36th Southern Forest Tree Improvement Conference

Overcoming Tree Improvement Bottlenecks with New Technologies

June 7 - 9, 2021

Proceedings

Booklet of Abstracts

36th SFTIC Conference Website

Proceedings of the 36th 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 36th Southern Forest Tree Improvement Conference was held virtually on June 7-9, 2021, under the auspices of the Southern Forest Tree Improvement Committee, and hosted by the Warnell School of Forestry and Natural Resources at the University of Georgia, in cooperation with Southern Regional Extension Forestry and the Schatz Colloquium for Tree Genetics Fund. This was the first time the conference has been conducted virtually in its 70-year history. The conference was attended by over 90 registered participants, representing forest industry, university forestry programs, and federal and state agencies with interests in tree genetics. The theme of the conference was "Overcoming tree improvement bottlenecks with new technologies," and this theme was reflected in the presentations made by the three invited speakers in the opening plenary session, Dr. Gerald Tuskan (ORNL), Dr. Ingo Ensminger (University of Toronto Mississauga) and Dr. Anna Conrad (USDA Forest Service HTIRC). Volunteer presentations were made in sessions focusing on: (1) pine genetics and breeding, (2) forest health and restoration, (3) hardwood genetics and breeding, and (4) pine pathogens, insects and stress. Since a traditional poster session was not possible, a "lightning presentation" session of 5-minute volunteer papers ended the regular portion of the conference. The 36th SFTIC also marks the first SFTIC to include a symposium sponsored by the Schatz Colloquium for Tree Genetics Fund, which constituted the closing session of the conference. The three invited speakers for the Schatz Symposium were Dr. Sally Mckenzie (Penn State University), Dr. Bode Olukolu (University of Tennessee), and Dr. Jill Hamilton (North Dakota State University), who covered specialized topics in the areas of tree epigenetics and genomics.

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 Lauren Eserman of Atlanta Botanical Garden for her presentation, "Conservation and population genetics of the federally endangered *Torreya taxifolia* (Taxaceae)" and Patrick Bewg from the University of Georgia for his presentation, "CRISPR/Cas9-knockout of trichome-regulating MYBs in *Populus* alter light sensitivity and wax composition".

The **Bruce Zobel Award** is given for the best oral presentation by a student. The winner was Matthew Huff from the University of Tennessee, for his presentation, "Assembly and annotation of the green ash genome".

The **Belle Baruch Foundation Award** is traditionally given for the best poster, but since the 36th SFTIC was virtual, the award was given for the best "lightning presentation." This year, the first place award went to Mason Richins of the University of Georgia, for his presentation, "Propagation and conservation of three rare North American ash species using somatic embryogenesis". Second place was won by Stephen Goodfellow of Mississippi State University, for his presentation, "Fusiform rust resistance of loblolly pine inter-provenance hybrids," and the

third place award went to Samantha Surbur of the University of Georgia, for her presentation, "A delve into the unknown of sulfate transporters' role in elemental movement and stress response in poplar."

Another "first" for the 36th SFTIC was a research support funding competition, with special funding for 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 research projects on which they made presentations at the 36th SFTIC. These grants were separate from the traditional SFTIC awards above. Those receiving the research support grants were Austin Thomas (North Carolina State University), Matthew Huff (University of Tennessee), Anna Conrad (USDA Forest Service), Lauren Eserman (Atlanta Botanical Garden) and Chen Ding (Texas A&M University). Recipients of the grants are expected to make a presentation at the next SFTIC in 2023 on their new research results, including those supported by the Schatz Colloquium funds.

The 36th SFTIC Planning Committee would like to thank the award judges: Meg Staton, Carol Loopstra, John Davis, Patrick Cumbie, Kathy Smith, Chris Rosier, Carrie Pike, Kurt Johnson, David Barker and Kevin Potter.

The 36th SFTIC was a success largely due to the outstanding efforts of a number of people, including the personnel of the Southern Regional Extension Forestry group, Leslie Boby, Darryl Outlaw and Jessica Shaklee, Warnell IT Specialist Brent Peterson, Warnell Continuing Education Coordinator Ingvar Elle and Warnell Program Coordinator Eva Levi.

SFTIC would like to offer special recognition and thanks to Dr. John Carlson (Penn State University), who arranged financial support for the Schatz Symposium and for the Research Support Grants from the Schatz Colloquium Fund.

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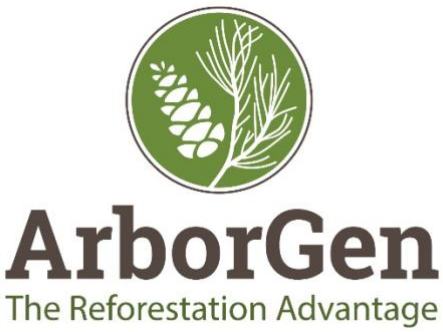

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Plenary Session

THE GENETIC (AND FUNDING) OPPORTUNITIES, CHALLENGES AND EXPECTATIONS FACING TREE IMPROVEMENT, EVEN WITH WORLD-CLASS GENOMICS RESOURCES: A *POPULUS* CASE STUDY

Gerald A. Tuskan[1]

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The current U.S. Administration has a focus on renewable energy, climate change and environmental justice. All of these issues contain forestry related opportunities and challenges. Application of molecular biology and genomics offers potential to ameliorate these problems but the quick delivery window for solutions for these global challenges is confounded by the long reproductive cycles of many long-lived perennial plants and the lack of foundational genomics resources. New sequencing technologies and applications of advanced computing offer hope, but for most species, the realization of that hope is not happening fast enough. In *Populus*, where we have a world-class genomics resources, including high-quality assemblies and annotations for multiple species, fully genotyped association mapping and QTL populations replicated in multiple common gardens, as well as a reliable transformation system based on CRISPR. Using these resources, we have made progress in identifying and validating genes and their associated phenotypes. Examples include EPSP synthase, a novel gene that functions as a transcriptional regulator in the lignin pathway, a lectin kinase that controls colonization by the mycorrhizal fungus *Laccaria bicolor*, and a series of receptor kinases that control Septoria disease resistance. These genes, and many additional SNP markers linked to growth and cell wall traits, are being used in a machine learning approach to stack favorable genes in a genomic selection context. Still, the path to delivering solutions to issues related to renewable energy, climate change and environmental justice remain challenging. Part of the answer is greater funding at all levels, inclusive of federal, state and private sources. Continued improvements in genomics resources, transformation efficiencies and development of early flowering technologies are desperately needed in the conifers and many other hardwood species. The *Populus* case study suggest that rapid progress can be made once such resources are in place. In *Populus*, and all forest tree species, we also need broader field testing of selected and improved plant materials, as well as integrated studies on soil health, carbon sequestration, and water quality. All of us need to become active advocates for the development of these resources. There will be no solution to issues related to renewable energy, climate change and environmental justice without forest trees.

SOMETHING IN THE AIR – DRONE-BASED HIGH-THROUGHPUT PHENOTYPING OF TREES

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The field of forest genomics has seen unprecedented advances during the past decade. A suite of genomic resources is now available for enhanced genomic selection. These resources can be used to accelerate breeding cycles and to select genotypes that are better adapted and more resilient to future climate change and diseases. The large-scale phenotyping of populations has become the bottleneck for identifying and connecting the different genomic resources with adaptive traits in populations with thousands of trees. Measuring leaf optical properties using spectral reflectance sensors carried by remotely piloted aircraft systems (drone systems) represent an innovative approach for large-scale phenotyping of tree responses to drought, monitor phenology, and assess differences between tree genotypes in large-scale field experiments. In this presentation I will give an overview of leaf-level, canopy–level and drone based observations of leaf spectral reflectance. I will demonstrate that some of the widely used vegetation indices such as the normalized difference vegetation index (NDVI) and photochemical reflectance index (PRI) vary in their ability to adequately track important traits such as phenology or photosynthetic efficiency. Finally, I will discuss some of the technical challenges of using optical sensors when monitoring complex canopies and why using carotenoid based vegetation indices are particularly useful in order to monitor evergreen conifer canopies.

SPECTRAL-BASED TOOLS FOR DISEASE RESISTANCE PHENOTYPING IN TREES

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Plant specialized metabolites play important roles in tree responses to abiotic and biotic stressors, e.g., plant pathogens and insect pests. For this reason, individual metabolites or groups of metabolites may be useful as biological markers for identifying trees with desired traits, like disease resistance. Infrared spectroscopy is one approach for measuring the complex chemical make-up of tree tissues and has the potential to be used for rapid and high-throughput phenotyping when combined with multivariate statistical analysis or machine learning. Real-time analysis of tree tissues under field conditions is now possible thanks to advances in spectral technology and the availability of low-cost developmental sensor units. Case studies were presented that demonstrate how infrared spectroscopy can be used to distinguish between disease resistant and susceptible trees. Constraints and opportunities for applying spectral-based tools for tree improvement was discussed.

Pine Genetics and Breeding

ESTIMATING INDIVIDUAL TREE HEIGHTS IN LOBLOLLY PINE BIOMASS PLANTATION USING DRONE IMAGERY AND LIDAR-DERIVED MEASUREMENTS

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Height estimation from Unmanned Aerial Systems (UAS) is an ever-increasing interest in natural resource fields. With the use of PhoDAR from drone imagery in combination with LiDAR data, we produced high-resolution digital surface models over two test sites. After individual tree identification, we computed individual tree heights on a young biomass plantation of loblolly pine. PhoDAR derived heights were compared with field measurements. Of the 7,200 study trees, the software was able to detect 7,143 trees. Overall correlations between field measured heights and PhoDAR derived heights among sites was low (0.18). Site 1 had a very low correlation (-0.10), but Site 2 had a very strong correlation (0.79). The difference in correlation was attributed the "bowl effect" commonly noted in some digital elevation models from vertical UAS imagery (James and Robson, 2014). With proper ground control and techniques to mitigate such board-scale deformations, the use of PhoDAR derived measurement can become a more efficient method than traditional field methods.

PITRO50K: A MULTISPECIES GENOTYPING ARRAY FOR TROPICAL PINES

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Using a combination of reduced representation sequencing methods, we performed targeted SNP discovery towards the development of a genome wide, multi-species genotyping array for tropical and subtropical pine species. Pooled RNA-seq data for five species of tropical pines, originating from pathogen challenge experiments, were used to identify transcript based SNP markers. Additionally, target capture sequencing was performed for six species, utilizing pooled DNA from 81 provenances that represented the natural range of the species across Mexico and Central America. Variant calling yielded a total of 1.8 million candidate SNP probesets, 1.3 million and 563K from RNA-seq and target capture respectively. In total, 300K RNA-seq and 120K target capture derived probesets were evaluated on a 420K screening array through the genotyping of 576 trees from eight species, representing the original 81 provenances and commercial breeding material. Evaluation of the screening array probesets resulted in the selection of 50K SNPs for inclusion on the commercial array. These markers included 20K polymorphic SNPs for *P. tecunumanii*, *P. patula*, *P. caribaea*, and *P. oocarpa*, 15K for *P. maximinoi* and *P. greggii*, 13K for *P. elliottii*, and 8K for *P. pseudostrobus*. Of the 50K markers, 75% are polymorphic in two or more species. The Pitro50K genotyping array represents the first high throughput and affordable genomic tool for these species of pines and their hybrids which represent the majority of tropical and subtropical pine plantations globally. Potential applications for the array include population genomics, genetic mapping, molecular breeding, species and hybrid identification, and genetic resource management.

APPLICATION OF MATESEL SOFTWARE IN A LOBLOLLY PINE BREEDING PROGRAM

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The use of the MateSel software is now standard in many livestock and aquaculture breeding programs and is increasingly used in forest tree breeding programs. The software uses a differential evolution algorithm to balance gain and diversity when selecting parents for the next generation and is subject to several logistical constraints that vary based on the biology of the species. The North Carolina State University Cooperative Tree Improvement Program first utilized this algorithm to develop a mating plan for the 4th-Cycle breeding program (Isik and McKeand 2019). The Cooperative is now using the software in a novel approach to make forward selections for the 5th cycle of breeding by modifying the mating plan output to create a list of progeny test trees to graft for future breeding. We have evaluated several constraints, such as accounting for recent selections in breeding orchards that are not yet producing strobili and accounting for potential selections from families in young progeny tests that are not yet measured. Results indicate that even when the nominal balance of gain and diversity is held constant, considerable impacts on the gain and diversity of the next generation can occur with the inclusion or omission of these logistical breeding constraints.

Isik, F., and S. E. McKeand. 2019. Fourth cycle breeding and testing strategy for Pinus taeda in the NC State University Cooperative Tree Improvement Program. Tree Genetics & Genomes. 15(5):70.

DEVELOPMENT OF ELITE LOBLOLLY PINE FAMILIES FOR THE PIEDMONT REGION USING CLONAL PROGENY TESTING AND GENOMICS

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The development timeline to breed, select and commercialize new genetic families for seedling production is a significant bottleneck for forest tree improvement. Reductions in the time required to progeny test and establish seed orchards have been valuable improvements for tree breeding programs in the past. In this study we evaluate the use of clonal progeny testing and genomics to accelerate the development of new selections for the Piedmont region of the southeastern United States. An elite population was created with selections from Alabama, Georgia, North Carolina, and South Carolina. A partial diallel mating design of 22 crosses was completed and 20 to 50 clones per full-sib family were established in 4 locations. After 3 growing seasons, mean height ranged from 10.3 feet to 14.5 feet across the 4 sites with single site h^2 ranging from 0.25 to 0.42. We will present results from current phenotypic and genomic analyses.

RANGEWIDE PATTERNS OF GENETIC DIVERSITY IN TABLE MOUNTAIN PINE (*PINUS PUNGENS),* AN APPALACHIAN ENDEMIC

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Table Mountain pine (*Pinus pungens*) is an imperiled tree species endemic to the Appalachian Mountains from Georgia to Pennsylvania. Generally reliant on fire for regeneration, its formerly fragmented but widespread distribution has dwindled to fewer than 30,000 acres. It typically occurs in small and geographically isolated populations at elevations of 1,000-4,000 feet in rocky soils on south- and west-facing ridgelines. The suppression of wildfire has allowed hardwoods to take over many sites formerly dominated by Table Mountain pine. We quantified the genetic diversity of 346 trees in 32 populations across the range of the species using data from seven highly polymorphic simple sequence repeat (SSR) loci. The species is relatively inbred (F_{IS} = 0.208) while differentiation among populations was relatively low (F_{ST} = 0.029). Areas with high genetic diversity included the Blue Ridge Mountains of west-central Virginia and of southwest Virginia and northwestern North Carolina, and the Great Smoky Mountains along the Tennessee-North Carolina border. Populations with unique alleles were scattered throughout the species range. We found a strong negative correlation between population isolation and heterozygosity and a strong positive correlation between population isolation and genetic differentiation. Few significant differences in genetic diversity metrics existed among the seed collection zones proposed for Table Mountain pine, except that populations in the most northern seed zone had lower heterozygosity than the southern seed zones, and that central seed zone populations are less genetically differentiated than the northern and southern seed zones. Genetic structure analysis suggested the existence of six to eight genetic clusters in Table Mountain pine. Some of these genetic clusters were associated with different parts of the species distribution. These results add to our limited knowledge of genetic variation across the distribution of this rare and threatened conifer.

VARIATION IN SALT TOLERANCE OF SLASH PINE *(PINUS ELLIOTTII)* FAMILIES

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The average temperature is going up, the glaciers are melting, and sea levels are rising! Salt tolerance will become an important factor in restoring vegetation in coastal environments. In Mississippi, Alabama, and Florida, slash pine *(Pinus elliottii)* occurs in coastal and barrier island environments that are often subject to salt-water exposure. No information is available on the inheritance of saltwater tolerance in slash pine though one might expect that the barrier island pines would be more tolerant than mainland sources.

Bayne Snyder, François Mergen, and Jeff Burley collected seed from the Mississippi Barrier Islands with companion collections from nearby mainland sources and found significant morphological differences between island and mainland sources (Mergen et al.1966). However, they did not test for saltwater tolerance. Sam Land (1973) studied salt-tolerance in loblolly pine *(P. taeda)* families from the North Carolina State University Tree Improvement Program. He included controls of pond pine *(P. serotina)* and slash pine. The only significant difference that he found was that salt tolerance was higher in slash pine than in loblolly pine or pond pine. Family differences among the loblolly pine were not apparent. It does not seem coincidental that slash pine is the only pine found on the Mississippi barrier islands.

We conducted several experiments to compare salt tolerance of island populations of slash pine with mainland populations (see map). We had two basic questions to answer: Is there a salt-tolerant island ecotype, and are there genetic differences among individual families in salt tolerance?

Adjusting our experimental conditions to attain a consistent response that allowed a separation of populations and families took some trial and error. Complete immersion of the seedlings in water containing salt was always fatal. Exposure of root systems to salt

concentrations approaching that of seawater (35 ppm salt) through irrigation from below killed the seedlings too soon to separate the responses of families or populations.

Nevertheless, we did find evidence of family differences and some indication of population differences in salt tolerances in these our early trials. We also found strong indication that loblolly pine was less salt tolerant than slash pine.

In the current experiment, we collaborated with the University of Southern Mississippi's Gulf Coast Research Laboratory (GCRL) to use their facilities to draw brackish water from the Mississippi Sound for our saltwater treatments.

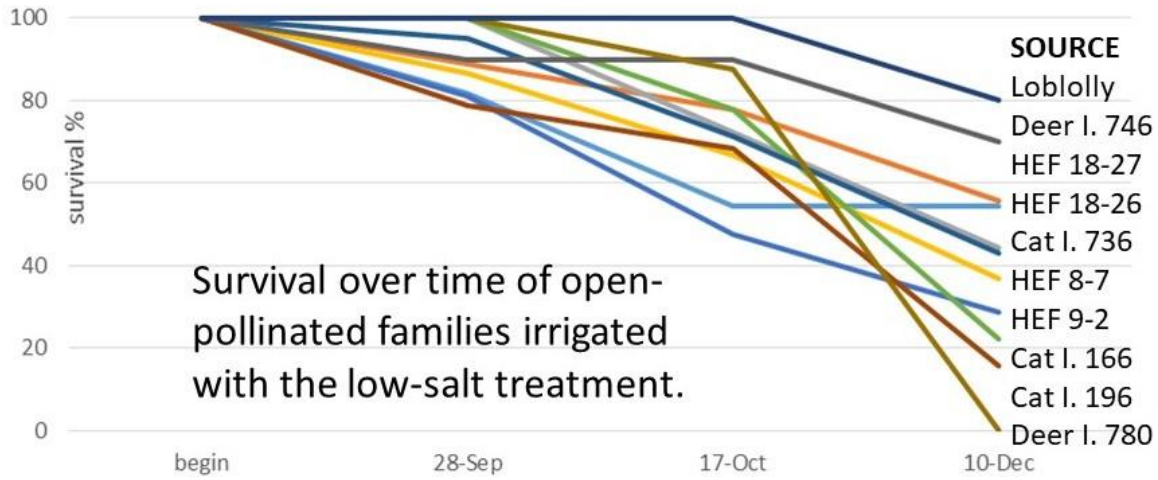
In addition, we used GCRL's immersion boxes for irrigating the plants with the drawn brackish water. We tested 16-month-old seedlings of open-pollinated (OP) families of natural slash pine from three populations: Cat Island (a barrier island 15 km offshore), Deer Island (a near-shore island 100 m off-shore) and inland, from the Harrison Experimental Forest (HEF, 55 km north of the coast) (refer to map above). Also included was one open-pollinated family of loblolly pine. We planted seed from 5 HEF, 3 Cat Island, and 4 Deer Island trees plus one loblolly pine. The seedlings of the 13 open-pollinated families were taken to GCRL 31 July 2019 and three treatments began 29 August 2019. The boxes were filled up to the ground line (root collar) of the seedlings with either fresh water or brackish water depending on treatment. The boxes were filled, then drained about an hour later, according to the schedule (0 salinity = fresh water):

Treatment	29Aug	4Sep	7Sep	11Sep	14Sep	18Sep	24Sep	29Sep	5Oct	11Oct
- Salinity – parts per thousand (ppt) -										
Control	0	0	0	0	0	0	0	0	0	0
Low Salt	12	0	0	0	19	0	0	0	19	0
High Salt	12	0	19	0	19	0	19	0	19	0

We found that the "low salt" treatment, i.e., three treatments of watering roots only from below at bi-weekly intervals with natural brackish water (12 to 19 ppt salinity) from the Mississippi Sound gave us overall survival around 50% and good family separation.
We found ample and significant family-in-source variation in survival (P=0.0385). Survival of families varied from 0 to 70% at the end of the study in the low-salt treatment, a range that appears to be useable in a breeding program.

The slash pine seedlings showed only small, non-significant differences among populations for survival (P = 0.0963). Loblolly pine was not included in the statistical analysis. There did not appear to be significant adaptation to salt-water inundation by the island sources versus the mainland sources.

In a related field study planted on Deer Island using Deer Island and HEF families we found significant family differences in survival after a storm flooded the planting with



brackish water (Schmidtling and Nelson 2019) but no seed source differences. Only the two sources were included in the study, but we had a large number of families (over 50).

In this study, the one difference that stood out was the better survival of the loblolly pine family. This was opposite the expected, based on previous studies (Land 1973) and our own studies. The loblolly family used, B-145-L, was from west of the Mississippi River. All other studies, including Land (1973), used families from east of the Mississippi River.

It has been established that loblolly pine from west of the Mississippi River are slower growing, more drought resistant, and more fusiform rust resistant than sources from east of the river (Schmidtling 2001). This could explain the difference we have observed in this study, i.e., if western loblolly seed sources are also more salt tolerant, a reasonable assumption

So far, the evidence for a salt-tolerant slash pine ecotype on the barrier islands seems lacking. There does, however, appear to be exploitable family variation in salt tolerance. The possible difference in salt tolerance in western versus eastern loblolly seed sources also may become important. Our next experiment will incorporate not only more island slash families, but also eastern and western loblolly pine sources. We are also setting up our own facilities at the HEF, similar to the GCRL facilities. This will give us better daily control without the necessity of driving 30 miles to tend the experiment.

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Cooperators: Gulf Coast Research Laboratory, University of Southern Mississippi
Department of Marine Resources (State of Mississippi)
Mr. George Boddie, property owner, Cat Island, MS

Forest Health and Restoration

CONSERVATION AND POPULATION GENETICS OF THE FEDERALLY ENDANGERED *TORREYA TAXIFOLIA* (TAXACEAE)

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Florida torreya, or *Torreya taxifolia* (Taxaceae), is one of the most endangered conifers in North America and is endemic to the ravines east of the Apalachicola river in the Florida panhandle. In the last century, populations have declined from nearly 700,000 trees in the early 1900s to around 700 trees today. This dramatic decline is the result of an invasive fungal pathogen, *Fusarium torreyae*. For the last 30 years, staff at the Atlanta Botanical Garden have been collecting cuttings for safeguarding, caging trees to prevent deer browsing, and tagging and monitoring wild trees in Torreya State Park and the Nature Conservancy's Apalachicola Bluffs and Ravines Preserve. Recently, partnership with the Florida Native Plant Society has allowed outreach to private landowners to locate, tag, and collect cuttings of trees on private lands. Using the collection of trees from across its range, we are performing conservation genetic studies of *Torreya taxifolia* using target gene capture to assess the level of genetic diversity and population structure remaining in the wild. Conifers are notorious for having extremely large and highly repetitive nuclear genomes, making typical population genomic techniques such as Genotype-by-Sequencing (GBS), restriction site-associated DNA sequencing (RADseq), and genome skimming unfeasible. Gene capture, in contrast, allows for targeted sequencing of specific loci in the genome and allows us to overcome the problem of the large, repetitive genome structure. This method is a cost-effective way to obtain DNA sequence variation necessary to distinguish among closely related populations. Together, these projects will advance conservation efforts for this critically imperiled conifer.

EVOLUTIONARY ORIGINS OF THE HEMLOCK WOOLLY ADELGID RESISTANCE-DISPARITY BETWEEN ASIAN AND NORTH AMERICAN HEMLOCKS (*TSUGA* SP.)

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Evolutionary theory provides a framework for understanding population and species level variation, which, when translated into a context for understanding the scope and magnitude of a conservation problem, can provide novel solutions. The hemlock woolly adelgid (HWA, *Adelges tsugae*) is a sap-feeding pest evolved in eastern Asia and introduced into Eastern North America in the 1950's that has nearly extirpated local populations of the eastern hemlock (*Tsuga canadensis*) across the central and southern portions of its range. The Chinese hemlock (*T. chinensis*), in contrast, has a long evolutionary history of exposure to the HWA and is largely resistant. Here, we seek to use evolutionary theory to understand the origins of the disparity of resistance to HWA by focusing on patterns of allele frequency variation in terpenoid biosynthesis pathway genes. Terpenoids are generally thought to be stress avoidance or tolerance mechanisms in gymnosperms, and are likely candidates to explain patterns of resistance to the HWA within *Tsuga*. We employ neutrality tests in gene coding regions to understand patterns of sequence evolution. This study will provide an important context for understanding the origin of epidemics after the introduction of novel pests as well as provide important genomic resources for an understudied and imperiled tree of North American forests.

DEVELOPMENT OF A NEW GENETIC TRANSFORMATION SYSTEM FOR WHITE AND GREEN ASH USING EMBRYOGENIC CULTURES

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All North American ash species are under threat from the emerald ash borer (EAB; *Agrilus planipennis*), an exotic wood-boring beetle that has already destroyed millions of ash trees in the U.S. and Canada. One potential approach to managing this destructive insect is to develop a host-induced gene silencing strategy employing transgenic ash that express RNA interference (RNAi) constructs targeting EAB-specific genes. Exogenous double-stranded RNA triggers the RNAi pathway and can silence key genes, disrupting protein function and causing insect mortality. An important prerequisite for advancing this technology is a reliable transformation/regeneration system for ash. We tested embryogenic cultures of white ash (*Fraxinus americana*) and green ash (*Fraxinus pennsylvanica*) as target material for Agrobacterium-mediated gene transfer with marker genes. In a series of experiments, cells of different suspension-grown white ash and green ash embryogenic cultures were collected and inoculated with *A. tumefaciens* strain AGL-1 carrying the pFHI-GUSi expression vector, which, in addition to the intron-GUS reporter gene, also includes the nptII selectable marker gene. After inoculation, cells were maintained on semi-solid medium that contained 35 mg/l geneticin and 300 mg/l timentin to select for transformed cells and eradicate Agrobacterium. Once putative transgenic events were identified, they were assayed at every developmental stage from callus to plantlets using the fluorometric assay for β-glucuronidase to confirm the presence of the transgene. When plants were obtained, DNA was isolated from leaf tissue and PCR was used to confirm the insertion of the intron-GUSi gene. To date, two genotypes each of white ash and green ash have been transformed and multiple events from both species have been successfully acclimated to soil.

MAPPING QTLS FOR BLIGHT RESISTANCE AND MORPHOLOGICAL TRAITS WITH COMPLEX CHESTNUT FAMILIES

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Chestnut blight (caused by *Cryphonectria parasitica, Cp*), together with Phytophthora root rot, has nearly extirpated American chestnut (*Castanea dentata*). In contrast to the susceptibility of American chestnut, many Chinese chestnut (*C. mollissima*) genotypes are resistant to blight. In this research, we performed a series of GWAS studies for blight resistance originating from three unrelated Chinese chestnut trees (Mahogany, Nanking and M16) and a QTL study on a Mahogany-derived F2 family. We evaluated trees for resistance to blight after artificial inoculation and scored nine morphological traits that are the hallmarks of species differentiation between American and Chinese chestnuts. Blight resistance and morphological trait QTLs that were identified in the GWAS and F2 mapping studies were compared. Results support a moderately complex genetic architecture for blight resistance, as 13 QTLs were found on 11 chromosomes across all studies, including two previously identified QTLs (Cbr1 and Cbr3). Additionally, blight resistance QTLs overlapped with 9 of 15 morphological trait loci indicating that it will be challenging, but still possible, to eliminate Chinese chestnut alleles for these distinguishing traits while still achieving high blight resistance in the backcross hybrid chestnuts. Finally, comparison between QTL intervals for blight resistance and those previously published for root rot resistance, revealed common disease resistance loci on chromosome A, E, and K.

EVIDENCE OF FRASER FIR PARTIAL RESISTANCE TO THE BALSAM WOOLY ADELGID

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The balsam woolly adelgid, *Adelges piceae* (BWA), was first reported on Fraser fir, *Abies fraseri*, on Mount Mitchell in 1955. A novel herbivore in North America, BWA was responsible for sharp declines in wild Fraser fir populations in the 1960's and 70's. Tree mortality is thought to be the result of systemic rotholz formation. Rotholz is dense and resinous wood that restricts water transport throughout the tree. Fraser fir is the premier Christmas tree species in North Carolina and plantations require frequent pesticide applications to prevent BWA related damage and mortality. Breeding Fraser fir for BWA tolerance or resistance traits may lessen or eliminate the need for pesticide treatments in the future. In this genetic study we evaluated 37 clones of improved Fraser fir for increased tolerance or resistance to BWA using tree growth, gouting, loss of apical dominance, systemic rotholz formation, and mortality as performance metrics. Additionally, we evaluated the lateral bud transcriptome and foliar terpenoid expression of select clones in response to BWA feeding. We found a broad range of responses among clones ranging from extreme susceptibility to significant levels of resistance. Increased foliar concentration of several terpenoid compounds in response to BWA feeding were observed across all clones but this response did not correlate with clone performance. Significant upregulation of genes related to plant defense, lignin synthesis, and traumatic resin duct formation observed in high performing clones, however, does suggest a targeted defensive response to BWA feeding. We believe these data provide strong evidence that some degree of BWA resistance is present in Fraser fir populations which could be further enhanced through selective breeding.

SAVING BUTTERNUT

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Butternut is a relatively uncommon hardwood tree native to eastern North America. Butternut abundance has declined over the past fifty years, primarily due to the invasive pathogen *Ophiognomonia clavigignenti-juglandacearum* and loss of suitable habitat for regeneration. Despite steep population declines, genetic diversity range-wide remains fairly high. While there is little evidence for even moderate resistance in native butternut, hybrids with Japanese walnut, a closely related species, display enough resistance to persist on the landscape without protection and bear abundant nut crops year after year. Cryostorage of native embryogenic axes has yielded promising initial results as a strategy for gene conservation, but additional action is needed to conserve the remaining native gene pool. We describe a practical recurrent selection strategy for resistance breeding in butternut, using sources of resistance from naturally occurring hybrids, hybrids in research orchards, sources of native trees from as many regions as possible, and targeted genotyping.

Hardwood Genetics and Breeding

WHITE OAK GENETICS AND TREE IMPROVEMENT PROGRAM: RANGE-WIDE COLLABORATIVE EFFORT AND EARLY RESULTS

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White oak (*Quercus alba*) occurs throughout the eastern US forests where it is important to the health and how these forests function. White oak also provides habitat for wildlife such as turkey and deer, and it has high value to the forest products industry. The White Oak Initiative (whiteoakinitiative.org) is working to ensure there is a never-ending presence of white oak in the eastern forests. It supports the sustainable growth and production of white oak for a wide range of environmental, social and economic benefits. White oak research and the role of genetics and tree improvement is a focus area of the White Oak Initiative in recognition of the importance this work plays in our ability to respond to increasing pressures on the white oak resource. The White Oak Genetics and Tree Improvement program (WOGTIP) (white-oak-genetics.ca.uky.edu) also supports the goals of the James B. Beam Institute for Kentucky Spirits.

Unimproved **Improved**

10-year-old white oak growing in adjacent rows, Indiana DNR Vallonia Nursery (photo by L. DeWald)

Limited research indicates there are good opportunities for improvement in white oak (see Figure 1). WOGTIP was developed as a collaborative program including industry (forest, wood, distilling), and agencies and organizations (forestry, conservation, wildlife) to: (1) quantify genetic variation in white oak and, (2) improve traits that have economic and ecological value. The project will support white oak users by: 1) providing a sustainable supply of high quality, improved white oak seedlings via a tree improvement program to meet current and future demands, 2) improving our ability to conserve and restore white oak in the forest to achieve a variety of ecological, conservation and economic goals at regional and national levels, and 3) providing genetic resources for academic and industrial research and development.

WOGTIP has three phases: 1) collecting and archiving genetic material, 2) progeny testing, and 3) acorn production and seedling deployment.

Phase 1 - Collecting and Archiving Genetic Material: White oak genetic material is being collected from the entire geographic range of white oak. Acorn collectors include federal and state agency personnel, academic institutions, woodland owners, NGO's and many citizen volunteers including those in the Master Naturalist programs. Acorns are planted and seedlings

are grown at the Kentucky Division of Forestry's (KDF) Morgan Co. nursery. Other than hand-planting, standard nursery operating procedures are followed. Scions from the trees that acorns were collected from are grafted onto swamp white oak root stock and out-planted to create a clone bank to conserve genetic material of the parent trees for creation of future seed orchards. Acorn and scion collecting will continue until the entire geographic range of white oak is represented in the program. Despite being a poor mast year, 91 collections from 9 states were obtained in 2019 and 17,000 acorns were planted at the KDF nursery. In 2020, 112 collections from 18 states and over 35,000 acorns were planted at the KDF nursery. 2021 is a good mast production year across the range of white oak and multiple collections from every state in the natural range will be collected. 1-0 seedlings varied significantly among seed sources in height, root collar diameter and branchiness. Acorn size and percent of acorns resulting in seedlings also varied significantly.

Phase 2 - Progeny Testing: 1-year-old seedlings are planted in progeny tests to evaluate parent tree traits of interest to stakeholders. Depending on the trait, identification of superior performance can occur within 7-15 years. A progeny test located at Maker's Mark Star Hill Farm in Loretto KY includes seed sources representing the entire geographic range of white oak. Planting began in March 2021. Many smaller regional progeny tests are also being established throughout the geographic range of white oak. These tests will allow us to describe genetic patterns to ensure seed sources do not get moved outside their range of adaption, and we can look for local and non-local genetic superiority. Partners hosting regional tests include academic institutions, USFS National Forests and Research Stations, and state natural resource agencies.

Phase 3 – Acorn Production and Seedling Deployment: Parents of superior progeny based on the progeny tests results are used to create grafted seed orchards using material stored in the clone banks. Pollen mixing among top parents within seed orchards creates genetically diverse, high quality offspring. Controlled pollination can be used to breed for insect and disease resistance, or for other traits that will support ecological success in the forest and/or increased economic value for wood products industries. Demand for white oak acorns is very high, and acorn production is highly variable in white oak. Therefore, to ensure a sustainable supply to the nurseries, two addition types of acorn production areas of genetically superior trees will be established. Poorer performing trees will be removed from the regional progeny tests after 15 years and these tests will be converted to acorn production areas. Superior seedlings will also be planted in small areas on private woodlands and within National Forests to become additional acorn production areas. The establishment of many acorn production areas using superior white oak throughout its range will supplement the grafted seed orchards to ensure a consistent annual supply of acorns is available to the nurseries for the production of superior white oak seedlings.

Reforestation using superior white oak seedlings will achieve the goals of the white oak genetics and tree improvement program.

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ASSEMBLY AND ANNOTATION OF THE GREEN ASH GENOME

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Once the most widely distributed ash tree in North America, the green ash (*Fraxinus pennsylvanica*) has faced high mortality as a result of the non-native invasive emerald ash borer (EAB; *Agrilus planipennis*). A small percentage of native green ash trees that remain healthy in long-infested areas, termed "lingering ash," display partial resistance to the insect, indicating that breeding and propagating populations with higher resistance to the pest may be possible. Genomic resources could assist green ash resistance breeding programs by enabling identification of genetic markers associated with resistance and other important traits. However, at present only scaffold-level genome assemblies, without gene or trait information, are available. As a first step toward providing information needed by tree breeders, we developed a 757 Mbp chromosome-scale assembly scaffolded with a newly expanded genetic linkage map containing over 4,000 SNP markers. Gene annotation of the assembled genome sequence identified 35,470 protein coding genes. Synteny and base-pair substitution analysis confirmed the presence of the previously reported Oleaceae family whole genome duplication. Interestingly, the chromosomes are only moderately rearranged since the duplication event and residual syntenic blocks are identified both within the green ash genome and between green ash and olive. In addition, we demonstrate further utility of the new assembly through reference-guided scaffolding of publicly available genomes from 28 other *Fraxinus* species and subspecies. These resources and analyses provide a new opportunity to characterize EAB response among species spanning from resistant to susceptible in phenotype and should benefit EAB-resistance breeding programs and ash restoration efforts.

FAST-TRACK TRANSFORMATION AND FLOWERING FOR RAPID-CYCLE BREEDING IN POPLAR

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Forest trees have a juvenile phase of several years to several decades. The long generation time is a major impediment to breeding and a bottleneck to fundamental research involving reproductive traits, transgenerational monitoring, and genetic containment. Several regulators of floral development, such as LEAFY and FLOWERING LOCUS T (FT), have been used to induce precocious flowering in annual models. Translating these findings into the poplar system has met with various challenges, including dwarfism and sterile flowers. While the use of heat-inducible promoters for floral gene expression has alleviated many of the developmental anomalies, tedious heat treatments over multiple weeks and months are detrimental to microsporogenesis. The efficacy is also season- and genotype-dependent, limiting widespread adoption of this method. For dioecious species like *Populus*, early flowering male and female genotypes must be available for cross-pollination. Building on the recent discovery of a single female-specific cytokinin response regulator (FRR) underlying sex determination in *Populus*, we reasoned that early flowering and sex switch can be engineered simultaneously to produce male, female and/or hermaphrodite individuals from the same genetic background to accelerate poplar breeding and research. We used CRISPR/Cas9 to knock out a negative regulator of floral initiation in a female hybrid aspen *Populus tremula* x *alba* INRA 717-1B4. *In vitro* flowering was readily observed within 3-4 months of *Agrobacterium*-mediated transformation. When *FRR* was also targeted, transgenic plants developed male and sometimes hermaphrodite flowers in tissue culture. With further development of an efficient early flowering and sex switch system like this, the promise of CRISPR to accelerate rapid-cycle breeding or rapid-cycle genomic selection via controlled crosses is finally within reach for long-lived woody perennials.

EXPLORING THE GENETIC DIVERSITY OF AN EXTINCT TREE SPECIES IN THE WILD, *FRANKLINIA ALATAMAHA*, USING GENOTYPING-BY-SEQUENCING OF THE SURVIVING CULTIVATED POPULATION

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Genetic diversity of an extinct tree species in the tea family (Theaceae), *Franklinia alatamaha*, has been of curious interest since this tree only existed as one remote population when growing in its native habitat on the Georgia Coastal Plain. *F. alatamaha* was first documented by American naturalists John and William Bartram during their travels to Florida in 1765. The only known population of *F. alatamaha* became extinct in the wild by the early 1800s. Fortunately, William Bartram collected seed from this population in 1773 and brought it back to Philadelphia, where he grew plants from the harvested seed, which has allowed *F. alatamaha* to be cultivated as an ornamental for nearly 250 years. All extant *F. alatamaha* trees in the world are derived from that seed collection. The only opportunity to infer the genetic variation that once existed in the original population was to examine the genetic variation in the cultivated population. Leaves from live and herbarium accessions were obtained from 42 sites worldwide. Genotyping-by-sequencing (GBS) was used to determine the genetic diversity and structure of 76 *F. alatamaha* accessions, including a 178-year-old herbarium specimen. STRUCTURE analysis with 9604 high-quality single-nucleotide polymorphisms (SNPs) identified two subpopulations within the cultivated accessions. This result was supported by UPGMA (unweighted pair group method with arithmetic mean) and principal component analyses. F statistics indicated that there was a moderate level of genetic diversity among the cultivated accessions (FST = 0.09), with more genetic diversity among accessions within a subpopulation than between the two subpopulations. The inbreeding coefficient of the cultivated accessions was low (FIS = − 0.4902), indicating that the sampled trees represent what was once a highly outcrossing population. The genetic differentiation identified in this study may be useful for further development of new horticultural traits such as disease resistance to *Phytophthora* root rot, which inhibits *F. alatamaha* from being cultivated in the region to which it was once native.

CRISPR/CAS9-KNOCKOUT OF TRICHOME-REGULATING MYBS IN *POPULUS* ALTER LIGHT SENSITIVITY AND WAX COMPOSITION

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In all habitats, plants face a variety of environmental stresses, and thus, have evolved an array of physiological and transcriptional coping mechanisms. Acting as a physical barrier between plant and its environment, hair-like trichomes act multifunctionally to provide pest defense and UV shielding whilst reducing transpiration rates. Additionally, they can also act as a locale for secondary metabolite synthesis and storage. The role and regulation of trichomes is well studied in herbaceous models, but less so in poplar, a woody perennial with bioenergy importance. Previous research identified the transcription factor PtaMYB186 as a positive regulator of trichome initiation during early stages of leaf development in *Populus tremula* x *P. alba* (IRNA 717-1B4). Here, the CRISPR/Cas9 system was utilized to target *PtaMYB186* and its close paralogs for knockout mutagenesis in poplar. The regeneration of trichomeless mutants confirmed the regulatory roles of the MYB transcription factors during trichome initiation. These trichomeless poplar had increased pest susceptibility, though unexpectedly, growth and leaf transpiration rates were not affected. Additionally, light-regulated genes were found to be differentially expressed and exposing the trichomeless mutants to a high-light environment significantly increased synthesis of anthocyanins, a class of known photoprotective metabolites. Notably, cuticle wax and whole leaf analyses found a complete absence of triterpenes in the mutants, suggesting biosynthesis and storage of triterpenes in poplar occurs in the trichomes. Together, these findings contribute further insights into the multifunctional role of trichomes in poplar as both a pest and light barrier, as well as a site of triterpene biosynthesis and storage.

RIBOSOMAL DNA ORGANIZATION AND COMPOSITION IN AMERICAN AND CHINESE CHESTNUTS

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Introduction

The American chestnut (*Castanea dentata*, $2n = 2x = 24$), once a foundation forest species over 800,000 km^2 in eastern North America, was decimated by chestnut blight caused by an introduced fungal pathogen, *Cryphonectria parasitica*. The devastating disease was first reported in 1904 by Hermann Merkel, a forester at the New York Zoological Park (Murril 1906). The disease spread rapidly, covering the entire species range by the early 1950s and killing nearly 4 billion trees. (Hepting 1974). Chinese chestnut (*Castanea mollissima*), a species closely related to American chestnut, is relatively resistant to the blight pathogen. Efforts are underway to transfer resistance from Chinese chestnut to American chestnut, including a backcross breeding program operated by the American Chestnut Foundation (Hebard 2006; *www.acf.org*) and a biotechnology-based program sponsored by the Forest Health Initiative (Nelson et al. 2014; www.foresthealthinitiative.org). Recently an integrated genetic/physical map of Chinese chestnut was published (Kubisiak et al. 2013) and the species genome has been sequenced (Staton et al. 2020); however, little cytogenetic data are available to confirm and complement these genomic resources. Fluorescence *in situ* hybridization (FISH) is an important cytogenetic technique for assigning and orienting genetic markers to specific chromosomes. In this study we assign the major 35S rDNA to LG_H and compare this linkage group chromosome between American and Chinese chestnuts.

Materials and Methods

Root tip collection and pre-treatment to accumulate chestnut metaphase chromosome spreads were carried out as described by Staton et al. 2020. Whole plasmid DNA including the 18S-26S insert of maize (Zimmer 1988) and four BAC clones [BB134N22, 1.3 cM (C5); BB171MO4, 6.3 cM (G6); BD176N08, 50.2 cM (F12); and BB055C18, 57.9 cM (F2)] were selected from LG_H BAC contigs that genetically mapped (9), two from each extreme end of the genetic map, were labeled with biotin-16-dUTP (Biotin Nick Translation Mix, Roche, USA) and/or digoxigenin-11-dUTP (Dig Nick Translation Mix, Roche, USA) following the manufacturer's instructions.

Fluorescent *in situ* hybridization coupled with epi-fluorescence microscopy to capture digital images and subsequent processing were performed as described previously (Islam-Faridi et al.

2009a, 2020). Three FISH experiments were conducted. In the first experiment, Chinese chestnut chromosome spreads were probed with four BAC clones (C5, G6, F12 and F2). In the second, BAC probes were washed off and the second FISH with 35S rDNA probe were carried out as described elsewhere (Islam-Faridi et al. 2020). In the third, American chestnut chromosome spreads were probed simultaneously with two BAC clones (G6 and F2) and 35S rDNA.

Results and Discussion

Earlier we reported the major 35S rDNA site is located terminally and sub-terminally in American and Chinese chestnut trees, respectively (Islam-Faridi et al. 2009b; Staton et al. 2020). For the LG_H chromosome identification, we used four BAC clones (see material and Methods). While analyzing BAC-FISH images for LG_H, we observed that all four BAC clones hybridized to the NOR-associated satellite pair of chromosomes, and these were concordant to the LG map but leaving about 25% of the physical (structural) body of the chromosome (Fig. 1), which has not been assigned to this genetic map. After visual microscopic identification of the NOR-associated satellite for this LG_H chromosome, we recorded the co-ordinates of a few good chromosome spread cells from this FISH slide and conducted a second FISH with the 35S rDNA probe (see materials and methods). The 35S rDNA probe hybridized at the NOR region, which is considered to be the major site for the 35S rRNA gene, and a proximal portion the satellite (Fig. 1b). In a third FISH experiment, we used two BAC clones (one from each end of the LG_H) including the 35S rDNA region on American chestnut chromosome spreads to check the physical positions of these DNA probes on the NOR-bearing chromosome of this species. As expected, all three probes hybridized to the NOR-bearing major 35S rDNA chromosome of American chestnut (Fig. 1d). The 35S rDNA hybridized terminally, distal to BAC G6 (6.33 cM), covering the entire NOR and the satellite. This indicates that the AC satellite is relatively much shorter than that of its counterpart in Chinese chestnut. Further, this comparative FISH result supports the gene and/or molecular marker(s) synteny of these two chestnut species on LG_H (Kim et al. 2005). Additional research is needed to identify the chromatin composition of the respective satellite since the 35S rDNA covered the entire satellite of the American chestnut and the proximal portion of the Chinese chestnut.

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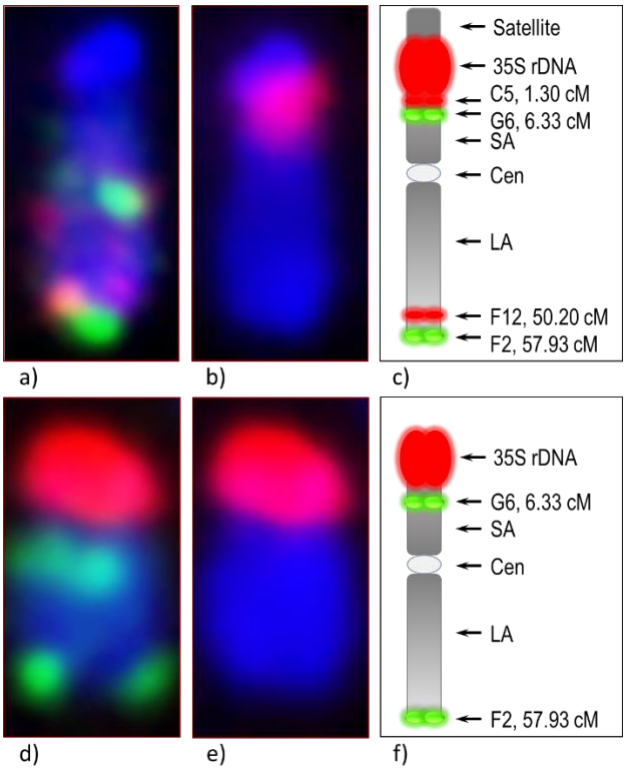

Fig. 1. The major 35S rDNA-bearing individual chromosome of American and Chinese chestnut show the physical location of the 35S rDNA locus and LG_H BAC clones. Four BAC clones on Chinese chestnut LG_H chromosome (a), the same chromosome with 35S rDNA (b, 2nd FISH); d) two BAC clones and 35S rDNA on American chestnut LG_H chromosome, e) AC LG_H chromosome with 35S signal; 'c' and 'f' are the diagrammatic illustrations of CC and AC LG_H chromosome, respectively. SA = short arm, Cen = centromere, LA = long arm.

Forest Health – Pine Pathogens, Insects, and Stress

RAPID-PHENOTYPING OF FUSIFORM RUST DISEASE RESISTANCE IN LOBLOLLY PINE THROUGH VIBRATIONAL SPECTROSCOPY

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Fusiform rust is one of the most economically devastating diseases in commercial pine stands in the southeast, causing massive losses in product and revenue. Significant progress has been made in selecting for rust-resistant families; however, the current method of determining fusiform rust disease resistance (i.e. phenotype) at the family level requires lengthy progeny screening trials, which usually need high levels of disease incidence to be effective. Further, this selection process does not directly test the recruitment population, but their progeny only. Thus, a more expedited approach to selection is needed. Here, we propose to use vibrational spectroscopy as an approach to real-time phenotyping for the selection of fusiform rust-resistance in loblolly pine seedlings. Vibrational spectroscopy tools such as Fourier-transform infrared (FT-IR) and near-infrared (NIR) spectroscopy allow users to obtain a comprehensive chemical fingerprint based on the bending and stretching of chemical constituents in a sample. Because pine trees mostly rely on chemical-based defense mechanisms against pathogens, the relationship between chemical makeup and resistance phenotype is promising. In this project, we are going to use a handheld NIR spectrometer to obtain chemical fingerprints from a common set of 40 different families (20 with higher rust incidence and 20 with lower rust incidence) originating from different geographic areas, sampling two non-infected trees per family at five different progeny test sites of the NC State University Cooperative Tree Improvement Program. Collected spectra will be analyzed through machine learning algorithms to build a predictive model that can discriminate the phenotype based on the chemical profile (i.e. chemotype) of a given tree. This project will establish a real-time phenotyping approach that can be used in the field to assess the recruitment population directly, regardless of the disease incidence in the area and without heavily relying on inoculation trials.

NLR DIVERSITY AND CANDIDATE FUSIFORM RUST RESISTANCE GENES IN LOBLOLLY PINE

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Fusiform rust causes substantial mortality and productivity losses in pine plantation yields across the southeastern United States. Breeding for genetically resistant families has reduced rust infection. Pine control resistance to the fusiform rust pathogen, *Cronartium quercuum* f.sp. *fusiforme,* in a classic gene-for-gene system. In gene-for-gene interactions, disease symptom expression is conditioned by pathotype-specific genetic interactions between resistance gene alleles and pathogen genotypes harboring specific (a)virulence alleles. Early resistance gene mapping in the loblolly pine (*Pinus taeda*) family 10-5 identified markers for a major fusiform rust resistance gene, *Fr1* (Wilcox et al. 1996; Kuhlman and Powers 1988). More recent work identified markers associated with resistance that mapped to a full-length gene model in version 1.01 of the loblolly pine genome encoding for an NLR protein (Neale et al. 2014). In plants, NLR proteins play key roles in disease resistance to biotrophic pathogens. Given the importance role of NLRs in gene-for-gene disease resistance in model and crop species, their identification and diversity are key targets of research directed at mining in other plant genomes. We hypothesize that elite loblolly pine families selected for disease-resistance and other desirable traits possess novel NLR genes that are not present in the reference genome and that a sequencing strategy targeting novel NLR genes will identify SNP alleles associated with disease resistance. We combined transcriptome mining with sequencing of targeted loci to identify novel NLR genes and map SNPs that reliably segregate with fusiform rust resistance in half-sibling loblolly pine progeny. The novel NLR genes identified here are the first characterization of intraspecies NLRs diversity in a gymnosperm. The SNPs segregating with rust resistance and in novel NLR genes derived from transcriptomes, can be applied to the breeding and deployment of resistant pine seedlings.

LONGITUDINAL STUDY ON IN-FIELD TRIAL RUST RESISTANCE AND SEEDLING SCREENING RESULTS

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The identification and selection of disease-resistant seedlings continues to be an important component of loblolly pine (*Pinus taeda* L.) breeding programs. Fusiform rust (caused by *Cronartium quercuum* (Berk.) Miyabe ex Shirai f.sp. *fusiforme*) is a prevalent disease across much of the southeastern United States and causes significant damage to loblolly pine plantations. Tree breeders can currently evaluate rust resistance through progeny testing in field trials or greenhouse inoculations at the USDA Forest Service Resistance Screening Center (RSC) in Asheville, NC. Both methods to evaluate rust resistance have been useful in breeding programs but the relationship between the two is not always clear. This study explores the relationships between a decade of field trial data and greenhouse inoculation results from the ArborGen population. We will compare how families perform for rust resistance across sites and determine if greenhouse inoculation results correlate to different rust incidence levels in the field. A total of 28 trials were analyzed for rust incidence with individual sites ranging from 4% to 72%. Individual families range from 0% to 80% in the field and 0% to 98% at the RSC.

PREDICTING GENETIC PARAMETERS AND TOLERANCE TO PITCH CANKER IN SLASH PINE

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Pitch canker is an increasingly important threat to Southeastern forests, especially in slash pine (*Pinus elliottii* var. *elliottii* Engelm.). Its increasing prevalence in the region due to mechanical damage from increased storm activity has led to concern from private and public stakeholders about poor characterization of tolerance from available germplasm. Traditional testing methodologies established to characterize the genetic mechanisms controlling pitch canker tolerance were limited by poor characterization of *Fusarium* isolate virulence and experiment design limitations. Here we describe an alternative experiment design intended to greatly expand the numbers of families that may be screened and increase the power of differentiating among pine families and pathogen isolates evaluated at the USFS Resistance Screening Center. Using this alternative design, genetic parameter estimates for pitch canker tolerance are presented and their implications for the future development of a deployment-oriented slash pine population are discussed.

HYPERSPECTRAL IMAGING FOR THE PREDICTION OF FREEZE DAMAGE AND MINIMUM WINTER TEMPERATURE AT SEED SOURCE ORIGIN OF LOBLOLLY PINE SEEDLINGS

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Freeze tolerance is the most important adaptability trait for deployment of loblolly pine in the southeastern US. Minimum winter temperature (MWT) at the seed source origin is the standard indicator of cold adaptability. In this study, we investigated a novel approach for the assessment of freeze-induced damage and prediction of MWT at seed source origin using hyperspectral imaging of seedlings. A population comprising 98 seedlots from Virginia to Florida was subjected to an artificial freeze. A custom-assembled hyperspectral imaging system was used for scanning the seedlings prior to the freeze and on four subsequent occasions after the freeze. On day 44 post the freeze event, seedlings freeze damage was visually scored and the logit link function was used to model the probability of freeze damage. The hyperspectral image data comprised spectra of each seedling that were then averaged for each family, and used to develop family-level predictive models for each scanning date. A significant positive relationship (R^2 = 0.28; $p < .001$) between the family MWT and logit scores for freeze damage was observed. Prediction accuracies of freeze damage and MWT based on hyperspectral data varied among scanning dates. The highest prediction accuracy of freeze damage (R^2 = 0.79) was achieved using hyperspectral data obtained 41 days after the freeze event. The highest prediction accuracy of MWT (R^2 = 0.78) was achieved using hyperspectral data obtained prior to the freeze event. In our study, the best hyperspectral model gave predictions of MWT with a root mean squared error of 2.45 °F, which suggests sufficient precision to rate families for seed source transfer well within a 10 °F plant hardiness zone. Therefore, this research demonstrates that hyperspectral imaging has the potential to serve as a rapid, nondestructive and objective tool for the prediction of MWT of origin of loblolly pine seedlings.

Lightning Presentations

GENOMIC ASSEMBLY COMPARISONS OF FOUR FLORIDA *FUSARIUM CIRCINATUM* ISOLATES

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Pitch canker disease incited by the fungus *Fusarium circinatum*, causes economic losses to the pine timber industry all over the world. Screening seedlings by measuring lesion length following controlled inoculation is used to provide estimates of pine tolerance and isolate virulence. Four isolates that demonstrated differences in virulence when screening slash and loblolly seedlings were selected for further study. To understand the genetic basis for the differences, we used Oxford Nanopore long-read sequencing combined with Illumina short-read sequencing to achieve high quality, nearly chromosome level, genome assemblies. The assemblies were checked for completeness using BUSCO (Benchmarking Universal Single-Copy Orthologs) and aligned to the publically available reference genome (NCBI: GCA_000497325.3). *Fusarium* species are known to transfer pathogenicity genes and whole "pathogenicity chromosomes" between and within species. Variation in virulence may derive from gene content as well as structural differences in the genome. We located genes in the assembled genomes using annotation software and employed various other techniques to a take a closer look at which genes differ between isolates. In addition, we examined the extent of structural differences among the genomes by locating and quantifying the number of sequence inserts, deletions and rearrangements across the genome. This study has identified genomic differences among *Fusarium circinatum* isolates that may be useful for characterizing isolate virulence, monitoring pathogen populations and identifying where different resistance mechanisms should be deployed across the landscape.

GENOMIC CHARACTERIZATION OF GROWTH AND DROUGHT TOLERANCE IN EAST TEXAS LOBLOLLY PINE

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The loblolly pine populations of east Texas are at the species' warmest range limit. Under future climate warming scenarios the health and productivity of these populations is expected to be negatively impacted. Forestry in east Texas generates billions of dollars in annual revenue and even small negative impacts on annual productivity will have substantial short and long-term economic consequences. Therefore, our research has two primary objectives in context of the east Texas loblolly pine breeding programs. Firstly, to assess the contemporary phenotypic and genomic variation in growth and drought-related traits, and secondly, to identify productive families or trees that are also candidates for increased drought tolerance and future breeding to maintain or increase timber yields. Our approach uses the growth data of 733 trees from 50 maternal families growing in a five-year old progeny trial near Livingston, TX. From these trees we have obtained stable carbon isotope ratios ($\delta^{13}C$) and nitrogen concentrations, as traits with established relationships to water use efficiency and photosynthetic capacity. In combination with these phenotypic data we are using genotypes from the recently developed Thermo Fisher Axiom™ Pita50K loblolly pine array, and 5000 custom targeted genotype-by-sequencing (GBS) SNPs from drought-related transcripts identified in our previous research. For the GBS we are working with Tecan Genomics to develop a repeatable protocol for using their multiplexed Allegro library preparation platform that has potential to substantially reduce the cost of targeted GBS in conifers. Our preliminary results indicate there is significant variation among families for growth and $\delta^{13}C$, and that array genotyping has been highly successful, yielding ~32,000 high-quality SNPs. More detailed quantitative genetic and association genetic analyses to dissect variation among families and individuals are forthcoming.

CAN SUCROSE PATHWAY MANIPULATION AFFECT REGROWTH OF COPPICED *POPULUS?*

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Perennial woody trees grown for short-rotation bioenergy production are often maintained by coppicing. While it is presumed that non-structural carbohydrate reserves contribute to regrowth, mechanisms for orchestrating remobilization of stored carbohydrates remain poorly understood. Sucrose transport proteins (SUTs) are known for their role in mediating the subcellular mobilization of sucrose from source leaves, yet their involvement in other physiological processes, such as carbohydrate remobilization from sink tissues, remains unclear. Here, we targeted *PtaSUT4* for CRISPR/Cas9-mediated mutagenesis to generate transgenic knock-out (KO) nulls in the *Populus tremula x alba* (INRA 717-1B4) hybrid clone. Amplicon-sequencing confirmed biallelic frameshift mutations in >30 independent transgenic lines; a subset of which was selected for further functional characterization. *SUT4*-KO had no obvious effect on mature plant appearance or growth, but subtle changes to biomass partitioning and metabolism were observed. We then coppiced mature greenhouse-grown poplar to perturb sink-source relations and examine how *SUT4*-KO impacts new vegetative growth. The KO mutants initially exhibited accelerated epicormic bud growth from the stool, but ultimately displayed a decline in new aboveground biomass accumulation after repeated rounds of collection. Metabolic profiling of stool bark and wood during bud flush and regrowth revealed a major shift in primary and secondary metabolism of the mutants. Consistent with vacuolar sequestration in the mutants, sucrose, hexoses, and phenylpropanoid compounds were detected at reduced levels following coppicing and initial bud growth in the mutants. In contrast, the *sut4* mutants accrued elevated levels of amino acids and Krebs cycle intermediates during initial bud burst. This data supports a scenario where carbon was diverted towards primary metabolism to fuel enhanced bud growth in the *sut4* mutants. Collectively, these findings point to a novel role for SUT4 in mobilizing soluble carbohydrates following coppicing and regrowth in *Populus.*

A DELVE INTO THE UNKNOWN OF SULFATE TRANPORTERS' ROLE IN ELEMENTAL MOVEMENT AND STRESS RESPONSE IN POPLAR

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Sulfur is one of the top six macronutrients essential for plant survival. Lacking this element, plants develop a yellow appearance and declines in biomass. Sulfate is the inorganic form of sulfur that is taken up by the roots and moved throughout the plant to the leaves where it is then assimilated into amino acids, lipids, and other metabolites. Sulfate movement throughout the plant is facilitated by transmembrane proteins known as sulfate transporters (SULTRs). The *SULTR* gene family is split into four groups. Group 1 are high affinity transporters involved in uptake of sulfate from the soil, group 2 are low affinity transporters that interact with group 1 and move the sulfate from the roots to the shoots, group 4 are tonoplast SULTRs for vacuolar efflux, and finally group 3 are the most diverse and lack a cohesive localization and function. Group 3 is made up of five genes, *SULTR3;1, SULTR3;2, SULTR3;3, SULTR3;4,* and *SULTR3;5. SULTR3;1* and *SULTR3;5* have been well studied. SULTR3;1 interacts with the chloroplast and is integral for assimilation of compounds. SULTR3;5 acts much like groups 1 and 2 in the movement of sulfur from roots to shoots. The other three proteins have conflicting subcellular localizations to the chloroplast or the plasma membrane in the literature. SULTR3;4 was recently recategorized to be a phosphate transporter in *Arabidopsis* and is phylogenetically related to SULTR3;3. This could indicate functional divergence within this subgroup of the SULTR family. In *Populus, SULTR3;2* and *SULTR3;4* are highly expressed in the xylem but had opposite sensitivities to development and stressors. CRISPR/Cas9 technology was employed to produce genetic knockouts of both *SULTR3;2* and *SULTR3;4.* These will be subjected to media stress experiments and observed for phenotypic changes such as yellowing. They will also be sent for elemental analysis to see metabolic shifts.

IN SEARCH OF SASSAFRAS WITH RESISTANCE TO LAUREL WILT DISEASE

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Laurel wilt disease has killed over 300 million redbay in the southeastern USA and is now spreading in sassafras as far north as Kentucky and Tennessee. Currently few if any options are available for managing laurel wilt disease and reducing its effects on forest health. Fungicide injections have been found effective but their costs and need for reapplying make them unsuitable to forests. Cost effective and sustainable options are needed if we are to maintain laurel wilt susceptible hosts as functioning parts of ecosystems. Variation in susceptibility has been identified in redbay and developing host resistance in this species is a promising management option. A collaborative team from the USDA Forest Service, Kentucky Division of Forestry, University of Kentucky, and University of Florida are developing the criteria (e.g., what level of mortality, time since LWD infection, survivorship of hosts in certain diameter classes…) for defining large surviving sassafras from high mortality stands that are likely to have some resistance to laurel wilt disease and not escapes. Using the tool/criteria, we will identify and propagate putatively resistant selections that can be used to develop resistance screening methods and identification of host resistance. Propagated material will be maintained by the University of Kentucky and SRS until disease resistance screening. The tool/criteria will allow others to make their own selections of putatively resistant sassafras from across the region. Ultimately this work will be the start of developing a sustainable and long-term management option using host resistance to maintain sassafras as a functioning member of forested ecosystems.

RECOVERING TWO ELITE HYBRID SWEETGUM CLONES FOR PROPAGATION BY SOMATIC EMBRYOGENESIS

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Somatic embryogenesis-derived hybrid sweetgum (*Liquidambar styraciflua* x *Liquidambar formosana*) clones were developed for pulp and paper and biomass energy applications over 15 years ago. Some of the clones displayed outstanding growth rates and enhanced wood density in field tests. These were propagated as rooted cuttings by ArborGen Inc. and grown by a small number of landowners in the southeastern US, with promising results. However, the clones are currently not being sold by ArborGen. Other clones that were not licensed by ArborGen have been grown by UGA in a small demonstration planting in Athens, GA, for the past 12 years and some of these have demonstrated superior growth rates. With help from consultants, we chose two of the most promising clones to be propagated for further field testing and possible commercialization. Only one of the two clones was still available as an embryogenic culture. Although a copy of this culture that had been maintained continuously by serial transfer no longer was capable of making somatic embryos, two copies of the same culture recovered following 15 years in cryostorage retained this ability. Trees of the other clone in the test planting that was not represented in cryostorage began producing staminate inflorescences in 2018. We collected dormant buds containing inflorescences from this clone in February 2020 and staminate inflorescence tissues were induced to initiate embryogenic cultures at a rate of 13.3%. Of the three plant growth regular treatments tested for induction, only explant material cultured on the basal medium supplemented with NAA produced somatic embryos. Somatic embryos produced from both clones have been germinated to produce somatic seedlings that can be used as the basis for scaled-up propagation of the clones.

PROPAGATION AND CONSERVATION OF THREE RARE NORTH AMERICAN ASH SPECIES USING SOMATIC EMBRYOGENESIS

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In addition to threatening North American ash species of major ecological and economic importance, such as green, white, and black ashes, emerald ash borer (EAB; *Agrilus planipennis*) also threatens other native ash trees, of which there are at least 18 North American species. Some of these ash species are comparatively rare. Although not much is known about the susceptibility of most of these species to EAB, some may be at higher risk of extirpation from their native ranges than the more common ash species, due to their smaller ranges and population sizes. Thus, there is a need to extend germplasm conservation efforts to these rare ash species. One approach is to establish new embryogenic cultures of rare ash species for which somatic embryogenesis (SE) has not yet been reported, with the goals using the cultures for propagation and germplasm conservation via cryostorage. We worked with cooperators to locate trees and collect seeds from three rare ash species, Carolina ash (*Fraxinus caroliniana*), Texas ash (*F. albicans*) and Mexican ash (*F. berlandieriana*), for use as explants for SE culture initiation. Immature seeds were harvested from source trees during late summers of 2019 and 2020, and immature zygotic embryos were dissected from them and cultured on media with varying concentrations of 2,4-dichlorophenoxyacetic acid (2,4-D) or picloram. SE was achieved in all three species, with the highest induction rate in Texas ash (12%). Carolina ash somatic embryos from cultures initiated in 2019 were successfully converted to somatic seedlings and hardened off to greenhouse conditions. Somatic embryos from the Texas ash and Mexican ash cultures initiated in 2020 are expected to produce plantlets following pre-germination cold treatment.

FUSIFORM RUST RESISTANCE OF LOBLOLLY PINE INTER-PROVENANCE HYBRIDS

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Fusiform rust (*Cronartium quercuum* [Berk.] Miyabe ex Shirai f sp. fusiforme) is a noxious pathogen that causes decreased productivity and significant economic loss to forest investments in the southeastern United States. Resistance to fusiform rust is a considerable factor in loblolly pine planting stock selection, particularly in areas with moderate to high rust hazard. Rust resistance has traditionally been evaluated using field trials as well as controlled inoculation trials from the USDA Resistance Screening Center in Asheville, North Carolina. Controlled inoculation screening offers an efficient method of rapidly identifying rust resistant families. Historically, rust resistance research has largely been geographically isolated within provenance. An inter-provenance mating strategy can incorporate rust resistance as well as geographic adaptability of loblolly pine. However, the relative resistance of inter- hybrids across the range remains unclear. This study examines rust resistance of select elite full-sib inter-provenance families, intro-provenance full-sib families and open-pollinated families from parental selections within the Piedmont, Coastal, and Western Gulf geographic origins. We evaluated rust resistance using controlled screening trials and discuss the resistance and deployment of elite inter-province hybrids across the range of loblolly pine.

COMBINED EFFECT OF TEMPERATURE AND WATER STRESS ON GERMINATION AND PLANT GROWTH OF *MAGNOLIA PUGANA*, *POPULUS LUZIARUM* AND *P. PRIMAVERALEPENSIS* ENDEMIC AND ENDANGERED SPECIES FROM WESTERN MEXICO

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Since 2016 we have conducted research on the conservation and germination of *Magnolia pugana*, *Populus luziarum* and *P. primaveralepensis* seeds. Our main objective is to know the response in germination and plant growth with the combination of high temperatures and water stress as these conditions are projected for the area where they are distributed. Since 2018 this research is part of the doctoral project of the student César Jacobo Pereira. Germination tests for *M. pugana* have been performed with the combination with three temperatures (24, 28 and 37°C) and five water potentials (0, -0.3, -0.6, -0.9 and -1.2 MPa). The interaction of temperature and water potential on germination percentages was significant (ANOVA: $F = 3.86$, $P < 0.001$). The highest number of germinated seeds (78%) was obtained at a temperature of 24°C and 0 MPa. These results indicate that germination of *M. pugana* is severely affected by the interaction between increasing temperatures and decreasing precipitation. In *P. luziarum* and *P. primaveralepensis* we tested germination and storage of their seeds at two temperatures. In the first 24 hrs after seed collection, germination was high (91 and 95%, respectively). Germination percentages decreased when stored at 21°C and for more than four weeks. The germination percentage of *P. primaveralepensis* decreased more slowly than that of *P. luziarum* at 4°C. This indicates that subtropical *Populus* subtropical seed storage conditions respond similarly to those in temperate climates. If approved, the grant for which I want to participate will serve to continue my studies and experiments to generate crucial information to establish in situ and ex situ conservation and repopulation programs.

SEARCHING ORPHAN GENES IN HYBRID POPLAR

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Orphan genes by definition have restricted taxonomic occurrence. They don't share sequence similarity to other known genes in the bioinformatic databases, thus making it difficult to predict their biological function using traditional approaches. Since orphan genes are unique to specific taxa, they may confer adaptive significance during evolution. We focus on the *Populus tremula* × *P. alba* INRA 717-1B4 hybrid (hereafter '717'), an important study system in poplar functional genomics because of its high transformation efficiency. To identify protein-coding orphan genes, a total of 15 genomes were selected, including well annotated model species and those closely related to our focal species for analysis. Orthogroups were constructed according to the amino acid sequence similarity among the selected proteomes. Genes belonging to orthogroups that only contain *Populus* sequences as well as those which cannot be assigned to any orthogroups were categorized as putative orphan candidates. Collinearity of certain 717-specific orphan genes can be observed between the two subgenomes in the current 717 draft genome assembly. The candidate orphans showed several properties, including shorter coding sequences and higher isoelectric points. Among these orphan genes, seven showed interesting expression patterns in different tissues and under drought stress treatment. Three genes showed higher expression in xylem. Two genes were only expressed in callus and the predicted polypeptides are shorter than 100 amino acids. The other two genes were induced under drought stress and were only expressed in roots. In addition, they were all predicted to contain intrinsically disordered regions. We hypothesize that these candidate orphans may act as chaperone proteins and contribute to the fitness of '717'.

ADDITIVE AND DOMINANCE GENETIC PARAMETERS OF MATURE-AGED TRAITS IN ARKANSAS POPULATIONS OF SHORTLEAF PINE (*PINUS ECHINATA* MILL.)

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Range-wide ecosystem restoration efforts are in progress for shortleaf pine (*Pinus echinata*). Genetic considerations must include provenance or seed source and genetic diversity as well as performance of the planted seedlings. Genetic information on seed source performance has been documented through provenance tests conducted in the 1950s-1980s. Genetic diversity studies have shown an increase in hybridization with loblolly pine from the 1950s to the 2000s. Only limited information is available on within source variation attributed to individual parents. We remeasured 15 R8 progeny tests representing the Ouachita and Ozark National Forests seed sources. Disconnected half-diallel crosses were made among the first-generation parents in the Mt Ida, AR seed orchard and established at tests sites in both national forests. Earlier measurements (ages 5 and 10 years), available on some of these tests, were merged with the current measurements that ranged from 31 to 40 years from planting. In total, 21,260 planted trees were evaluated for survival through the current assessment age, with all surviving trees measured for DBH and scored for damage and straightness, and a sample of these trees were measured for height. This data set provided progeny test performance information on 126 parents and 330 full-sib families. We found substantial additive and dominance genetic variation in growth and straightness, while the proportions of these variances differed by traits and ages. Additive and dominance genetic variances increased with age resulting in larger narrow- and broad-sense heritabilities for height at the latest measurement age. Predicted genetic gain is promising to advance tree growth and straightness for reforestation using both open- and control-pollinated families. Moderate age-age correlations suggest selection of high-performing parents in older trials can be done at younger ages.

CONSERVATION GENETICS AND RESISTANCE BREEDING AT THE HARRISON EXPERIMENTAL FOREST

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Genetic resistance offers a promising, sustainable option for managing introduced pests and pathogens in forestry. To counter two such introduced pathosystems, chestnut blight and laurel wilt, we are initiating conservation genetics and resistance breeding projects in the host species. Chestnut blight (caused by *Cryphonectria parasitica*) has virtually eliminated American chestnut (*Castanea dentata*) from the landscape since being introduced more than a century ago. We are searching throughout Mississippi for American chestnuts to use in cooperative resistance breeding. Mississippi is a desirable search area, since it represents the most southern portion of the species native range. American chestnuts will be vegetatively propagated, planted in two breeding orchards in Mississippi and genotyped to help validate species identity. To date, 20 putative American chestnuts have been discovered and propagated for orchard planting and genotyping. Laurel wilt (caused by *Raffaelea lauricola*) has killed over 300 million host trees since being discovered in Georgia in 2002. The main host tree has been redbay (*Persea borbonia*) in the southeast, but the disease is now impacting sassafras (*Sassafras albidum*) in areas as far north as Tennessee and Kentucky. Tolerance to the pathogen has been identified in redbay and we will take a similar approach to identify tolerance in sassafras. Specifically, we are propagating putatively tolerant selections of redbay and sassafras and will begin screening them for resistance to identity individuals for our breeding orchard. Currently, eight LW-tolerant redbay selections from the University of Florida are being established in the breeding orchard in Mississippi. A multiagency team is establishing a study to select individual sassafras trees with potential tolerance to laurel wilt. Disease resistance screening will be conducted once selections are propagated. Trees showing levels of resistance will be mated to evaluate the trait's inheritance and to identify candidate trees for breeding and seed orchard development.

SUSTAINABILITY GENETICS FOR AMERICAN WHITE OAK (*QUERCUS ALBA*)

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White oak (*Q. alba*) is a keystone forest tree species distributed over much of the eastern US. The species is important ecologically for watershed and wildlife health, and economically for lumber, veneer, and specialty products such as barrel staves. Because of the species importance, there are sustainability concerns primarily driven by forest mesophication, limiting regenerated seedlings' recruitment into the canopy, and climate change. The recently formed White Oak Initiative speaks to these concerns and looks to partner on key research themes encompassing white oak's biological potential in forest management. An important determinant in biological potential is a species' genetic diversity and how this diversity might be characterized and utilized in forest management. Our stakeholder-supported research program is addressing white oak genetics in three ways— (1) germplasm collection, conservation, and improvement; (2) genomic, transcriptomic and metabolomic assessment of genetic diversity; and (3) characterization of adaptive genes through analysis of genotype-phenotype associations. Initial work has resulted in two years of range-wide acorn collections, including a first-year nursery crop with subsequent field test plantings, and completion of a diversity study of white oak stands in the Daniel Boone National Forest. We are currently developing a high-quality, white oak genome sequence and comprehensive transcriptome and metabolome resources. Subsequent work will continue range-wide acorn collections and field test plantings, as well as finalizing and annotating the genome and initiating trait phenotyping and genotyping.

INTRODUCTION, EVALUATION, AND BREEDING OF NOVEL *POPULUS* FOR SUBTROPICAL CLIMATES

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Since 2008 the Forest Pathology Lab at the University of Florida has established and evaluated *Populus* germplasm with potential adaptation to subtropical climates such as Florida. We aimed to examine disease resistance, photoperiod response, growth rate, ease of propagation, adaptability to heat and lack of chilling. To this end, the focus has been to grow species from Mexico, naturally and allopatrically occurring within seven different biogeographic provinces in subtropical low-latitude locations (18°48'-30°34' N), including *P.* aff. *fremontii* var. *mesetae* (Jalisco origin), *P. luziarum*, *P. primaveralepensis*, *P. mexicana*, *P. guzmanantlensis*, *P. monticola*, *P. simaroa*, *P. tremuloides* (Jalisco origin) and evergreen clones originating from the mutant form of Lombardy poplar, *P. nigra* 'Chile', first discovered over 100 years ago near Santiago, Chile as a sport mutation on *P. nigra* 'Italica'. We carried out progeny trials (100 seedlings each) with *P. mexicana* ssp. *mexicana* using three open-pollinated families from near Monterrey, Mexico. Results from year one (from seed) suggested significant potential with this species, with mean height growth of > 4 m and very high disease and insect pest resistance. Seed propagation for *P. luziarum* and *P. primaveralepensis* was highly successful and although height growth for both species after six months was not significantly different, the means were 22.91 cm (± 2.19 cm) and 25.78 cm (± 3.45 cm), respectively. Early flowering was observed for the white poplar *P. luziarum*, with both male and female clones flowering within the first year from cuttings. Pollen was collected from *P. luziarum* and *P. monticola*, and crosses with *P. alba* were attempted, but so far no viable seed has been produced. This is the first report on our observations and early results suggest there is significant potential within these species for trait discovery, adaptation and future development within the genus for warmer climates.

GENETIC TESTING BASED ON PEDIGREE RECONSTRUCTION AND SPATIAL ANALYSIS IN A PLANTATION OF *LARIX KAEMPFERI*

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Larix kaempferi is one of the major timber species in Northeast Asia because of their rapid growth and straightness. The demand for the reforestation of the species has been increased in Korea due to the promoted timber utilization by recent advances in wood processing technology. The genetic testing is the prerequisite process for the evaluation of genetic value and gain, and in turn for the supply of genetically improved seed. However, the genetic testing of *L. kaempferi* has not been performed yet because it is an introduced species in South Korea. The establishment of progeny trial of the species is even difficult because of the irregularity and the large variation in seed production. In this study, the genetic testing was simulated using the pedigree reconstruction and the spatial distribution analysis in a plantation originated from seed orchard crops in order to replace progeny trials. The adequacy of utilizing the plantation as a testing population was confirmed based on the comparable level of the genetic variation in the offspring to that in the group of mother trees. The pedigree reconstruction was conducted by maternity analysis using microsatellite markers. The genetic testing of diameter growth was performed subsequently using both the animal model and the model accounting for spatial autoregression. The improved fitness of the latter presented the usefulness of the spatial analysis in genetic testing with plantation lacking the prior experimental design. To understand the environmental characteristics of the heterogeneous site, the environmental effects on the growth were investigated by geographically weighted regression analysis. The changes in the main effects allowed it possible to propose a zoning scheme for the management of the plantation as breeding material. In addition, it could be expanded to be utilized in the genetic testing of the other tree species without progeny trials.

Schatz Symposium

EPIGENETIC INFLUENCES ON PLANT GROWTH RESPONSE, AND POTENTIAL FOR ENHANCING STRESS RESILIENCE IN TREE SPECIES

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Understanding plant environmental response, and the heritability of that response, may prove vital to dissecting modes of plant adaptation. Our group has developed a system for investigating plant epigenetic behaviors that centers on plastid triggers of epigenetic reprogramming. These plastid-based epigenetic effects appear to be well conserved in plants and rely on histone modifying, DNA methylation and small RNA components. Implementation of epigenetic reprogramming in grafting experiments permits the identification of RNA-directed DNA methylation targets within the genome that involve graft transmissible and transgenerational signaling and lead to enhanced fitness phenotype changes. Two of the genetic components of this system, MSH1 and PPD3, were first identified and characterized in Arabidopsis, but are conserved in plants and offer important potential strategies for enhancement of growth potential and resilience in tree species. We have developed a model for plant environmental response that may apply to both seasonal and perennial growth habits, with a plan for implementation in poplar for identifying environmentally altered gene networks and alignment with longer-term genome evolutionary paradigms.

A QUANTITATIVE REDUCED REPRESENTATION SEQUENCING (QRRS) OF GENOMES; A PARADIGM SHIFT IN NGS-BASED GENOTYPING

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Next Generation Sequencing (NGS) is an extensively used tool for massive parallel sequencing of genomes. However, several applications such as NGS-based genotyping still suffer from pitfalls that limit their accuracy and utility. To mitigate these pitfalls, we have developed a significant advancement in short-read NGS library preparation. Presented here is an inexpensive quantitative reduced representation sequencing (qRRS) approach for dosage-sensitive genotyping and quantitative strain-level metagenome/microbiome profiling. The scalable, ligation-free and double-stranded DNA-protection assay eliminates off-target annealing temperature-dependent hybridization. This is achieved by using single-stranded barcoded adapters for isothermal strand displacement of double-stranded DNA templates with restriction site overhangs as the only priming site. As much as 9,216 samples can be multiplexed. Novel features in this protocol include a paradigm shift in adapter design that prevents chimeric reads and barcode swapping, a flow cell cluster enhancer that generates about 50% more yields, and consistent high-quality base calling scores. The library preparation workflow is optimized for ease-of-use and can be completed in one to two days. To accommodate these novel features during data pre-processing and downstream analytics, we have developed bioinformatic and analytical pipelines for empirical-based NGS data quality filtering (ngsComposer), haplotype-based variant calling and filtering (GBSapp), and quantitative metagenomic alignment and taxonomic exact matching (Qmatey). The qRRS approach establishes new standards in high-fidelity quantitative genotyping, minimizes missing data and allelic dropout, and makes functional microbiome studies more accessible. Compared to 16S amplicon sequencing, which uses a single gene for microbiome profiling, qRRS provides multiple genome-wide sequences for strain-level taxonomic delineation and quantification. We are now exploring its utility as a diagnostic tool for scoring multiple diseases (and disease complexes) based on titer levels of pathogens in a single assay. We envision that the enhanced quality and quantity of qRRS-derived markers will improve genomic-assisted breeding efforts.

GENOMICS IN RARE SPECIES CONSERVATION: TEASING APART EVOLUTIONARY HISTORY AND ADAPTATION

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Species evolutionary potential is tightly linked to both the amount and distribution of genetic variation available through which natural selection may act. Indeed, the genetic consequences of isolation and population size may be exacerbated in rare species, limiting species' ability to adapt to ongoing change. Thus, in a rapidly changing environment, maintenance of genetic variation within and across populations becomes an increasingly important target for species conservation. Here, we discuss the importance of understanding species' evolutionary history, and the role different evolutionary processes may play influencing neutral and adaptive processes both across space and time. We discuss the importance of these data to establishing conservation collections and designing species management strategies that preserve species' evolutionary potential. Providing a case study, we focus on Torrey pine (*Pinus torreyana* Parry) a critically endangered pine endemic to California. The combination of small population size, extremely low genetic variation, and abiotic and biotic challenges associated with climate change indicate Torrey pine may have reduced evolutionary potential to adapt to change. Thus, Torrey pine may be a potential candidate for inter-population genetic rescue. Pairing genomic data with phenotypic data from natural populations and common garden experiments, Torrey pine provides an ideal system to evaluate the contribution of demographic history, gene flow, and natural selection to population differences. These data become essential to considering potential risks associated with management decisions and identifying short and longer-term conservation strategies necessary to preserve species' evolutionary potential.