NORTH CAROLINA STATE UNIVERSITY

COOPERATIVE TREE IMPROVEMENT PROGRAM

59th Annual Report | May 2015

DEPARTMENT OF FORESTRY & ENVIRONMENTAL RESOURCES

COLLEGE OF NATURAL RESOURCES

NC STATE UNIVERSITY

TREE IMPROVEMENT PROGRAM
NC STATE UNIVERSITY

59ᵗʰ Annual Report

May 2015

Department of Forestry and Environmental Resources

College of Natural Resources

North Carolina State University

Raleigh, NC

	Email	Phone
Steve McKeand, Director	steve_mckeand@ncsu.edu	919-886-6073
Fikret Isik, Associate Director	fikret_isik@ncsu.edu	919-449-6891
Graham Ford, TIP Manager	gaford@ncsu.edu	919-374-0847
Chris Ball, Data Analyst	cmball3@ncsu.edu	919-792-8472
Austin Heine, Operations Manager	ajheine@ncsu.edu	910-977-5506
J.B. Jett, Professor Emeritus	jb_jett@ncsu.edu	919-408-7331
Ross Whetten, Professor	ross_whetten@ncsu.edu	919-515-7578
Tori Brooks, Admin	tori_batista@ncsu.edu	919-792-8472

Fax: 919-324-3620

http://www.treeimprovement.org

EXECUTIVE SUMMARY

SELECTION, BREEDING, AND TESTING

Based on conelet and seed counts following the first two years of breeding, the 4th Cycle is about half completed. Preliminary reports for the 2015 breeding season were also good.

Cooperative members have successfully planted two years of 4th-Cycle tests. Everyone has responded well to the new testing strategy and pre-planned test designs. The 2016 tests will be the biggest yet.

Big BLUP analyses were run for the Coastal, Piedmont, and Northern breeding populations. Based on updated breeding values, Coastal and Northern *PRS*™ 2014 versions were released on the TIPRoot Cooperative database in February 2015. The Piedmont *PRS*™ update is in progress.

The TIPRoot online database has been up and running for less than a year, and already there have been changes made to improve its organization and utility to members of the Cooperative.

Wood density and bending strength measurements were taken at age 8 or 9 years for three Coastal (280 families) and one Piedmont (74 families) PMX test series. Breeding values and *PRS*™ scores will be generated this summer.

SEED PRODUCTION

Cooperative members harvested more than 71,000 pounds of loblolly pine seed from operational orchards in 2014. This is more than twice the amount of seed from the 2013 harvest.

RESEARCH

As part of the PINEMAP Project, we surveyed seedling vendors to determine the genetics of southern pines currently being planted. For loblolly pine, 95% of plantations are now being established as genetically more homogeneous stands: 84% OP families, 8% full-sib families, 2% clonal varieties, remainder with mixtures.

An alternative approach to estimating breeding values within families by sequencing DNA copies of messenger RNA is under investigation.

For the PINEMAP project, a spatial Bayesian statistical approach was developed for modeling the expected height of trees using climate variables associated with the seed source and the planting site as predictors.

At SETRES2, the Lost Pines Texas (LPT) provenance had higher wood density than the Atlantic Coastal provenance at all ages. When the higher wood densities of the LPT provenance were combined with its greater volumes, the superior biomass yield of the LPT provenance at ages 12 years and beyond becomes more evident.

Progress is being made toward the goal of cost-effective genotyping applied to informative markers. New protocols have been developed for high-throughput extraction of either DNA or RNA from foliage samples.

In the Atlantic Coastal Elite clonal tests, factor analytic formulation combined with a block diagonal covariance structure (FA-BD) for the residuals was robust and informative for estimating genetic variances and breeding values.

Temperature dipped to 9ºF at the Hort Field Lab, and survival of the rooted cuttings in the Northern Elite Population was 30%. Families ranged in survival from 2% to 80%; survival was related to the winter temperature origin of the parents.

Based on June conelet survival counts, compared to the standard Lawson pollinations bags, 25% more full-sib seeds could be produced with PBS International's bags with more rigid material and the Lawson bags with wire frames.

At the Butner, NC biomass genetics/cropping study, Coastal families were substantially taller than the Piedmont families at age 3 years but were prone to more forking.

In recent years, there has been an important shift from the information in the data being used to increase the value of a commodity to the information itself becoming a commodity.

ASSOCIATED ACTIVITIES, GRADUATE STUDENTS, COOPERATIVE STAFF, MEMBERSHIP

We gained two new Contributing Members. There are now 11 Full Members, 17 Contributing Members, and 4 Research Associate Members. Graduate students' contributions to the program continue to be critical. We continue to teach short courses and workshops to members and colleagues at NCSU and around the world. Chris Ball has started as the new tree improvement analyst replacing Jarrod Morrice who resigned in October.

TABLE OF CONTENTS

TREE IMPROVEMENT AND SUSTAINABILITY

A MESSAGE FROM THE DIRECTOR

Foresters and Tree Breeders Do Good Every Day

As foresters, we are taught to practice sustainable and ecologically sound forest management to achieve land-owner objectives. Today we hear concerns and criticism about what is and is not ecologically sound or what is or is not sustainable. Almost 30 years ago, the United Nations World Commission on Environment and Development, commonly known as the Brundtland Commission, wrote a beautifully simple description of sustainability in the report entitled "Our Common Good":

Sustainable development is development that meets the needs of the present without compromising the ability of future generations to meet their own needs.

While the politically charged implications and messages of the report extend far beyond my simple world, this description of sustainability has resonated with me for years. As breeders, we are taught to manage populations to meet consumer needs for today but not compromise what future breeders can do for future consumers. By the future, I mean for dozens or even hundreds of generations down the road, and for pine trees, that means centuries to millennia.

In many respects, tree breeders are models for sustainability. It is what we do; it is the essence of our work. In our loblolly pine breeding programs in the southern US, companies and state agencies manage large and diverse populations that yield high-valued varieties that are established on over one million acres of plantations each year. When the best genetics are combined with sound and intensive silviculture, landowners can realize additional net present values of up to $500/acre or more, which in turn provides them with motivation to keep their lands forested, with attendant benefits to the ecosystem and to society. To sustain these capabilities for future generations, we manage our breeding populations to assure that genetic diversity is maintained and additional gains will be achieved for many generations to come. The Cooperative's 4ᵗʰ-cycle breeding strategy is an excellent example of managing tradeoffs of current gain with the need for genetic diversity to assure future genetic gains. Cooperative members and TIP staff have embraced this strategy, and excellent progress is being made in the breeding and testing program. Over half of the breeding is done for the 4ᵗʰ cycle, and testing is progressing very well. In just a few more years we will be making our first 5ᵗʰ-cycle selections!

I like to remind myself that, as foresters and breeders, we are the good guys. We have a positive impact on the environment and society every day. Not only do we enhance the value and productivity of forests for the economic benefit of landowners, and concomitantly their communities, but we defer the need for harvesting ecologically sensitive and natural forests by producing more wood, fiber, and fuel from genetically improved plantations. These more productive plantations have also had a huge impact on ecosystem services, such as significantly increasing carbon sequestration in the South (e.g. Aspinwall et al. 2012. Forest Science 58:446–456). The benefits of our forestry and tree breeding work extend far beyond the boundaries of the plantations.

In this year's Annual Report, we summarize several projects funded by PINEMAP and IBSS[1]. These large USDA Coordinated Agricultural Projects have allowed us to collaborate with other scientists throughout the South to do more research and develop new products and analytical tools. For example, we have estimated the impact that improved loblolly pine has on productivity, dry weight yields, and adaptive response to climate change. We are developing better analytical tools, cost-effective genotyping methods and alternative ways of estimating breeding values within families. Networking with scientists with diverse backgrounds and expertise allows us to do even more good today and in the future.

It is good being a card-carrying forester (NC Registered Forester 1246) and a tree breeder!

Steve McKeand

May 2015

Pictured left: New 4th-cycle seed orchards, like the one Hancock Timber Resources Group established in 2013, will produce the next level of genetic improvement to landowners in the next 5 to 7 years. Maintaining and sustaining a healthy and diverse breeding population is critical for making continued genetic gain for all future generations.

Pictured right: The New Breeding Orchard at the Arrowhead Breeding Center is the repository for most of the germplasm for the future of the Cooperative's breeding program. The partnership we have with the Georgia Forestry Commission allows us to manage the breeding program for the benefit of today's landowners and for landowners and citizens for generations to come.

Pictured left: Vigorous, healthy, young forests will provide more wood, fiber, and energy for landowners when they are harvested. They also sequester substantially more carbon than forests planted with slower-growing genetics of the past.

SELECTION, BREEDING, AND TESTING

Fourth Cycle breeding and testing progress

Breeding

The principal plan in the 4th Cycle is to breed and test 800 Coastal, 500 Piedmont, and 300 Northern crosses for a total of 1600 crosses, Cooperative-wide. Fortunately, at the outset of the 4th Cycle, we were able to draft in some full-sib breeding that was done but not tested in the 3rd Cycle. These are great crosses, so we did not want to throw away that effort, especially since the crosses fit into the 4th-Cycle crossing scheme. There are approximately 170 of these "substitute" crosses in both the Coastal and Piedmont regions. The Northern region's crosses are constituted of a larger proportion of substitute crosses, with approximately 140 of the 300 total crosses being substitutes. True 4th-Cycle crosses were designed, assignments were made, and breeding began in spring 2013. Our expectations were to have the 4th-Cycle breeding completed after the fourth breeding season in 2016 (Figure 1), which is next year. How time flies when you are having fun!
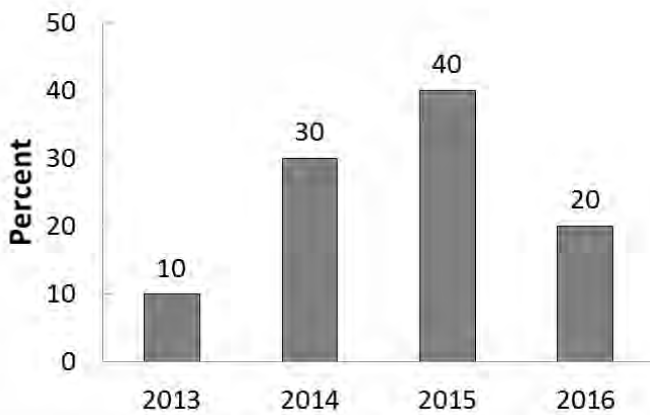

Figure 1. *Anticipated percent breeding per year for the 4th Cycle. For planning, we expected all breeding to be completed over four years, with most of the breeding being completed in 2014 and 2015.*

The Cooperative members and staff have just completed the third breeding season for the 4th Cycle. In talking with members during and after this breeding season, everyone is confident that this was another very productive year. Since breeding season is just getting wrapped up and summaries of crosses made and pollen collected are just coming in for 2015 breeding, we can

only assuredly report on breeding done in 2013 and 2014. After the 2014 breeding season, the Cooperative is on or ahead of schedule for the breeding in each region. "On schedule" is a good place to be at the halfway mark in our breeding cycle.

Progress in the Coastal breeding was slightly below the 40% completed mark after the 2014 season (Figure 2). This is due to the overall size of the Coastal breeding population; the Coastal region is as large as the Piedmont and Northern regions combined. Starting with grafting and pollen collection in 2013, and then a lot of breeding last year in 2014, Cooperative members have done an amazing job in the transition to the 4th Cycle. A big year of breeding this year should be just what we need to stay on schedule.
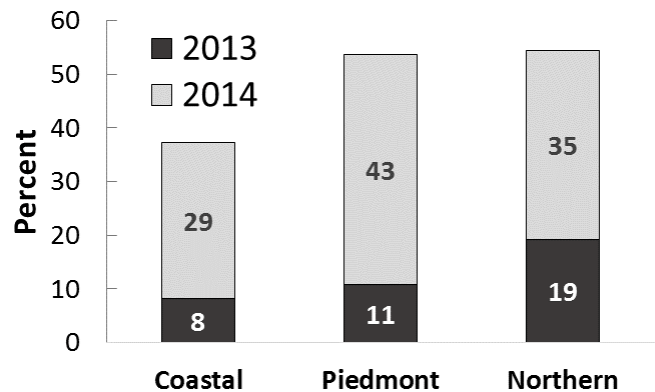
Figure 2. *Actual percentage of 4th-Cycle breeding completed in the 2013 and 2014 breeding seasons. Numbers are based on seed and conelet counts.*

Piedmont and Northern breeding, respectively, have progressed better than we had originally projected (Figure 2). Our members doing Piedmont and Northern breeding worked very hard in 2013 and 2014 at grafting, pollen collection, efficient pollen exchange, and breeding. Another round of successful breeding this year will propel our Piedmont and Northern breeding regions closer to completing the 4th-Cycle breeding on schedule.

Testing

Being aggressive with breeding progress is imperative for installing the 4th-Cycle tests in an efficient manner. As crosses are bred and seeds are collected, they are eligible to be included in the progeny tests. Our approach within the 4th-Cycle testing strategy is to include each cross in 60 reps within its native region. Each year, in each region, we are deploying 30 reps, with approximately 150-180 families per rep (dependent upon seed availability and region). To provide connection and comparison of tests across years, families will be included in the testing strategy across multiple years. For example, if a cross's testing effort was initiated with 30 reps in the 2014 tests, then that cross will be included in another 30 reps in the 2015 tests. The crosses that are first tested in 2015 will connect the 2014-15 crosses with future families.

Figure 3 illustrates our approach for connecting the testing across regions within the 4th-Cycle testing. There will be approximately 20% of the crosses from each region tested in other regions. The only exception is that within the Coastal region, only Coastal material will be tested. This regional connection will provide us with direct comparisons of families from different regions, an analytical endeavor that has been lacking in the Cooperative until this cycle. By testing material from all three regions side by side, we will now know how the top selections of each region compare to the top selections in each other region. The new regional overlapping testing scheme will also provide a means to consider merging the Piedmont and Northern populations.

The 4th-Cycle test designs are different than in previous cycles of testing, where the placement of the seedlings within each rep was truly random. In the 4th Cycle, there is very strict control of the seedling coordinates within each rep before tests are planted. For increased analytical power, each row and column is being regarded as an incomplete block. Seedlings from each cross are assigned to specific rows and columns to optimize test efficiency (reducing the residual variance) and increase direct comparisons among crosses. The location of seedlings in one rep is tied to the location of seedlings in all the reps within a year, both at the same site and at other sites. To facilitate test establishment, data measurement sheets and rep maps are generated by the TIP staff in Raleigh before the planting season. Both staff and Cooperative members work diligently to ensure that the correct seedlings go into the correct planting order in the tests.

Cooperative members have planted two years of 4th-Cycle tests, and the results have been good thus far. Everyone has responded well to the new testing strategy and pre-planned designs. The 2015 tests will be the biggest yet. We are pushing the members to plant tests with as many crosses in each rep as is feasible. The number of reps in each year for each region has been set at 30, but this will need to increase in the coming years as more crosses are ready to test.

The TIP staff has just completed the preliminary designs of the next round of tests to be planted in the Fall/Winter of 2015/2016. There will be approximately 180 families in each Coastal rep, 170 in the Piedmont reps, and 150 families in the Northern reps. The members have been great in pushing the TIP staff to find the efficiencies needed to complete the 4th-Cycle testing in an expedient time frame. It's hard to imagine we will be measuring the first 4th-Cycle tests in two years and making 5th-Cycle selections soon after.
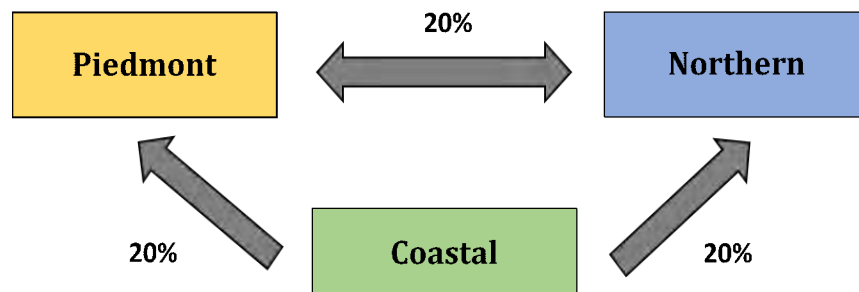

Figure 3. *Schematic of regional testing overlap within the 4th-Cycle testing. The percentages and the arrows show the percent of a region contributing to another region.*

Big BLUP, *PRS*™, and TIPRoot updates[1]

In 2014, Big BLUP analyses were run for the Coastal, Piedmont, and Northern breeding populations. Based on updated breeding values, Coastal and Northern *PRS*™ 2014 versions were released on the TIPRoot Cooperative database in February 2015. The Piedmont *PRS*™ update is in progress.

New data for the Coastal Big BLUP came from the addition of tests and updated measurements (age 6 years vs. age 3 years) of coastal polymix tests CPMX3 and CPMX4 series. In the *PRS*™ 2014 version, there were 663,954 data points analyzed from 571 tests (up from 564 tests). There were an additional 7 new selections from polymix tests. Some selections included in the previous version (2012) were dropped from this version of Big BLUP analysis because of updated pedigree, mostly related to RR3 test series established by the Georgia Forestry Commission. Correlations of breeding values for various traits between the 2012 and 2014 versions were r = > 0.99.

The new data for the Northern 2014 Big BLUP analysis were from the NPMX1 series. Analysis was done in late 2013, but the *PRS*™ scores were developed in 2014. Heritability estimates from some tests sites in the series were unusually low. One difference from the previous (2010) Northern big BLUP analysis was that raw data were standardized in this version to have consistency with the Coastal and Piedmont breeding populations' analyses. The number of sites included in the 2013 Big BLUP analysis was 263 (up from 258). A total of 293,198 data points were included in the analysis. Correlations between breeding values of traits from 2010 and 2014 were all perfect (r = 1.00).

In the Piedmont 2014 Big BLUP analysis, one test series (4 sites) was added to the 2012 version to update breeding values and produce *PRS*™ scores. In addition, the 3-year data of the polymix test PPMX2 series was replaced by the 6-year data in the 2014 analysis. A total of 539,638 data points were included (up from 533,605). There were 59 new families from the PPMX3 series in the 2014 version. Trait correlations between the different versions for breeding values ranged between 0.98 and 0.99. These high correlations signify that the adjustments in the family values were small between the two different versions.

TIPRoot database

The TIPRoot online database has been up and running for less than a year, and already there have been changes made to improve its organization and utility to members of the Cooperative. Here is a summary of new and improved tools:

Main headings on the side tool bar (**Starting Points**) are now drop-down tabs (see screen shot of the TIPRoot home page). Most main headings are bold with a "+" beside them. Click on this main heading and the sub-sections will appear below. As we add more tools and information to TIPRoot, these drop-down tabs will help keep the webpage tidy.

+Organizations: Lists Cooperative members and their membership type (Full, Contributing, Research Associate)

+Pedigree Information: Houses several tools for inspecting the ancestors and descendants of any selection within the Cooperative. Also reported within this same page are the relatives of the selection entered. There is now a query to check the relatedness of two selections and visualize their pedigrees (see example of how two trees, selections *A* and *U* are related). This information is critical for full members to ensure they do not breed related selections or put closely related trees as neighbors in new seed orchards.

+PRS Reports: Generates *PRS*™ Spec Sheets for an individual family, full-sib cross, or a list of families

+PRS: Used to generate tables of *PRS*™ values, breeding values, and genetic values of tested families and full-sib crosses

+Data Upload: New to the TIPRoot tool bar, and is only relevant to full members who measure progeny tests. Members will now directly upload progeny test data to the database, instead of attaching data sheets in an email to send to the TIP staff. As progeny test data are uploaded, their quality is checked through back-

---

[1]We love acronyms in TIP. Big BLUP is the Best Linear Unbiased Prediction analytical procedure used to analyze all the Cooperative data. *PRS*™ is the Performance Rating System used to quantify the value of the loblolly pine families. TIPRoot stands for Tree Improvement Program Rapid online output tool and is the name for our online database.

ground scripts. The database administrator then inspects the data and summary information to ensure there are no data issues before allowing the new data to populate the database.

The TIP staff has been without a full-time database administrator /data analyst since October 2014. Therefore, progress on some aspects of database development have been slowed. When the new data analyst starts in May 2015, we will be able to intensify the database development and testing. For example, the Breeding Logistics Database is in development and will be the mechanism to efficiently manage the wealth of information related to the Cooperative's breeding plans. Using TIPRoot, members will be able to access their breeding assignments, track breeding and pollen collection progress, and upload breeding activity. The Cooperator section of the Breeding Logistics Database will be overseen by the tree improvement manager. There will also be a portion of the Breeding Logistics Database the TIP staff will use for the Arrowhead Breeding Center. With so much breeding at Arrowhead, this section of the Breeding Logistics Database will serve to track breeding activities, pollen collection, grafting, orchard establishment and maintenance, and various inventories.

Pictured above: Screen shot of TIPRoot homepage
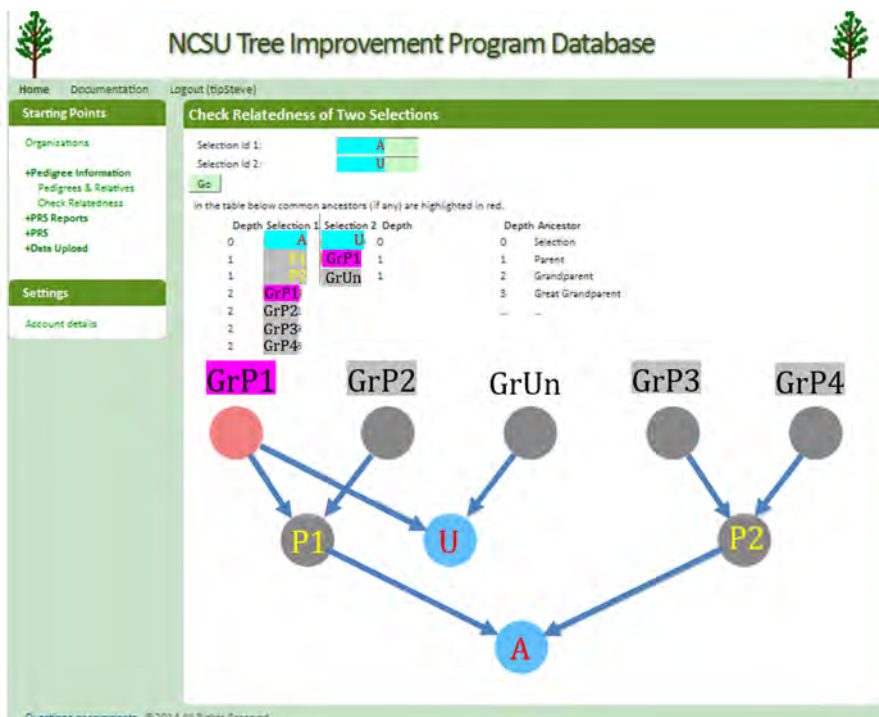
Pictured left:

*Example of pedigree visualization to easily see how two trees are related. Selections **A** and **U** have **GrP1** as a common ancestor. **U** is an aunt or uncle (second degree) ancestor of **A**.*

SEED AND CONE YIELDS

Cooperative members harvested more than 71,000 pounds of loblolly pine seed from operational orchards in 2014 (Table 1). This is more than twice the seed yield from the harvest in 2013. Average yield of seed per bushel also increased relative to 2013. All provenances and generations had incredible increases in amount of seed collected. Northern 2.0, Piedmont 3.0, and Coastal 2.0/2.5 orchards had more than twice as much seed collected as in 2013. Piedmont 2.0 orchards had nearly triple the amount of seed collected in 2014 compared to 2013.

At approximately 12,000 seedlings produced per pound of seed, last fall's harvest can result in over 858,000,000 seedlings being grown this year. That is enough to plant approximately 1,700,000 acres of pine plantations!

Table 1. Fall 2014 cone and seed yields compared with the figures from 2013 harvest

Provenance / Generation	Bushels of Cones		Pounds of Seed		Pounds per Bushel	
	2014	2013	2014	2013	2014	2013
Coastal 2.0/2.5	15,282	7,139	22,066	9,054	1.44	1.27
Coastal 3.0	9,511	6,851	11,474	8,107	1.21	1.18
Piedmont 2.0	12,598	4,224	18,418	5,479	1.46	1.3
Piedmont 3.0	11,101	4,567	14,893	5,446	1.34	1.19
Northern 2.0	1,319	550	1,511	482	1.15	0.88
Northern 3.0	2,966	1,685	3,175	1,730	1.07	1.03
Totals	**52,777**	**25,016**	**71,537**	**30,297**	**1.28**	**1.14**

Mist House Repairs

Pictured left: *The mist house at the Horticulture Field Labs was gutted and nearly rebuilt this past summer as it was in desperate need for repair. New greenhouse plastic, polycarbonate end walls, support poles, wet wall motor and ventilation system, and a Wadsworth environmental control system were installed. The new mist house is now capable of producing rooted cuttings using an irrigation system run off of vapor pressure gradient (VPG). This system uses the current relative humidity and temperature to determine when to water. With this system ,cuttings are watered when they truly need water versus being watered at a set time interval.* **Pictured right:** *The same view of the mist house after renovations.*

RESEARCH

Trends in Deployment of Advanced Loblolly Pine Germplasm[1]

Loblolly pine is the most widely planted forest tree species in the world. In the southern US, about 80% of the more than 30 million planted acres are loblolly pine, and virtually all of those have been established with genetically improved seedlings. Advanced tree breeding and seedling deployment programs now provide landowners with options to plant specific families that are 40% to 60% more productive than unimproved loblolly pine. A wide range of planting stock is available to landowners with different costs, benefits, and levels of genetic diversity.

As part of the *Pine Integrated Network: Education, Mitigation, and Adaptation Project* (PINEMAP[2]), we surveyed seedling vendors who are members of the three pine tree improvement cooperatives in the South to determine the genetics of loblolly pines currently being planted. We wanted to quantify how many seedlings of various types are being planted as well as determine evidence of risks associated with planting genetically more homogeneous seedlots such as specific full-sib families or clonal varieties. We asked vendors to respond for each planting zone where they sell seedlings. These zones varied by organization, but generally are within 200 to 300 miles of a given nursery.

In the 2011, 2012, and 2013 planting seasons, 31 cooperative members planted an average of 843.5 million tree seedlings per year[3]. This is 37% fewer than the 1.35 billion seedlings grown yearly during the previous survey period (see McKeand et al. 2003, J. For. 101 (3):32-37.). Reduced seedling production was expected given the economic downturn and reduction in plantation establishment during last 10 years. Of these 843.5 million seedlings, 87.1% were loblolly pine, 6.1% slash pine, 5.7% longleaf, and 1.1% other conifers and hardwoods.

For loblolly pine, the most dramatic change from the previous survey period is that 95% of plantations are now being established as genetically more homogeneous stands to capture greater gains in yield from improved genetics. In the previous survey, 59% of loblolly stands were planted with open-pollinated (OP) families and the rest with mixtures of seedlings from different mother trees. Today, the vast majority of stands are planted with OP families (84%); about 8% of stands are planted with specific crosses or full-sib families; and about 2% are planted with clonal varieties where every tree is genetically identical. Landowners can now choose families that best match their management goals. They can also select families that may be better suited to future climate scenarios.

Although most individual plantations are relatively genetically homogeneous, there is substantial genetic diversity across the landscape. Within a planting zone, up to 354 different OP families and 86 full-sib families were planted, although some of these families were undoubtedly the same for different companies and agencies. On average within a planting zone, there were 24 different parent trees grafted in seed orchards, with a minimum of 14 and a maximum of 36.

To determine possible risks associated with establishing plantations with relatively homogeneous germplasm, we asked respondents if they were aware of any unexpected environmental or pest problems (e.g., diseases, insects, cold, or storm damage) encountered in single family plantings. One of the 31 respondents experienced freeze damage with one southern coastal family planted in northern environments that were colder than recommended by Schmidtling (2001. Southern pine seed sources. Gen. Tech. Rep. SRS-44. Asheville, NC: U.S. Department of Agriculture, Forest Service, Southern Research Station.), and another family consistently showed about 10% lower survival compared to others. No respondents reported outright plantation failures due to the use of family blocks.

Investment in forest management have had large, positive impacts on stand productivity, landowner returns, and carbon budgets. In the southeastern United States, relative to no genetic improvement, planting genetically improved loblolly pine over the past 40 years has resulted in an additional 3.7 billion m^3 (17% increase) in volume of wood and 1,100 Tg C (13%) in carbon sequestration (see Aspinwall et al. 2012 For. Sci. 58: 446–456 for details). As older plantations are replaced with newer, faster growing plantations with families planted in blocks and managed with good silvicultural practices, increased yields have the potential to increase carbon sequestration by an additional 10% or more than the stands they are replacing, thus becoming an important carbon mitigation strategy in the region.

Alternative approaches to estimating breeding values within family[1]

The goal of this study is to increase the selection intensity within loblolly pine breeding programs by assessing the relationship between unique patterns of family gene expression and parental breeding values (BV). We hypothesize that selection intensity can be increased in pine breeding programs under two conditions: first, that there are genetic differences among families in gene regulatory networks, and second, that those differences are correlated with family mean performance in field tests of progeny.

Currently, selections for advanced generations of loblolly pine breeding are made on the basis of family mean and individual-tree phenotypes, where phenotypically superior individuals are selected from top-performing families. Individual-tree phenotypes are an unreliable guide to breeding value, so progeny of each selection are tested to identify trees that have the best BVs. This requirement to progeny test all candidate selections limits the size of breeding populations, and therefore limits the selection intensity that can be applied.

In order to estimate the BV of a tree, linear mixed models are conducted where phenotype and pedigree data are utilized to help define the genetic covariance among a set of families from a mating design. It is reasonable to propose that genetic variation in family mean phenotypes can be partially accounted for by differences in gene structure, and partially by differ-

ences in gene regulation. Recent published work with maize described the use of pair-wise comparisons of gene expression levels among inbred parents of full-sib F1 hybrid families to identify a set of genes that are differentially-expressed in at least one pair-wise comparison. Gene expression data was then used to create covariance matrices, either using the quantitative differences or simple binary comparisons (Frisch et al, TAG 120:441-450, 2010; Fu et al, TAG 124:825-33, 2012). Genetic differences among F1 hybrid progeny in field performance were accurately predicted using statistical models trained with a subset of the data in a cross-validation study.

An alternative approach would be to use a covariance matrix based on family-mean levels of all gene expression values from sequencing DNA copies of messenger RNA, whether differentially expressed or not, as a measure of genetic covariance in gene regulation. This approach uses no information about differentially-expressed genes, but is a simpler approach to capturing similarities as well as differences among families. The sequencing approach will also provide a means to collect information on genetic covariance among families in gene structure as well as gene regulation patterns. Utilizing covariance structures based on these types of genetic variation in a BLUP analysis instead

of, or in addition, to the standard numerator relationship matrix, may provide a higher prediction accuracy of BVs. New software tools allow incorporation of multiple covariance matrices, with variable weights on each matrix, in statistical modeling (Wheeler et al., Genetic Epidemiol 38:402-15, 2014).

To test this hypothesis, we have chosen 43 different parents from a wide geographic distribution with existing progeny data available from field tests across multiple sites. Seeds (37 open-pollinated or pollen-mix families and 6 controlled-crosses) from each of these parents were grown in a greenhouse, and seedlings were harvested at 3 months for RNA extraction and sequencing, which is still underway. The data on gene structural variation and gene expression levels from these seedlings will be used to create covariance matrices reflecting genetic variation in coding sequences on one hand, and variation in gene regulatory networks on the other. Cross-validation models using these covariance matrices, as well as a standard numerator relationship matrix, will be used to test the hypothesis that phenotypic variation can be accurately modeled by covariance of these classes of genetic variation.

In summary, this is a pilot study, with the objective of assessing the relative contribution of separate covariance matrices modeling gene structure and gene expression in cross-validation studies to predict full-sib progeny performance from crosses among a small number of parents. It is unlikely that this study will provide definitive evidence that using gene expression differences will result in better estimates of parental genetic value, but there are several practical questions to be addressed: (1) can we obtain reasonably reproducible results from triplicate samples of seedlings from open-pollinated, polymix and controlled-pollinated families with respect to estimating family-mean levels of gene expression for a set of parents; (2) can we identify methods for combining those family-mean estimates of gene expression levels into covariance estimates for pairwise-combinations of parents that show utility in cross-validation studies for modeling phenotypic variation, and (3) do covariance matrices based on coding sequence SNP variation, gene expression level variation, or pedigree-based estimates of allele sharing have independent value for modeling phenotypic variation, or are they redundant so that one approach has the same information present in the other two? Overall, the resulting analyses should provide insight into the value of using RNA expression patterns as another screening effort in selecting individuals as parents for future breeding populations.

Q: What do the Arrowhead Breeding Center and Disney World have in common?

A: The happiest place on Earth that everyone, from far and wide, comes to visit!

The Arrowhead Breeding Center has become a popular place for visitors to see southern US tree improvement in action. This year brought visitors from Canada, France, Sweden, China and many domestic visitors as well. Below are pictures of students and faculty from SLU learning about breeding techniques and enjoying time at the chalet.

Bayesian Spatial Modeling for Loblolly Pine Seed Source Movements[1]

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

In the mid 1990s, the Cooperative initiated the Plantation Selection Seed Source Study (PSSSS) to determine the performance of loblolly pine seed sources for multiple traits and to describe genotype by environment (G×E) interactions. Each parent tree was selected from plantations (Figure 1) based on growth, stem quality, and fusiform rust incidence. For the study, 20 families from each of seven geographic regions were used. Each selection was mated with a pollen mix from 40 individuals sampled from the same geographic region. Half-sib progeny of 140 families were planted at 25 test locations throughout the southeastern United
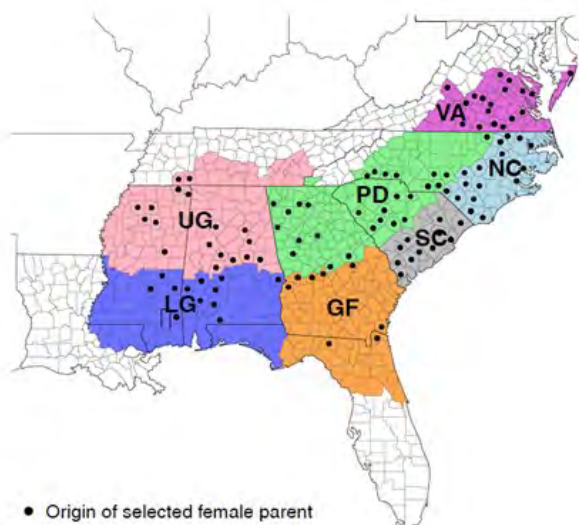
States (Figure 2). A randomized complete block experimental design with single-tree plots was used at all locations. There were 24 blocks per location. Height, diameter, stem straightness, and fusiform rust incidence were measured at ages 4 and 8 years at 16 sites for this study.
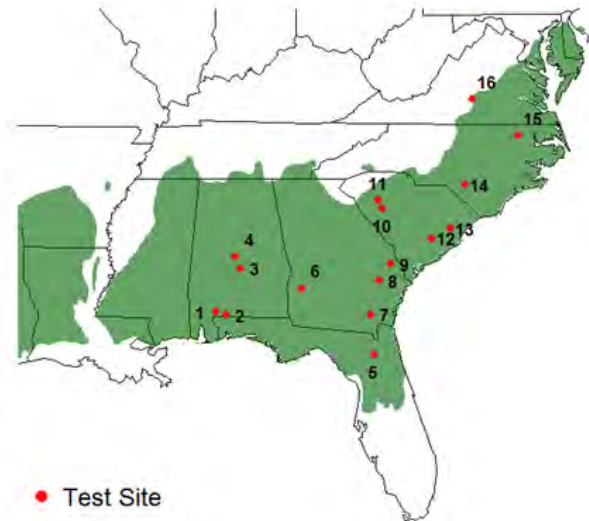

Figure 2. *Location of test sites across the Southeastern US; natural range of loblolly pine is shaded in green.*

Coastal sources grew faster compared to inland or northern sources, which is consistent with previous works. Lower Atlantic Coastal regions (SC and GF in Figure 1) seed sources outperformed the Virginia, the Gulf Coast, and inland seed sources.

In this work, a spatial Bayesian statistical approach was developed for modeling the expected height of trees using climate variables associated with the seed source (Figure 1) and the planting site (Figure 2) as predictors; the growth potential of the planting site was also used as a predictor. The modeling technique introduces a separable Matern covariance structure that provides flexible means to describe and estimate effects associated with the origin and planting site locations (See Matern, B. 1986. *Spatial Variation*, 2nd Ed. Berlin: Springer-Verlag). The Matern function is used to define the statistical covariance between measurements that are distant from each other. The distance

Figure 1. *Seven seed sources sampled (shaded regions) and locations of the plantations where the selections were made (black dots).*

between the observations was generalized and written in terms of three sets of predictors: the geographic location of the test sites and source of seed, the climate variables associated with the test sites and seed sources, and a combination of both geographic and climate variables associated with the test sites and the seed sources. The performance of the proposed models was assessed through cross-validation based on their capacity to make predictions at unobserved locations and also by their ability to predict the expected height of non-tested seed sources.

Two examples are presented to illustrate possible applications of the model as a seed deployment tool. Since the model uses climate variables as predictors (minimum and maximum temperature and precipitation), a hypothetical climate scenario was created from historical data assuming that future climate conditions would be the same as the average of the last 20 years.

The first example (Figure 3) depicts the predicted eight-year height of trees planted in Marion County, Florida, as a function of seed source across the Southeast. From the estimated values, it can be inferred that seed sources from the neighboring counties perform very well in Marion County. The top performing sources are predicted to be from the Georgia-Florida coastal plain region. In contrast, if northern seed sources are used, they grow slower.

The second example (Figure 4) shows the predicted height of loblolly pine seed sources planted in Wayne County, North Carolina, under the modeled climate scenario across the southeastern United States. The best seed sources for planting in Wayne County can be found in multiple locations. However, the Coastal Plains and Piedmont regions of South Carolina seed sources stand out. Note that the contour line clusters the top 1% of performing sources.

The statistical model can be used as a quantitative tool to model the effect of climatic variables on the performance of loblolly pine seed sources. Furthermore, the model could be used to identify superior performing sources and to estimate their growth potential for a given planting site under a given climate scenario.

Figure 3. *Predicted eight-year height (m) in Marion County, FL (highlighted in gray) as a function of seed source. The red color indicates the top performing seed sources under the modeled climate scenario. The arrows show the direction of the optimal seed source movement based on the top 3% locations.*
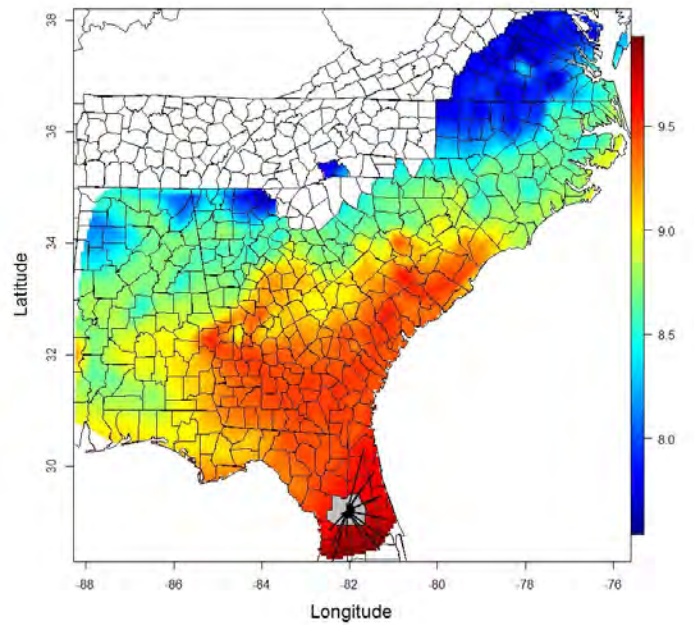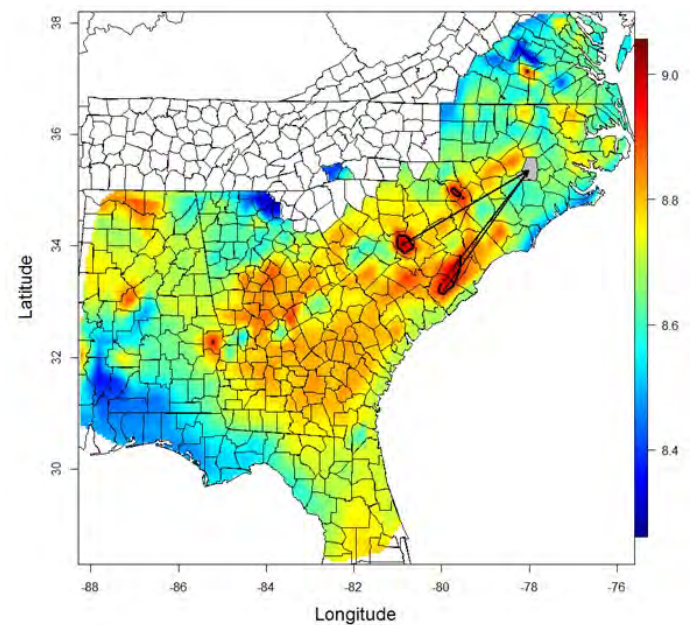

Figure 4. *Predicted eight-year height (m) in Wayne County, NC (highlighted in gray) as a function of seed source. The red color indicates the top performing seed sources under the modeled climate scenario. The small contour lines specify the top 1% of locations, and the arrows show the direction of the optimal seed source movement.*

Effects of Provenance Variation and Fertilization on Dry Biomass Yield in Loblolly Pine[1]

The emerging biofuels industry in the Southeast may stimulate a market for trees whose mechanical wood traits have been genetically improved. Loblolly pine has been proposed as a potentially viable biofeedstock in the region, since it is abundant, is not a competing food source, and a substantial forest industry based on loblolly pine already exists. Developing a biofuel-specific population of loblolly pine would require breeding for productivity traits (height and diameter) and possibly for wood density to increase dry weight production. Fortunately, loblolly's wood properties vary across its native geographic range and can be manipulated for genetic gain due to high heritability of wood property traits.

This research aims to characterize the effect of genetics and fertilization on dry biomass yield of loblolly pine. The research was conducted at SETRES2, a large block-plot study established in the winter of 1993-1994 in the Sandhills of Scotland Co., NC. Originally designed to evaluate genotype by environment interactions, the study is a split-split plot design composed of nine experimental blocks. Each block has fertilization (optimally-fertilized and non-fertilized) as main plots, genetic provenance [Atlantic Coastal Plain (ACP) and Lost Pines Texas (LPT) seed sources] as sub plots, and family (five open-pollinated families per provenance) as sub-sub plots of 100 trees nested within provenance. For the current study, a subsample of five trees were randomly selected from each family plot for assessment. Only six replications could be considered, due to severe crown scorching from a fire in 2011. At age 21 years, 600 trees (6 replications x 2 provenance x 2 treatment x 5 families/provenance x 5 trees/family) were cored using a gas powered drill and bore. Whole-core wood density values were obtained using the volumetric density method. Following the volumetric calculation, dried cores were prepared for densitometry analysis to obtain ring-by-ring wood density estimates. Densitometry values were paired with previously-recorded volume per hectare data (see Smith et al. 2014. Can. J. For. Res. 44: 1453–1461) to obtain an index of dry weight per hectare[2] for ages 6, 8, 10, 12, and 14. Stand-level plot means for dry weight per hectare, volume per hectare, and wood density were analyzed using the model described in Smith et al. (2014).

The fertilization treatment effect was highly significant ($p < 0.0001$) at all ages for volume, wood density, and dry weight. Provenance effect was highly significant ($p < 0.0001$) for wood density, but non-significant for dry weight. The interaction of treatment and provenance was marginally significant to highly significant for wood density and dry weight at most ages. For wood density, the treatment by provenance interaction was not statistically significant ($p = 0.0597$) at age 8, significant at age 10 ($p = 0.0453$), significant at age 12 ($p = 0.0409$), and non-significant at age 14 ($p = 0.0604$). For dry weight, the treatment by provenance interaction was not significant at age 8 ($p = 0.0624$), significant at age 12 ($p = 0.0305$), and significant at age 14 ($p = 0.0097$).

Trends in volume per hectare (Figure 1) are essentially the same as described by Smith et al. 2014. In the non-fertilized plots, the ACP provenance had greater volume per hectare at all ages, but in the fertilizer treatment, a provenance rank shift occurred about age 12 (Figure 1). The primary reason for the increase in volume of the LPT trees compared to the ACP trees in the fertilized treatment was higher mortality in ACP trees starting at about age 8 years (see Fig. 8 in Smith et al. 2014).

The provenance ranking shift in dry weight occurred a bit earlier (Figure 2) than for volume (Figure 1), suggesting the genetic differences in wood density are responsible for the altered response in dry weight production. Lost Pines Texas provenance had significantly higher wood density than the Atlantic Coastal provenance at all ages (Table 1). When the higher wood densities of the Lost Pines Texas provenance were combined with its greater volumes per hectare, the superior yield of the Lost Pines Texas provenance at ages 12 years and beyond becomes more evident (Figure 2).

These results support the findings of previous studies, showing the significant negative impact of fertilization on wood density (Table 1), but the greater volume due to fertilization results in a substantial increase in dry weight yield compared to the non-fertilized trees. Although dry weight per hectare has not been extensively

---

[2]Since wood cores were only taken at breast height, and wood density in the entire stem was not estimated, the dry weight is a surrogate for the whole-tree dry weight.

studied in relation to biofuel production in loblolly pine, previous carbon allocation studies have indicated fertilization would have a significant positive impact. The significance of the treatment by provenance interaction effect on dry weight per hectare suggests it may be plausible to selectively breed a biofuel specific population of loblolly pine. Additional analyses are underway to compare family differences within the provenances and to evaluate growth curve differences for dry weight yield.

Table 1. Mixed model output for wood density showing least square means by age. Treatment or provenance means for a given year followed by the same letter are not significantly different at p = 0.05.

Treatment	Level	Wood Density (kg/m^3) at Age				
		6	8	10	12	14
Fertilizer	Control	536^a	543^a	547^a	548^a	552^a
	Fertilized	485^b	491^b	497^b	500^b	505^b
Provenance	LPT	517^a	523^a	528^a	530^a	535^a
	ACP	505^b	511^b	516^b	518^b	522^b

Figure 1. *Volume per hectare for the Atlantic Coastal Plain (ACP) and Lost Pines Texas (LPT) provenances for the fertilized and control treatments by age. Significant provenance by fertilizer treatment interactions started to occur at about age 12 years and was primarily due to higher mortality in the ACP provenance compared to the LPT provenance in fertilized plots (see Smith et al. 2014 for details). The provenance rank shift occurred somewhere after age 12 in the fertilized treatment.*

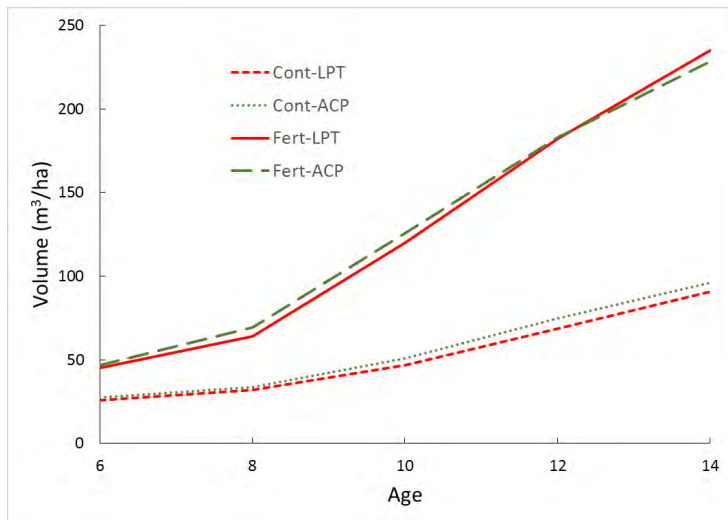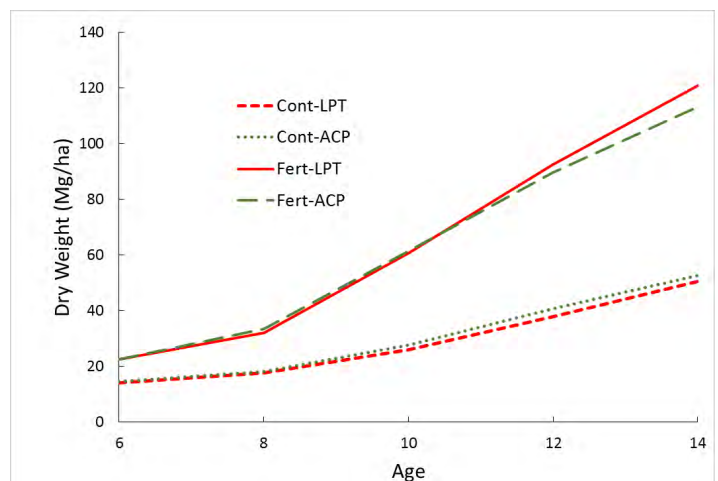**Figure 2.** *Dry weight per hectare for the Atlantic Coastal Plain (ACP) and Lost Pines Texas (LPT) provenances for the fertilized and control treatments by age. Significant provenance by fertilizer treatment interactions started to occur at about age 10 years. The provenance rank shift occurred somewhere after age 10 in the fertilized treatment.*

Progress toward the goal of cost-effective genotyping, applied to informative markers[1]

The potential applications of molecular markers to solve practical problems in applied tree breeding programs have been a subject of active research for the past 25 years. Some successes have been achieved, such as the use of microsatellite and SNP markers to verify clone identity and confirm parentage, but the vision of using molecular marker genotypes to increase genetic gain or reduce the time required to complete a breeding cycle has not yet been realized. Several reasons account for this – one is the high cost of marker genotyping assays, and another is the challenge of finding which markers to genotype in order to accurately model phenotypic variation as a function of genotype. Recent technical advances have the potential to reduce genotyping costs, and a growing understanding of fundamental aspects of gene regulation (driven largely by investments in human biomedical research) may provide guidance to solve the problem of choosing which markers are likely to be informative. The Pine Integrated Network: Education, Mitigation, and Adaptation Project (PINEMAP) is a USDA-supported five-year Coordinated Agricultural Project initiated in 2011 (USDA NIFA Award #2011-68002-30185; see http://pinemap.org/ for details). This project has supported research to test methods for cost-effective genotyping in loblolly pine and to find ways to identify markers associated with traits of interest to breeders. Laura Townsend has been supported in her M.S. research project by PINEMAP funds, and her research addresses these two topics.

PINEMAP researchers chose to focus on DNA sequencing as the method for discovery and analysis of genetic variation in loblolly pine for two reasons. First, the cost of DNA sequencing (expressed in dollars per million bases of DNA sequence) has dropped by over 10,000-fold in the past ten years, due to technological advances driven by the Human Genome Project; and second, DNA sequencing is the only genotyping method capable of detecting any genetic variant that is present in the DNA sequence, regardless of whether it was previously known to exist. Costs for other methods of detecting genetic variation, such as "SNP chip" microarray assays, have also decreased, but by much smaller proportions, perhaps on the order of 5- to 10-

fold. Such assays also require extensive background information for assay design, and are therefore not well suited to the detection of novel genetic variation in previously-unstudied species. Loblolly pine is one of the most extensively-studied conifer species, but at the time the PINEMAP project started in 2011, pine lacked the kind of detailed information on gene structure and genetic variation that is available for many other agricultural and experimental organisms.

This shortcoming was addressed at the same time as the PINEMAP effort began in 2011 with the initiation of another USDA Coordinated Agricultural Project to determine the DNA sequence of the loblolly pine genome - all the DNA in all the chromosomes, including not only the genes but also the DNA between genes that contains sequences important for controlling the location, timing, and level of gene expression. The PineRefSeq project (http://pinegenome.org/pinerefseq/) has produced draft genome assemblies not only of loblolly pine but also of sugar pine as a representative species of white pines and of Douglas-fir. The availability of a draft genome assembly for loblolly pine creates new opportunities for research and also poses significant challenges for data analysis due to the size and complexity of the pine genome.

Laura's effort to discover genetic variation used a method called "genotyping by sequencing", which is designed to sample a reproducible set of DNA fragments from each of many different trees for DNA sequencing. She conducted her research using material collected from the Plantation Selection Seed Source Study site in Screven County, Georgia, with the goal of obtaining genotype data from 1,536 progeny from the pollen-mix crosses planted in the single-tree-plot test. The DNA fragments sampled using genotyping by sequencing are sampled somewhat at random from different regions of the genome, although they should be slightly enriched for sequences in or near functional genes. This more-or-less random distribution of fragments means that there is potential to detect both major classes of genetic variants – those that affect gene structure and function, and those that affect patterns of

---

[1]*This summary is by Ross Whetten, based on results from Laura Townsend's M.S. research.*

gene regulation. Other PINEMAP researchers at the University of Florida and at Texas A & M University carried out experiments using a method that collects DNA sequences from regions of the pine genome known to encode proteins, so the genetic variation detected can be evaluated for potential effects on protein structure and gene function. Both types of DNA sequencing experiments were successful in detecting genetic variation; Laura identified over 1.5 million potential genetic variant loci while the groups in Florida and Texas identified over 3 million candidates. In parallel, the PineRefSeq project requested seeds of first-cycle selections from natural stands across the range of loblolly pine from the Western Gulf and NC State Tree Improvement Programs and is now conducting whole-genome DNA sequencing of sample seed from each of about a dozen parent trees. These data are likely to yield tens of millions more candidate sites of genetic variation.

The loblolly pine research community now has a wealth of information regarding candidate sites of genetic variation in the pine genome, but unfortunately it is not easy to determine which of these genetic variants affect traits of interest to breeders. The sites where candidate genetic variants fall in coding sequences of genes can be examined using computational methods to identify a subset likely to affect the structure and perhaps the function of the gene product, which covers one of the two major types of genetic variation. Candidate sites of genetic variation that do not lie within coding sequences of genes are not easily classified with respect to potential effects on gene regulation, so additional experiments are underway to test alternative methods for identifying differences in patterns of gene expression (see research summary by Adam Festa). Another key question that remains unanswered is the proportion of phenotypic variation that is due to a few relatively common genetic variants as opposed to a diverse collection of many variants, each of which is relatively rare. This information is critical to the design and use of genotyping assays for use in tree breeding, if the goal is to develop methods for predicting breeding value based on genotype data.

The classical model for quantitative genetics, developed by Sir Ronald Fisher in 1918, is that variation in a complex trait, such as stem volume in forest trees, is based on genetic variation in thousands of genetic loci,

each of relatively small and approximately equal effect. Detailed studies of the molecular basis of height variation in humans, using genotypes at 2.5 million variant sites for study populations of over 100,000 individuals, support this hypothesis. Many different variants in many different genes can contribute to the genetic variation that underlies phenotypic differences in human height. The challenge for tree breeders, then, is to find a way to use molecular marker data cost-effectively to sample what is likely to be an enormous range of genetic variation, to organize that information, and to use it to accurately predict differences in breeding values. A key aspect of this challenge is that the most important need is for a method to improve the accuracy of selection within families. Existing methods for analysis of progeny test data are quite accurate with respect to identifying the best families, but making forward selections within those families is limited by the low individual-tree heritability of traits such as volume or stem quality. Each family is likely to have a somewhat different array of genetic variants that account for phenotypic variation in those traits, so identifying which variants are responsible for variation within each family and accurately modeling the genetic basis of superior phenotypes will not be an easy task. The Atlantic Coastal Elite population, consisting of clonally-tested offspring from three partial-diallel mating groups with seven parents per group, will be an excellent subject for an experiment to test alternative approaches to overcome this challenge.

One important decision to be made in moving forward with molecular marker applications in tree breeding is which genotyping technology to use. DNA sequencing has the advantage that it detects any variation that exists in the fragments of DNA that are sequenced, but it has the disadvantage that there is sampling variation in which fragments of DNA are sequenced among individuals in an experimental population. For example, of the 1536 trees that Laura Townsend analyzed from the PSSSS site near Oliver, Georgia, only about 1300 had useful data on DNA sequence variation, and there was substantial variation among those 1300 in the number of genetic sites at which data were obtained. The alternative to DNA sequencing methods is array-based methods, such as those widely used in cattle breeding. The array-based methods target specific individual nucleotides for assay, and typically yield data from 95%

to over 99% of the individuals in a population for assays that are properly designed. These low levels of missing data are an important asset, but come with the disadvantage that array-based assays do not provide any information on other nearby sites, so the choice of which specific sites to assay is even more important for these methods.

Researchers working in biomedical genetics have developed a strategy for identifying genetic variants that are more likely to be functionally-related to phenotypic variation than a random sample of sites from anywhere in the genome. This approach takes advantage of the fact that DNA in higher organisms is highly organized inside the cell, and DNA sequences involved in gene expression are organized differently from DNA sequences that lack known functions. These differences in DNA organization can be detected by a variety of different approaches, and the resulting information about the locations of functional DNA sequences are used as a means of enriching for genetic variants that affect phenotypic variation. Laura tested

one approach to detect differences in DNA organization, using a set of replicated samples of four different tissues and organs from three different trees. Comparison of the regions identified showed that the accessible regions of DNA detected in triplicate samples of each tissue or organ (differentiating wood, phloem, shoot tips, or needles) differed from those detected in other tissues/organs, and that each tissue has thousands of unique DNA regions likely to be involved in gene regulation in that tissue type. A follow-up experiment is planned during the final year of the PINEMAP research effort, to confirm these results in another set of samples and extend the results by incorporating more analysis of the genetic variation discovered in the putative regulatory regions. The results of these experiments will provide guidance to future decisions to be made in choosing a genotyping platform, and choosing the variant sites to be genotyped with that platform, as we move closer to the goal of developing technology to accurately predict breeding value based on genotype.

A Cost-Effective, Automation-Friendly, High-Throughput Protocol for Isolating DNA or RNA from Forest Trees[1]

Extraction and purification of DNA and RNA from tissue samples is one of the first and most important steps of many molecular genetics methods. There are hundreds of protocols for these steps that have been published over the last few decades. These protocols can be carried out using traditional laboratory preparation (homemade extraction protocol) or one of the many commercially available nucleic acids extraction kits (Csaikl et al., 1998, Pl Mol Biol Rep 16:69). DNA extraction in our laboratory from fresh tissues of different forest tree species has been carried out using a modified version of a protocol from The Canadian Centre for DNA Barcoding (CCDB, www.ccdb.ca). We have made additional modifications to the CCDB protocol, incorporating some features from other commonly-used methods such as those reported by Dellaporta et al. (1983, Pl Mol Biol Rep 1:19) and Doyle and Doyle (1987, Phytochem Bull 19:11). The new protocol is also useful for RNA extraction from fresh or frozen leaf samples (from -80 $^{\circ}$C), with minor modifications of the procedure.

The current version of our DNA isolation protocol uses foliage dried over silica desiccant as the starting material. Phloem disks are a more convenient tissue sample to collect from mature trees, and testing the procedure with that tissue type is an objective for later this spring. Collected leaves or needles are cut into smaller pieces and put into a 6 × 9 cm paper envelope which is labeled with sample name, number, and collection date. The paper envelope is closed and placed into a 10 × 16 cm plastic zip-closure bag containing nontoxic orange silica desiccant beads. The amount of silica gel should be at least enough to cover half of the paper envelope, or 1:10 ratio of leaf tissue:desiccant (Photo 1). The paper envelope prevents sample contact with the silica desiccant, avoiding contamination of the tissue with dust from the desiccant. The status of the desiccant in the plastic bags should be checked every day; orange silica desiccant beads turn dark green when saturated

---

with moisture. Beads must be replaced when they turn dark green, in order to keep the tissue from retaining too much moisture for safe long-term storage. Beads are replaced until the orange color remains stable.

The process of drying foliage samples with silica desiccant beads takes from hours to days, depending on many factors such as the size of the leaf sample, the amount of silica desiccant, the moisture content of the sample, and the humidity in the laboratory. Silica desiccant can be reactivated by placing it into an oven for 2-3 hours at 150-200 °C; it can be reused many times. Leaf sample drying of forest trees in silica gel is generally considered more effective than other methods due to rapid desiccation, and the low-cost reusable nature of silica desiccant (Chase and Hills, 1991, Taxon 40:215; Semagn, 2014, Methods in Molecular Biology vol:1115).

The DNA and RNA extraction procedures were tested on leaf samples of many angiosperm and gymnosperm forest tree species; the foliage samples were obtained from the JC Raulston Arboretum and Camcore program at NC State University. The DNA extraction protocol was also tested on dried samples stored for long periods (from 2 to 6 years) at room temperature with silica desiccant; these samples were obtained from the University of California, Davis. The 96-well plate format is used for nucleic acids extraction.

High quality and high molecular weight nucleic acids were obtained from all species tested based on UV absorbance spectra. Additional tests of the suitability of these DNA samples for molecular marker genotyping are planned. The yield and quality of DNA obtained from the two- to six-year-old silica dried samples were similar to that of freshly-dried tissues, suggesting that silica dried samples can be stored at room temperature in a moisture free laboratory many years. The variation in nucleic acids quality and quantity between species can be explained by species-specific differences in the anatomy and chemistry of mature leaf tissue complexity (Semagn, 2014, Methods in Molecular Biology vol: 1115), differential homogenization of samples (Drabkova et al., 2002, Pl Mol Biol Rep 20:161), and the efficiency of the protocol to species (Csaikl et al., 1998, Pl Mol Biol Rep 16:69).

Nucleic acids can be extracted from four 96-well plates at the same time. One extraction (4 × 96) process takes about 5 hours. In one work day with careful timing of overlapping steps in the protocol, a laboratory equipped with one water bath and a plate centrifuge that accepts four plates can extract DNA from up to twelve 96-well plates (1152 samples). This scale of throughput is a big advantage for projects with large numbers of samples. Also, speed of method, consuming labor, per extraction cost and broad taxonomic applicability are encouraged to work with large number of samples. The estimated cost of consumable supplies and reagents for the extraction procedure is less than $1.00 per sample at the current scale of operation, but that does not include labor. A more detailed economic analysis of the cost of labor, equipment maintenance, and supplies will be carried out.

In conclusion, the new extraction protocol is an efficient and high-throughput method for extraction of either DNA or RNA from foliage samples of forest trees, including hardwood genera such as *Corymbia*, *Eucalyptus*, and *Quercus* as well as softwood genera such as *Abies*, *Picea*, and *Pinus*.

Pictured left: The orange silica gel beads (left) turn to dark green (right) when they become saturated with moisture (Photos by Yusuf Kurt)

Predictions of Breeding Values in a Large Cloned Population of Loblolly Pine[1]

The elite breeding populations of the Cooperative Tree Improvement Program are subsets of the mainline population that are intensively managed for short-term genetic gain. The Atlantic Coastal Elite (ACE) population is the elite breeding population of loblolly pine from the Coastal Plain region. For this population, clonal tests of the progeny were established to provide more reliable predictions of individual-tree breeding values compared to traditional seedling progeny testing. Since the individuals are cloned and replicated across trials, the accuracy of the genetic merit of individuals within family is increased. This is an improvement in the selection process and is expected to be superior relative to traditional seedling progeny testing and forward selection, but reliable estimates of breeding values are crucial for realizing this genetic gain. The objectives of this study were 1) to analyze the ACE cloned progeny test data for identifying superior genotypes for breeding, and 2) to explore approaches to obtain more accurate estimates of breeding values.

The initial genetic material consisted of 76 crosses produced from 24 elite parents from the Atlantic Coastal Plain. Seedlings were first screened for fusiform rust disease at the USDA Resistance Screening Center in Asheville, NC with a broad-base field inoculum that covered the expected deployment range. After six months, non-diseased seedlings were selected. Seedlings with rust galls were considered susceptible to rust and were eliminated from the study. The underlying assumption was that seedlings with a "no gall" response would be more likely to be resistant in the field than seedlings with galls. Based on the inoculations, 51 out the initial 76 crosses were selected for progeny testing in field trials. From each full-sib family, an average of 46 progeny were cloned using rooted cutting techniques. A total of 2362 clones were tested at seven test sites across the southeastern United States (Table 1). An incomplete block row-column experimental design was used in the field trials. Individual clones were represented once at each test. Growth measurements (height and diameter at breast height), stem straightness, fusiform rust incidence, forking, ramicorn branching, and survival were assessed at age 4 years. In total, over 15,000 trees were measured. The rust incidence frequency across test sites was only around 2% (Table 2), suggesting that the inocula-

tion and selection process carried out at the Resistance Screening Center was effective. Although several traits were measured, the trait used in this work is height.

Table 1. Test site IDs, cooperator names, and location.

SITE ID	COOPERATOR	LOCATION
1	Plum Creek	Wayne County, GA
2	CellFor	Colleton, SC
3	Westervelt	Greene County, AL
4	GA Forestry Commission	Pulaski County, GA
5	Rayonier	Pierce County, GA
6	Weyerhaeuser	Beaufort County, NC
7	ArborGen	Colleton, SC

The multi-environmental trial analysis was accomplished using linear mixed effects models. Predictions for genetic merit of clones were obtained using various variance-covariance structures that account for heterogeneity and genotype by environment interactions. Multi-environmental trial analyses usually require the estimation of separate residual variance for each trial, and the estimation of a genetic variance-covariance matrix for the environments under consideration. Such analyses give multiple predictions of the genetic merit for each tested clone. In fact, different predicted genetic merits are obtained for each trial in the experiment. The challenge then is how to appropriately weigh multiple predictions for the same genotype to obtain an aggregate genetic merit for each clone.

To calculate aggregate clone breeding values, a weight factor for each site was obtained from separate analyses of trials. The ratio of the genetic variance to the residual plus genetic variance at each site was used to define the set of weights. Defined this way, the weights provide a measure of the quality of each site to make inferences about genetic merit.

The overall models were assessed relative to each other through information criteria (AIC, BIC) to take into account both the goodness of fit and model complexity. In addition, model predictions for the genetic merit of the clones were compared using overall and within-family rank correlations, Pearson's correlations, and

[1]*Alfredo Farjat, PhD student in the Cooperative, has done most of the analyses of the ACE data. This is a summary of one of his dissertation chapters.*

Trait	MEAN	N	MIN	MAX	SE
HT (ft)	14.91	13,222	1.1	24.5	3.02
DBH (in)	3.02	13,157	0.1	6.7	1.03
RUST freq	0.02	13,222	0	1	0.13
STRT score	3.31	12,812	1	6	1.26
VOL (ft³)	0.36	13,157	0.03	1.7	0.24
FORK/RAM freq	0.24	13,219	0	1	0.42

Table 2. Overall summary statistics for the ACE population. Sample mean, number of observations, minimum, maximum, and standard error of the measured traits at age 4 years.

overall standard error of predictions. The overall rank and Pearson's correlations between the genetic merit of the clones for the explored models ranged from 0.88 to 0.99, respectively. The within-family rank correlation between the predicted genetic merit of the clones ranged from 0.82 to 0.99.

Among the fitted models for the multi-environmental trial analysis, the factor analytic model combined with a separable autoregressive process of first order (FA-AR1) for the residuals (spatial model) was superior. However, the model was computationally demanding, and it required good starting values to reach convergence, which will likely be impractical for large data sets. On the other hand, the factor analytic formulation combined with a block diagonal covariance structure (FA-BD) for the residuals was robust and informative for modeling genotype by environment interactions. In this case, the resulting model was parsimonious and could be fit without running into convergence problems, since the computational burden of the mixed model analysis is greatly reduced. The overall rank and Pearson's correlation between the factor analytic spatial and block diagonal models was around 0.91 (Figure 1), and the average of the within family rank correlations was 0.85 (Figure 2).

With the comprehensive statistical analysis, best linear unbiased predictions for the individual clone breeding values can be produced while accounting for the multiple sources of variation present in multi-environment trails. The resulting breeding values will be more accurate than those obtained by using overly simplified variance-covariance structures, which can potentially lead to biased estimates, and as a result, valuable selections may be overlooked.
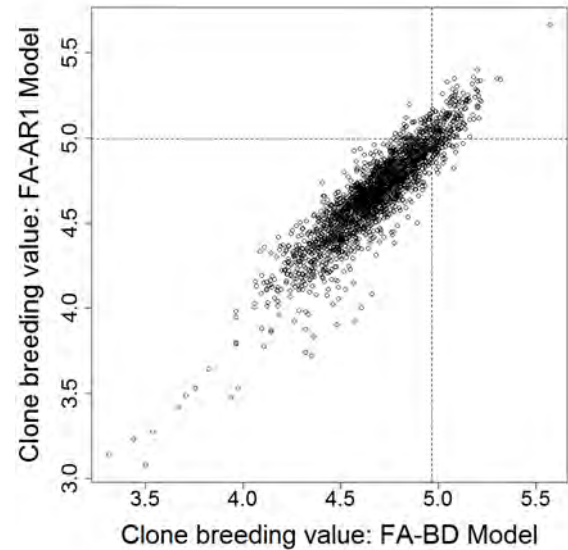

Figure 1. Scatter-plot of predicted height between factor analytic spatial and factor analytic block diagonal models. The dashed lines indicate the 90% quantile for each model. The rank and Pearson's correlation are about 0.91.
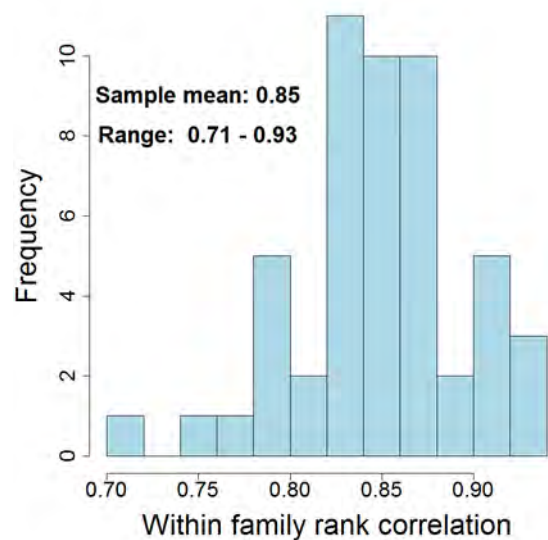

Figure 2. Histogram of family rank correlations based on genetic merit between the factor analytic spatial (FA1-AR1) and factor analytic block diagonal (FA1-BD) models. The within family rank correlations range from 0.71 to 0.93 with an average of approximately 0.85.

Analysis of wood properties in 3rd– Cycle PMX Tests

Wood properties are inherently time-consuming and expensive traits to include in routine evaluations of tree improvement progeny trials. In the 2014 Annual Report, we reported on a pilot study that assessed the use of non-destructive technologies to efficiently sample wood properties traits from third-cycle polymix progeny trials. Following the successful results of the pilot study, we were confident in initiating a more extensive sampling effort for the rest of the population.

In winter 2014, the top two test sites in each of the Coastal PMX series 2, 3, and 4 and Piedmont PMX series 1, were measured for wood quality traits. The pollen mix families were half-siblings from the 3rd-Cycle breeding population. The most recent height and diameter measurements were taken at each of the sites at age 5 or 6 years. Resistograph and TreeSonic measurements were taken at age 8 or 9 years for each site. A total of 280 families in the Coastal population and 74 families in the Piedmont population were sampled. Acoustic stress wave time-of-flight, measured by the TreeSonic, was converted to velocity in m/s, where the interpretation is that a higher velocity implies a higher bending strength (denoted "Velocity"). Relative amplitude from the Resistograph readings was converted into one mean amplitude for each tree (denoted "Resistograph").

ASReml was used to fit the mixed model and produce estimates for individual-tree and family-mean heritabilities (Table 1). Family breeding values for acoustic stress wave velocity and mean relative amplitude were also produced. Additionally, a multivariate model was utilized to estimate genetic correlations among all pairs of traits (Table 2).

There was a positive genetic correlation between relative amplitude (surrogate for wood specific gravity) and growth traits, especially in the Piedmont (r_G=0.70). This is a curious result in view of most previous studies with loblolly pine, where wood quality traits are generally unrelated to volumetric growth traits. These positive correlations suggest that bigger volume trees have higher wood density, which we have never seen in our breeding populations.

Heritability estimates from this round of data collection confirm once again that wood quality traits have a high degree of additive genetic control. Thus, breeding values produced in this analysis can be used for accurate characterization of these families. Breeding values will be uploaded to TIPRoot and can be used by members who want to select families based on wood density and bending strength.

Table 1. *Narrow-sense individual-tree (h^2_{ns}) and family-mean (h^2_{fm}) heritability estimates for each of the four traits in each test series.*

Trait	CPMX1		CPMX2		CPMX3		CPMX4		PPMX1	
	h^2_{ns}	h^2_{fm}	h^2_{ns}	h^2_{fm}	h^2_{ns}	h^2_{fm}	h^2_{ns}	h^2_{fm}	h^2_{ns}	h^2_{fm}
Height	0.30	0.65	0.23	0.48	0.31	0.65	0.15	0.49	0.23	0.49
Volume	0.20	0.54	0.15	0.33	0.17	0.50	0.15	0.46	0.35	0.63
Velocity	0.34	0.73	0.50	0.72	0.18	0.44	0.36	0.76	0.34	0.71
Resistograph	0.15	0.50	0.35	0.70	0.60	0.78	0.25	0.67	0.23	0.53

	Height	Volume	Resistograph	Velocity
Height	-	0.91	0.54	0.17
Volume	0.94	-	0.37	-0.13
Resistograph	0.70	0.70	-	0.09
Velocity	0.09	-0.13	-0.02	-

Table 2. *Estimates of genetic correlations among all traits from fitting the multivariate model. Genetic correlations among traits in Coastal PMX (all 4 series combined) are given above the diagonal and the correlations for Piedmont PMX are given below the diagonal and are shaded gray.*

Freeze Damage and Cold Tolerance of Clones in the Northern and Piedmont Elite Breeding Population[1]

The goal of this project was to look at survival of rooted cuttings from the Northern/Piedmont Sawtimber Elite Population (SEPop) and determine if there were significant genetic differences with respect to cold tolerance. Rooted cuttings from 27 elite crosses and checklots were produced in the spring of 2013 (see p.4 of the 2014 Annual Report). Once the cuttings were well rooted, they were moved outside to the container pad at the NCSU Horticulture Field Lab (HFL) and were grown until the fall. In the fall to early winter 2013, the cuttings remained outside to transition from high summer temperatures to low winter temperatures. In December, the container pad was covered with plastic to protect the trees from the extreme cold temperatures that can occur during winters in Raleigh. As luck would have it, in January 2014, the protective plastic cover on the container pad ripped off during a wind storm. The plastic could not be repaired, nor were we able to obtain a replacement cover. The unusually harsh winter exposed the unprotected cuttings to multiple days of snow, ice, and temperatures down to 9ºF. Mortality became evident during spring 2014 as the cuttings began their second year of growth (Figure 1).

Figure 1. After the January 2014 temperature dipped to 9ºF at the HFL, survival of the rooted cuttings in the Northern/Piedmont Sawtimber Elite Population was 30%. Families ranged in survival from 2% to 80%.

In April / May 2014, survival of the cuttings was assessed for each clone and family. Overall survival was 30%, and families ranged in survival from 2% to 80%. Not only were the differences among families highly significant, but clones within families also varied sig-

nificantly for survival. The origin of the parent trees for the crosses also had an effect on the rooted cuttings' tolerance to cold stress (Figure 2). Families with origins from warmer regions did not survive the winter extremes as well as families with origins from cooler regions.

Fortunately, enough rooted cuttings for 6 reps of the Northern/Piedmont Sawtimber Elite Population clonal tests were protected at the HFL and were delivered to cooperators and established in the field in the winter/spring of 2014. These same clones will be assessed for adaptability, growth, and stem form in the field trials established by Virginia Department of Forestry, Tennessee Division of Forestry, and the North Carolina Forest Service. It will be interesting to see if the clone and family survival at the HFL is related to growth and/or survival in the field.
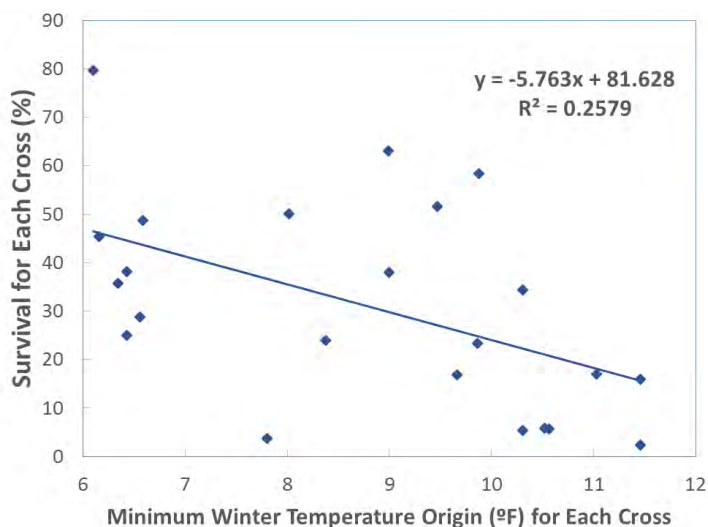

Figure 2. Relationship between the minimum winter temperature origin and survival for each cross in the study.

Comparison of Pollination Bags for Mass Production of Control Cross Seeds in Loblolly Pine

In the spring of 2014, the members and staff of the Co-operative designed and installed a study to compare four pollination bag prototypes. Bags from PBS International were compared to the industry-standard Lawson pollination bag with and without a support wire. Open pollinated (OP) flowers were also added as a control treatment to the study. The main objective is to compare seed yields and seed efficiencies of cones produced from these bags to determine the optimal bag for maximizing production efficiency of control cross loblolly pine seed.

To quantify the utility of each bag, total seed and seed efficiency per bag will be counted after cone harvest in fall 2015. At this point in the study, we have assessments of female strobili (flower) survival at time of bag removal and after the period of "June drop". Based on these preliminary results, there was a highly significant difference between two of the PBS prototype bags and the industry-standard Lawson bags. PBS bags constructed of more rigid material and the Lawson bags with a support wire had the greatest survival of female strobili in June (Figure 1). If the preliminary conelet survival results hold true for seed yield and seed efficiency per bag, these better bags could produce about 25% more seed over the Lawson bags with no support wires. This 25% increase in seed production could also have a large economic impact on the mass production of control cross seeds.

In addition to the cone and seed counts that will be done this fall, another round of testing for this study has already begun to evaluate 4 new pollination bags and a revised pollination bag from last year (Figure 2). These new bags could potentially have an even larger impact on conelet survival and seed yield and could have a big impact on the future of mass production of control cross seeds.

Figure 2. (above) One rep from the 2015 round of the bag study at the Arrowhead Breeding Center. Shown are PBS bags E, F, G, H, B2, and A, and the Lawson bags with and without a wire. The OP treatment is on the lower right hand branch.
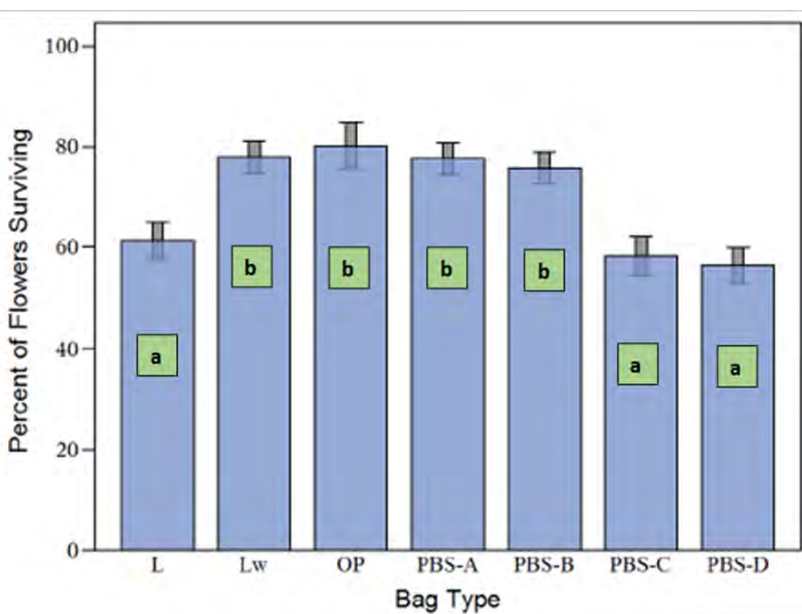

Figure 1. (left) Percent of flowers surviving at the time of the June drop check. The bag type means with the same letter are not significantly different from each other ($P \leq 0.05$).

# Loblolly Pine Biomass Genetics/Cropping Study[1] - Year Three Summary

Loblolly pine is a rapid-growing tree that does not compete with America's food production and may be an ideal feedstock for the developing biofuels industry. Almost 1 million acres of loblolly pine derived from the Cooperative are established each year across the South on wide ranges of sites. Substantial genetic differences exist that will influence biomass/biofuel production. By planting genetically superior trees with desirable biomass/biofuel traits, it may be possible to substantially increase the amount of biomass produced at any given site.

At the NC Department of Agriculture & Consumer Services (NCDA & CS) Umstead Farm in Butner, NC, 20 of the Cooperative's fastest-growing families, each with varying potential for bioenergy feedstock are being tested. Ten of the top volume families adapted to the Coastal and Piedmont regions are included in the study. Five of the 10 families for each seed source were selected based on biomass production only, and the other five were selected for high sawtimber quality as well as for biomass. The Coastal families typically are faster growing than the Piedmont families but are less well adapted to this Piedmont site. By incorporating high-risk Coastal material, will allow evaluation of the risk/reward of planting potentially ideal biomass/biofuel material that are not normally planted in this region.

Additionally, a thinning and no-thinning treatment is incorporated in the experiment. The no-thin plots can be harvested at age 8-10 years to assess genetic differences for biomass/biofuel production, while the thin plots will be thinned to allow the stand to develop into sawtimber. The different thinning regimes will be valuable to demonstrate to forest landowners that planting loblolly pine as a dedicated energy crop will provide options both for early harvest for biomass and/or longer rotations for sawtimber production.

Third year measurements were completed for all 5 reps this year. Height, straightness, and presence of rust, forking, and ramicorns were the traits assessed. Diameters were not taken due to the small size of some trees. As expected, differences between Coastal and Piedmont families, specifically for height and forking, were large (Figures 1 and 2). Most Coastal families were substantially taller than the Piedmont families but

were prone to more forking. For biomass production, poor form is not a major concern. However, if landowners want to have flexibility in managing stands for biomass as well as for sawtimber, then families that survive and grow well with good form will be the most valuable.
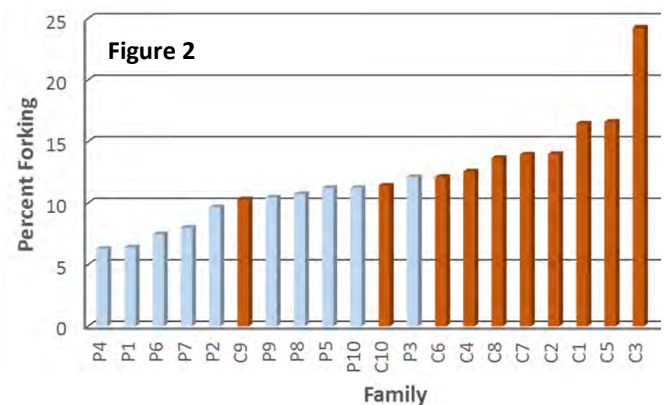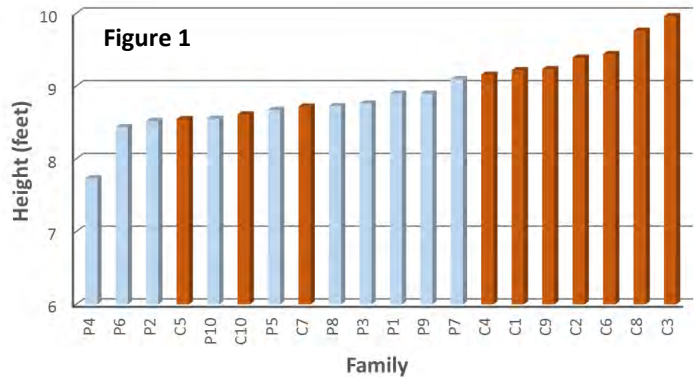
Figures 1 & 2. *Mean height by family(**1**) and mean percent forking by family (**2**). Dark bars represent Coastal families and light colored bars represent Piedmont families.*

Although cold damage was not an issue in the first two growing seasons, shortly after completing measurements this winter, this study site faced two severe ice/snow storms and brutally cold temperatures. Assessments for cold damage and stem breakage will be performed this spring.

The Butner trial is a long-term study that will be maintained through rotation. Harvesting/thinning is planned for about age 10 years so that the biomass and sawtimber yields can be predicted.

Tree Improvement Derivatives[1]

My dissertation research takes an anthropological approach to studying the "institutional ecology" of scientific forestry in the southeastern United States. I am analyzing the relationship between specific *life forms*, in this case loblolly pine trees, and the various social, cultural, symbolic, economic, and institutional *forms of life* that organize and pattern human interactions and their environments. Last year, I spent 6 months with NC State's Cooperative Tree Improvement Program, and I also conducted research in the Bruce Zobel archives at NC State's D.H. Hill Library and at the Forest History Society. In this short article, I would like to analyze aspects of the Cooperative through the concept of the derivative.

Derivatives have at least three forms or meanings: that of a piece of work that is based on another work; that of an instantaneous rate of change; and that of a financial instrument that locks in future prices in the present. As an anthropological study of tree improvement, my research is the first type of derivative in that much of my data is based on things I have heard from stakeholders and members of the Cooperative. I write down and analyze what I have heard or read about the Cooperative Tree Improvement Program and the ways that stakeholders and members represent themselves in brochures, websites, or even Arbor Day programs at the Raleigh Farmers Market. The second meaning of derivative is arguably what tree improvement has always been about, increasing the rate of change in the size of a tree. For my larger project, I am more interested in the second derivative of this rate of change, that is, the acceleration of tree breeding over time. What components of the tree improvement process have changed in speed, and what methodological advances have allowed this to happen? This is an interesting and useful exercise because it now situates tree improvement in other histories, that of the history of genetics in general, that of the history of agricultural science, and that of the history of resource management and economic development. The first meaning of derivative also applies here. "Genomic selection is *derived from* work on pigs," as an example, or "PINEMAP funding is *derived from* concerns over climate change." This approach looks at the external relations of tree improvement rather than internal ones. For the remainder of this article, I will focus on one such external relationship, finance, which is fundamentally concerned with derivatives of the third type, dealing with "future price in the present".

Since the beginning, tree improvement has been a promise of gains in the future. For the companies that made the initial investments in tree improvement, the payout would not be immediate but would come after the trees were cut and turned into a final product. However, the vertically-integrated companies that funded this tree improvement paradigm either no longer exist or no longer exist in their same form. The type of systems thinking that informed the vertically-integrated model put the genes, trees, land, and every other component of the production chain in a part-whole relationship with the organization as a whole. For example, in an International Paper Company publication from 1956 titled *The Forest is the Future*, we see the call for a model that "fixes [the industry] permanently on the land in a program of perpetual development". That is, "a program of perpetual development" relied on the notion of a "fixed" relationship between the land and the industry. In the same year that *The Forest is the Future* was published, the University-Industry Cooperative Tree Improvement Program was founded at NC State. As described by Zobel and Sprague in their 1993 book, *A Forestry Revolution: History of Tree Improvement in the Southern United States*, "the cooperative enabled (and exemplified) a symbiotic relationship between university and industry". What we see here is a set of interrelated relationships - that between the land and the industry, and that between the industry and the university.

Since the 1980s, we have seen the vertically-integrated model break down in many industries as they have been increasingly financialized, which is an organizational model based on the logic of derivatives. This process hit forestry a little later than other industries, beginning in the early 1990s and culminating in International Paper Company selling off its entire land base in the US in 2006. Stated simply, a financial derivative

[1]Kevin Burke is a PhD student in the Department of Anthropology, University of Pennsylvania. This is a brief summary of his dissertation research.

is a contract to exchange something in the future at a price agreed upon in the present. While this may seem straightforward enough, what derivative markets ultimately do is disassemble the part-whole relationships that characterized an earlier moment of industrial capitalism and reassemble the fragments in portfolios traded and priced by levels of "risk" (e.g. Martin, 2013. After Economy? Social Logics of the Derivative. Social Text 31:83-106.). Risk and uncertainty become the defining features of these new bundles whose component parts have no functional interrelationship with each other. This is exactly what happened in the forestry sector, and it is the operating model of Timber Investment Management Organizations (TIMOs) and Real Estate Investment Trusts (REITs).

How did this change in the organization of the industry affect the "symbiotic" relationship with university researchers and the Tree Improvement Cooperative members? Without going into too much detail or mapping the various responses, I would like to highlight the role of the Performance Rating System (*PRS*™) in adapting to this shifting economic landscape. It is no coincidence that in 2006, when International Paper announced its massive land sale, that the Cooperative released the *PRS*™. When the characteristic feature of the organization of an economy is risk and uncertainty, then information takes on new value. It was at this

time that the Cooperative staff and members realized that "there was value in the data" that could be used to attract new non-traditional members (e.g. the Contributing Member option that started in 2007). However, it is not that there was not *always* "value in the data" in the sense that it could be monetized, but that the new institutional arrangements *gave monetary value to the data*. That is, it used to be that there was no "value in the data" that circulated independently of the ownership of land and trees. What we ultimately see is the shift from the information in the data being used to increase the value of a commodity to the information becoming a commodity, where the value is *derived from* a historically-contingent set of institutional and economic relationships. This movement of information becoming a commodity in itself *within* tree improvement is part of larger trends in the global information economy.

Pictured left: *Kevin Burke contemplating Tree Improvement derivatives during a field trip to NCFS during his visit in Spring 2014.*

Grants

New Grant

Steve McKeand, Fikret Isik, and Ross Whetten on a North Carolina Department of Agriculture, Loblolly Pine Biomass Cropping Study: Years 3-5. $40,513. 1/2015-12/2016 with funding likely for 2017.

Continuing Grants

Fikret Isik is a co-principal investigator with Chiang, Ralf, Sederoff, and Ducoste on a NSF Plant Genome grant, Regulation and Modeling of Lignin Biosynthesis. TIP portion is $73,728. 7/2009-6/2015.

Ross Whetten, Steve McKeand, and Fikret Isik. Subcontract with the University of Florida, USDA Coordinated Agricultural Project (CAP) "Integrating research, education, and extension for enhancing southern pine climate change mitigation and adaptation". TIP portion: $867,665. 3/2011 to 2/2016.

Ross Whetten, Steve McKeand, and Fikret Isik. USDA via subcontract with the University of Tennessee. Integrated Biomass Supply Systems (IBSS). TIP portion: $652,369. 8/2011-7/2016.

Fikret Isik is a co-principal investigator with Vincent Chiang on a U.S. Department of Energy grant, Transcription Factor Regulatory Network for Bioenergy: Genotype to Phenotype TIP Portion: $20,223. 9/2011-8/2015.

ASSOCIATED ACTIVITIES

Meetings, Workshops, and Short Courses

Tree Improvement faculty can be in high demand when it comes to teaching and extension around the world. Both Steve and Fikret traveled to Sweden in May 2014 for an extensive workshop hosted by the Swedish University of Agricultural Sciences, Department of Forest Genetics and Plant Physiology and the Umeå Plant Science Centre, Umeå, Sweden. Steve taught Applied Tree Improvement, a course for PhD students, during a 10 day winter blast in Sweden. Fikret followed the next 10 days, with beautiful warm weather, teaching a Quantitative Genetics course along with Christian Maltecca (NCSU Animal Science) to most of the same students. This intensive short course was attended by 11 graduate students, and most of those students and Harry Wu traveled to the US in late February 2015. They stayed a week in the Southeast, where they visited with NCSU faculty, traveled to field sites to see tree improvement in action, and even got to stay in the Arrowhead Chalet as breeding season was ramping up. Steve, with a host of other forestry leaders, spent a week in April at the University of Concepción in Chile and taught sections of a short course on Tree Improvement techniques. Almost 40 students were in attendance for the 2 week short course, so it was a large crowd learning about the benefits of tree improvement and forest management. Steve was also a keynote speaker at the IUFRO– Forest Tree Breeding Conference in Prague, Czech Republic in August 2014.
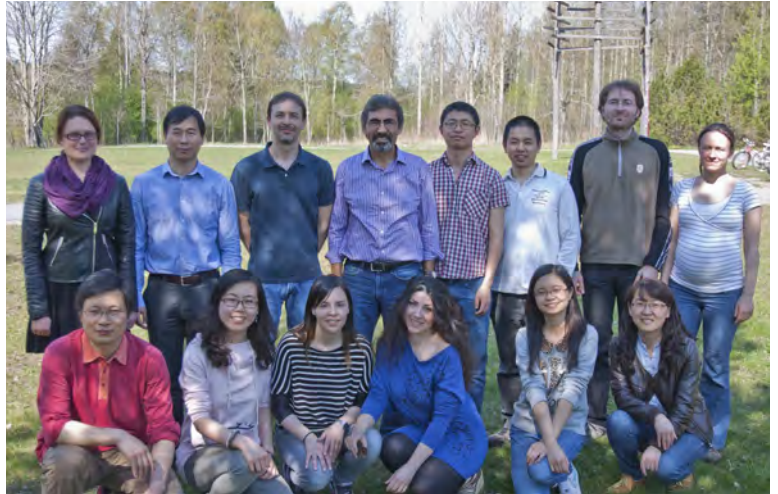
Pictured above: (left) *During the TIP Contact Meeting the group saw a tractor trailer full of wood chips being emptied into an underground transport system that eventually makes it to a transport ship bound for far away places.* ***(right)*** *The workshop taught by Fikret Isik, Christian Maltecca, and Steve McKeand (not pictured) to students at the Swedish University of Agricultural Sciences.*

The annual TIP Contact meeting was held in Savannah, GA with Plum Creek hosting the field tour. As members of the tree improvement community, we LOVE to go into the woods and stare at progeny tests and seed orchards, but this year we shook things up a bit and took a tree improvement tour with a different spin. The group looked at the results of all of their hard work being sent off on ships to faraway places. We had a great tour of the Peeples Industries, Inc., a maritime shipping facility at the Savannah riverfront. There were mountains of pine chips, a truck full of pine chips being unloaded at an 85 degree angle, and a large vessel being loaded with chips and headed for Turkey; we had to hold Fikret back from jumping on the ship to visit the motherland! After the shipping tour, the group headed back to the famous Savannah squares for a walking tour of the history of the squares and urban forestry. Savannah, "The Forest City", is serious when it comes to protecting and treasuring their trees. It was really neat to understand how forestry has played a part in the history of Savannah, and how trees are being protected for future generations…sound familiar?
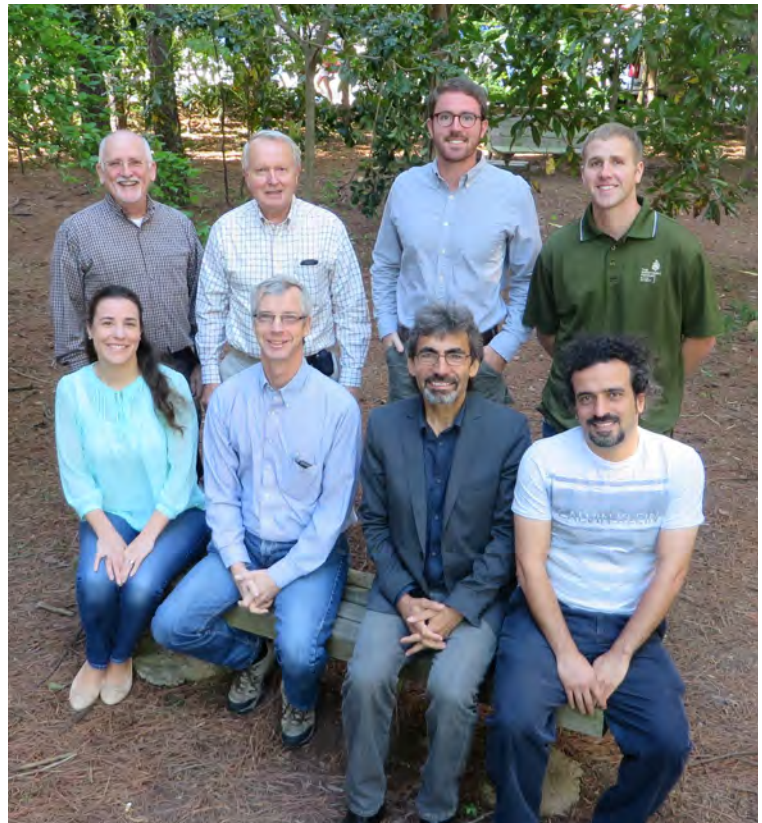
Staff

Last year's annual report was left with "an open search is currently underway for Jadie's replacement titled TIP Operations Manager." Austin Heine started in May 2014 as the new Operations Manager. The minute he walked in everything broke, so he has been repairing, replacing, troubleshooting, and really learning a lot along the way. Austin has really been put through the ringer but has managed to survive his first year of overseeing the greenhouse, lab, and field sites. He manages a great staff of undergraduates who have made significant contributions to our program.

We are happy to report that Fikret did return from his sabbatical in France. We were a little worried the wonderful food and wine would keep him there, but it seems his heart belongs to the southern pines. Upon his return, he was full of ideas and things to do, so we have been chasing him ever since. It is also our honor to announce that Dr. Isik is being promoted to Full Professor in FY 2016, a much-deserved promotion. The group also welcomed the addition of Dr. Yusuf Kurt to the program. Yusuf is filling a yearlong post doc position shared with the Christmas Tree Genetics Program that started in January.

And just as we were thinking all was well and finally getting into a familiar groove, someone came and snatched Jarrod away. In October 2014 we lost Jarrod Morrice, who is still at NC State but is working with the Integrated Pest Management group…he mentioned something about a "dream job" that he went to school for this opportunity, etc. We are sad he is gone but happy he was not far when some database emergencies arose! So after another open search, Jarrod's replacement was found completing his masters degree at UNC-Charlotte in bioinformatics. Chris Ball started May 2015 and has some big shoes to fill, but we know he will do a great job. We are excited to have his skill set around the office!

There are another few folks who are really excited to have Chris onboard. Two grad students with the program really stepped up and helped ease the loss of Jarrod and the arrival of Chris in May. The first student we want to recognize is Andrew Sims. He worked with Jarrod as an undergraduate student and finished his bachelor's degree in Statistics last spring. Andrew has been instrumental in keeping TIPRoot online and moving along with plans we had for additions to the database. The next student is Trevor Walker, who only started in January, and has already jumped in head first to really help out with database implementation and assisting with preliminary designs for the 2016 4th-cycle tests. And one more BIG shout out to Graham Ford, who has had to learn more about database management than he ever wanted. Graham has worked in combination with Andrew and Trevor to keep TIP operations moving. These folks have been vital to the stability of the office during this time and we just want to recognize their over-and-above efforts.

TIP Staff from R to L *(back row): Steve McKeand, J.B. Jett, Graham Ford, Austin Heine, (front row) Tori Brooks, Ross Whetten, Fikret Isik, Yusuf Kurt*

Visitors

When most folks go abroad, they bring back trinkets or food for the group, but Fikret returned from his sabbatical in France with two human beings! The first visitor to arrive was Marjorie Vidal, a graduate student at the French National Institute for Agricultural Research (INRA) and the French Institute of Technology for Forest Based and Furniture Sectors (FCBA) in Bordeaux. She was brave enough to sit in on both of Fikret's data analysis and quantitative genetics courses, and she even fully subjected herself to all homework and exams during her stay. The next visitor was Jerome Bartolome. Jerome had recently completed his PhD at the University of Bordeaux and was working with Fikret while he was at INRA in Bordeaux. As with all of our international students, we enjoy introducing them to American cuisine, but Jerome took it to another level when it came to soft drinks. His love of Coca Cola led him on a search for something new in America...not sure if he found what he was looking for but not for lack of trying. Marjorie was also treated to some hard labor at Arrowhead Breeding Center. She found that she rather enjoyed the Georgia climate, so we may have found our next Arrowhead breeder.

Pictured left: Marjorie Vidal, Jerome Bartolome, and Fikret Isik

Teaching

Each year the faculty associated with the Cooperative teach various courses at both the graduate and undergraduate level. Fikret Isik made up for the lost Spring semester during sabbatical and taught not one, but two high level courses, NR 554 (Introduction to Data Analysis) and FOR/ANS/CS 726 (Advanced Topics In Quantitative Genetics and Breeding) with Jim Holland from Crop Science. Steve McKeand and Ross Whetten teach FOR 411 (Forest Tree Genetics and Biology), our undergraduate tree improvement course, and graduate level FOR 725 (Forest Genetics). Ross also teaches FOR 350 (Professional Development III: Ethical Dilemmas in Natural Resource Management) a discussion-based class on ethical perspectives and issues in natural resource management, and BIT 815 (Analysis of Deep Sequencing Data Analysis) a hands-on introduction to analysis of high-throughput DNA sequencing data in the Linux command-line environment.

Pictured above: Steve's FOR 411/725 class at a field trip hosted by Bobby Smith with NCFS Claridge Nursery in Goldsboro, NC

Graduate Students

Tree Improvement student Jesse Spitzer defended in January 2015 and received his master's degree in May. Congratulations to Jesse for completing his MS thesis entitled "Broad-Based Testing Compared to Local Testing of Families of Loblolly Pine for Resistance to Fusiform Rust and Growth Traits".

We additionally welcomed both a masters and PhD student, Andrew Sims and Trevor Walker, this year. Listed below are the current students:

Greg Albert, MS - Greg joined us in spring of 2012 and has conducted his work at SETRES2. His research is, "Effects of provenance and management on dry biomass yield in loblolly pine".

Alfredo Farjat, PhD candidate in Statistics and Forestry - Alfredo is continuing his efforts on the PINEMAP project titled "The effect of climatic variables on pine growth". He is expected to complete his studies in the summer of 2015.

Adam Festa, PhD – Adam decided to supersize his degree and go straight for a PhD. He continues his research titled, "Modeling methods for prediction of genetic value and breeding efficiency in tree improvement".

Austin Heine, MS - Austin started May 2013; his research is titled "Impact of Improved Loblolly Pine Seedlings in the Southeastern United States since the Conception of the NCSU Tree Improvement Cooperative".

Andrew Sims, MS- Andrew began his graduate program in August 2014. With Jarrod's departure, he set aside his research direction to help out the Cooperative staff and keep up with classes. He hopes to zero in on a project over the summer.

Laura Townsend, MS - Laura's research is titled "Genomic analysis of loblolly pine adaptability to climate conditions". She took a lab position at Expression Analysis, a Quintiles company in RTP in August but is still expected to complete her degree in 2015.

Trevor Walker, PhD- Trevor comes to us from Hancock Forest Management. He started in January 2015, so he has yet to decide on a project but has lots of great ideas, so stay tuned.

Pictured from L to R:

Trevor Walker, Adam Festa, Andrew Sims, Alfredo Farjat, and Greg Albert

Membership in the NCSU Cooperative Tree Improvement Program

It was another busy year for membership in the Cooperative. TIP staff have been busy attending association meetings and meeting with potential members throughout the year. The Contributing Membership level added two new members to the list with the addition of Charles Ingram Lumber Company, Inc. and Meeks Farms & Nursery, LLC. We did say goodbye to a Contributing Member this year, Pacolet Milliken Enterprises, Inc. As always, we deeply appreciate the support of our research and breeding activities by our Cooperative partners listed below.

Full Members

ArborGen, Inc.

Georgia Forestry Commission

Hancock Timber Resources Group

International Forest Company

North Carolina Forest Service

Plum Creek Timber Company, Inc.

Rayonier, Inc.

Tennessee Forestry Division

The Westervelt Company, Inc.

Virginia Department of Forestry

Weyerhaeuser Company

Research Associate Members

American Chestnut Foundation

Arauco-Bioforest, SA

PBS International

US Forest Service

Contributing Members

American Forest Management, Inc.

Campbell Global, LLC

Charles Ingram Lumber Company, Inc.

Dougherty & Dougherty Forestry Services, Inc.

F&W Forestry Services, Inc.

Jordan Lumber & Supply Company

Meeks Farms & Nursery, LLC

Milliken Forestry Company, Inc.

Molpus Woodlands Group, LLC

North Carolina Natural Resources Foundation

ProFOR Consulting

Resource Management Service, LLC

Scotch Land Management, LLC

South Carolina Forestry Commission

Timberland Investment Resources , LLC

White City Nursery, LLC

Z.V. Pate, Inc.

Publications of Special Interest To Members (2012-2015)

From our inception in 1956, there have been over 900 publications written by Cooperative Scientists, Students, and Associates. Below is a list of publications from the last 3 years. The entire list is available at our web site: http://treeimprovement.org/public/publications/publications

2015

Egbäck, S., B.P. Bullock, F. Isik, and S. McKeand. 2015. Height-diameter relationships for different genetic planting stock of loblolly pine at age six. For. Sci. 61(3): 424-428.

Farjat, A.E., F. Isik, B.J. Reich, R.W. Whetten, and S.E. McKeand. 2015. Modeling climate change effects on the height growth of loblolly pine. For. Sci. http://dx.doi.org/10.5849/forsci.14-075.

Ford, G.A., S.E. McKeand, J.B. Jett, and F. Isik. 2015. Effects of inbreeding on growth and quality traits in loblolly pine. For. Sci. 61(3):579-585.

Kim, T.J., B.P. Bullock, and S.E. McKeand. 2015. Spatial autocorrelation among different levels of genetic control and spacings in loblolly pine. For. Sci. 61(3):438-444.

McKeand, S. 2015. The success of tree breeding in the southern US. Editorial in: BioResources 10(1): 1-2.

2014

Chen, H-C, J, Song, J.P. Wang, Y-C Lin, J. Ducoste, C.M. Shuford, J. Liu, Q. Li, R. Shi, A. Nepomuceno, F. Isik, D.C. Muddiman, C. Williams, R.R. Sederoff, and V.L. Chiang. 2014. Systems biology of lignin biosynthesis in *Populus trichocarpa*: heteromeric 4-coumaric acid: coenzyme a ligase protein complex formation, regulation, and numerical modeling. Plant Cell, DOI 10.1105/tpc.113.119685.

El-Kassaby, Y.A., F. Isik, and R.W. Whetten. 2014. Modern advances in tree breeding (Chapter 18). In: T. Fenning (Ed.), Challenges and opportunities for the World's Forests in the 21st Century. Series: Forestry Sciences, Vol 81. 818p.

Isik, F. 2014. Genomic selection in forest trees: prospects and challenges (review paper). New Forests, DOI 10.1007/s11056-014-9422-z.

McKeand, S., J.B. Jett, Tom Byram. 2014. Good wood. Forest Landowners. 73(2): 14-19

Moraga-Suazo, P., L. Orellana, P. Quiroga, C. Balocchi, E. Sanfuentes, R. W. Whetten, R. Hasbún, and S. Valenzuela 2014. Development of a genetic linkage map for *Pinus radiata* and detection of pitch canker disease resistance associated QTLs. Trees Structure and Function 28(6): 1823-1835. DOI 10.1007/s00468-014-1090-2.

Neale, D.B., J.L. Wegrzyn, K.A. Stevens, A.V. Zimin, D. Puiu, M.W. Crepeau, C. Cardeno , M. Koriabine, A.E. Holtz-Morris, J.D. Liechty, P.J. Martínez-García, H.A. Vasquez-Gross, B.Y. Lin, J.J. Zieve, W.M. Dougherty, S. Fuentes-Soriano, L-S Wu, D. Gilbert, G. Marçais, M. Roberts, C. Holt, M. Yandell, J.M. Davis, K.E. Smith, J.F.D. Dean, W. W. Lorenz, R.W. Whetten, R. Sederoff, N. Wheeler, P.E. McGuire, D. Main, C.A. Loopstra, K. Mockaitis, P.J. deJong, J.A. Yorke, S.L. Salzberg, and C.H. Langley. 2014. Decoding the massive genome of loblolly pine using haploid DNA and novel assembly strategies. Genome Biology 2014, 15:R59 doi:10.1186/gb-2014-15-3-r59.

Ögüt, F., C. Maltecca, R.W. Whetten, S.E. McKeand, and F. Isik. 2014. Genetic analysis of diallel progeny test data using factor analytic linear mixed models. For. Sci. 60, 119-127.

Plomion C., E. Chancerel, J. Endelman, J-B. Lamy, E. Mandrou, I. Lesur, F. Ehrenmann, F. Isik, M.C. Bink, J. van Heerwaarden, and L. Bouffier 2014. Genome-wide distribution of genetic diversity and linkage disequilibrium in a mass-selected population of maritime pine. BMC Genomics, 15:171.

Smith, B.C., B.P. Bullock, F. Isik, and S.E. McKeand. 2014. Modeling genetic effects on growth of diverse provenances and families of loblolly pine across optimum and deficient nutrient regimes. Can. J. For. Res. 44: 1453–1461.

Xiong, S.J., S.E. McKeand, R.W. Whetten, and F.T. Isik. 2014. Genetics of stem forking and ramicorn branches in a cloned loblolly pine family. For. Sci., 60(2): 360-366.

Wood, E.R., B.P. Bullock, F. Isik, and S.E. McKeand. 2014. Variation in stem taper and growth traits in a clonal trial of loblolly pine. For. Sci. 61(1): 76-82. http://dx.doi.org/10.5849/forsci.12-068

Zapata-Valenzuela, J., F. Ogut, A. Kegley, W.P. Cumbie, F. Isik, B. Li, and S.E. McKeand. 2014. Seedling evaluation of Atlantic Coastal and Piedmont sources of *Pinus taeda* L. and their hybrids for cold hardiness. For. Sci. 61(1): 169-175. http://dx.doi.org/10.5849/forsci.12-610

2013

Alberto, F.J., S.N. Aitken, R. Alia, S. C. González-Martínez, H. Hanninen, A. Kremer, F. Lefèvre, T. Lenormand, S. Yeaman, R. Whetten, and O. Savolainen. 2013. Potential for evolutionary response to climate change - evidence from tree populations. Global Change Biology. 19:1645-1661.

Aspinwall, M.J., J.S. King, and S.E. McKeand. 2013. Productivity differences among loblolly pine genotypes are independent of individual-tree biomass partitioning and growth efficiency. Trees 27:533–545.

Frampton, LJ., F. Isik, and D.M. Benson. 2013. Genetic variation in resistance to *Phytophthora cinnamomi* in seedlings of two Turkish Abies species. Tree Genetics & Genomes. 9:53-63.

Nelson, C.D., G.F. Peter, S.E. McKeand, E.J. Jokela, R.B. Rummer, L.H. Groom, and K.H. Johnsen. 2013. Pines. *P.* 427-459, *In*: B.P. Singh (ed.). Biofuel Crops: Production, Physiology and Genetics, Chapter 20. CABI Wallingford, UK.

Zapata-Valenzuela, J., R. W. Whetten, D. B. Neale, S. E. McKeand, and F. Isik. 2013. Genomic estimated breeding values using genomic relationship matrices in a cloned population of loblolly pine. G3: Genes Genomes Genetics. doi:10.1534/g3.113.005975.

2012

Aspinwall, M.J., S.E. McKeand, and J.S. King. 2012. Carbon sequestration from 40 years of planting genetically improved loblolly pine across the southeast United States For. Sci. 58:446-456.

Cumbie, W.P., F. Isik, and S.E. McKeand. 2012. Genetic improvement of sawtimber potential in loblolly pine. For. Sci. 58:168–177.

Espinoza, J.A., H.L. Allen, S.E. McKeand, and P.M. Dougherty. 2012. Stem sinuosity in loblolly pine with nitrogen and calcium additions. For. Ecol. and Manag. 265: 55–61.

Isik, F., H.V. Amerson, R.W. Whetten, S.A. Garcia, and S.E. McKeand. 2012. Interactions of Fr genes and mixed-pathogen inocula in the loblolly pine-fusiform rust pathosystem. Tree Genetics & Genomes. 8(1): 15-25.

McKeand, S. and R. Pohl. 2012. The Arrowhead Tree Breeding Center, $483 million present value to the citizens of Georgia and the South. Georgia Forestry Today. 8(1): 24-29.

Zapata-Valenzuela, J., F. Isik, C. Maltecca, J. Wegrzyn, D. Neale, S. McKeand, and R. Whetten. 2012. SNP markers trace familial linkages in a cloned population of *Pinus taeda* – prospects for genomic selection. Tree Genetics and Genomes DOI 10.1007/s11295-012-0516-5.

Loblolly pine is the most widely planted forest tree species in the world. Today, 95% of plantations in the South are established with seedlings from specific individual families; capturing greater gains in yield and value from cooperative breeding programs. In this year's Annual Report, we update results from our USDA NIFA grants that evaluate these loblolly pine families for climate change adaptation (PINEMAP) and bioenergy/biofuel potential (IBSS). Research support like this allows our group to provide more value to landowners and helps ensure a sustainable, reliable, and affordable source of wood for future generations. For more information about these projects please see inside, and go to www.pinemap.org and www.se-ibss.org.

Cover: Seedlings of individual open-pollinated families of loblolly pine at the Virginia Department of Forestry nursery.