were grouped into series (CYCLE4C, CYCLE4P, and CYCLE4N) that correspond to the set of families from the corresponding breeding zone (Coastal, Piedmont, and Northern, respectively), but there is overlap both in the geography of test sites and in the families tested. Around a third of the families in the CYCLE4P tests are from the Coastal and Northern breeding zones, and around a third of the families in CYCLE4N are from the Piedmont and Coastal breeding zones, although care was taken to exclude maladapted families. The overlap in families was done to connect the series so that breeding values for all three populations could be estimated on the same scale.

The 5th-cycle selections were made based on an index calculated from individual-tree breeding values for stem volume, straightness, and disease resistance using weights of 60%, 20%, and 20%, respectively, from a standard normal transformation for each trait (where bigger values are more desirable). The MWT has a positive correlation (*r* = 0.32) with the index breeding value (Figure 2 (b)), driven by the correlation between MWT and volume breeding values.

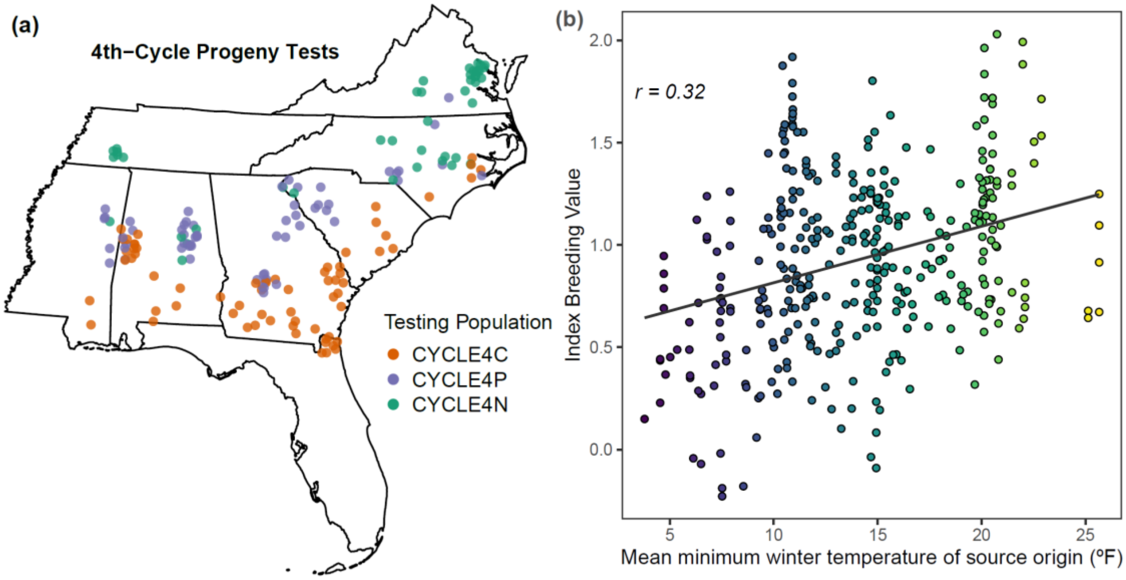

Figure 2. (a) Locations of 4th-cycle progeny tests in the southeastern United States. (b) Relationship between the index breeding value and mean minimum winter temperature for the 369 5th-cycle selections considered in this analysis.

The 369 selections varied in their cold hardiness, and the distribution of MWT was selected to reflect the distribution of area in the deployment region, ranging from 3.8°F to 25.7°F (Figure 3).

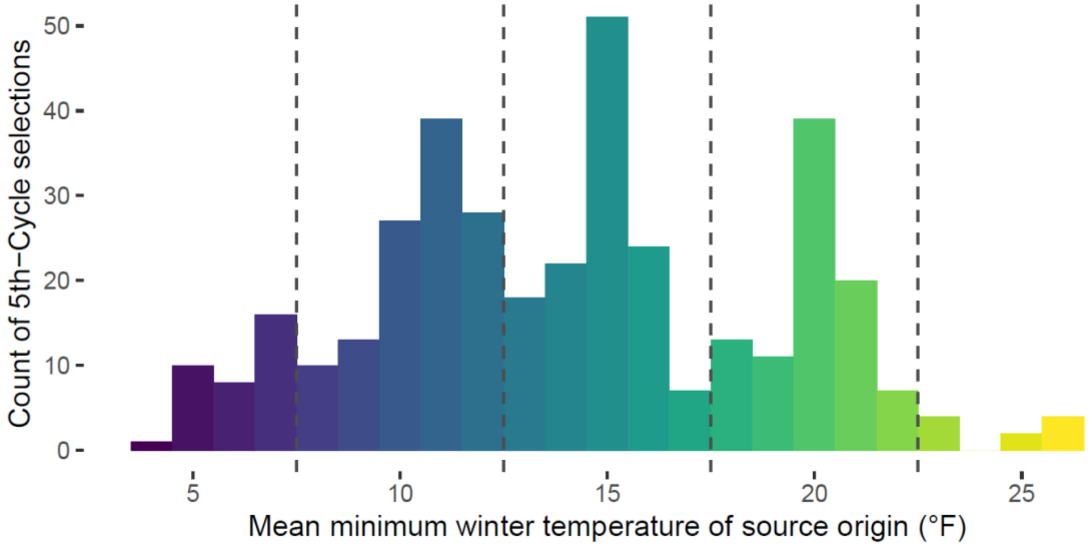

Figure 3. Distribution of mean minimum winter temperatures for 369 5th-Cycle selections used in the study. Vertical lines represent bins of 5°F that were used as mating groups in the flexible breeding zone scenario.

Mating scenarios

Three mating scenarios were compared: 1) no breeding zones, 2) strict breeding zones, and 3) flexible breeding zones. The first scenario represents a do-nothing approach and illustrates the consequences of ignoring the need for cold hardiness. The second scenario represents the traditional approach, and selections were assigned to three separate breeding zones based on MWT breaks of 8°F and 13°F. The third scenario represents an attempt to generalize the breeding zone scenario to match the clinal nature of cold hardiness.

For the flexible breeding zones scenario, we utilized the mating group functionality of MateSel, which was originally designed to place logistical restrictions on mating livestock from different farms. In our application, selections were assigned to five MWT bins with widths of 5°F that corresponded to mating groups (Figure 3). A target number of matings within each mating group was provided. A mating permission matrix was designed that allowed crosses among adjacent mating groups (MWT bins), but not more distant groups. For example, matings between selections from the <7.5°F and 7.5°F – 12.5°F bins are allowed, but not between selections from the <7.5°F and 12.5°F – 17.5°F bin. The exception is that the permission matrix allowed matings between the third group (12.5°F - 17.5°F) and the fifth group (MWT > 22.5°F), because the geographic region corresponding to these MWT bins can be considered a single breeding zone (the Coastal region). A weighting parameter is used by the algorithm to indicate how much deviation from the target number of matings within each group. A weight of 0 places no emphasis on the targets and is equivalent to the no constraints scenario (scenario 1). Preliminary analysis indicated that a weighting factor of 0.70 provided a desirable solution, so that was the value used in this analysis.

In all three scenarios, 500 crosses were requested and the number of crosses per breeding zone/mating group was made proportional to the number of selections in that group. The target degrees parameter was set at 35 (a typical value for the Cooperative's breeding program) and selections were allowed to be used as both female and male. No more than 9 matings per selection were allowed.

Metrics considered

Each scenario produced a mating list (list of trees to cross and their mating pairs). To evaluate the cold hardiness of the resulting mating lists, the mid-parent MWT was calculated for each cross, and the distributions were evaluated in a figure. This can also be considered the distribution of the progeny. To evaluate short-term gain, the mid-parent index value for each cross was calculated, and the distributions were evaluated. To evaluate the diversity of each mating list, we reported the number of parents used in the mating, the number of founders represented, and the mean coancestry coefficient among progeny. Also presented is the status number, an estimate of the effective population size (Lindgren et al. 1996).

RESULTS

Maintenance of cold hardiness

Ignoring the adaptability requirements (no breeding zones) resulted in a mating list where all but one progeny/cross had a mid-parent MWT greater than 8°F (Figure 4). The strict breeding zones and flexible breeding zones scenarios had similar distributions that were trimodal. The minimum mid-parent MWT for the strict breeding zones and flexible breeding zones were the same (both were 5.3°F). However, the mean for the strict breeding zones was lower (14.3°F) compared to that of the flexible breeding zones (15.2°F), which was actually slightly higher than the mean for the no breeding zones scenario (15.1°F). In all three scenarios, the distribution of cross MWT values had a warmer minimum than that of the breeding candidates, although the scenarios with strict and flexible breeding zones had appropriate values for the target deployment range.

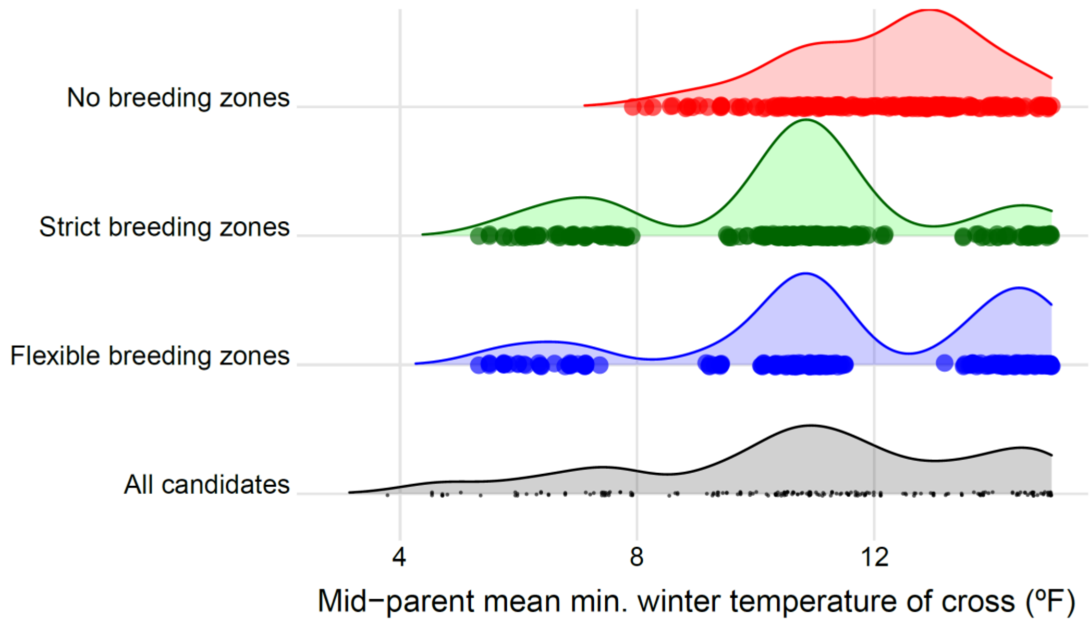

Figure 4. Distribution of progeny/cross (mid-parent) mean minimum winter temperature values for three mating scenarios. The distribution of all candidates for mating is shown on the bottom in black.

Short-term gain

Improvement in mean progeny index breeding value was highest for the scenario without breeding zones and lowest for the strict breeding zones scenario (Figure 5). The flexible breeding zones scenario was intermediate. All of the scenarios made considerable gain over the population from which the parents were selected.

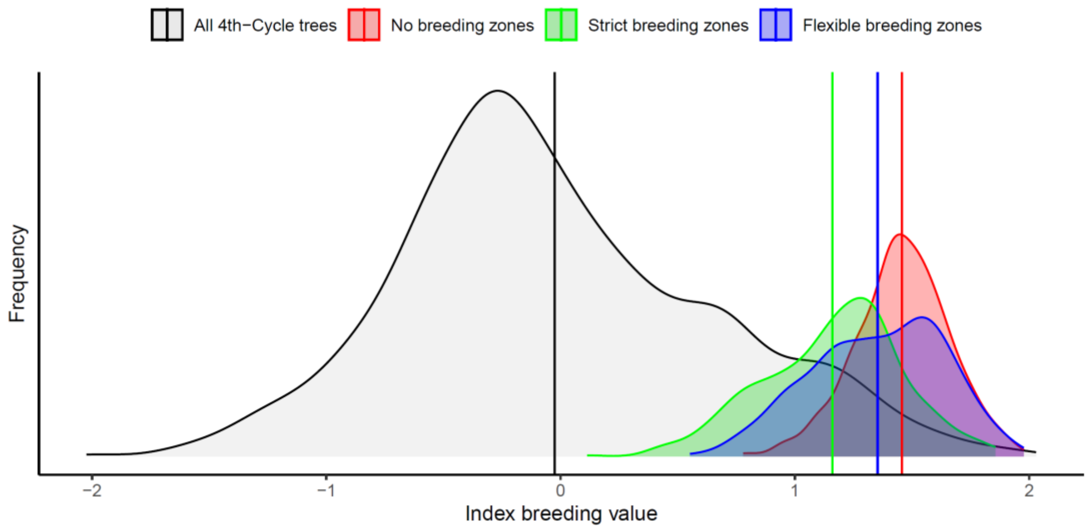

Figure 5. Distribution of progeny/cross (mid-parent) index breeding values for three mating scenarios. The distribution the 4th-Cycle population is shown in gray. Index breeding value units are on the standard normal scale.

Diversity

For all of the diversity metrics considered, the flexible breeding zones scenario was intermediate between the no breeding zones scenario (least diverse) and the strict breeding zones scenario (most diverse). For the number of parents used and the number of founders represented, the flexible zones tended to be more similar to the strict breeding zones scenario. For the status number and mean relationship among progeny, the flexible zones scenario tended to be more similar to the no breeding zones scenario.

Table 1. Diversity metrics for three mating scenarios.

Scenario	Number of parents used	Number of founders represented	Mean coancestry among progeny	Status number
No breeding zones	126	171	0.01469	31.9
Strict breeding zones	236	290	0.00656	66.3
Flexible breeding zones	191	253	0.01250	37.1

DISCUSSION

Ignoring cold hardiness in this population of *Pinus taeda* will result in breeding plans that choose warmer-source parents and, ultimately, the loss of adaptability for the colder part of the deployment range. The use of breeding zones is an effective tool to maintain cold hardiness, but it has a cost of short-term gain and increased breeding load (more parents are required to participate in breeding). The work we present here sheds light on quantifying these costs.

The flexible breeding zones approach produced a solution that met the cold hardiness needs and appeared to strike a good balance between short-term gain and diversity needs. However, this work did not elaborate on the choice of the weighting parameter on the target number of crosses per breeding zone. The value used (0.70) was “backed into to” by running several preliminary analyses. Breeders that use the flexible zones approach need to carefully re-evaluate this weight to ensure the mating list solution is adequate. Another limitation of our analysis was that it included just one cycle of breeding. Future research should evaluate the scenarios over multiple breeding cycles to better understand their impact. Further, we only considered the cold hardiness, short-term gain, and diversity impacts on the breeding population. Future research should also evaluate the impacts on gain and cold-hardiness on the best set of unrelated parents, as the deployment population will consist of a much smaller set of parents from the breeding population.

There has been considerable research to develop methods for defining appropriate breeding zone boundaries (Raymond et al. 1990; Johnson 1997; Thomson et al. 2010; Ukrainetz et al. 2018; Yu et al. 2022). Owing to the fact that most tree breeding programs are in their infancy, with few cycles of breeding completed, there is little research on improving the use of breeding zones in advanced cycles. More research is needed to generalize the breeding zone strategy when adaptability traits are clinal.

ACKNOWLEDGEMENTS

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Genetic Variation in Growth and Survival Through 40 Years in a Southern Mississippi Longleaf Trial

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In a long-term, 13-parent diallel of longleaf pine (*Pinus palustris* Mill.) tested in southern Mississippi, there was significant variation among full-sib families for growth traits at ages 3, 7, 17, 30, and 40 years and for survival at later ages. The variation was under genetic control, with a trend of increasing importance of dominance variance with age. Genetic values for height and volume were most strongly correlated between ages 17 and age 40. Survival was more strongly heritable than either height or volume, and full-sib genetic values for survival were also correlated across ages. Conservation efforts for partial recovery of the longleaf forest cover type continue through government cost-share programs. Establishment of new longleaf pine forests should ideally be with tested seed sources, to ensure that the performance of longleaf plantations meets conservation and forest management goals.

Rotation-Aged Genetic Parameters for Shortleaf Pine (*Pinus Echinata* Mill.) and Their Implications for Tree Improvement, Disturbance Response, and Species Restoration in a Changing Climate

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Local adaptations are commonly observed in naturally regenerating tree populations, with diverse abiotic and biotic environmental factors influencing both phenotypic and genetic variations in adaptive and growth traits (Keller *et al.*, 2011; Wang *et al.*, 2014; Zhang *et al.*, 2019; Ding and Brouard, 2022; Hu *et al.*, 2022). At the same time, local maladaptation, when present, and associated tree mortality are typically related to multiple abiotic and biotic stresses exacerbated by the increasing frequency of extreme weather events (Worrall *et al.*, 2013; Anderegg *et al.*, 2015; Aitken and Bemmels, 2016; Ware *et al.*, 2021). To address this situation, tree improvement may be utilized to provide the needed adaptability and productivity traits for trees planted under future environmental conditions. Clearly, ensuring adaptability to extreme weather events while maintaining competitive growth increments for long-lived tree species is critical for successful reforestation efforts, including species restoration. As a precursor to tree improvement, provenance tests (seed source studies) are often conducted on multiple sites to investigate genetic and genetic × environmental responses of growth and adaptive traits. In recent years, these studies have become important to further study the association between phenotypes, genotypes, and environments, where climate variables at the source location of the genotypes as well as the test planting locations are considered (Isabel *et al.*, 2020).

Shortleaf pine (*Pinus echinata* Mill.), an important commercial tree species that often co-occurs with loblolly pine (*Pinus taeda* L.), is under considerable threat with significant range-wide declines (Hossain *et al.*, 2021). The species which plays an important role in many forest ecosystems across the southeastern US, is usually found on drier sites where fire is more frequent (Stewart *et al.*, 2016). However, the biology and genetics of shortleaf pine have not been broadly investigated, including its ecological adaptations and traits important to stand productivity (Stewart *et al.*, 2016). Shortleaf pine is typically managed for sawlog production and planted for ecosystem restoration at cooler and drier sites compared to those managed for and planted to loblolly pine. However, as reforestation of loblolly pine expands into the shortleaf pine range due to its faster growth rate, the potential for hybridization between the species will likely increase

(Stewart et al. 2012) as well as changing the stand dynamics of the two species growing under novel conditions.

Utilizing long-term genetic trials and accumulated phenotyping data to better understand the genotype-phenotype-environment associations within and between seed sources should help to inform the development of management strategies optimized for projected climate scenarios. For example, guided with this information, tree improvement programs can produce productive and resilient tree families for planting in commercial- and conservation-oriented projects, thereby increasing the overall resilience of the forested landscapes and the associated local and regional communities. To better understand the potential for tree improvement in shortleaf pine, we estimated genetic parameters of growth and adaptive traits (e.g., ice storm damage) using data collected at rotation age (30 to 40 years) on about 20,000 planted trees, representing 330 full-sib families formed by a series of disconnected half-diallel crosses. The parents of the crosses originated on National Forests selected from two environmental zones (Ouachita Mountains and Ozark Plateau of Arkansas) and the crosses were assessed in 11 and 4 field trials, respectively, in the same two zones (Fig. 1.). The parents tested are maintained by the USFS Southern Region's Genetic Resources Program at the Mt. Ida, AR seed orchard.

Notes, a) The symbols of 15 USDA-FS progeny trial locations and natural species range of shortleaf pine in the USA with the extreme minimum temperature (EMT); b) The progeny tests and the five delineated zones based on the climate and geographical locations in Arkansas. The species range is based on the USDA-FS forest inventory data (Peters *et al.*, 2019); the climate data were retrieved from the ClimateNA (http://climatena.ca/, Wang *et al.*, 2016). The climatic variables include MAT, Mean annual temperature (°C); MAP, mean annual precipitation (mm); MWMT, mean warmest month temperature (°C); MCMT, mean coolest month temperature (°C); EMT, extreme minimum temperature over 30 years (°C); DD5, degree-days above 5°C; bFFP, date-of-year as the beginning of frost free period; eFFP, date-of-year as the end of frost free period (Ding *et al.,* 2023).

Fig. 1. Study area and trial locations in Arkansas in the context of the species range of shortleaf pine with x-axis as the longitude, and the y-axis as the latitude.

Both individual tree- and family-based models provided robust genetic parameter estimates for stem growth and taper; with narrow-sense heritability of height increment from age-5 years to the rotation-age measurement reaching a moderate level (0.1 to 0.4) across the trials that were not highly impacted by ice storms (Ding *et al.*, 2023). Although, the trials were differentially impacted by ice storms, as indicated, we found that these severe weather events changed the pattern of genetic parameters, with those trials most highly impacted providing an opportunity for selection for tolerance to ice damage (Ding *et al.*, 2023). Overall, the results underscore the potential of tree improvement for developing shortleaf pine planting stocks with enhanced resilience to extreme weather events, such as ice storms, thus, benefitting ongoing species conservation and ecosystem restoration efforts across the southern US.

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Concurrent Session 2A:

Hardwood Genetics and Tree Improvement II

Conserving Mississippi-Origin American Chestnut Genotypes Through Nut-Grafting Surviving Stump Sprouts
– *Charles Burdine, USFS-SRS*..pg. 28

Cytogenomic Characterization of rDNA and the Chromatin Composition of NOR-Associated Satellite in American and Chinese Chestnuts
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A Comparative Approach to Understanding Adventitious Root Induction in Recalcitrant Species
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Improved GWAS Methods Refine the Model of Multi-Genic Blight Resistance in Hybrid Chestnut
– *Austin Thomas, ORISE Fellow to USFS-SRS*..pg. 38

Conserving Mississippi-Origin American Chestnut Genotypes— Initial Results with a Modified Nut-Grafting Method

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Often considered the greatest ecological disaster in North America, the loss of the American chestnut (*Castanea dentata*) to chestnut blight (caused by *Cryphonectria parasitica*) greatly impacted the Appalachian forests along with local and regional economies (Clark et al. 2019, 2023). Thanks to tree breeding efforts made by various entities; blight-resistant seedlings are anticipated to become widely available in the next few years. However, before reintroduction to the forest, it is necessary to develop and produce both resistant and locally adapted seedlings. Fortunately, coppice regenerating stumps in the wild provide an opportunity to preserve local genotypes by establishing germplasm conservation orchards through clonal propagation. In addition, it is expected that American chestnut genotypes that evolved in the most southwestern area of the species' range (i.e., northeast Mississippi) will prove to be valuable sources of germplasm for breeding trees that are adapted to warmer environments. In the last couple of years, the Southern Research Station (SRS) has made substantial progress in developing a modified nut-grafting technique that is resulting in the conservation of rare American chestnut genotypes located in natural forests in Mississippi and adjacent Alabama.

Methods: In November 2022, we obtained seeds from Chinese chestnuts (C. *mollissima*) and American chestnuts from two cooperators, respectively-- Ron Revord, University of Missouri and Steve Hartman, Scottsville, Kentucky. During 2022/2023, additional cooperators provided scions for grafting in addition to scions we collected from the Mississippi and Alabama trees. In total, scions from 76 genotypes were collected from four areas within the southern US (northwest Alabama, northern Georgia, western Kentucky, and northeast Mississippi) (Table 1). The seeds for rootstock were germinated in December 2022 using peat pots and potting media (Fig. 1A). Once the seedlings were around 5 cm tall, they were cut for grafting using a razor blade (Fig. 1B-C) and grafted, with the graft unions being secured with fly-fishing line (Fig. 1D). The grafts were maintained in a growth-room with high relative humidity and artificial light (Fig. 1E). After 2-3 weeks in the growth-room, the surviving grafts were transplanted into half-gallon pots and transferred to a shadehouse (Fig. 1F). At various times during the season, the number and percentage of successful grafts for the two rootstock species and the four scion-areas were scored.

Results: We found that the peat pots and media allowed for good seedling growth and development and our environmental settings (temperatures, relative humidities, and light) in the growth-room (Fig. 1E) and shadehouse (Fig. 1F) provided adequate conditions for graft survival. Also, we noted that transplanting to one-gallon pots (Fig. 1F) at the time of transition to the shadehouse tended to be beneficial for survival (data not shown). Overall, for the 2022/2023 grafting season, 67.4% of the grafts survived through the transition to shadehouse stage, with modest differences observed among scion origins, ranging from 76.7% for north Georgia to 61.1% for northeast Mississippi (Table 1). In terms of rootstocks, Chinese chestnut resulted in a higher percentage of successful grafts overall, at 78% compared to 42% for American chestnut.

Fig. 1. Preparation of the American and Chinese chestnut rootstock and the modified root-grafting method. **A.** Rootstock germination using peat pots and potting media. **B.** Preparing the rootstock using a razor blade to cut the tissue. **C**. Scion ready to be grafted. **D**. Securing graft union using fly-fishing line. **E**. Acclimating the grafts from higher to lower humidity and appropriate light quality in the growth-room. **F**. Transferring the surviving grafts into half-gallon pots and placing them in the shadehouse.

Table 1. Results achieved in 2023— attempted and successful grafts and number of scion genotypes by state of scion-origin.

State of scion-origin	Surviving grafts	Attempted grafts	% Surviving grafts	No. genotypes grafted
Alabama	157	224	70.1	20
Georgia	165	215	76.7	19
Kentucky	126	187	67.4	14
Mississippi	248	406	61.1	23
Total	**696**	**1,032**	**67.4**	**76**

Discussion: In comparison to our results, researchers in New Zealand observed a significant range in success rates (0 to 100%) in grafting 18 Castanea spp. selections (Oraguzie et al. 1998). The choice of rootstock is essential for successful chestnut grafting and graft failure can be attributed to scion genotype and environmental conditions (Ackerman and Jayne 1980; Oraguzie et al. 1998). In our case, it took several attempts to modify the standard nut-grafting method for chestnut. Key adjustments included timing of scion collection, 1- or 2-bud scion preparation, rootstock preparation, type of cutting blade, and acclimation of grafted plants from one stage to the next. Previous studies have supported the importance of the choice of blade and scion preparation for grafting success (Ackerman and Jayne 1980; Xie et al. 2022). Through experience, we found that maintaining adequate post-grafting microclimates was critical for graft survival. Most importantly, was high relative humidity in the growth-room during the post-grafting acclimation phase (Fig. 1E). In our hands, the modified nut-grafting method has proven quite robust over the initial two seasons of scaling-up the number of grafts (the overall survival rate in 2021/2022 on over 1000 grafts was 63%, Nance and Parker, unpublished data). In addition, grafting on Chinese chestnut rootstock appears to be a good option (Ackerman and Jayne 1980), as survival rates were higher than grafting on American chestnut and the grafts will likely be resistant to Phytophthora root rot (Crandall et al. 1945).

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Cytogenomic Characterization of rDNA and the Chromatin Composition of the NOR-Associated Satellite in American and Chinese Chestnuts

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Abstract: Several genetic linkage maps of American and Chinese chestnuts are being utilized to accelerate the American chestnut breeding program. Both chestnut genomes have recently been sequenced, facilitating gene discovery and assisting in molecular-based breeding and genetic engineering. To complement and extend this genomic work, we analyzed the distribution and organization of their ribosomal DNAs (35S and 5S rDNA), and the chromatin composition of nucleolus organizing region (NOR)-associated satellites. Two loci of 35S (one major and the other minor) and one 5S rRNA locus were identified using fluorescent *in situ* hybridization. An additional 5S locus was observed intermingled distally with the major 35S site in Chinese chestnut. The major 35S site is located terminally and sub-terminally in American and Chinese chestnut, respectively. The 35S site originates at the end of the short arm of its chromosome, extending through the secondary constriction (the central region of the NOR) and into the proximal region of the satellite in Chinese chestnut, while in American chestnut the 35S site covered the entire satellite. In Chinese chestnut, a distinct demarcation was observed separating the distal region of the satellite, which is highly heterochromatic (i.e., gene-poor), from the proximal region containing the rRNA gene(s). This region may be euchromatic (i.e., gene-rich). This heterochromatic DNA could be species and/or chromosome specific. A striking accumulation of high 35S rDNA gene copy number was noticed at one of the minor 35S sites in a Chinese chestnut line, suggesting an evolutionary strategy that may favor the emergence of a new variant within this species.

Introduction: The American chestnut tree, *Castanea dentata* [(Marsh.) Borkh., $2n = 2x = 24$], once revered as the 'Majestic Forest Tree' of eastern North America, covered an impressive expanse of over 800,000 sq. km., ranging from Maine to Mississippi. However, it was tragically decimated, first in the 19th century by Phytophthora root rot in the Southeast and then by the middle of the 20th century across its entire range by chestnut blight. These devastating diseases are respectively caused by invasive pathogens *Phytophthora cinnamomi* Rands., and *Cryphonectria* *parasitica* (Murr.) Burr. In contrast, the Chinese chestnut (*Castanea mollissima* Blume, $2n = 2x = 24$) exhibits resistance to both pathogens, having co-evolved with them and consequently suffers little damage (Anagnostakis 1992; 2012). Efforts spearheaded by the American Chestnut Foundation and the State University of New York are currently underway to identify and transfer the resistance genes from the Chinese chestnut to the American species, through backcross breeding and from wheat by genetic engineering (Powell 2020). Several genetic maps of Chinese chestnut have been developed (Kubisiak et al. 1997, 2013; Zhebentyayeva et al. 2019), integrated with a physical map (Fang et al. 2013) and more recently, the genomes of both chestnut species have been sequenced (Staton et al. 2020; Westbrook et al. 2022). These valuable resources are expected to significantly bolster genomic research and breeding efforts aimed at the development of resilient American chestnut varieties. Despite these advancements, knowledge of chestnut cytogenetics is limited, especially when compared to other plant species, such as Arabidopsis, rice, sorghum, and maize, and others (see review by Garcia et al. 2017; Jiang 2019). Knowledge of structural organization of the chestnut genome is important for inter-species breeding, particularly when target genes are located on rearranged chromosomes. Fluorescent *in situ* hybridization (FISH) can precisely locate specific DNA sequences on individual chromosomes and shed light on the structural and compositional organization of a genome and its molecular evolution (Cerbah et al. 1998; Heslop-Harrison 2000; Islam-Faridi et al. 2002; Jiang 2019).

In this study, we report:

i) compositional details of the major 35S rDNA at the NOR region in both American and Chinese chestnuts; and
ii) repositioning of the major 35SrDNA locus from a major site to a minor site in the Chinese chestnut.

Materials and Methods: Root tip collection and pre-treatment to accumulate chestnut metaphase chromosome spreads were carried out as described by Islam-Faridi et al. 2023. Fluorescent *in situ* hybridization coupled with capture of digital images by epi-fluorescence microscopy and subsequent processing were performed as described previously (Islam-Faridi et al. 2023). Two experiments were performed; in the first experiment, American and Chinese chestnut chromosome spreads were probed with 35S, 5S and telomere repeat Oligo probes (Islam-Faridi et al. 2020). In the second, a 35S rDNA probe was used in a Chinese chestnut x American chestnut F_1 hybrid spread as described elsewhere (Islam-Faridi et al. 2023).

Results and Discussion: In plant cells, ribosomes play a crucial role in protein synthesis, and their production is driven by ribosomal genes, namely 35S and 5S rRNAs (Rosselló et al. 2022). We observed that both American (AC) and Chinese chestnuts (CC) contained two 35S rDNA loci (one major and one minor) and a single 5S rDNA locus, each independently located on three different chromosomes. Additionally, a second 5S locus was identified in Chinese chestnut located on the satellite one (SAT-1). The major 35S (mj-35S) locus was positioned at the terminal end in AC, while it was sub-terminal in CC. The minor 35S (mn-35S) and the 5S loci were proximally located in both species [Fig. 1 (AC) and Fig. 2 (CC)]. Interestingly, our findings regarding the Chinese chestnut contrast with those reported by Ribeiro et al. 2011, in which the mj-35S and the second 5S loci in *C. mollissima* were identified as pericentromeric on the short arm. They proposed that these rDNA loci underwent restructuring during the evolutionary history of *Castanea* species. A detailed cytomorphological analysis of the rDNA loci in AC and CC, along with numerous evolutionary insights, was recently reported in Islam-Faridi et al. (2023).

From an analysis of their length, we identified two types of satellites (SAT) in Chinese chestnut, viz., SAT-1 and SAT-2 (Fig 2; for details see Islam-Faridi et al. 2023). We observed that the satellites in both AC and CC appear detached from their respective mother-chromosome [mo-ch, see Fig. 1 (AC) and Fig. 2 (CC)] until they reach full condensation (Fig. 3a; the chromosome spread was from a CC x AC F_1 hybrid). A second 5S locus was identified on the SAT-1 of CC (see enlarged image in lower-right box, Fig. 2) (for additional details see Islam-Faridi et al. 2023).

Telomere length in plants, which ranges from 1 to 50 kb, plays a crucial role in safeguarding the chromosome ends and maintaining chromosomal integrity (Fuchs et al. 1994). Using a telomere-specific probe in FISH, we confirmed their presence at the chromosomal termini (Figs. 1 and 2). The telomere signals on AC satellites and SAT-2 of CC are initially overshadowed by the mj-35S signal (green). Notably, no telomere signals were detected at the end of the short arm of the mj-35S mother-chromosome [mo-ch, arrowheads, Fig. 1 (AC) and Fig. 2 (CC)]. However, they were visible on the satellites that extend beyond the short arm when the green signals were dimmed and the red signals enhanced through image analysis [see inserts, enlarged images of the satellites, Fig. 1 (top-left) and Fig. 2 (lower middle-right)].

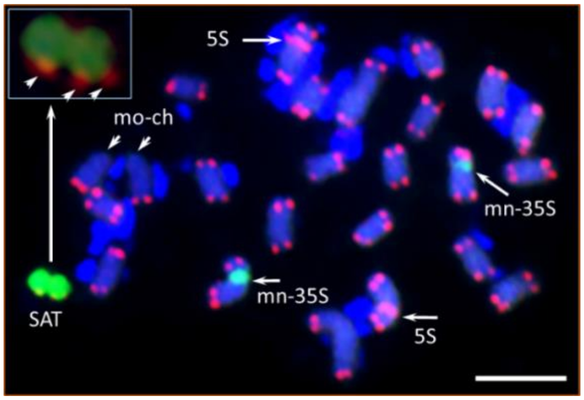


Fig. 1. Somatic chromosome spreads of American chestnut, showing two 35S rDNA loci [one major (mj-35S), one minor (mn-35S), green signals] and one 5S rDNA locus (red signals) and telomere repeat sites at each chromosomal end (red signals). Both satellites appeared detached from their respective mother chromosomes (mo-ch, arrows). The image was captured under a 100x objective. Scale bar = 5 µm.

Based on DAPI staining, we have recently reported that the AC satellite is likely euchromatic, while the proximal region of the CC satellite's might be euchromatic and its distal region heterochromatic. In CC, the mj-35S originates at the end of the short arm, passes through the secondary constriction (SC) and extends into the proximal region of the satellite [CC mj-35S, Fig. 3a and Fig. 3a1 (an enlarged image)]. The same path is observed for AC, but it covers the entire satellite (AC mj-35S, Fig. 3a). The SAT-2 of CC appeared the same as that in AC, suggesting it might have been contributed by a chestnut species other than Chinese chestnut (for details see Islam-Faridi et al. 2023).

Expressed DNAs are predominantly or preferentially euchromatic and stained weakly or less intensely with DAPI. In contrast, heterochromatic regions, which are AT-rich, stain strongly or more intensely with DAPI and appear bright blue under a UV-filter (Schweizer 1976; de Jong et al. 1999). Surprisingly, we observed a robust 35S signal on one of the mn-35S site homologues (an enlarged image shown in top-right box, Fig. 2). This accumulation of additional rDNA copies, potentially an evolutionary strategy, might result from jumping events and/or unequal

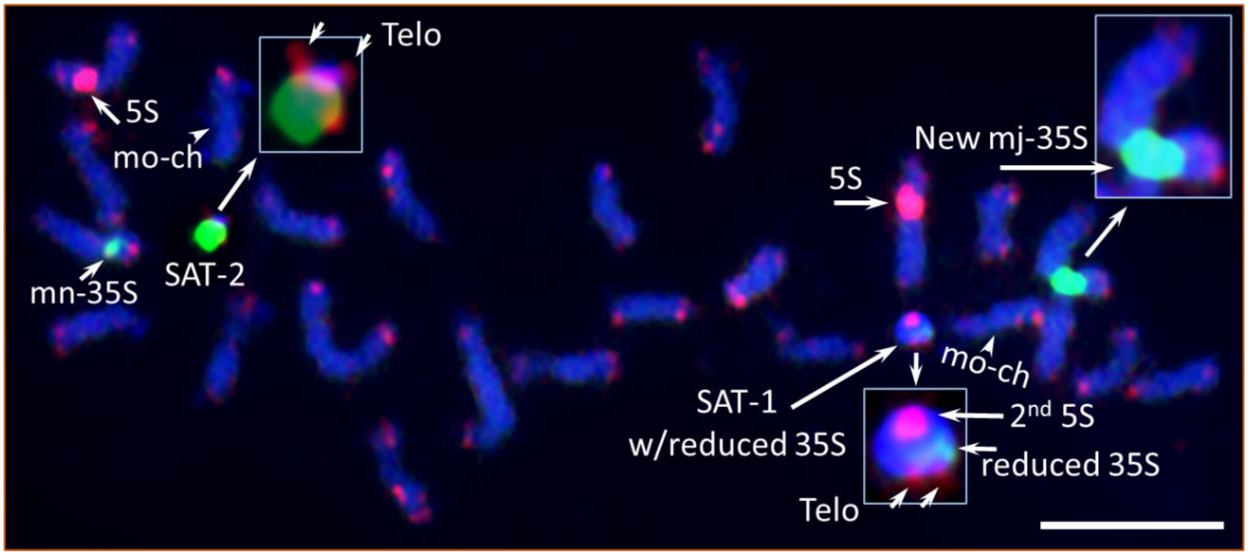
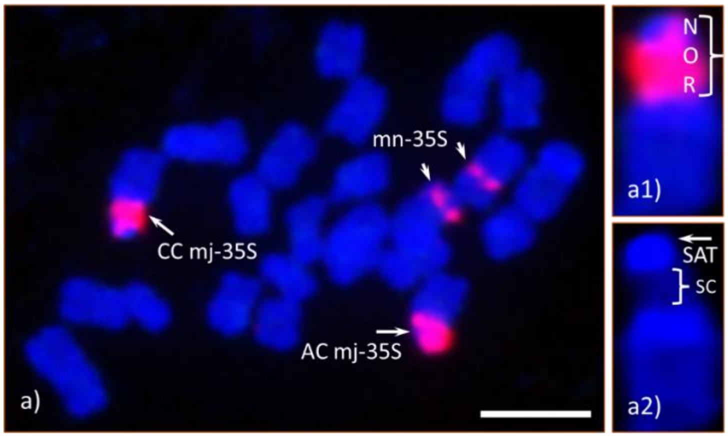


Fig. 2. Somatic chromosome spreads of Chinese chestnut, showing two 35S rDNA loci (green signals) and one 5S rDNA locus (red signals) and telomere repeat sites at each chromosomal end (red signals). Both satellites appeared detached from their respective mother chromosomes (mo-ch, arrowheads). An enlarged image of the new mj-35S locus at minor 35S site shown in upper-right box. The image was captured under a 63x objective. Scale bar = 5 µm.



a1): Enlarged image (RGB) of Chinese chestnut mj-35S rDNA bearing chromosome.
NOR = nucleolus organizing region

a2): DAPI image, same as “c1” under UV-filter
SAT = satellite
SC= secondary constriction

Fig. 3a. Somatic chromosome spread of Chinese x American chestnut F_1 hybrid hybridized with 35S rDNA probe, illustrates the expected two 35S sites. Notably, one of the mj-35S signals shows a prominent satellite, contributed by the CC parent, while the other was from AC. The image was captured under a 100x objective. Scale bar = 5 µm.

crossing-over between the mj-35S mo-ch and the mn-35S rDNA chromosome [Schubert 1984; Mohannath et al. 2019; observed in maize and ash, Islam-Faridi (unpublished)]. Consequently, this event might lead to the emergence of a new Chinese chestnut variant with an interstitial major 35S locus, while the original satellite (SAT-1), possibly carrying fewer 35S gene copies at the NOR (an enlarged image shown in lower-right box, Fig. 2), may remain unchanged. Alternatively, the satellite might eventually be lost over time. In Arabidopsis, significant variation in the copy number of the rDNA genes was associated with changes in gene expression profiles of several hundred genes (Lopez et al. 2021). Whether variation in the cytogenetic organization of the 35S locus affects the chestnut transcriptome remains to be elucidated.

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Disclaimer: The findings and conclusions presented here are those of the author(s) and should not be construed to represent any official USDA or US Government determination or policy.

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A Comparative Approach to Understanding Adventitious Root Induction in Recalcitrant Species

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Adventitious roots (ARs) are plant roots that form from tissues other than roots via an organogenic process. For numerous plants, AR formation is crucial for successful vegetative propagation. However, many species, such as American chestnut (*Castanea dentata*) and golden camellia (*Camellia nitidissima*) are recalcitrant to adventitious root formation, imposing a major limitation in the clonal propagation of elite germplasms. The AR formation is a complex process affected by numerous factors. With auxin induction, stem cuttings of American chestnut and golden camellias could form ARs in six weeks, albeit the rooting rate was low. Calli were often found in American chestnut cuttings, while this was not the case for golden camellia cuttings. Compared to easy-to-root poplar (*Populus* × *euramericana*), American chestnut cuttings and golden camellias had a low level of indole-3-acetic acid (IAA) and a high level of cytokinin (CK), abscisic acid (ABA), salicylic acid (SA), jasmonic acid (JA), and oxylipin 12-oxo-phytodienoic acid (OPDA). Hormone distribution between leaves and stems in American chestnut and golden camellias also differed from poplar. For example, IAA showed an almost even distribution between poplar leaf and stem, while American chestnut and golden camellia had a much higher IAA in stem and leaf, respectively. American chestnut and golden camellia showed a much higher JA level in stem than poplar, when compared to leaf. For cytokinin, American chestnut and golden camellia had the opposite leaf/stem distribution as poplar did. For ABA, American chestnut and golden camellia had a lower stem/leaf ratio than poplar. When secondary metabolites that are known for promoting AR formation were compared, American chestnut cuttings had zero being up-regulated and six being downregulated, and golden camellia cuttings showed two being up-regulated and six being downregulated. Poplar cuttings also contained seven AR-promoting secondary metabolites that were not found in American chestnut and golden camellia. Our results indicate that the unfavorite endogenous hormone and secondary metabolite profiles may contribute to American chestnut and golden camellia cuttings' recalcitrance to rooting.

Improved GWAS Methods Refine the Model of Multi-Genic Blight Resistance in Hybrid Chestnut

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The American chestnut (*Castanea dentata*) once spanned the Appalachian region of North America before chestnut blight (*Cryphonectria parasitica*) led to the tree's functional extinction. The chestnut blight fungus persists in the environment throughout the American chestnut's historical range. The potential to resurrect the American chestnut through interspecific hybridization and backcross breeding with Chinese chestnut (*Castanea mollissima*) holds promise but largely depends on the underlying genetics of the Chinese chestnut's resistance to chestnut blight. To expand our understanding of the genetics of blight resistance, we used improved GWAS techniques to analyze data combining blight inoculation phenotypes across several families of hybrid origin. Our analysis identified twenty peak QTNs, eleven with well-defined QTL regions. When analyzed jointly, these QTNs and QTL regions account for roughly 2.8 to 5% of the genome, while explaining 13 to 23% of the phenotypic variation in canker size, the standard measure of blight resistance. Several QTN candidate genes are directly related to the regulation of reactive oxygen species and, more broadly, to a pathogen defensive response. These results suggest that several genes across the chestnut genome contribute to blight resistance but that the trait is not quantitative in the traditional sense, where hundreds or thousands of genes contribute equally to the observed phenotype.

Concurrent Session 2B:

USFS Programs Update & SFTIC Update

A Washington Office Update on the USDA Forest Service Genetics R&D
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Forest Service, National Forest System, Southern Region, Genetic Resource Management Program
– *Marcus Warwell, USFS-R8*...pg. 41

The Forest Service National Genetic Resources Management Program and Integration with the National Forest and Grasslands
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Futuring The Southern Forest Tree Improvement Conference
– *C. Dana Nelson, USFS-SRS*...pg. 43

A Washington Office Update on the USDA Forest Service Genetics R&D

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Within the USDA Forest Service, the National Program Lead for Genetics & Biodiversity Conservation Research and Development is housed within Sustainable Forest Management. This position supports policy formation, provides input on priorities and processes, facilitates inter-agency genetics communication, and responds to legislative and executive branch inquiries related to genetics. More importantly, it serves about 30 Forest Service geneticists and genetics-users by facilitating communication and through leadership that addresses national-level research concerns. In that capacity, I will review the process and results of the recent $20 M BIL solicitation for reforestation and revegetation. I will also review progress on concerns raised by FS geneticists, including data storage and archiving, high-performance computing, staffing and the genetics infrastructure of the National Forests, and coordination with National Forests (NFs) and Tribes concerning seed sources and management. Although the mandates of the Endangered Species Act and National Environmental Policy Act are hardwired into public lands management, I hope to facilitate a strategic vision for how USDA-FS R&D Genetics & Biodiversity Conservation supports species conservation as part of a new strategic plan for R&D Genetics. Any strategic plan must consider the rapid changes in genetics technologies, the diversity of the American public, and the needs of both the NFs and SP&T forestry - including plant and animal pathology - on 'All Lands' public and private. In helping to shape the future of USDA-FS genetics, the ideas and concerns of geneticists and genetics-allies attending SFTIC are especially important and earnestly requested.

Forest Service, National Forest System, Southern Region, Genetic Resource Management Program

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Genetic principles are fundamental to the USDA Forest Service's mission to sustain the health, diversity, and productivity of the nation's forests and grasslands to meet the needs of present and future generations. The Forest Service, Southern Region encompasses 13 states and Puerto Rico and consists of 14 National Forest units and two special units. The objectives of the Forest Service, Southern Region, Genetic Resource Management Program (GRMP) are to 1) provide leadership and expertise to incorporate genetic principles into ecosystem management; 2) provide high quality, genetically diverse, and source-appropriate seed for reforestation and restoration and 3) to conserve genetic diversity of at-risk plant populations in accord with National Forest planning. The GRMP focuses primarily on the support of longleaf pine, shortleaf pine, northern red oak, and white oak reforestation. The program also supports genetics management actions for more than 20 additional forest tree species and several native plants. Program components include the active management of the Ashe Seed Extractory and Seed Bank in Brooklyn, Mississippi and 7 Genetic Resource Management Areas (GRMAs) located on National Forests in 6 states. GRMAs are special administrative areas designated for intensive tree seed production. Combined, the GRMAs encompass nearly 3,700 acres and contain 100 orchards. Additional tree seed orchards and seed production areas apart from the GRMAs are also maintained on the National Forests. Most notably the Watauga Northern Red Oak seed Orchard in northern Tennessee is the largest of its kind and is presently administered through an agreement with the University of Tennessee. To meet the objectives of the GRMP, the Forest Service is reliant on external partnerships with state, local, private, tribal, and federal organizations including CAMCORE, Global Conservation Consortium for Oak, Georgia Forestry Commission, Longleaf Alliance, NC State Tree Improvement Cooperative, Western Gulf Forest Tree Improvement Program, and others.

The Forest Service National Genetic Resources Management Program and Integration with the National Forest and Grasslands

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The Forest Service's Genetic Resource Management Program is critical for providing genetics expertise and training to support the National Forests and Grasslands ensuring ecosystems resilience and health while conserving biodiversity. Our national program supports the Forest Service Mission "to sustain the health, diversity, and productivity of the nation's forests and grasslands to meet the needs of present and future generations." It contributes to successes in restoration, revegetation, reforestation, species conservation, mitigating impacts of non-native pathogens and insects, fire recovery and climate change adaptation efforts. Environmental problems, climate change, pest and diseases, wildfire and other natural disasters are part of the threats impacting the agency's forests and grassland resources. The National Forest System (NFS) Geneticists routinely work collectively on commercial and non-commercial conifer and hardwood tree species, as well as native grasses and forbs. The major responsibilities of the NFS Geneticists are to: (1) Coordinate tree improvement and plant breeding programs to develop better adapted, climate change resilient, and disease/pest resistant genetic resources for planting, (2) Manage orchards for operational seed production and breeding arboreta as *ex situ* conservation reserves, (3) Select disease and pest resistant germplasm with durable genetic resistance to endemic and introduced diseases of forest trees, (4) Implement strategies for genetic conservation of threatened and endangered tree species or populations for *ex situ* and *in situ* preservation, (5) Develop and apply seed zones, breeding zones, and seed transfer guidelines to ensure adaptability and successful reforestation or revegetation with proper seed deployment recommendations, (6) Provide planting programs with assisted migration guidance on the proper relocation of species or populations to ensure resilient landscapes under climate change, (7) Establish evaluation plantations to assess the genetic worth of plant materials for long-term adaptation in lieu of climate change, (8) Plan, coordinate, and train for seed procurement activities to ensure seed supply with genetically diverse and adapted planting materials, and (8) Collaborate in genetic related activities with research, academic, non-profit, private sector, tribal, and international entities in cooperative efforts, consultations, reviews, information and germplasm exchange. The Forest Service Genetic Resources Management Program also provides genetic and genomic services for forest and grassland management with the National Forest Genetics Laboratory (NFGEL). NFGEL provides baseline genetic information, determines the effect of management on the genetic resource, supports genetic improvement programs, and contributes to conservation and restoration programs, especially those involving native and threatened, endangered, and sensitive species. State-of-the-art molecular genetic information is provided to National Forests and grassland managers as well as to other cooperating agencies to assist with the proper management of our nation's genetic resources.

Futuring the Southern Forest Tree Improvement Conference

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Abstract: The Southern Forest Tree Improvement Conference (SFTIC) was first held in 1951 as the Conference on Forest Genetics Research in the Southern Pines, while the precursor to the Committee on Southern Forest Tree Improvement (CSFTI) was formed at the close of that initial conference. The second and subsequent conferences were titled the Second (Third, etc.) Southern Conference on Forest Tree Improvement, with SFTIC being adopted by about the 12th conference in 1973. Early proceedings suggested that the committee and the conference would consider forest genetics broadly in terms of research questions and applications, while having a special focus on southern pine improvement. Over the decades, the conference grew and evolved to a large meeting on forest genetics of southern species and allied biotechnologies, while maintaining a focus on loblolly and slash pine improvement. This evolution seems natural, and it appears to have met the committee's initial expectation. However, recent decades have seen declines in conference attendance, apparently caused by competition among meetings, but possibly due to a reputation of being a meeting on southern pine improvement, and not forest genetics more broadly. Given the current, urgent need for genetics information in forest management, specifically to address climate change and invasive species, SFTIC clearly has the potential to reverse this trend. We will explore options on re-branding and re-positioning SFTIC to better reflect the initial and our continuing aspiration to be the go-to conference on forest genetics and tree improvement— south-wide and beyond.

Background and Context

The Southern Forest Tree Improvement Conference (SFTIC), as we now know it, was first held in January of 1951 as the Conference on Forest Genetics Research in the Southern Pines (Haig, 1951). The conference was organized by the precursor of the soon to be formed Committee on Southern Forest Tree Improvement (CSFTI). The second conference was titled the Second Southern Conference on Forest Tree Improvement, although C.E. Ostrom writing in the proceedings of that meeting (Ostrom, 1953) referred to the conference as the Southern (Forest) Tree Improvement Conference. In reviewing SFTIC proceedings (at https://sftic.org/) it appears that SFTIC was mostly adopted for the committee by the 10th conference (1969) and for the biennial conference by the 12th conference (1973).

The early SFTIC proceedings suggested that the committee and the conference would consider forest genetics broadly in terms of research questions and applications in forest management, while having a special focus on southern pine improvement (Haig, 1951). The imperative to genetically improve the southern pines was clear in the founding and early development of the committee and the conference (Haig, 1951), while there were concerns expressed about starting too late (Stone, 1951) and not having the resources to obtain success. Later proceedings clearly show the steady progression of southern pine improvement, and the growing demand for improved seedlings by forest landowners. The founding committee likely would have been amazed by the progress made in loblolly and slash pine genetic improvement and possibly a bit dismayed by the lack of improvement in the other southern pines and hardwoods.

In the following sections we provide a brief history of the SFTIC committee and the biennial SFTIC conference, including their purposes and trends (conference attendance and topics discussed) over the decades. For additional context, we provide information on forest genetics and tree improvement associations initiated and conferences convened in the US and Canada over their respective histories. Following this, we move on to envisioning the future of the SFTIC conference and committee, providing recommendations for both as well as a summary of feedback received on this topic from an organized discussion session convened during the final day of the conference. One persistent point of discussion along these lines is how to coordinate scheduling the biennial SFTIC conference with other similar conferences in the US and Canada. Using available information on these conferences' schedules, we develop and present a putative SFTIC conference schedule that provides opportunities for joint meetings with these organizations while reducing the competition for meeting attendance.

History, Purpose, and Trends

The first SFTIC was held January 9-10, 1951, in Atlanta as the Conference on Forest Genetics Research in the Southern Pines (Haig, 1951). An advisory committee on forest tree improvement was formed at the conclusion of the initial SFTIC. In its first meeting (June 20, 1951), the advisory committee revised its name to the Committee on Southern Forest Tree Improvement (CSFTI) (Kaufman, 1971). Early in committee deliberations it appears that a decision was made to hold a biennial conference, with committee meetings held at least annually. By the 10th conference (SFTIComm, 1969), held in Houston, the CSFTI became widely known as SFTIC (as approved by the committee in 1968, Kaufman 1971), while the general use of SFTIC for the conference appears to be associated with the 12th biennial meeting (Dinus, Thielges, & Wells, 1973). The original SFTIC logo (Fig. 1) was adopted for the 14th conference in Gainesville, FL, resulting from a design competition organized by the committee (SFTIComm, 1977). The current logo (Fig. 1) was adopted specifically for the 21st conference (SFTIComm, 1991) in Knoxville, TN, and then by default (or lack of action, otherwise), adopted for subsequent conferences.

Soon after SFTIC's formation, additional regionally-based forest genetics and tree improvement associations or committees organized in the US, with two of those remaining to date— Northern Forest Genetics Association (NFGA) and Western Forest Genetics Association (WFGA). The NFGA was formed in 1991 and consists of four previous forest tree improvement committees (FTIC) or associations (FTIA); namely Northeastern FTIC (1953-1990), Lake States FTIC (1953-

1976), Central States FTIC (1958-1978), and Northcentral States FTIA (1979-1990). The WFGA began in 1954 and currently includes the interests of the Rocky Mountain Forest Genetics Association that had formed in 1979. In Canada, the Canadian Forest Genetics Association (CFGA) formed in 2000 and consists of two previous forest tree breeding committees (FTBC) and one tree improvement association (TIA). The earlier FTBC was initiated by the National Research Council in 1937 and operated until transitioning into the Canadian FTBC in 1953, which in 1973 transitioned into the Canadian TIA, and finally into the CFGA in 2000. More recently, the North American Forest Genetics Society (NAFGS) organized convening their first conference in 2022 at the Asilomar Conference Center in California. This group (see https://treegenesdb.org/nafgs) was designed to have broad appeal to forest geneticists from tree breeding and species conservation to quantitative and population genetics to genomics and biotechnology, while being inclusive of species and issues across the three countries of North America.

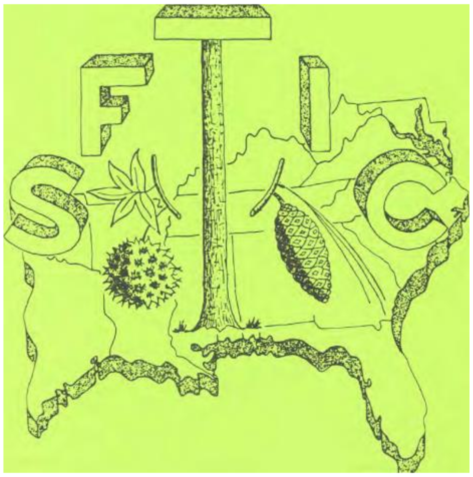
 SFTIC logo modified for the 21st SFTIC (1991)	 SFTIC logo adopted for the 14th SFTIC (1977)

Fig. 1. Current (1991) and original (1977) SFTIC logos

The purpose of the SFTIC committee as approved in 1953 consisted of four functions (Ostrom, 1953) as follows (and as modified in 2011). First, to advise and assist those interested in the improvement of southern forest trees in arranging and conducting research and development programs (to advise and assist those interested in the improvement of southern forest tree species). Second, to provide a clearing house for information on forest tree improvement (no change). Third, to provide for or assist in coordination in the conduct of a South-wide program of tree improvement research and development (to provide for and to assist with conducting south-wide programs in tree improvement). Fourth, to foster and encourage the advancement of knowledge of southern forest tree genetics (to foster and encourage the advancement of knowledge, training and education related to southern forest tree genetics). It seems that the SFTIC conference directly supports the fourth purpose (advancing knowledge, through the biennial meeting) and to some degree the second purpose (providing information, through the conference proceedings), but it’s not clear how the committee supports the first (advising and assisting tree improvement) and third (coordinate south-wide tree improvement) purposes. Clearly a reassessment of the SFTIC committee’s purpose is needed to either revise the purpose to better reflect the committee’s activities or implement activities that more fully meet the purpose.

The SFTIC committee’s make-up consists of a specified formula including four groups: Group A—forestry schools, state forestry agencies, forest industry; Group B—US Forest Service (NFS, S&PF and R&D); Group C—technical specialists; and Group D—tree Improvement cooperatives (Squillace, 1983). The committee’s current roll includes 23 members across these four groups, with more than one-third being technical specialists (e.g., geographic variation, pathology, entomology, pollen management). The original committee, consisting of 12 members, formed four sub-committees to report on progress in specific areas of concern (Ostrom, 1953)—Geographic Source of Seed, P.C. Wakeley, chair; Genetic Control of Seed, Floyd Cossitt, chair; Tree Selection and Breeding, Keith Dorman, chair; and Progeny Testing, E.G. Wiesehuegel, chair. Over the years’ there are records of numerous temporary sub-committees formed (e.g., Squillace, 1983) to address specific topics (e.g., pathology, cone and seed insects, seed certification).

As described by I.T. Haig (Haig, 1951), the purpose of the SFTIC conference was to consider forest genetics broadly in terms of research questions and applications in forest management, while having a preferential focus on tree improvement of southern pines. The first conference attracted about 80 registrants, surprising the organizers and demonstrating the need for such a meeting (Stone, 1951). Conference attendance rose steadily through the mid- and late-1970s peaking at 229 for the 14th conference in 1977 (Table 1). Over the first 11 biennial meetings, conference attendance averaged 140, then 180 during the next 15 meetings, dropping to 100 and 80 in the most recent two biennium. For this biennium’s conference, the 37th SFTIC, the number of registered attendees (74) was quite close to the average (80) of the previous five conferences.

Early conferences (1950s-70s) emphasized tree improvement topics, including the prospects for tree improvement (with a focus on loblolly and slash pines), seed source studies (provenance tests) to determine geographic variation (i.e., Southern Pine Seed Source Study, SPSSS) (Wakeley, 1959; Wells, 1969), polyploids and inter-species hybrids, early flowering induction techniques, data on genetic variation, heritability in species and traits, progeny test design (plot types, family sizes, etc.), genotype x environment interactions, early selection (age-age correlations), evidence of progress (realized genetic gain), seed orchards (cone yield and seed quality) and clonal propagation (grafting and rooting cuttings). In the mid-1970s and 1980s the conference topics evolved to include isozyme markers for pollination studies, fusiform rust and other diseases to varying degrees, wood quality (pulpwood vs. saw timber, juvenile vs. mature wood), long-term breeding strategies and maintaining population sizes, and BLUP development and implementation for breeding value estimation. Into the 1980s new biotech approaches arrived with tissue culture, genetic transformation, and DNA markers (RFLPS, RAPDs) leading the way. Other topics becoming more apparent, including clonal performance and potential deployment strategies, full-sib family deployment through supplemental mass pollination, and short-rotation traits and practices to maximize woody biomass production.

Through the 1990s and 2010s, SFTIC conference topics continued to evolve, especially towards genetic mapping various species, QTL mapping and marker-assisted selection (MAS), transcriptomics for differential gene expression studies and genetic marker development (e.g. EST-

Table 1. Information on Southern Forest Tree Improvement Conferences (1951 through 2023)

SFTIC #	Year	Host(s)	Location		Number of Attendees
			City	State	
1	1951	USDA-Forest Service (USDA-FS) Southern Region (Region 8)	Atlanta	GA	80
2	1953	USDA-FS Region 8	Atlanta	GA	n/r[1]
3	1955	USDA-FS Southern Forest Experiment Station (SFES)	New Orleans	LA	115
4	1957	School of Forestry, University of Georgia (UGA)	Athens	GA	215
5	1959	School of Forestry, North Carolina State University (NCSU)	Raleigh	NC	n/r
6	1961	School of Forestry, University of Florida (UF)	Gainesville	FL	n/r
7	1963	USDA-FS Institute of Forest Genetics	Gulfport	MS	155
8	1965	Continental Can Co.; Georgia Forestry Research Council	Savannah	GA	135
9	1967	Department of Forestry, University of Tennessee (UT-K); Division of Forestry Relations, Tennessee Valley Authority	Knoxville	TN	n/r
10	1969	Texas Forest Service (TFS)	Houston	TX	n/r
11	1971	USDA-FS Southeastern Area, State & Private Forestry	Atlanta	GA	n/r
12	1973	Division of Continuing Education, Louisiana State University (LSU); USDA-FS SFES	Baton Rouge	LA	150
13	1975	School of Forest Resources and Division of Continuing Education, NCSU	Raleigh	NC	176
14	1977	School of Forest Resources & Conservation, UF; USDA-FS Southeastern Forest Experiment Station (SEFES)	Gainesville	FL	229
15	1979	Department of Forestry, Mississippi State University (MSU); USDA-FS SFES	Starkville	MS	204
16	1981	Department of Forestry and Extension Division, Virginia Polytechnic Institute & State University (VA Tech)	Blacksburg	VA	199
17	1983	School of Forest Resources, UGA; USDA-FS SEFES	Athens	GA	203
18	1985	USDA-FS Southern Institute of Forest Genetics (SIFG); University of Southern Mississippi, Gulf Park	Long Beach	MS	169
19	1987	Texas Forest Service; Texas Agricultural Research Station	College Station	TX	173
20	1989	Westvaco Corp.; Clemson University	Charleston	SC	208
21	1991	Department of Forestry, UT-K; Tennessee Division of Forestry	Knoxville	TN	161

22	1993	Institute of Paper Science & Technology (IPST); Georgia Forestry Commission; USDA-FS Region 8; Cooperative Extension Service, UGA	Atlanta	GA	176
23	1995	NCSU-Industry Cooperative Tree Improvement Program (NCSU-ICTIP)	Asheville	NC	145
24	1997	School of Forest Resources and Conservation, UF	Orlando	FL	200
25	1999	School of Forestry, Wildlife & Fisheries, LSU	New Orleans	LA	166
26	2001	Warnell School of Forest Resources, UGA	Athens	GA	122
27	2003	Department of Forestry, Oklahoma State University (OSU)	Stillwater	OK	94
28	2005	Department of Forestry & Environmental Resources, NCSU	Raleigh	NC	131
29	2007	Western Gulf Forest Tree Improvement Program (WGFTIP), TFS; Inland Empire Tree Improvement Cooperative (joint meeting with the Western Forest Genetics Association, WFGA)	Galveston	TX	118
30	2009	Department of Forest Resources & Environmental Conservation, VA Tech	Blacksburg	VA	64
31	2011	USDA-FS SIFG; Department of Forestry, MSU	Biloxi	MS	109
32	2013	ArborGen, LLC; School of Agricultural, Forest & Environmental Sciences, Clemson University (Clemson)	Clemson	SC	79
33	2015	Department of Forestry, University of Arkansas-Monticello (UAM); Weyerhaeuser Corp.	Hot Springs	AR	84
34	2017	Cooperative Forest Genetics Research Program (CFGRP), UF	Melbourne	FL	83
35	2019	Department of Forestry and Forestry Cooperative Extension, University of Kentucky (UKY); USDA-FS Southern Research Station (SRS)	Lexington	KY	89
36	2021	Warnell School of Forestry and Natural Resources, UGA	Athens	GA	73
37	2023	US Department of Energy (USDE), Oak Ridge National Laboratory (ORNL); Department of Entomology & Plant Pathology, UT-K	Knoxville	TN	74

[1] n/r, number of attendees was not reported

based SSRs), and eventually genome sequencing, genome-wide marker development (e.g., GBS, SNPs), genome-wide association studies (GWAS), genomic prediction and selection (GS), and candidate gene (CG) discovery based on gene expression and QTL- or GWAS-based map positions. These topics culminated in the promise of more efficient tree improvement using a combination of MAS and GS to improve selection efficiency and shorten the generation time and CGs for applications in cis-genic genetic transformation for engineering improved trees. SFTIC conferences in the 2010s and 2020s further evolved to include topics beyond tree improvement per se, such as resistance breeding for invasive pests, gene conservation for threatened species, restoration genetics of diminished species and ecosystems, seed orchards and seed collections for minor species, and adaptation to climate change. Now, 72 years since the founding of SFTIC, the promise of the early aspirations from the committee (Haig, 1951), summed up by keynote speaker Scott Pauley[1] in 1955, has been and is clearly being met—genetically selected southern pines for planting and a continuing discussion of forest genetics broadly.

Envisioning the future of SFTIC

The current realization is that forest management in the south and beyond needs forest genetics and tree improvement now-- and in many ways, more than ever (e.g., Wheeler et al. 2015). Three major forces, all on the increase, are in play in this regard: habitat fragmentation, invasive species (pests, pathogens, and plants), and climate change. As we have seen through a review of the topics covered by SFTIC, forest geneticists and tree breeders have a good track record for delivering on practical needs in southern forestry, and at this juncture, SFTIC has the potential to be the must-go-to conference for forest geneticists, tree breeders, and related professionals and practitioners. To achieve this, the focus of the committee and conference should be on solving practical problems in (i) tree improvement, (ii) gene conservation, (iii) species and ecosystem restoration, (iv) resistance to invasive pests and pathogens, and (v) adaption to climate change.

With this vision in mind for SFTIC, we can list several recommendations for future conference organizers— stay with the basics and focus on solving practical problems, while embracing change in people, organizations, funding, and technology. Continue to consider forest genetics broadly and the increasing urgency to address many species, traits, and environments. Emphasize forest genetics and tree breeding and biotechnology with applications in gene conservation, tree improvement, and species restoration as situations demand. Consider trait improvement for wood production and carbon sequestration, species restoration and climate change, including resistance and tolerance to abiotic and biotic stress.

Further recommendations are more organizational in nature that will serve to encourage interest and subsequent growth in conference attendance and diversity, such as committing to biennial meetings in June of odd years, determining hosts for the next two conferences (e.g., now for 2025 and 2027), and considering a name change for the conference. Two name change suggestions that

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[1] Quote from Scott Pauley (Pauley, 1955), “If at the end of the next fifty years all of the forest nursery stock produced in the South is grown from seed derived from wild trees of proved genetic superiority, I think this Committee will have served its purpose with notable success. There is, however, good reason to believe that within fifty years the utilization of the best wild seed available in the southern pine region will be looked upon as an antiquated practice. Surely by that time the logical procedure of combining superior genotypes within a species by controlled crossing will have long since occurred. By the utilization of the seed orchard technique as a means of making such crossings, there is good reason to believe that not only can the genetic quality of the seed be improved over that of the best wild stock, but the cost of production may actually be reduced through increased facility of collection.”

may better align with the conference's evolving content while maintaining SFTIC continuity and brand recognition include Southern Forest Genetics and Tree Improvement Conference (SFGTIC) and Southern Forest Genetics Conference (SFGC). Both are minor variations from SFTIC, but more significant changes should be considered being more descriptive of the current and anticipated content of the conference.

Other recommendations involving the content of the conference can be listed, specifically offering field tours and increasing workshops. Field tours of local/ongoing work can be beneficial to attendees, and they might be best placed in the middle of the conference to encourage wider participation and further facilitating networking for new and veteran attendees. This year's field tour of ORNL's woody biomass research center was outstanding but suffered low attendance (about 25% of conference registrants), likely due to it's being described as 'optional' and being placed on the morning after the conference concluded. Pre-conference meetings have been a regular part of SFTIC week over the years, especially with the North American Quantitative Forest Genetics (NAQFG) workshop and the annual meeting of the SFTIC committee. More recently, workshops on state agency needs and a short course of the basics of tree improvement (Tree Improvement 101) have been offered. Other ongoing and more topical ideas for workshop content include bioinformatics methods, tree propagation techniques, seed orchard management, and population genetics.

A final group of recommendations can be made around communications and attendee experiences. Having a social media presence is a must for timely engagement with the content and features of the conference, and possibly the committee's activities throughout the year. In addition, we recommend that SFTIC organizers continue student travel stipend opportunities, poster and speaker awards, industry-hosted career panels, graduate student mixers, and the SFTIC 5K. For the 36th SFTIC, the Schatz Symposium was added as the conference's closing session (SFTIComm, 2021). This symposium, designed as a plenary session with invited speakers, was funded as a collaboration with the Schatz Center for Tree Molecular Genetics at Pennsylvania State University. We encourage a continuation of this collaboration and the Schatz Symposium. We also encourage discussions on re-instating the Schatz student research awards that were offered in 2021, but not at this year's conference.

One persistent issue concerning the future of regional-based forest genetics and tree improvement groups (committees, associations, societies) has been coordinating conference schedules—specifically, keeping them local and often enough to effectively serve their constituents, while providing opportunities for exposure to a wide range of topics and issues. Given our geographic proximity to the NFGA (eastern US), we suggest holding a joint conference with them every other SFTIC, i.e., every 4 years. Joint conferences with the other groups are encouraged as opportunities arise. Maintaining SFTIC's June of every odd year schedule also coordinates well with the current schedule of meetings in terms of minimizing competing conferences happening at nearly the same time. As an example, we developed the following potential schedule of the US regional meetings and the NAFGS:

2023 SFTIC 37, Knoxville, TN, June 20-23, 2023 (this meeting)
2024 NAFGS 2, Oaxaca, Mexico, June 10-14, 2024 (already scheduled)
2025 SFTIC 38 jointly with WFGA & NFGA (hosted by Penn State University)
2026 NAFGS 3, TBD, Canada
2027 SFTIC 39 (hosted by Auburn University and Alabama A&M University)
2028 NAFGS 4, TBD, United States
2029 SFTIC 40 jointly with NFGA (Louisville, KY, hosted University of Kentucky and SRS)
2030 NAFGS 5, TBD
2031 SFTIC 41 (hosted by Mississippi State University and SRS)
2032 NAFGS 6, TBD
2033 SFTIC 42 jointly with NFGA (Columbia, MO, hosted by University of Missouri and NRS)

Finally, we offer some recommendations for the SFTIC Committee. Annual full committee meetings should be re-instated to provide continuity in the flow of information to constituents, communications with technical specialists, and coordinating with current conference organizers. This may encourage additional alternate-year biennial meetings or workshops such as those offered through the information exchange group (IEG) construct. For example, IEG-40 workshops have provided valuable meeting opportunities for SFTIC stakeholders over the years, with topics ranging from fusiform rust screening to genetic impacts on productivity of southern pine forests, as well as emergent issues in seed orchard management. Other committee functions could include regular monitoring of the SFTIC web site (www.sftic.org), inspecting and updating the SFTIC mailing list, and coordinating with other mailing lists (e.g., NAFGS).

On the final day of this year's conference, we asked participants seated at each lunch table to brainstorm ideas for improving SFTIC, namely, to grow the conference and ultimately the field (numbers and diversity) of forest genetics. We obtained written notes from 7 of the 9 tables and have summarized these results into Table 2. The notes indicate wide support for considering a SFTIC name change, with the next most mentioned ideas being south-north joint conferences, student engagement (e.g., representation, travel stipends, poster plenaries, presentation awards, early career research grants), social media presence, and conference swag. We also saw clear support for a new logo, standing up sub-committees, and regular communications from the SFTIC committee. Clearly some of these ideas mesh with our recommendations presented above, but there are some new perspectives provided as well. For a start, the SFTIC committee should take these ideas up in their meeting(s) this year and plan to report back to the full constituency in a timely manner.

Summary and Conclusions

SFTIC has a long and successful history dating back to 1951. However, in the past 10 plus years, conference attendance has been falling to a level that is now only marginally sustainable. At the same time, the need and opportunity for forest genetics and tree improvement in many respects is rising. The needs fall along the lines of monitoring, understanding, and predicting the genetic consequences of emerging phenomena such as habitat fragmentation, invasive species, and climate change, and the impact of these consequences on forest management planning, implementation, and outcomes. The opportunities can be seen in tailoring traditional tree improvement methods for the current situations, applying new genomic technologies to solve long-standing and emerging problems (e.g., seed sourcing for future climates) and merging candidate gene discovery with new biotechnologies (e.g., gene editing to engineer parental breeding lines with enhanced traits). Given the emerging needs and opportunities for forest genetics in forest management, we suggest that SFTIC can become the “go-to” conference for scientists, students, and practitioners to present their latest work in the field and learn from leaders across the region and beyond.

Disclaimer: The findings and conclusions presented here are those of the author(s) and should not be construed to represent any official USDA or US Government determination or policy.

Table 2. Summary table of working lunch discussion groups on ideas to improve SFTIC conference and committee.

	Discussion Group							
Idea to consider	1	2	3	4	5	6	8	9
Rename SFTIC[1]	Not necessary	SFGA, EFGA		SFTIC+	SFTIC Gen Conf	SFTIC+, SFTIC Cons Conf, ETIA		EFG Conf
New logo					Yes	Yes	Yes	
Stand up Sub-comms		Yes		Yes			Yes	
Regular info from SFTIC			Yes			Yes	Yes	
Forest Gen certificate	Yes[2]				Yes[3]	Yes[4]		
Pre-conf Workshops	Yes	Yes						Yes
Social media, SFTIC-toc				Yes	Yes	Yes	Yes	
Conf swag			Yes	Yes	Yes		Yes	
Invite vendors		Yes		Yes				
Invite granting agencies	Yes					Yes		
North-South joint meetings		Yes	Yes			Yes		Yes
Student reps, travel grants	Yes			Yes		Yes	Yes	Yes
Early career research grant	Yes						Yes	
Poster plenary, short talks	Yes						Yes	Yes

[1] SFGA (Southern Forest Genetics Association), EFGA (Eastern Forest Genetics Association), SFTIC+ (Southern Forest Tree Improvement Committee or Conference Plus), SFTIC Gen Conf (SFTIC Genetics Conference), SFTIC Cons Conf (SFTIC Conservation Conference), ETIA (Eastern Tree Improvement Association), EFG Conf (Eastern Forest Genetics Conference)

[2] USDA NIFA could possibly fund a multi-university effort (Tree Genetics and Improvement); needs to be SAF accredited

[3] SAF accredited certificate with three areas of emphases (1) genetics, seed sources; (2) tree improvement, breeding; and (3) seed orchard, nursery. Explore what this might look like as an undergraduate certificate or a graduate level course in Forest Genetics and Tree Improvement shared across participating SAF accredited university programs.

[4] SAF certified course in Forest Genetics and Tree Improvement

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Plenary Session 2:

Poplar Genomics to the Rescue – Bioenergy and Climate Mitigation

Leveraging Plant-Microbe Interactions to Build Sustainable Ecosystems
– *Melissa Cregger, ORNL*...pg. 56

Population-Level Genomics Enable Bioengineering of Biomass Cell Wall Properties and Sustainability Traits
– *Wellington Muchero, ORNL*...pg. 57

Harnessing the Power of Synthetic Biology to Enhance Tree's Natural Ability to Rebalance the Planet's Carbon Cycle
– *Yumin Tao, Living Carbon*...pg. 58

Leveraging Plant-Microbe Interactions to Build Sustainable Ecosystems

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Over the past two decades, it has become increasingly clear that symbiotic host–microbe interactions alter the way in which plants grow and respond to abiotic and biotic stress. Harnessing diversity within these plant–microbe associations in managed ecosystems, provides an opportunity to create sustainable, multipurpose bioeconomies whereby globally important plant feedstocks can be produced while simultaneously maximizing soil health and mitigating adverse impacts to climate. Therefore, to increase sustainability within Department of Energy relevant biofeedstock plantations, I am leveraging genetic variation in the plant host to select for plants that are tolerant to abiotic stress and pairing these plants with belowground microbial partners to alter plant performance and ecosystem processes. Specifically, my research leverages a suite of molecular, physiological, and ecosystem level techniques and experiments to identify natural variation within diverse genotypes of Populus and characterize associated microbial communities under varied environmental conditions. As I identify these complex associations, I aim to build Populus-microbial associations within ecosystems that alter plant growth, plant tolerance to drought, and ecosystem carbon cycling.

Population-Level Genomics Enable Bioengineering of Biomass Cell Wall Properties and Sustainability Traits

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Undomesticated and outcrossing plant species such as *Populus trichocarpa* exhibit high levels of allelic variation across their species ranges. As such, high-resolution genome-wide association mapping (GWAS) utilizing these populations provides an unparalleled opportunity to resolve loci with large allelic effects on complex cell wall and adaptive traits that have been shaped by decades to centuries of genome evolution. Here, we summarize results of GWAS studies leveraging a panel of > 60 million single nucleotide polymorphisms (SNPs) segregating in population of 1,500+ *P. trichocarpa* genotypes established in replicated field sites in California and Oregon. We reveal allelic variants that are associated lignin and carbohydrate properties, root and shoot architecture, bud phenology, photosynthetic efficiency, disease resistance and tolerance to biotic stresses including drought. CRISPR-Cas genome editing targeting these alleles is providing systems-level understanding of the function of these variants and their specific roles in bioengineering biomass for industrial applications.

Harnessing the Power of Synthetic Biology to Enhance Tree's Natural Ability to Rebalance the Planet's Carbon Cycle

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Forest trees have played a pivotal role in maintaining the planet's carbon cycle, both in terms of carbon drawdown through photosynthesis and of carbon sequestration through wood production. The drastic increase in atmospheric CO_2 concentration since the industrial revolution presents a significant challenge for trees to quickly evolve an efficient mechanism to rebalance the carbon cycle. Recent advancement in synthetic biology opens the door to bioengineering plants to fix carbon more efficiently and to preserve carbon for longer. We strive to apply these learnings and tools in trees to enhance their natural ability in carbon cycle maintenance to increase net biome productivity. Using hybrid poplar as a model system, we have built a technology platform beyond poplar for tree bioengineering and provided proof-of-concept in several focus areas. An example of proof-of-concept using the technology platform was provided with testing in hybrid poplar INRA717-IB4 of a photorespiration bypass pathway design selected from literature reports showing efficacy in tobacco (South et al. 2019; Cavanagh et al. 2022). The design includes an RNAi strategy to reduce the transportation of the photorespiration byproduct, glycolate, out of the chloroplast and a shunt pathway to metabolize the retained glycolate back to CO_2 for fixation through the Calvin-Benson cycle. The shunt pathway includes a gene from algae encoding glycolate dehydrogenase and a gene from pumpkin encoding malate synthase. Working together, and alongside the endogenous enzymes in the malate metabolism cycle, these two enzymes located in the chloroplast convert glycolate to CO_2 for added carbon fixation by RuBisCO carboxylation. Molecular and physiological data collected from two separate growth experiments indicates that transgenic plants expressing genes in the photorespiration bypass pathway have reduced transportation of glycolate, increased photosynthetic efficiency, faster plant growth and elevated biomass production. One lead transgenic event accumulated 35-53% more above-ground dry biomass over four months of growth in a controlled environment (Fig. 1). Field testing of the performance of these genetically engineered poplar trees for photosynthesis enhancement is in progress. We have since made progress on providing proof-of-concept in several project areas directed towards increased carbon sequestration including wood decay resistance using our technology platform.

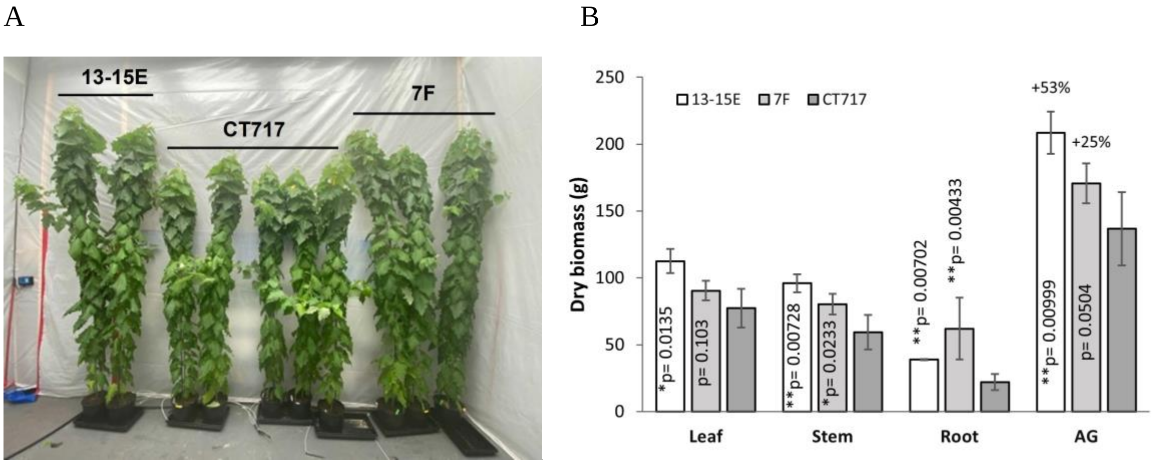

Figure 1. Plant growth and biomass analysis in selected transgenic events. A. Plant height difference between transgenic plants and CT717 non-transgenic control plants before harvesting for biomass measurement at week 21. Left: 13-15E, 2 ramets; Right: 7F, 3 ramets; Middle: CT717, 5 ramets. **B**. Dry biomass of harvested C1 experimental plants, including leaf, stem, root, and above-ground (AG; leaf + stem). Number of ramets: CT717 control plants, 5; Event 13-15E, 2 (one of the three initial ramets died earlier in the experiment); Event 7F, 3. All *p*-values were calculated using t-test and represent the difference compared to the non-transgenic CT717 control plants. * and ** represents a significant difference at $P < 0.05$ and $P < 0.01$, respectively when compared to non-transgenic CT717. For more data and information, see Tao et al. 2023.

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Concurrent Session 3A:

Pine Genetics and Tree Improvement II

A Comparison of Bareroot and Containerized Loblolly Pine Planting Stock – 6-Year Results From a Series of Trials Planted Over 3 Years by Resource Management Service
– *Christopher Rosier, Resource Management Service*.................................pg. 61

Nursery Inventory System in Bareroot Pine Seedlings: Possibility of Automated Technologies
– *Jessica Maynor, ArborGen*..pg. 62

Biomass Cropping and Effects on Thinning on Different Provenances of Loblolly Pine in the Piedmont of NC
– *Austin Quate, NC State University*...pg. 63

Estimation of Genetic Parameters and Breeding Values Using ABLUP and HBLUP in *Pinus taeda* L. Piedmont Rooted Cutting Trials
– *Colin Jackson, ArborGen*...pg. 66

A Comparison of Bareroot and Containerized Loblolly Pine Planting Stock – 6 Year Results from a Series of Trials Planted Over 3 Years by Resource Management Service

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Resource Management Service (RMS) established a series of trials between 2013 and 2016 to compare survival and growth of containerized and bareroot seedling stock. The main objective of the trials was to see if the added cost of planting containerized seedling stock is recovered through improved seedling survival and or growth. Two trials were established in each of three operating regions—North Carolina (2014 and 2015), South Carolina (2014 and 2015) and Alabama (2015 and 2016). For each trial, both control pollinated (MCP), and varietal seedlings were planted (as bareroot and container stock) and they were planted on two dates – October and January or February. After 6 growing seasons each trial was assessed for survival, height and dbh. The difference in survival and growth between bareroot and containerized stock was dependent on the operating Region. There were no significant differences between containerized and bareroot seedlings in the four trials planted in NC and SC. In AL, seedling type significantly influenced survival, height and plot volumes with containerized seedling growing better than bareroot. The differences (when significant) between container and bareroot seedlings were greater for October planting dates than for Jan/Feb dates. October planted seedlings were almost always significantly larger than Jan/Feb planted seedlings for height, dbh, volume index and plot volume. In contrast, time of planting did not significantly affect seedling survival for 5 of the 6 test sites. The two genetic sources and plant origin types (MCP and varietal) behaved similarly as bareroot or containerized planting stock for all tree growth characteristics (height, dbh and volume). In 2 trials, bareroot MCP seedling survivals at one planting time were lower than containerized MCP and both bareroot and containerized varietals resulting in a significant seedling type x genetics interaction. Varietals grew better than or equal to MCP seedlings in all studies except for the noncommercial variety AGV-105 planted in 2014 trials. The results from this series of trials suggest that there is no advantage to planting containerized seedlings in Atlantic Coastal Plain sites for either plantation survival or tree growth. However, for upper coastal plain sites in AL there can be opportunities for better survival and growth using containers when planting early in the season. Planting seedlings early in the season (October) can significantly improve tree growth through 6 years. The results were similar whether planting seed-based propagules or vegetative propagule planting stock.

Nursery Inventory System in Bareroot Pine Seedlings: Possibility of Automated Technologies

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In 2020-2021 the southern United States grew 1,147,700,000 seedlings, of which 74.4% were bareroot conifers (854,128,000) (SFNMC Spring Newsletter, 2022, p.2). For seedling providers, accurate inventory of seedlings within a nursery is critical for selling seedlings to landowners and other industry customers. A collaborative project between the University of Georgia and ArborGen sought to develop an automated inventory system for bareroot nursery beds. Using videos of nursery beds were captured over three years (2018-2021), a deep-learning model was created to detect and count seedlings at various growth stages. To examine variation in nursery beds and sampling intensity needed to confidently estimate bed inventory, sample plots were installed in six beds at two nursery facilities in Summer 2022. The automated inventory system on average was 0.53 standard deviations from the sample mean across both nursery facilities. To further test the detection accuracy, 15-foot hand counted control plots were installed in 2022 in production nursery fields and recorded model accuracy from May to August. The accuracy ranged from 42% at the lowest in May to 98% at the highest in late July. Automated inventory system shows promising results in hand counted control plots as well as estimates to bed means. Model improvements are needed to further develop the technology before deployment industry wide.

Biomass Cropping and Effects of Thinning on Different Provenances of Loblolly Pine on the Piedmont of North Carolina

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A loblolly pine biomass/cropping study was planted by TIP staff near Butner, North Carolina in the spring of 2012. The purpose of the study was to evaluate different genetics (provenances and families) under various silviculture regimes to guide landowners looking to maximize their financial returns in both the bioenergy and sawtimber markets. The study site is in the Piedmont of NC, and a feature of the study is the inclusion of Coastal-source families to determine if the risk of cold damage is offset by their faster growth and the presence of a bioenergy market (where damaged trees can be commercially thinned). The study includes ten families from the Coastal Plain provenance and ten from the Piedmont. The test was established at a high planting density (1,037 trees per acre). In the fall of 2020, three thinning treatments were applied: no thin, moderate thin (519 residual trees per acre), and heavy thin (288 residual trees per acre). These thinning scenarios were designed to allow comparison of scenarios focusing on biomass production versus biomass and sawtimber production.

Evaluating the no-thin treatment at age 11 years, families from warmer sources tended to have more green tons per acre (**Figure 1**). The colder-source families tended to have better sawtimber potential (**Figure 2**). Seed sources moved northward often outperform the local source, but if they are moved too far northward, they suffer cold damage and do not perform as well as the local source (Wells and Wakeley 1966; Lambeth et al. 2005). This seems to be the case for the families in the Butner trial. Out of the six top families for the no thin treatment, five of them are from a coastal source. The top five families that have the highest proportion of trees with sawtimber potential are from a piedmont source. Coastal families tend to have higher wood volume while piedmont families tend to have better sawtimber potential, on this piedmont site.

Figure 1. Green tons per acre at age 11 years.

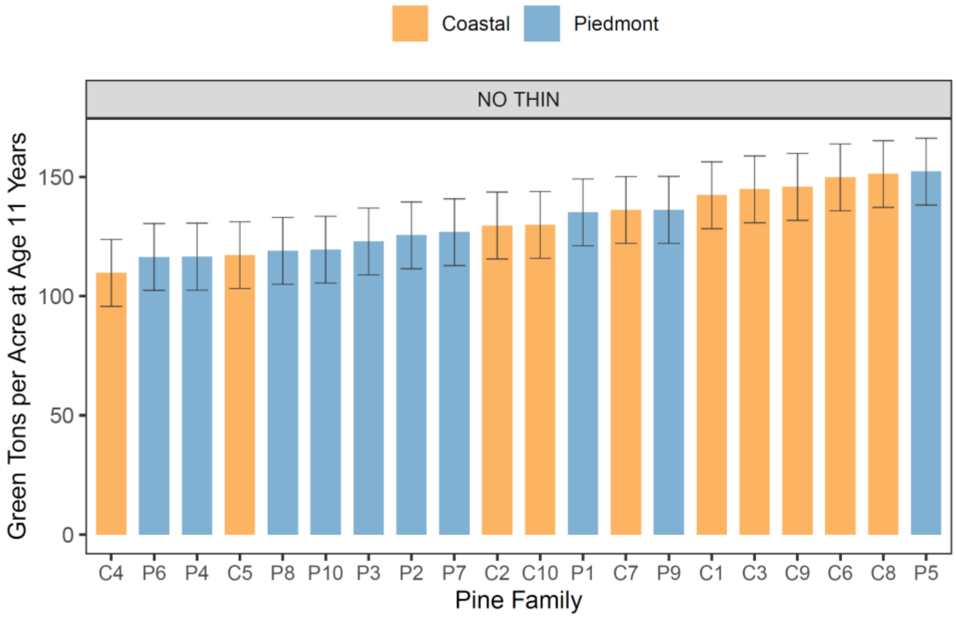
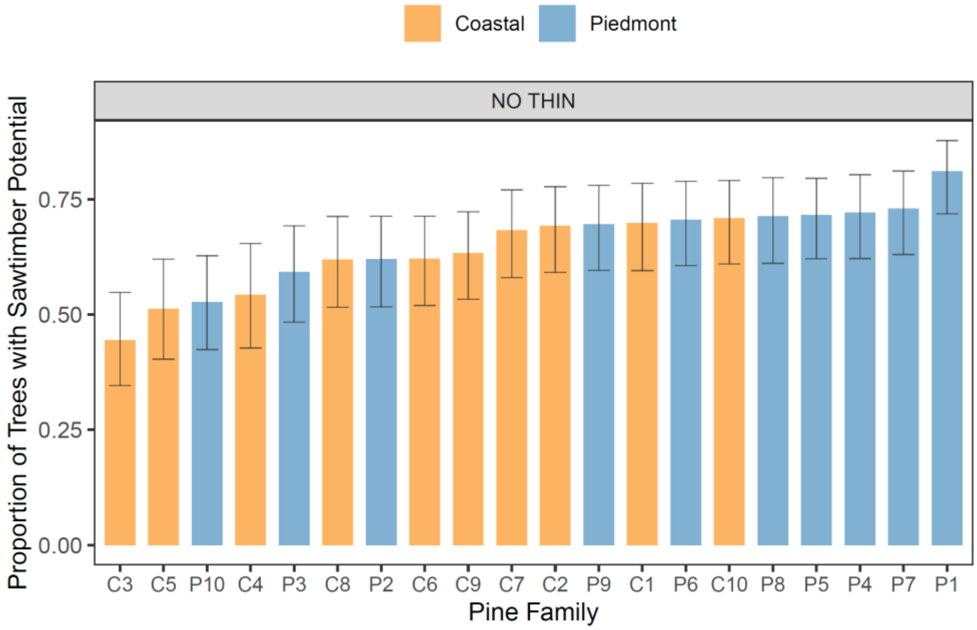
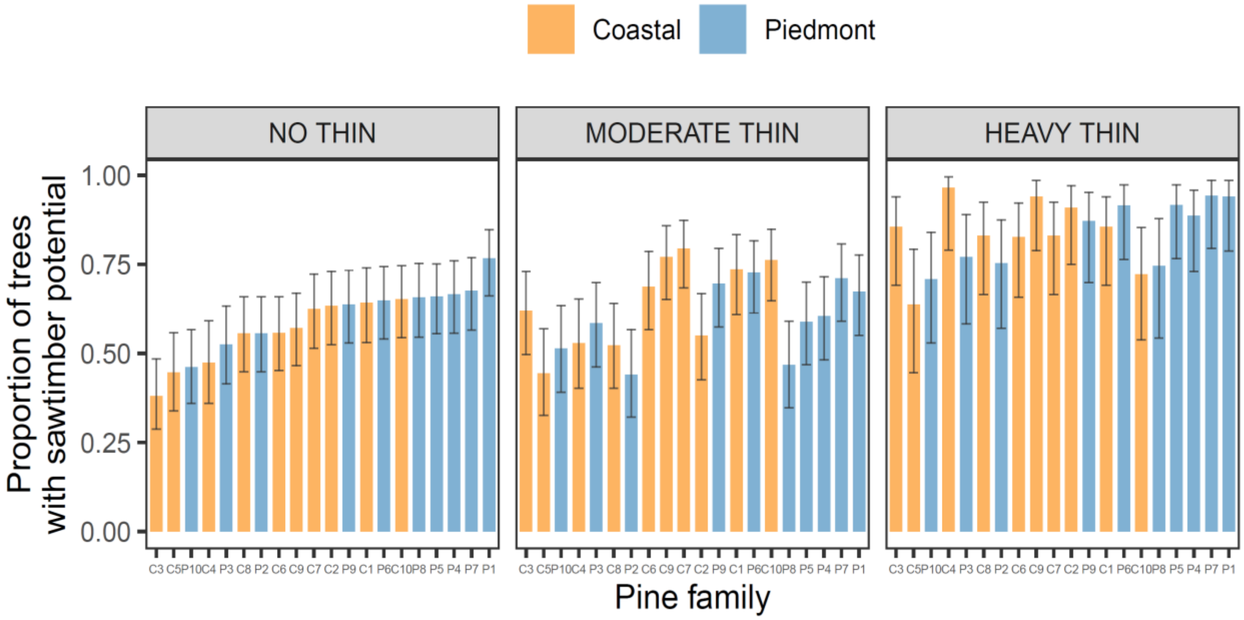


Figure 2. Proportion of trees with sawtimber potential at age 11 years.



The no-thin treatment has fully occupied the site and has begun to experience mortality from self thinning, as it has entered the "stem exclusion" stage of forest stand dynamics. In the thinned areas, the form of the trees tends to be much better, likely due to thinning from below. Stem form tends to be best in the heavy-thin treatment, but trees with repeated cold damage can be found in all the thinning treatments and provenances. Figure 3 shows how the proportion of sawtimber potential for each family increases in the heavy thinning treatment. The families are in order from smallest proportion to largest for the no-thin treatment. This is to be expected, because in the thinning we removed most of the defective trees that did not have good sawtimber potential. Further work needs to be done to understand seed transfer distance and how that distance for each family affects green tons per acre and sawtimber potential.

Figure 3. Proportion of sawtimber potential for each thinning treatment at age 11 years.



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Estimation of Genetic Parameters and Breeding Values Using ABLUP And HBLUP in *Pinus taeda* L. Piedmont Rooted Cutting Trials

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Multi-environmental trials are the standard for estimation of genetic parameters and breeding values in pine tree improvement programs. Traditionally, analysis of multi-environmental trials has been performed using pedigree information to estimate relationships between individuals for ABLUP analysis. However, growing accessibility to genomic tools for pine species has made the implementation of genomic based analysis methods such as HBLUP more commonplace. In this study, five trials located across the Piedmont region of the southern United States were analyzed at age six using pedigree based ABLUP and genomic based HBLUP to estimate genetic parameters and breeding values in a population of clonally propagated *Pinus taeda*. Analysis of the growth traits height, diameter at breast height, and volume showed individual tree heritabilites ranging from 0.18-0.25 using ABLUP to 0.22-0.34 using HBLUP. Type B genetic correlations for each trait were high, between 0.89-0.92, indicating minimal genotype by environment interaction. Breeding value accuracies were high for both analysis methods, between 0.87-0.95. Utilization of HBLUP, increased the mean breeding value accuracy values across all traits for genotyped, ungenotyped, and parental breeding values compared to ABLUP. These results indicate that while clonal progeny testing alone can generate breeding value estimates with high accuracies, incorporating genomic relationships through a blended relationship matrix using HBLUP further improves breeding value accuracies while increasing heritability estimates for growth traits.

Concurrent Session 3B:

Hardwood Genetics and Tree Improvement III

Update on EAB-Resistance Breeding Programs
– Jennifer Koch, USFS-NRS..pg. 68

Establishment and Application of Embryogenic Cultures for Conservation and Restoration of Multiple North American Ash Species
– Scott Merkle, University of Georgia..pg. 69

Development of a Sensitive and Rapid Detection Tool for the Detection of the Laurel Wilt Pathogen
– Meher Ony, University of Tennessee...pg. 70

Natural Pruning Varying With Sweetgum Variety and Density
– Joshua Adams, Louisiana Tech University...pg. 71

Update on EAB-Resistance Breeding Programs

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The invasion of emerald ash borer (EAB, *Agrilus planipennis*) threatens the survival of ash trees (*Fraxinus)* in the United States, where it is a common hardwood species especially in riparian and wetland forests. Ash is also used extensively for soil conservation (including wind breaks) and in urban green spaces and streets. "Lingering" ash trees, defined as surviving mature ash trees in natural forests long infested by EAB that maintain healthy canopies for at least two years after all other large ash trees have died, were first identified as a result of annual monitoring of plots. EAB egg bioassay experiments on grafted ramets of lingering green and white ash selections confirmed that these trees possess an increased level of resistance due to several types of defense responses, including mortality of early instar larvae, larvae with significantly lower weights, and larvae with delayed development. Controlled cross pollinations have been used to produce full-sibling progeny from varied combinations of lingering ash parents. Bioassay data indicate an increased frequency of resistance (measured as the proportion of larvae killed by a host defense response) in these families. The level of resistance measured in some of the 2-year-old progeny was greater than either parent, suggesting a tree-improvement program is likely to be successful in producing EAB resistant seed. A pilot study screened unselected, open-pollinated families of blue ash and found that the level and frequency of EAB-resistance in these families was similar to full-sibling families of select lingering green ash. This indicates that screening open-pollinated blue ash seed may be an effective approach for resistance breeding. Longer term goals include combining the best performing progeny from many families into second-generation seed orchards, to produce seed appropriate for restoration plantings and evaluation of the best performing genotypes for cultivar development.

Establishment and Application of Embryogenic Cultures for Conservation and Restoration of Multiple North American Ash Species

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The development of systems for mass clonal propagation systems for North American ash (*Fraxinus)* species would significantly aid ash germplasm conservation and restoration efforts in the face of the devastating losses of these trees to the exotic insect pest emerald ash borer (EAB; *Agrilus planipennis*) during the past two decades. We have initiated somatic embryogenic (SE) cultures of multiple ash species over the past several years and are applying them for clonal propagation of putatively resistant genotypes for EAB resistance testing, for conservation of ash germplasm via cryopreservation, and as targets for gene transfer for eventual testing of candidate EAB resistance genes. SE cultures have been initiated from open-pollinated and control-pollinated seeds of putatively EAB-resistant "lingering" green ash (*F. pennsylvanica*) and white ash (*F. americana*) trees provided by Ohio State University and US Forest Service Northern Experiment Station cooperators. Trees regenerated from these SE cultures will facilitate clonal testing for EAB resistance and, potentially, development of EAB-resistant ash varieties. We have optimized a cryopreservation protocol for the ash embryogenic cultures that will allow us to store the cultures while trees regenerated from the cultures are tested in the field. We have also initiated embryogenic cultures of multiple rare North American ash species, including Texas ash (*F. albicans*), Mexican ash (*F. berlandieriana*) and Carolina ash (*F. caroliniana*), and cryostored copies of the cultures to conserve germplasm of these species. With the recent appearance of EAB in Oregon in 2022, we have initiated a project with USFS Dorena Genetic Resource Center collaborators to develop SE cultures of Oregon ash (*F. latifolia*) and have already shown that this species can be clonally propagated via SE. Embryogenic ash cultures also make excellent target material for gene transfer via Agrobacterium-mediated transformation. Working with scientists at the University of Kentucky and the USFS, Southern Research Station, we have demonstrated that we can stably transform green ash and white ash embryogenic cultures to produce transgenic trees expressing marker genes. Work on testing RNAi technology as a means of conferring EAB resistance to ash trees is in progress. Overall, the research demonstrates the multiple contributions that SE technologies can potentially make to ash conservation and restoration.

Development of a Sensitive and Rapid Detection Tool for the Detection of the Laurel Wilt Pathogen

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Laurel wilt (LW), caused by the ambrosia fungus *Harringtonia lauricola* (HL), is a destructive vascular wilt disease affecting many Lauraceae hosts, including important forest species in the southeast United States and commercial avocado trees in Florida. In the past several years, Laurel wilt has expanded its range rapidly through numerous host jumps, which has warranted the development of early detection and monitoring protocols. Current molecular-based detection methods lack the sensitivity to detect HL DNA from complex samples (i.e., host and vector tissues), especially when the titer is low in host tissue in the early and asymptomatic post-infection stages. Our goal is to develop a cost-effective highly sensitive detection method with a simple visualization and interpretation of results for end-users with limited training. A published species-specific IFW SSR region was used to design a TaqMan probe to perform qPCR and traditional PCR amplification. Our cost-effective visualization tool comprises of an inexpensive blue flashlight and barrier filter glass. Using this system, fluorescence can be visualized when positive PCR amplicons are present and thus bypassing costly qPCR protocols. The sensitivity using pure HL DNA of the primer and probe pair with qPCR was 0.32 pg/uL (compared to the lowest sensitivity of 1.6 ng/uL in conventional PCR). Using conventional PCR with blue flashlight, we can detect 0.04 ng/uL of pure HL DNA. Screening susceptible hosts and potential vectors for the detection of the LW pathogen will be an effective tool to control the spread and establishment of this lethal disease in new areas.

Natural Pruning Varying with Sweetgum Variety and Density

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A sweetgum (*Liquidambar spp.)* genotype study was planted at two sites in Louisiana in the winter of 2015-2016. Two taxa, including six total genotypes, were planted including the native sweetgum, *L. styraciflua*, and a hybrid, *L. styraciflua x formosana*, in 25-tree block plots and have been measured for diameter and height each year. Crown closure appeared to begin during the mid-fourth year in those blocks that had near complete survival and growth under competition stresses has been occurring for at least three full years in most cases. Overall the hybrids grew faster and thus had a greater stocking than the native counterparts prior to crown closure. Gains of over 50% in dbh have been observed when using the best hybrids and genotype ranks are stable across sites. We will further explore the growth trajectories of the genotypes now that there are three years post crown closure.

Concurrent Session 4A:

Populus Genetics

Exploring Cytonuclear Interactions and Their Phenotypic Outcomes in a Poplar Hybrid Zone
– *Michelle Zavala-Paez, Penn State University*...pg. 73

Combined GWAS And eQTL Analysis Uncovers Novel Genetic Regulatory Networks for Lignin and Carbohydrate Biosynthesis in *Populus trichocarpa*
– *Mengjun Shu, ORNL*..pg. 74

CHX20 Mediates Stomatal Opening to Enhance Carbon Assimilation Under Water-Deficit Conditions
– *Amith Devireddy, ORNL*..pg. 75

Exploring Cytonuclear Interactions and Their Phenotypic Outcomes in a Poplar Hybrid Zone

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Nuclear and cytoplasmatic genomes, including both chloroplast and mitochondrial genomes, interact synergistically to maintain essential functions across plant species. The maintenance of these functions largely depends on the coevolution of cytoplasmic genes with co-functioning nuclear genes, including mitochondrial-nuclear (mt-N) and chloroplast-nuclear genes (cp-N). Thus, cytonuclear interactions, or the interplay between mt-N/cp-N genes with cytoplasmic genes, have likely coevolved at the species level. However, interspecific hybridization can influence co-evolved cytonuclear interactions, with impacts to phenotypic trait variation important to adaptation. *Populus* trees provide an excellent model to study cytonuclear interactions due to its weak barriers to reproduction and extensive hybridization in nature. In this study, we leveraged whole genome resequencing of 576 *Populus* trees across seven latitudinally distributed transects spanning the natural hybrid zone between *Populus trichocarpa* and *P. balsamifera*. We used genotypes sourced from across the hybrid zone transplanted to three replicated common garden environments to ask how cytonuclear interactions contribute to phenotypic variability across novel environments. We estimated admixture proportions from nuclear data and assigned chloroplast identity using phylogenetic analyses. We first tested the role of climate to changes in ancestry for nuclear genes, cp-N genes, and chloroplast genes across the hybrid zone. Preliminary clinal analysis reveals similar clinal variation, but different centers and widths of clines across gene groups, suggesting fine-scale selection may play a role influencing cytonuclear interactions across the hybrid zone. Using our common garden experiments, we assessed the role cytonuclear interactions to phenotypic traits important to adaptation. We found evidence that chlorophyll content was influenced by varying cytonuclear interactions. These findings emphasize the need for further evaluation of the role cytonuclear interactions where species hybridize to understand how these interactions may influence phenotypic outcomes needed to predict fitness across novel environments.

Combined GWAS And eQTL Analysis Uncovers Novel Genetic Regulatory Networks for Lignin and Carbohydrate Biosynthesis in *Populus trichocarpa*

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Lignocellulose, primarily composed of cellulose, hemicellulose, and lignin, constitutes the main components of plant cell walls. Poplars (*Populus* spp.), due to their rapid growth and broad geographic distribution, have become an economically important tree species, providing lumber, paper, and bioenergy feedstocks. Despite the economic and ecological significance of poplar, the genetic regulation of lignocellulose biosynthesis remains poorly understood. In this study, we utilized an integrative approach, combining 1) robust genome-wide association study (GWAS) using multiple traits measured in three distinct techniques, 2) expression quantitative trait loci (eQTL) analysis, and 3) co-expression analysis, to investigate the genetic regulation of lignocellulose biosynthesis in the model species *Populus trichocarpa*. Our study identified four key genes significantly involved in lignocellulose formation, with one known gene and three novel genes discovered. Among these genes, Potri.005G116800 has been previously established as a critical regulator of secondary cell wall formation. Genes Potri.012G130000, Potri.004G202900, and Potri.002G216300 exhibit both upstream and downstream networks involved in cell wall biosynthesis and defense responses. Furthermore, we uncovered the importance of candidate genes in lignocellulose formation and present a gene network based on information in the eQTL analysis. The integration of these methodologies enabled the discovery of new putative regulators of lignocellulose development. Our findings provide valuable insights into the genetic regulation of lignocellulose development in *P. trichocarpa* and lay the foundation for future genetic engineering of cell wall properties to optimize biomass traits.

CHX20 Mediates Stomatal Opening to Enhance Carbon Assimilation Under Water-Deficit Conditions

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Drought is a primary limiting factor for plant growth and development impacting agricultural productivity worldwide. Water deficit conditions beyond the plant's physiological optimum can trigger significant physiological perturbations, reduce the rate of photosynthesis, and accelerate leaf senescence leading to a decrease in canopy size and yield. One way to enhance drought tolerance in plants is by delaying drought-induced leaf senescence, retaining photosynthetic activity, and maintaining leaf water potential. In this study, genome-wide association studies (GWAS) in *Populus* identified a genetic locus that is highly associated with drought-induced leaf senescence. This genetic locus is predicted to encode a member of the putative Na^+/H^+ antiporter family CATION/H^+ EXCHANGER (*CHX20*). To validate the function of *CHX20*, we developed transgenic poplar lines with altered expression of *CHX20* and tested these lines under water deficit conditions. The Populus *CHX20* overexpression (OE) lines retained not only photosynthetic activity (albeit at a reduced level) but also maintained high water potential compared to wildtype (WT) or KO lines during the drought treatment without significant yield penalties. Furthermore, testing the role of *CHX20* using *Arabidopsis thaliana* plants indicated that *AtCHX20* transgenic overexpression (OE) plants had greater stomatal aperture size, enhanced photosynthetic activity, and higher osmolyte contents compared to WT or KO under water deficit conditions.

Concurrent Session 4B:

Tree Genetics and Improvement Tools

Cartograplant: Cyberinfrastructure to Improve Plant Health and Productivity in the Context of a Changing Climate
– Jill Wegrzyn, University of Connecticut..pg. 77

Historical Genetic and Tree Improvement Trials: An Untapped Resource That is Quickly Disappearing
– Rebekah Shupe, Purdue University...pg. 78

Research Frontiers in North American Conifer Orchard Management
– Andrew Sims, University of Florida...pg. 79

Cartograplant: Cyberinfrastructure to Improve Plant Health and Productivity in the Context of a Changing Climate

Jill L. Wegrzyn[1], Irene Cobo-Simon[1], Risharde Ramnath[1], Emily Grau[1], Sean Buehler[1], Meghan Myles[1], Victoria Burton[1], Madison Gadomski[1], Emily Strickland[1], Isabella Harding[1], Shay Muhonen[1], Vlad Savitsky[1], Gabriel Barrett[1], Abdullah Almsaeed[2], Nic Herndon[3], and Margaret Staton[2]

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CartograPlant (https://cartograplant.org/) is the first web-based application that integrates genotype and phenotype data for model and non-model plant systems with global environmental layers. This Tripal-based (tripal.info/) field to analysis framework connects data collection, data submission, ontology-based metadata annotation, and analytics directly to high performance computing resources. Genotype and phenotype metrics are collected through direct submission of studies at the time of publication via the FAIR compliant Tripal Plant PopGen Submit data submission module, and through the biocuration efforts of the affiliated databases (TreeGenes, BIEN, Dryad). CartograPlant also integrates with the mobile forest health application, TreeSnap (https://treesnap.org/). Tripal employs the Chado relational database schema (gmod.org/wiki/Chado) which is itself designed to use ontology terms to describe both primary data and metadata. This ontology-centric structure allows CartograPlant to seamlessly filter and analyze genotypic, phenotypic, and environmental data across multiple independent studies from a variety of sources. Phenotypes are composed of several separate elements including structure, observed trait, and units, all of which are described with ontology terms from a number of ontologies including PATO, PO, CHEBI, ENVO, and Woody Plant Ontology. This allows CartograPlant to standardize and group phenotypes despite differing sources.

Today, CartograPlant houses genotype, phenotype metrics from over 300 studies describing 636 plant species from 278 genera. The associated environmental metrics describe abiotic and biotic descriptors including range maps, seed zones, canopy height, land use, forest fragmentation, soil type, precipitation, temperature, aridity, fire, and biotic damage. The underlying analysis framework, developed in Galaxy, provides a mechanism to span studies (meta-analysis) to estimate diversity, calculate population structure, and perform association mapping/landscape genomics.

Historical Genetic and Tree Improvement Trials: An Untapped Resource That is Quickly Disappearing

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Federal, state, and tribal governments spent millions of dollars during the mid-late 20th-century to install and maintain thousands of genetic and tree improvement trials, including seed orchards, throughout the eastern U.S. Many of these plantings were established to study the genetic and phenotypic variation for hardwood and conifer tree species. As time progressed, many of these plantings have been abandoned or forgotten due to the retirement of key scientists, lack of funding, a shift in priority, the degradation or loss of plantings, or the loss of data. Though some plantings have been removed, many still exist along with accompanying data. This untapped resource is in demand by 21st-century scientists to help overcome challenges like the lack of seed availability, and decimation of forests due to pests and to address key questions about assisted migration of forests. Locating these plantings and their data is essential to restore the services that these studies were established to provide.

We were able to verify the existence of a list of historical plantings, by contacting affiliated scientists, using Google Earth Pro™, and visiting several universities and facilities to collect physical data. Across 18 states, we verified the existence of 1,172 plantings. Of this total, 466 plantings were verified to be alive, 261 had been removed, 5 were verified to be abandoned but alive, and 440 have been confirmed to be alive but without location information to verify. Out of the 1,172 plantings that have been confirmed, only 416 have accompanying datasets. To prevent data from becoming destroyed or lost, we plead for researchers to keep updated records, update successors on these historic plantings, and retain these datasets for future use. We also encourage researchers to standardize and share information for newer plantings to ensure the data can be used in the future.

Research Frontiers in North American Conifer Orchard Management

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Orchards are the principal vehicle by which tree improvement is translated into deployment. In the US Southeast, virtually all loblolly and slash pine (*Pinus taeda* and *Pinus elliottii* var. *elliottii*) seedlings (800m+ planted annually) are sourced from grafted orchards with material deriving from cooperative breeding programs. This is also the case for improved genetic material in the US and Canadian Pacific Northwest, BC Interior and Eastern Canada, South America, South Africa, Australasia, and other world regions.

Although these precious resources are the lynchpin of translating product development into value for stakeholders, very little public knowledge has been gained from research efforts in the last 20 years. In late Fall 2022, the UF|IFAS Cooperative Forest Genetics Research Program (CFGRP) hosted an IEG40 meeting to gather orchard managers, tree breeders, and geneticists to discuss emergent issues in orchard management. The group of more than 60 scientists met to share insight into present and perceived future challenges facing orchards across several coniferous and deciduous forest tree taxa on four continents.

In this presentation we will summarize the conclusions gathered from the IEG40 meeting, focusing on the discussion that is primarily relevant to the US Southeast. We will report the status of known efficacy of contemporary orchard management techniques, gaps in knowledge, and requirements for designed experiments to equip future orchard managers to meet oncoming challenges. We will report the status of [ongoing] follow-through communications with stakeholders, especially those representing the membership and leadership of the Southeastern cooperative tree improvement and research programs. Our aim in presenting these results is to report on the outcomes of the IEG40 meeting for the SFTIC community, to present a working agenda for obtaining new knowledge, and to invite stakeholders to join in this vital research.

Schatz Symposium

Synching Forest Genetics with Climate Change

Identification of a Locus of Sex Determination in *Fraxinus pennsylvanica*
– *Matthew Huff, University of Tennessee*...pg. 81

Using Genomics to Predict Future Maladaptation – Potential for Assisted Migration and Restoration
– *Vikram Chhatre, USFS-NRS*..pg. 82

The Role of Provenance Trials and Common gardens in Understanding Intraspecific Genetic Variation in Responses to a Changing Climate
– *Laura Leites, Penn State University*.................................no abstract submitted

Identification of a Locus of Sex Determination in *Fraxinus pennsylvanica*

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Green ash (*Fraxinus pennsylvanica*) is dioecious, with distinct male and female individuals. At present, the mechanisms of dioecy in green ash and other species in the *Melioides* section of *Fraxinus* are unknown, and trees cannot be sexed until after they first flower. Current efforts to breed green ash with enhanced resistance to the invasive emerald ash borer (EAB; *Agrilus planipennis*) would be improved by finding molecular markers linked to sex determination, allowing researchers to sex trees at the seedling stage. Over 200 samples were collected from ash species in section *Melioides* from 5 states in their native range and from a Forest Service breeding program. An initial double-digest genotyping-by-sequencing (ddGBS) experiment yielded no SNPs in linkage disequilibrium with the sex-determining region, but it did yield a region of interest more likely to be sequenced in female samples along chromosome 8. Whole genome sequence (WGS) data of a subset of these samples was analyzed with two different bioinformatic approaches. Both testing for differential read coverage and presence of unique k-mers identified the same region of Chromosome 8. Annotation of nearby genes provides candidates for further study. Though the exact mechanism of sex determination is still under study, we have a putative genomic location determining sex in green ash, which can be used for marker design and testing in a breeding program.

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Using Genomics to Predict Future Mal-Adaptation: Potential for Assisted Migration and Restoration

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In most widely distributed taxa, the distribution of Standing Genetic Variation (SGV) is shaped by the combined action of post-glacial phylogeography and local environments resulting in local adaptation (greatest fitness in native environments). This adaptation (*GxE* relationships) is vulnerable to disruption by the cascading effects of rapidly warming climate thereby rendering the populations mal-adapted. Pre-adaptation is also likely given that many northerly environments under climate warming may mimic those at lower latitudes making direct transfer of genotypes a consideration. Assisted migration and restorative seed transfer protocols have been discussed as potential avenues for mitigating the disrupted ecosystems. The key to enabling these approaches is to first understand where along the landscape these disruptions may occur. Advent of fine scale climate projections, inexpensive genome-scale genotyping and artificial intelligence (machine learning) has made it possible to (a) identify populations most vulnerable to climate change by assigning a genomic offset metric to them, (b) identify regions of the genome involved in phenological adaptations, and (c) inform assisted migration approaches based on this knowledgebase. In this talk, I discuss the latest advances in these areas and their potential to solve contemporary issues surrounding the effects of climate change. I showcase examples from studies on widely distributed tree taxa and discuss how species restoration can be informed by genomic prediction of mal-adaptation.

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Genetic Variation in *Pinus taeda* L. Populations for Fusiform Rust Disease Incidence from Artificial Inoculations and Correspondence to Field Trials

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Fusiform rust, caused by the fungus *Cronartium quercuum* f. sp. *fusiforme*, is the most damaging disease affecting the health and productivity of loblolly pine (*Pinus taeda* L.), the most commercially important pine in the United States. Planting genetically improved stock is the only efficient way to mitigate disease incidence. Artificial inoculation of seedlings and progeny testing can be used to select genetically resistance germplasm in loblolly pine breeding populations in the Southeastern U.S. This study was conducted in a loblolly pine population to assess genetic variation for resistance to fusiform rust disease. In this study, 24 loblolly pine parents were mated to produce 76 full-sib crosses using a three disconnected eight-parent diallel mating design. Approximately 120 full-sib seedlings of each cross was challenged with 50,000 spores per milliliter of a broad-based inoculum from across the expected deployment region. The overall disease incidence from the artificial inoculation was 48%. The narrow-sense heritability of full-sib family means was 0.90, suggesting strong genetic control of the disease incidence on the family level. For further testing, all seedlings with fusiform rust galls and 25 entire crosses with high disease incidence were discarded. The remaining 2362 seedling progeny of the 51 crosses were clonally propagated via rooted cuttings. One genetically identical copy of each clone (ramet) was planted at eight test sites across the Southeastern U.S. The disease mean from the field tests was 5.3% while the nonscreened seedling checklot family had a mean of 23%. Narrow-sense clone mean heritability for disease incidence was 0.47 while broad-sense clone mean heritability was 0.69. The correlation between the disease incidence from the artificial inoculation and field trials was 0.44 for the full- sib families, while correlation was 0.74 for half-sib families (not truncated during artificial inoculation or cloned for the disease when tested in the field trials).

White Oak Genetics and Tree Improvement Program: Range-Wide Collaborative Effort

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White oak (*Quercus alba*) occurs throughout the eastern US forests where it is important to the health and ecological function of these forests, and it has high value to the forest products industry. The White Oak Genetics and Tree Improvement Program (WOGTIP) supports the goals of the White Oak Initiative in working to ensure there is a never-ending presence of high-quality white oak in the eastern forests. Limited research indicates that there are good opportunities for genetic improvement in white oak and the WOGTIP was developed as a collaborative program to: (1) quantify range-wide genetic variation and (2) improve traits that have economic and ecological value. The WOGTIP has three phases to achieve these goals: 1) collecting acorns from mother trees, 2) progeny testing the mothers, and 3) producing genetically improved acorns for seedling deployment. The poster presentation describes each of these phases. At present phase 1 is completed, phase 2 is well underway, and phase 3 is yet to come. Access to plant materials and data collected by the network of collaborators are available for research purposes, while widespread reforestation using genetically improved white oak seedlings remains the overarching goal of the program.

A Protocol for Rooting Softwood Cuttings of *Fraxinus pennsylvanica*

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North American ash trees are suffering the devastating impact of the invasive emerald ash borer (EAB). The US Forest Service is developing a traditional breeding program based on the selection of surviving ash trees with a healthy crown after peak EAB infestation, termed “lingering ash”. Currently, lingering ash are grafted for use in the program, but because rootstock plants are often susceptible to EAB, propagules on their own roots would be more resilient. The techniques described have produced vigorous *F. pennsylvanica* (green ash) propagules to use for breeding, experimentation, and restoration. In the first trial year, 2019, twelve different treatments were defined from combinations of four factors: environment, media, copper root drench, and hormone application. Five different ash species and hybrids were tested: *F. quadrangulata*, *F. pennsylvanica*, *F. nigra*, *F. pennsylvanica* x *americana*, and *F. mandshurica* x *nigra*, each assigned to at least one of the twelve treatments. Overall species rooting success rates were calculated—10%, 14.9%, 26.7%, 17.3%, and 0%, respectively—and then used to refine the protocol to use the following year on 2- and 3-year-old green ash seedling stump sprouts. Use of the updated techniques increased rooting rate from 14.9% to 83.6%. Total overwinter survival increased from 81% to 95%. Environment, hormone application and timing, and species were all found to significantly affect rooting. Results are now being used to further refine the softwood rooted cuttings protocol, with a focus on its use on older green ash materials and additional species including black, blue, and Manchurian ash.

Genetic Variation in the Endangered Florida Torreya (*Torreya taxifolia* Arn.) and Implication for Species Conservation and Resistance Breeding

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Florida torreya (*Torreya taxifolia* Arn.) is a critically endangered (IUCN 3.1) conifer with a very limited native range in the USA-- limited to two counties in Florida and one in Georgia where it persists in ravines along the Apalachicola River. The species suffered a major decline, ~99% loss, beginning in the 1930s with a total population (trees >2 cm dbh) estimated at 357,500 in the early 1900s to less than 1,500 individuals currently in the wild. The initial decline was attributed to an unknown fungal disease with a canker disease, caused by *Fusarium torreyae*, identified in 2011. The objectives of this work were to develop genetic markers to uniquely identify torreya individuals, examine the structure of the *in situ* population, and use an *ex situ* germplasm to determine if reproduction without fertilization (apomixis) occurs. We developed a panel of microsatellite markers, sampled individuals from three natural, *in situ*, populations and found structure at all levels tested. The markers were next applied to *ex situ* mothers, potential fathers, and progeny to investigate the occurrence of apomixis. Although one, of 29, progeny seedling and its mother had identical multi-locus genotypes, the probability of observing this genotype by chance, given the mother and the two possible fathers' genotypes, is approximately one in ten. Thus, we cannot adequately reject the hypothesis that apomixis occurs. Our finding of population structure suggests genotypes from as wide an area as possible are required to capture the diversity of the species in conservation efforts. The multiplexed marker panel can be used to identify individual accessions and help with managing *ex situ* collections, however, the panel is not sensitive enough to differentiate all closely related individual genotypes in the *ex situ* population.

AgriSeq^{TM} Genotyping Panel for Quality Control in *Pinus taeda* Breeding

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The Cooperative Tree Improvement Program at North Carolina State University, in partnership with Thermo Fisher, developed an AgriSeq^{TM} targeted genotyping-by-sequencing panel for breeding in *Pinus taeda*. This panel contains 995 single nucleotide polymorphic markers selected from the Pita50K array across the genome using the linkage map (Lauer and Isik, 2021). This technology aims for a cost-efficient genotyping solution for routine applications in breeding. In this research, we present preliminary results of the AgriSeq genotyping for pedigree errors using the second-generation Atlantic Elite Population (ACE2). We first calculated genetic relationships between individuals using pedigree and AgriSeq markers. Then, for any given pair of individuals, we measured the difference between realized (G_{ij}) and expected (A_{ij}) genetic relationships, referred as the discrepancy. A significant discrepancy was defined when the standardized discrepancy exceeded three standard deviations. The frequency of the significant discrepancies among individuals were counted. Four trees displayed a noticeably high number of significant discrepancies than others in the population, which are likely caused by the errors in their pedigree. A principal components analysis (PCA) with genotypes from AgriSeq^{TM} was conducted. According to the PCs plot, trees within same full-sib family were clustered together. The same four individuals deviated away from their pedigree-based families, which showed agreement with the previous approach. We also investigated the utility of AgriSeq markers for parentage assignment with CERVUS. A reduced number of markers (50 to 500) were selected based on high minor allele frequency and high genotype call rate for parentage assignments. We compared the parental pair assignments from CERVUS with the pedigree and calculated the consistent assignment accuracy. The accuracy was lowest at 0.69 when 50 markers were used, and the accuracy reached 0.79 when 300 markers were used. In conclusion, the results are encouraging for operational breeding program and seed orchard/nursery quality control.

Development of Embryogenic Cultures for Conservation and Restoration of Redbay, Swamp Bay and Sassafras

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Since the effects of the vascular disease laurel wilt on redbay (*Persea borbonia*) trees were first described in 2002, it has spread rapidly throughout redbay and swamp bay (*P. palustris*) populations in the southeastern U.S., with very high mortality rates. The disease, caused by the redbay ambrosia beetle (*Xyleborus glabratus*) and its fungal symbiont (*Harringtonia lauricola*), has also rapidly increased mortality of sassafras (*Sassafras albidum*) trees in the region. One approach to potential restoration of these species is to employ natural genetic tolerance to the ambrosia beetle and/or fungal pathogen, which may already exist in populations of the affected species. Researchers at the University of Florida have identified redbay genotypes that appear to be tolerant of the pathogen. We are developing somatic embryogenic (SE) culture systems for all three species for eventual use for mass propagation of laurel wilt-tolerant genotypes. Immature fruit were collected by University of Florida cooperators from multiple redbay clones in their collection, and from swamp bay trees on the UGA campus in July and August 2020 and 2021. Immature fruit were collected from four sassafras source trees growing in a USFS test planting in Athens, GA in June 2022. Immature embryos were dissected from the fruit and cultured with associated endosperm on woody plant medium (WPM) containing either 2 mg/L 2,4-D or 0.2 mg/L picloram. Most redbay and swamp bay explants either made small amounts of non-morphogenic callus or turned black in culture within several weeks. Repetitively embryogenic cultures were only recovered from one redbay explant and one swamp bay explant cultured in 2021. Four repetitively embryogenic cultures were established from sassafras explants representing two source trees. All the embryogenic cultures were induced on medium with 0.2 mg/L picloram. Repetitive somatic embryo production has been maintained in the redbay and swamp bay cultures for almost two years and in the sassafras cultures for one year by monthly transfer to fresh medium. The cultures also have produced developing somatic embryos with cotyledons on the same medium. Transfer of sassafras somatic embryos to liquid basal medium lacking picloram promoted production of large, spherical somatic embryos resembling mature zygotic embryos following ten weeks in suspension culture in the dark. Some of these embryos produced roots and short shoots following transfer from suspension to semisolid basal medium with activated charcoal and incubation in the light. Cold pre-germination treatments are now being tested to see if they will enhance sassafras somatic embryo germination. With further development, we believe that SE technologies will aid in the conservation and restoration of North American Lauraceae species affected by laurel wilt.

Celebrating Fifty Years of the USDA Forest Service Resistance Screening Center

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Fifty years ago, in 1973, the Resistance Screening Center (RSC) was established with cooperation from industry, universities, and the USDA Forest Service to manage a fusiform rust epidemic on southern pines. With few options for chemical or cultural controls and evidence of genetic resistance within pine populations, breeding and selection for host resistance became a plausible method for integrated pest management. Efforts to evaluate rust resistance in field-grown pines were expedited through greenhouse-based seedling inoculations using standardized disease loads and rating assessments. Performance of artificially inoculated seedlings was highly correlated to phenotypes observed under field conditions and results could be obtained in less than one year. Interdisciplinary cooperation championed the establishment of the RSC as a centralized high-throughput seedling screening facility serving any organization needing disease resistance information for application in the field. Administration of the RSC was tasked to the Forest Service as a public institution that could unite all interests and receive the legislative support necessary to maintain operations over the long-term. Information derived from the RSC has been used to structure seed orchards, select breeding parents, aid in planting decisions, evaluate fungicides for nursery stock protection, quantify heritability of resistance, characterize mechanisms of resistance, and genetically map resistance at the molecular level. With increasing impacts from invasive pests, there is renewed interest in tree resistance breeding programs in the Southeast and nationwide, as a long-term strategy to enhance forest health and resilience. Furthermore, genetic improvement remains among the most socially, environmentally, and financially sound strategy to ensure that forests remain healthy against invasive pests in an ever-changing climate. The mission of the RSC has never been more relevant, nor more vital, than it is today. This creates opportunities to galvanize the user communities to implement a compelling business model to ensure the long-term success of the RSC.

The American Chestnut Founder Line *OxO* Transformation Project

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Production of American chestnut (*Castanea dentata*) trees expressing the wheat oxalate oxidase gene (*OxO*) to provide resistance to the chestnut blight fungus (*Cryphonectria parasitica*) has been adopted by The American Chestnut Foundation (TACF) as a promising means to restore the tree to forests of the eastern U.S. The primary path chosen by TACF for spreading the transgene to multiple genetic backgrounds for restoration is via pollinating American chestnut trees with pollen produced by Darling 58 transgenic OxO trees. An alternative approach is to directly insert the *OxO* gene into multiple American chestnut genotypes representing the natural genetic diversity of the species. The resulting trees would already be adapted for growth in their native regions. We began pursuing this approach by initiating new somatic embryogenic (SE) culture lines ("Founder Lines") from nuts collected by TACF cooperators from large surviving American chestnut trees (LSAs) growing in different parts of the range from Maine to Georgia. In 2020, over 100 new embryogenic cultures representing eight source trees from five regions (New England, Pennsylvania, Maryland, Virginia, Georgia) were captured. Copies of all the new Founder Lines were placed in cryostorage. The cultures were then screened for their abilities to produce abundant somatic embryos and high-quality somatic seedlings, to facilitate choosing those to target for transformation with *OxO*. The selected Founder Lines showed a range of sensitivities to the selection agent geneticin in liquid medium, necessitating that selection be customized for each line. Transformation experiments with these lines using the pFHI-OXO vector have produced over 80 PCR-positive events in nine culture line backgrounds thus far. Some of these PCR-positive events are currently being grown up in suspension culture for somatic embryo and somatic seedling production. Future plans call for the transgenic lines to be screened for *OxO* transgene copy number, mRNA expression level and production of a functional OxO enzyme. We have also begun working on transforming selected Founder lines with the pWIN3.2-OXO vector to produce events that will have *OxO* expression under the control of a wound-inducible promoter. We hope these Founder Line transformations will eventually lead to restoration of regionally-adapted, blight-resistant American chestnut trees.

Forest Invasions: The Brutal Paradigm of the Bradford Pear

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Pyrus calleryana Decne. (Callery pear) includes popular ornamental cultivars in commercial and residential landscapes. The species has increasingly naturalized across portions of the eastern and southern US, yet the mechanisms behind its spread are not well understood. The relationship of genetics among present-day *P. calleryana* cultivars and escaped Callery Pear populations, to the genetics presented by their Asian counterparts (native trees from China, Japan, and Korea), are largely unknown. We developed and used 18 microsatellite markers to analyze DNA samples from populations of these groups and to articulate the status of genetic diversity within Asian *P. calleryana* and US cultivars. Analyses encompassed 36 specimens of Asian *P. calleryana* and 21 samples of 7 USA commercial cultivars of *P. calleryana*, as well as 6 escaped populations of 30 tree specimens each from across the southeastern US occurring within a ~177 km radius. We hypothesized that Asian *P. calleryana* specimens and US cultivars and escaped populations would be genetically diverse but would show genetic relatedness. Our data revealed high genetic diversity, high gene flow, and presence of population structure in *P. calleryana* both in native range and in the invaded areas, potentially relating to the highly invasive capability of this species. Our results revealed that *P. calleryana* populations had differentiated shortly after the introduction to the US, most likely from specimens imported from Asia, consistent with historical records and our prior findings. Evidence of pervasive cultivar mislabeling assessed using the microsatellite markers, S-locus genotyping, and Sanger sequencing data highlighted one of the possible invasion mechanisms for non-native plants. Our data argues for more broad-scale assessment of genetic diversity in the invasive areas, to uncover molecular mechanisms underlying such behavior.

Characterization of Molecular Interactions Between *Geosmithia morbida* and *Juglans nigra* Using Dual RNA-Seq

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Thousand cankers disease results when the fungus *Geosmithia morbida* is vectored by walnut twig beetles to susceptible *Juglans* host species. At a molecular level, influence of host-pathogen interactions and effects of disease management actions on host-pathogen interactions are not understood. To address these knowledge gaps, dual RNA-seq was used to identify host and pathogen gene expression changes following exposure to chemical (PHOSPHO-jet) and biological (RootShield) management strategy. These treatments were compared to water-only controls using 24 trees per treatment (n=72). One week after treatment, 12 trees per treatment were inoculated with *G. morbida*, and the remaining trees were inoculated with potato dextrose agar (PDA) as a negative control. At 14-, 28-, and 56-days post-treatment application (PTA), RNA was extracted from canker-bordering phloem tissues and sequenced. In *J. nigra*, differentially expressed genes (DEGs) declined across time between *G. morbida* and PDA trees, dropping from 1,897 DEGs (day 14 PTA) to 60 DEGs (day 56 PTA). In *G. morbida*, gene expression also changed with 1,499 DEGs between days 14 and 56 PTA, with reduced expression of putative effectors, including pectate lyase genes. This study provides deeper understanding about interaction mechanisms related to *G. morbida* pathogenicity and provides guidance about how available management strategies influence pathogen virulence at a molecular level.

Systems Genetics in Poplar: Identification of Candidate Genes for the Transport and Deposition of Cell Wall Precursors During Wood Formation

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Lignin is a phenolic polymer that plays critical roles, stiffening secondary cell walls for structural support to aerial organs while also protecting polysaccharides from microbial degradation, which reduces amenability of biomass for certain bio-energy applications like biofuels. The initial steps required for monolignols monomers production through the phenylpropanoid biosynthetic pathway have been extensively studied in different plants including some woody species. The subsequent steps required for monolignol export to the apoplast and polymerization into the cell wall however remain poorly characterized in a biomass crop like poplar. In model plants, members of multiple protein families were shown to contribute to lignification. This includes large families of transporters, oxidases such as laccases and peroxidases that mediate radical coupling of the monolignols to the lignin polymer, and non-catalytic dirigent proteins that are thought to guide stereoselective coupling of monolignols at the initiation sites of lignification in the cell wall. In poplar, genome-wide association studies pointed to multiple transporters, proteins involved in vesicle trafficking, and dirigent proteins as potential key determinants of biomass traits such as lignin composition and structure. By integrating population-wide genetic and transcriptomic information into systems genetics approaches, we identified a subunit of the Exocyst complex, a synaptobrevin, and several members of the dirigent protein family that could be directly involved in the transport and biosynthesis of cell wall polymers and their precursors during wood formation in poplar. We further investigate the function of these genes using reverse genetics approaches to explore the potential of these candidate genes for biomass engineering in poplar.

Transcriptome Analysis of Two *Populus trichocarpa* Genotypes with Contrasting Responses to In Vitro Regeneration Treatments

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Advances in plant molecular biology, and the use of recombinant DNA-based biotechnologies, depend heavily on the ability of species to undergo genetic transformation and regeneration. However, for most species the rate of regeneration is a limiting factor, and is highly variable between plant species and genotypes. Widespread failure to regenerate (known as recalcitrance) can severely constrain the gene pool available to scientists for modifying clonal varieties using genetic engineering or gene editing (GE). To help understand why regeneration varies so widely among genotypes, we are using "transcriptome" analysis in the model tree black cottonwood (*Populus trichocarpa*). Changes in gene expression throughout the genome were characterized while explants (pieces of plant stem used for transformation) regenerated into plants under *in vitro* conditions similar to those of plants undergoing GE. We report on two genotypes selected because their rates of regeneration vary widely. RNA was collected from tissues every three days for about six weeks and subjected to high intensity DNA sequencing by the Department of Energy Joint Genome Institute in California. From raw RNAseq files, several terabytes of data were processed and organized for mapping to the *Populus trichocarpa* reference genome. HISAT2 and STRINGTIE programs were used to filter and map reads to a host genome, subsequently consolidating them into gene count matrices for differential gene expression analysis (DGE) within R. The expression patterns of known developmental regulator genes such as *WUSCHEL* and *SHOOT MERISTEMLESS* are being studied to help characterize tissue developmental states. PCA and cluster analysis is underway to analyze data quality. The identities of the genes that differ in expression among stages and genotypes, and the developmental processes that these genes take part in, should provide insights into recalcitrancy and ways to overcome it.

Beyond Rotholz: Systemic Changes in Fraser Fir Xylem Induced by Balsam Woolly Adelgid

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The balsam woolly adelgid, Adelges piceae, (BWA), was first reported on Fraser fir, Abies fraseri, on Mount Mitchell in North Carolina in 1955. A novel herbivore in North America, BWA was responsible for sharp declines in wild Fraser fir populations throughout its Southern Appalachian distribution during the 1960s and 1970s. BWA induces dense and resinous wood growth in the host fir which is reddish in color and called rotholz. Rotholz restricts water transport in Fraser fir and is a major factor contributing to mortality in BWA-infested trees. Our microscopy study* of wood from BWA-infested trees demonstrates that, in addition to rotholz formation, tracheids appearing normal in color and free from resin deposition also have thickened walls and restricted diameters. Additionally, a previous study identified several Fraser fir clones that exhibited high tolerance to BWA feeding with very low mortality. Analysis of BWA-infested wood from these putatively tolerant clones revealed a larger average tracheid size with reduced cell wall thickening, as well as reduced resin deposition in rotholz. These findings suggest that tolerant Fraser fir genotypes infested with BWA may not experience water stress to the same extent as susceptible trees.

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Private Landowner Perception and Willingness to Grow Short-Rotation Hybridized Sweetgum in the Western Gulf

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In the forests of the eastern half of the United States, sweetgum (*Liquidambar styraciflua*) has long been disregarded, either as a 'trash tree' of little economic value or seen as a competitor for higher value species that must be controlled in the landscape at a cost to the forest landowner. However, with the transition of the fiber market from fine paper to craft paper and biofuels, the value of sweetgum is being given a second look. New breeding programs that combine the native sweetgum species of North America with its Asian counterparts, such as the Formosan gum (*Liquidambar formosana*) have yielded some promising results for the industry. Now the question arises, will landowners be willing to plant and grow hybrid sweetgum as a short-rotation woody crop? In a joint undertaking, researchers with Oklahoma State University and Louisiana State University Agricultural Center are studying the growth potential of several hybrid sweetgum varieties and attempting to quantify if landowners in the Western Gulf region would be willing to consider these varieties as options for timber production in the future. This synopsis will cover landowner perceptions and their willingness to convert to hybrid sweetgum. As a result of the survey, it was determined that 1/3 of the landowners were interested hybrid sweetgum production. The majority of these same landowners currently manage their lands for pine production and indicated a need for further information on growing and managing hybrid sweetgum as well as a reliable market before fully committing to conversion.

The Role of Winter-Biased Genes in Biomass Productivity of Hybrid Poplar

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Renewable biomass is a promising source of bioenergy that can help to reduce reliance on fossil fuels. Biomass accumulation is a year-round process for perennial trees that contribute a significant amount of material to the bioenergy industry. However, the molecular processes behind wood growth tend to only be studied in summer and greenhouse conditions. Investigating winter protection in trees is crucial for understanding and enhancing year-round wood productivity, especially with impending climate changes. We performed seasonal sampling of xylem tissues from poplar trees for RNA sequencing to identify genes and their genome duplicates that are preferentially expressed in different seasons. Genes with winter-biased expression are involved in a wide range of metabolic processes, including carbohydrate metabolism. Carbohydrates stabilize cellular membranes and proteins in stressful conditions to provide protection across the plant, and carbohydrate storage reserves fuel spring regrowth before photosynthesis resumes. Candidate genes involved in carbohydrate processes were identified, prioritizing those with winter-biased expression between paralogs. We designed gRNAs to target individual or duplicated genes for CRISPR-Cas9 knock-out in *Populus tremula* × *alba* INRX 717-1B4. Transgenic trees have already been generated and will be utilized in a series of experiments aimed at exploring the effects of the gene mutations on carbohydrate metabolism and poplar growth phenology during seasonal transitions. This study takes a step towards understanding the molecular basis of winter protection in trees. The results could be used to develop new strategies for improving the cold hardiness of trees, which could lead to increased wood productivity and a more sustainable source of renewable bioenergy.

Development of a Higher Throughput EAB Egg Transfer Bioassay

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The United States Forest Service, Northern Research Station in Delaware, OH has been developing ways to screen ash trees for resistance to the emerald ash borer (EAB). The current egg transfer bioassay protocol is conducted in an environmentally controlled greenhouse, in which EAB eggs are placed on 2-3-year-old grafted trees or seedlings, and the larvae are left to feed for 8 weeks before being dissected from the trees. Outcomes measured include larvae killed by host tree defenses and the developmental instar and weight of live larvae. Hand dissection of these 8-week bioassays is labor intensive so we are developing a 1-year bioassay to decrease labor time and increase screening capacity. In the 1-year bioassay, eggs are transferred to trees in an outdoor growing area covered with bird exclosure netting. After a year, larvae that were able to complete their life cycle without being parasitized or killed by defense responses of the tree will have emerged as adult EAB. The number of EAB exit holes and parasitoid exit holes are counted and relative to the number of hatched eggs with a larval entry hole present. The health of the canopy is also assessed. The egg density used for the 8-week bioassay is 400 eggs/m^2 of bark surface area, but this density causes high mortality in trees undergoing the 1-year bioassay, which may not allow trees with partial resistance to be distinguished from susceptible trees. Experiments were performed to optimize the density of eggs. Lower densities of 200 and 100 eggs/m^2 of bark surface area were tested in a sample of grafted green ash trees. Mortality rates at the end of one year were 19 % at 100 egg/m^2 and 40 % at 200 egg/m^2 densities, an improvement over mortality rates previously observed at 400 eggs/m^2.

eQTL Mapping Identifies Ptrxbat35 as a Regulator of Adventitious Root Development in *Populus*

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Plant establishment requires the formation and development of extensive root systems whose architecture is modulated by complex genetic networks. Here, we report the identification of the *PtrXBAT35* gene as an eQTL hotspot, mapped using 390 leaf and 444 xylem Populus trichocarpa transcriptomes. Among predicted targets of this trans-eQTL were genes involved in plant hormone responses and root development. Overexpression of *PtrXBAT35* in Populus led to significant increase in callusing and formation of shoot-borne roots and wound-induced adventitious roots. Omics studies revealed that genes and proteins controlling auxin transport and signaling were involved in *PtrXBAT35*-mediated adventitious root formation. Protein-protein interaction assays indicated that PtrXBAT35 interacts with components of endosomal sorting complexes required for transport (ESCRT) machinery, implying that *PtrXBAT35* regulated root development may be mediated by regulating endocytosis pathway. Taken together, this work identified a crucial root development regulator and shed lights on the discovery of other plant developmental regulators through combining eQTL mapping and omics approaches.