

# NORTH CAROLINA STATE UNIVERSITY COOPERATIVE TREE IMPROVEMENT PROGRAM

*58<sup>th</sup> Annual Report | May 2014*



DEPARTMENT OF FORESTRY & ENVIRONMENTAL RESOURCES  
COLLEGE OF NATURAL RESOURCES

**NC STATE UNIVERSITY**



# 58<sup>th</sup> Annual Report

May 2014

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
Jarrold Morrice, Data Analyst	jjmorric@ncsu.edu	919-972-8470
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

As always, the Cooperative continues to evolve, but the big change during the last 12 months was with staffing. Jarrod Morrice, Graham Ford, and Austin Heine are all new additions to the team. Jarrod is Tree Improvement Analyst; Graham is Tree Improvement Manager; and Austin is the brand new Tree Improvement Operations Manager.

### SELECTION, BREEDING AND TESTING

Breeding for the 4<sup>th</sup> Cycle really took off this past year. While the 4<sup>th</sup>-Cycle breeding effort started in 2013 with 51 crosses made in the Coastal region, 32 in the Piedmont region, and 29 crosses in the Northern region, the breeding in 2014 should significantly exceed this. While final numbers are not yet available, some members report as much as 80% of their assigned crosses were done this year.

Final selections coming from 3<sup>rd</sup>-Cycle full-sib block plots to be used in the 4<sup>th</sup> Cycle breeding were made in February of 2014. Based on the 4-year measurements of the Atlantic Coastal Elite (ACE) clonal testing effort, 48 new selections were made based on growth, rust, and stem form. All of these selections were topgrafted at the Arrowhead Breeding Center and should be ready for breeding in 2015 and 2016.

The first of the 4<sup>th</sup>-Cycle progeny tests were planted this past fall and winter with 110 families planted in Coastal tests, 95 families in the Piedmont, and 93 families in Northern tests. The first six reps of the Northern/Piedmont Sawtimber Elite Population (SEPop) clonal test were established by the Virginia Department of Forestry, Tennessee Division of Forestry, and the North Carolina Forest Service. Several series from the 3<sup>rd</sup>-Cycle testing have wrapped up, and wood quality measurements were completed on eight 3<sup>rd</sup>-Cycle tests.

The new **TIPRoot** (Tree Improvement Program Rapid online output tool) database is ready for release so that all **PRS**<sup>TM</sup> databases can be accessed from a single location. **TIPRoot** is being designed as a data archiving tool and will contain the data related to all breeding and testing efforts.

### SEED PRODUCTION

Cooperative members collected more than 30,000 pounds of loblolly pine seed in 2013, a 24% increase in seed yield from the harvest in 2012.

### RESEARCH

We have worked with other PINEMAP investigators to develop a cost-effective, easily-implemented, and high-throughput genotyping method called genotyping-by-sequencing or GBS. We are now using this method to test the hypothesis that genomic regions controlling climate adaption can be identified in families in the Plantation Selection Seed Source Trials.

We are creating a computer simulation program that can simulate multiple generations of a given breeding strategy utilizing marker and transcriptome data of parents to predict: 1) progeny genetic values (phenotype) and 2) potential long-term risk of inbreeding depression.

When comparing half-sib families that were tested for resistance to fusiform rust in local regions as well as across broad regions, it appears that local testing for rust resistance is a reliable predictor for resistance across a broad range of sites. Locally derived rust breeding values generally hold true across a broad range of sites.

A new model to predict climate change effects on the growth of loblolly pine families across the Southeast will allow us to identify the most productive and resilient families for deployment to balance risk and potential productivity on specific sites.

### ASSOCIATED ACTIVITIES, GRADUATE STUDENTS, COOPERATIVE STAFF, MEMBERSHIP

We gained one new Research Associate member. There are 11 Full Members, 16 Contributing Members, and 4 Research Associate Members in the Cooperative. Students were taught in numerous classes. We continue to teach short courses and workshops to members and colleagues around the world.

## TABLE OF CONTENTS

<b>Executive Summary</b> .....	i
Table of Contents .....	ii
<b>Tree Improvement Cooperative-Tree Improvement and Sustainability</b> .....	<b>1</b>
<b>Selection, Breeding, and Testing</b> .....	<b>2</b>
Fourth Cycle Breeding and Testing Progress .....	2
Clonal Testing of Northern and Piedmont Elite Populations .....	4
Progeny Testing Status .....	5
<b>PRS<sup>TM</sup></b> Updates and introducing <b>TIPRoot</b> .....	7
Analysis of wood properties of Coastal Polymix 1 test series .....	9
Clonal Selections from ACE Population .....	11
<b>Seed and Cone Yields</b> .....	<b>12</b>
<b>Research</b> .....	<b>13</b>
Genomic Analysis of Loblolly Pine Adaptability to Climate Conditions .....	13
Modeling methods for predictions of genetic value and efficiency in tree improvement.....	15
Broad-based testing compared to local testing of loblolly pine families for resistance to fusiform rust .....	17
Modeling climate change effects on growth of loblolly pine families across the southeastern U.S. ....	19
Grants Received.....	22
<b>Associated Activities</b> .....	<b>22</b>
Meetings, Workshops, and Short Courses.....	22
Staff .....	24
Visitors.....	25
Teaching.....	25
Graduate Students.....	26
Membership .....	27
Publications of Special Interest to Members .....	28

## TREE IMPROVEMENT AND SUSTAINABILITY

### *A MESSAGE FROM THE DIRECTOR*

Our graduate students in the Cooperative Tree Improvement Program recently submitted a nomination to a University program to recognize the Cooperative for its long-term and dedicated commitment to sustainability and forestry in the southeastern US. I was so impressed by this effort that I thought I would share it verbatim.

The Cooperative and its diverse group of members have been committed to increasing the productivity and value of southern pine forests for today and for future generations since the mid 1950s. The Cooperative conducts research and manages breeding programs that are responsible for developing the genetic material that is planted on approximately 800,000 acres every year in the Southeast. While these planted pine forests compose about 15% of forest lands in the South, they provide over 50% of the timber harvested in this region. These managed forests are a renewable and sustainable source of timber for our homes, paper for our schools and businesses, biofuels to run our factories, and jobs for our communities. Genetically improved plantations promote sustainability by allowing more wood to be produced on less land, thus reducing harvest demands on more ecologically-sensitive forests and enhancing the ecosystem services that they provide. On top of the additional 3.7 billion m<sup>3</sup> or 17% increase in wood production that landowners have realized from the last 40+ years of genetic improvement of southern pines, the Cooperative estimates that these improved plantations have sequestered an additional 1,100 Tg or 13% more carbon compared to non-improved plantations (see Aspinwall 2012, *Forest Science* 58(5):446–456).

Another excellent example of sustainability in the Cooperative is the work of our tree breeders in the breeding program. We have a very large and diverse population of trees that are managed for both short-term economic benefit and for long-term gain over many generations. Breeders actually improve the base resource and do not constrain the opportunities of future generations. While one of the main goals of the Cooperative is to increase productivity for classic timber products, much research is done in the areas of biofuels, climate change, genomics, and species restoration as evidenced by our work with longleaf pine.

The Cooperative not only works with its members but also forest managers, researchers, and community leaders in North Carolina, the Southeast, and internationally. In addition to educating forest landowners, private industry, and state agencies, undergraduate and graduate students at NCSU work and study with the Cooperative. These students are trained and educated to become leaders that will promote, sustain, and advance this legacy for future generations.

With 70% percent of the forest land in the Southeast owned by non-industrial private landowners, the increased productivity from improved genetics provides landowners with an economic incentive to keep their land in working forests and out of real estate development. The Cooperative's work is an extremely important part of the larger forest management effort in the Southeast that ensures there will be working and native forests for future generations to enjoy and derive benefits.

Thank you Austin, Adam, Alfredo, Greg, Laura, Jesse, and Graham for leading this effort. It's comforting to know that our future will be in good hands with leaders like you.

*Steve McKeand- May 2014*

## SELECTION, BREEDING, AND TESTING

### Fourth Cycle breeding and testing progress

#### *Breeding*

The breeding season of 2013 was the starting point for the 4<sup>th</sup> Cycle in the Cooperative. With the transition away from the 3<sup>rd</sup> Cycle, plans to begin the 4<sup>th</sup> Cycle became a reality last year. However, before the 4<sup>th</sup> Cycle breeding assignments could be accomplished, the moving pieces needed to be assembled and readied for action, so the breeding season of 2013 was predominantly a staging year. There was a large effort in locating ramets of the clones desired for breeding. Clones that were not already in breeding orchards needed to be topgrafted so crosses could be made. Members of the Cooperative also began to collect the pollen required for the breeding plans.



As for actual breeding toward the 4<sup>th</sup> Cycle in 2013, 51 crosses were made in the Coastal region, 32 in the Piedmont region, and 29 crosses in the Northern region. Of the approximately 1600 planned crosses to be done, 51 may not sound like much. But, if one lesson is to be learned from the long breeding cycles the Cooperative has seen in the past, time is invaluable. Small strides add up to significant amounts of work accomplished through the course of a breeding cycle. Every little bit of effort and time spent will pay large dividends with the approach we are taking in the 4<sup>th</sup> Cycle.

We are coming to the end of a breeding season that will go into the record books. After a long winter, breeding in 2014 did not begin until two to three weeks later than in most years. Several cooperators in the Northern region were wrapping up their breeding efforts well into late April. Despite the significant challenge of cold weather during this breeding season, Cooperative members had their breeding assignments and were ready to get to work. Thankfully, the preparation that was done last year in collecting pollen and locating clones for breeding allowed our members to accomplish a significant portion of 4<sup>th</sup>-Cycle breeding this year! Some members report as much as 80% of their assigned crosses were done this year. This is outstanding!

As topgrafts begin to produce strobili and bear pollen in the upcoming breeding seasons, the Cooperative should wrap up the majority of the 4<sup>th</sup>-Cycle breeding by 2016. Assigned crosses still yet to be completed will hinge on production of strobili from top-grafts and obtaining the needed pollen. Throughout the tough breeding season this year, the Cooperative members and staff have been working tirelessly on the logistics of the breeding program to guide us towards completing the breeding within the next two years.

*Pictured left: Despite the fact that Russ Pohl retired in January, he can't get breeding out of his system. Here he is testing pollen for moisture content before using for 4<sup>th</sup>-Cycle pollinations.*

### 4<sup>th</sup>-Cycle Selections

The majority of block-plot forward selections were made in January and February of 2013. Families were selected by indexing the breeding values of volume, straightness, and rust (VSR). Within-family selection was performed after ranking individual trees by their VSR values. Final selections coming from these full-sib block plots to be used in the 4<sup>th</sup> Cycle were made in February of 2014. The handful of selections made in February 2014 were subsequently grafted at the Arrowhead Breeding Center at the Georgia Forestry Commission's Arrowhead Seed Orchard in Cochran, GA. Breeding on these and last year's block plot selections will take place as they produce strobili.

Following the analysis of the Atlantic Coastal Elite (ACE) clonal progeny tests, a total of 48 selections were made from this population. In the field, scion were collected from the ACE test sites on Georgia Forestry Commission and Plum Creek land. Scions were also collected from ramets in the Breeding Ramet Orchard (BRO) at the Arrowhead Breeding Center.

Selections from the ACE tests were topgrafted at the Arrowhead Breeding Center in February and March of 2014. This was a large effort during this year's breeding season. A total of 336 topgrafts from ACE clones were made. Along with routine archiving and re-grafting important selections, the Cooperative staff made over 500 topgrafts in February and March this year. As the grafts of these superior genetics begin to produce strobili, the large breeding effort with these selections will begin. We have high hopes for the future of the progeny that will come from these exceptional crosses!

### Testing

The first progeny tests of the 4<sup>th</sup> Cycle were planted by cooperators this fall and winter. The 2014 testing effort was comprised of 8 Coastal, 5 Piedmont, and 7 Northern tests. In the Coastal tests, there were 110 families. The Piedmont tests were made up of 95 families. And in the Northern tests, 93 families were tested (more details on page 5).

To adhere to the new testing approach in the 4<sup>th</sup> Cycle, assembling the seed that will produce the tests for sowing in 2014 and field planting in 2015 was quite a logistical undertaking. The plan is to test families across



*Pictured above: Austin Heine completing his first topgraft in the New Breeding Orchard (NBO) this spring.*

multiple regions and across multiple years while linking the series in a region within a given year. In the Coastal region, only Coastal crosses are being tested, so Coastal tests are straightforward for the most part. In the Piedmont and Northern regions, families from all three regions will be present in the tests. Therefore, the testing load in the Piedmont and Northern regions expanded to the point that two series are needed to handle all of the families. Within a given year, the test series within a region will be linked by common families to uphold the need for connectivity throughout the 4<sup>th</sup> Cycle testing effort.

Initially, to achieve the 60 planted seedlings for each cross, we planned to test 40 in the first year and 20 in the second year. A slight change to this approach was borne out of necessity for simplicity. Our approach in forthcoming years will be to get 60 seedlings per cross to the field by planting 30 seedlings in each of two years.

All told, 2015 will be a large testing year. Our cooperators are growing 137 families for the Coastal tests, 178 families for the Piedmont tests, and 170 families for the Northern tests.

## Clonal Testing of Northern and Piedmont Elite Populations

In winter/spring 2014, the first 6 reps of the Northern/Piedmont Sawtimber Elite Population (SEPop) clonal tests were delivered to cooperators and established in the field. The Virginia Department of Forestry, Tennessee Division of Forestry, and the North Carolina Forest Service each planted two reps this year, and the plans are for four more reps to be planted next year. Rooting in the winter/spring of 2013 was generally good (Table 1), and we had excellent quality trees ready for the field.

**Table 1.** Average rooting and number of cuttings rooted per clone.

	Avg. Rooting Percentage	Avg. Number of Cuttings Rooted Per Clone
<b>Northern</b>	81%	19
<b>Piedmont</b>	64%	11

An alpha cyclic row-column incomplete block experimental design is being used for the clonal trials. For each rep, there were 700 trees that included between 645-647 cuttings from 19 crosses (16 Northern and 3 Piedmont), 43 seedling family checks, 4 northern cutting checks, and 6-8 fillers in each rep.



*Pictured above: Northern/Piedmont Elite test in the back (green) and 4<sup>th</sup>-Cycle seedlings (purple) at the VDF planting Site. As you can tell it was cold in Virginia!*



*Above: Josh Steiger, former Tree Improvement Manager, working with our undergraduate crew (Harris Kelly, Todd Labarr, and Teresa Bobbitt) sorting and inventorying SEPop cuttings at the HFL in June 2013. Thanks to Josh, Jadie Andrews, and all the crew, we had excellent propagules for the 2014 field trials.*

In each test installation there were 35 rows by 20 columns with a 6ft x 10ft spacing giving us about one acre per rep plus any additional space needed for borders.

The original plan for this study was to send out the final four reps next year, but Mother Nature changed these plans. Shortly after covering the container holding pad at the Horticulture Field Lab, where the cuttings that were not being sent out this year were being stored, strong winds caused the plastic to rip, and it had to be removed. The winter this year was brutal, and several nights close to 9°F took their toll on the cuttings. The new plan is to retake cuttings for the final four reps this spring and deliver the final reps to cooperators next spring.

The reason for using this clonal testing is to increase the within-family heritabilities of the Northern/Piedmont Sawtimber Elite Populations. By testing these clones and having more confidence in their within-family performance, we will have even more confidence that any selections made from this test will have superior sawtimber qualities.



## Progeny Testing Status

This past year was another big one in terms of test establishment and measurement collection. The first set of 4<sup>th</sup>-Cycle tests were established, several series from the 3<sup>rd</sup>-Cycle testing have wrapped up, and wood quality measurements were taken on several 3<sup>rd</sup>-Cycle tests.

### Test Establishment

Testing for the 4<sup>th</sup> Cycle has officially begun with the establishment of the first 20 tests in 2014. The number of tests established is more than originally planned because of a slight design change in the 4<sup>th</sup>-Cycle testing. More of an emphasis has been put on reducing the size of individual tests, thus increasing the overall number of tests. There were 8 tests established for the Coastal breeding region, 7 for the Northern region, and 5 for the Piedmont. Although tests are being assigned to specific breeding regions, selections within a test may have crosses from outside that region. This has been done to unify the breeding regions, so that comparisons of selections between regions can be made.

The majority of the remaining **PRS**<sup>TM</sup> Calibration Tests for the Performance Rating System (**PRS**<sup>TM</sup>) were established this year. This series of tests is a long-term study to calibrate the values within the **PRS**<sup>TM</sup> database. This study will link the 4- to 6-year data that we routinely collect for progeny trials to rotation age measurements. In turn, this will provide a better **PRS**<sup>TM</sup> value for estimating the performance of rotation age trees. There is only one Calibration Test that remains to be established in 2015. Measurements on these tests are scheduled to occur at ages 1, 3, 6, 12, 18, and 24 years.

Genetic Value - Breeding Value (GV-BV) tests were established this year. This set of tests is being used to validate the assumption that a genetic value derived from clonal testing is highly correlated with a breeding value for the parents that were selected based on clonal performance and whose breeding value is then derived from open-pollinated testing. These tests are planned to be measured at ages 5-6 years.

We have established the first 6 replicates (out of 10) for the Northern Elite (NE) clonal test. This test, which is similar in scope to the Atlantic Coastal Elite (ACE) test, clonally tests families from the Northern and Piedmont regions. By clonally comparing the siblings

within a family, we are able to increase the accuracy of our assessment on a single genotype, which will help ensure the selection of the best individuals from a family.

Other than the 4 NE replicates and the single **PRS**<sup>TM</sup> Calibration test to be established in 2015, establishment of tests in the foreseeable future will be related to 4<sup>th</sup>-Cycle testing (see Table 1). About 30 4<sup>th</sup>-Cycle tests are expected in 2015. The number of 4<sup>th</sup>-Cycle tests that will be established beyond 2015 is dependent on the progress of breeding and the number of seed that will be in hand for any particular year.

**Table 1.** Status and Plan for Test Establishment

Series	Pre-2014	2014	2015	2016
North. Elite		6	4	
GV-BV		4		
4 <sup>th</sup> -Cycle		20	30	?*
555C	9	1	1	
555P	5	2		
555N	4	1		
Total	18	34	35	?

\* The number of tests to establish for the 4<sup>th</sup>-Cycle testing in 2016 and future years will depend on the progress of the breeding. Series 555 is the **PRS**<sup>TM</sup> Calibration Study.

### Test Measurements

Standard measurements for height, DBH, rust, forking, ramicorn branches, and straightness have been taken for several of the 3<sup>rd</sup>-Cycle polymix (PMX) series. The test measurements collected were from series 3 and 4 in the Coastal PMX (i.e. CPMX3 and CPMX4, respectively) and series 2 and 3 in the Piedmont PMX (i.e. PPMX2 and PPMX3, respectively). All the tests within the series CPMX3, CPMX4, and PPMX2 have been measured at age ~6 years. Three tests from PPMX3 still require measurements, which will be done within the next 2 years.

Measurements for the Atlantic Coastal Elite (ACE) clonal series were performed this year at age ~4 years. A summary of these data can be found on page 11.

The first round of wood quality assessments have been taken on the 3<sup>rd</sup>-Cycle progeny tests this past year. TreeSonic and Resistograph measurements were taken to obtain estimates of bending strength and wood density for each tree. The series that have been measured include the Coastal PMX series CPMX1, CPMX2, CPMX3, and CPMX4 and PPMX1 series from the Piedmont PMX testing effort. Two tests from each series are being measured, and we plan to sample wood quality from the remainder of the 3<sup>rd</sup>-Cycle PMX series. We will use growth, survival, and rust information to determine which tests within the series are optimal for performing wood quality measurements.

The number of tests that are planned for measurement (not including year 1 survivals) in the upcoming years is shown in Table 2. The column 'Orig. # Tests' shows the number of tests that were established for a particular series. The 'Expected Tests' column is similar, but removes tests that had survival issues and were abandoned. This plan is based on 4 year measurements except for Northern and Tennessee tests which are at 5 years, and the **PRS**<sup>TM</sup> Calibration measurements which have an alternate schedule. Series 555 is the **PRS**<sup>TM</sup> calibration study and the letters following indicate the region.

**Table 2.** Measurement schedule for Cooperative tests in the coming years.

Series	Pre- 2014	2014	2015	2016	2017	2018	2019	Orig. # Tests	Expected Tests
CPMX1	14							15	14
CPMX2	7							10	7
CPMX3	3	5						10	8
CPMX4	1	5						8	6
CPMX5				5				5	5
CPMX6					3			3	3
PPMX1	7							8	7
PPMX2		7						7	7
PPMX3		3	2	1				6	6
PPMX4					2			2	2
NPMX1	4							4	4
NPMX2			4					4	4
ACE		7						8	7
NE							6	12	12
555C		9	3	9	3		9	12	12
555P		5	2	5	2		5	7	7
555N		4	1	4	1		4	5	5
GV-BV						4			
4 <sup>th</sup> Cycle						13	7	20	?
<b>Total</b>	<b>36</b>	<b>45</b>	<b>12</b>	<b>24</b>	<b>11</b>	<b>17</b>	<b>31</b>	<b>146</b>	

## **PRS**<sup>TM</sup> Updates and introducing **TIPRoot**

The Loblolly Pine Performance Rating System (**PRS**<sup>TM</sup>) database has become an important tool for many of our Cooperative members. The **PRS**<sup>TM</sup> database summarizes all the test data that the Cooperative has collected to provide a standardized platform to compare selections of loblolly pine. Simply put, **PRS**<sup>TM</sup> shows which selections are ‘bad’ and which are ‘good’ depending on deployment needs. This system has become an important tool for marketing genetics to landowners as well as a reference for our cooperators in determining which trees to plant or establish in seed orchards.

There is a **PRS**<sup>TM</sup> database for the ‘Atlantic Coastal’, ‘Piedmont’, and ‘Northern’ regions, and the selections within each of the databases have largely been separated from the other regions. The boundaries between these databases will eventually be broken as we start receiving data for 4<sup>th</sup>-Cycle tests, but for now we are keeping them separate. As we receive new data from field tests, we continually update the **PRS**<sup>TM</sup> databases to reflect the recent analyses we have performed with the new data. Within the last year, we have released an updated version of the Piedmont **PRS**<sup>TM</sup> database, and we plan to release three more in the coming year.

In fall 2013, we released the Piedmont **PRS**<sup>TM</sup> 2013 Version 1 database in Microsoft Access. This database included additional information from the first 2 Piedmont PMX test series in the 3<sup>rd</sup> Cycle. This database contained an additional query, compared to previous versions of this database, that allows the user to determine the appropriate counties to plant a selection based on the county’s minimum temperature and the user’s desired level of cold-damage risk. This query is a good solution for those who have an exceptional selection and need to find the appropriate locations, in terms of temperature, to plant it.

The next **PRS**<sup>TM</sup> database to be released will be for the Northern region. This will be the first database to be released exclusively on our new online database system, **TIPRoot** (Tree Improvement Program Rapid online output tool). As of this writing, we are in the process of developing **TIPRoot** so that all **PRS**<sup>TM</sup> data-

bases can be accessed from a single location. As soon as this functionality has been built and tested, the new Northern **PRS**<sup>TM</sup> database will be released to our Cooperative Members. Cooperators who are interested in the results of the current Big BLUP analysis (see the 52<sup>nd</sup> and 53<sup>rd</sup> Annual Reports for a description of the “Big BLUP” analysis) for this Northern region can obtain the breeding and genetic values on our website ([TreeImprovement.org](http://TreeImprovement.org)).

In addition to releasing the Northern **PRS**<sup>TM</sup> on **TIPRoot**, we will migrate the previous **PRS**<sup>TM</sup> databases, released in Microsoft Access, into the online database. By keeping all the regional **PRS**<sup>TM</sup> databases within the same platform, (i.e. **TIPRoot**), NCSUCTIP will be able to ensure that the same queries and functions are maintained for all the **PRS**<sup>TM</sup> databases that we have implemented. This should make it simpler for those who use multiple regions to switch between databases. In addition, this platform will also enhance the updating process of the **PRS**<sup>TM</sup> values. The results from the Big BLUP analysis can be quickly uploaded to the system without the need to redevelop or copy queries from other databases, as was done with previous versions in Access.

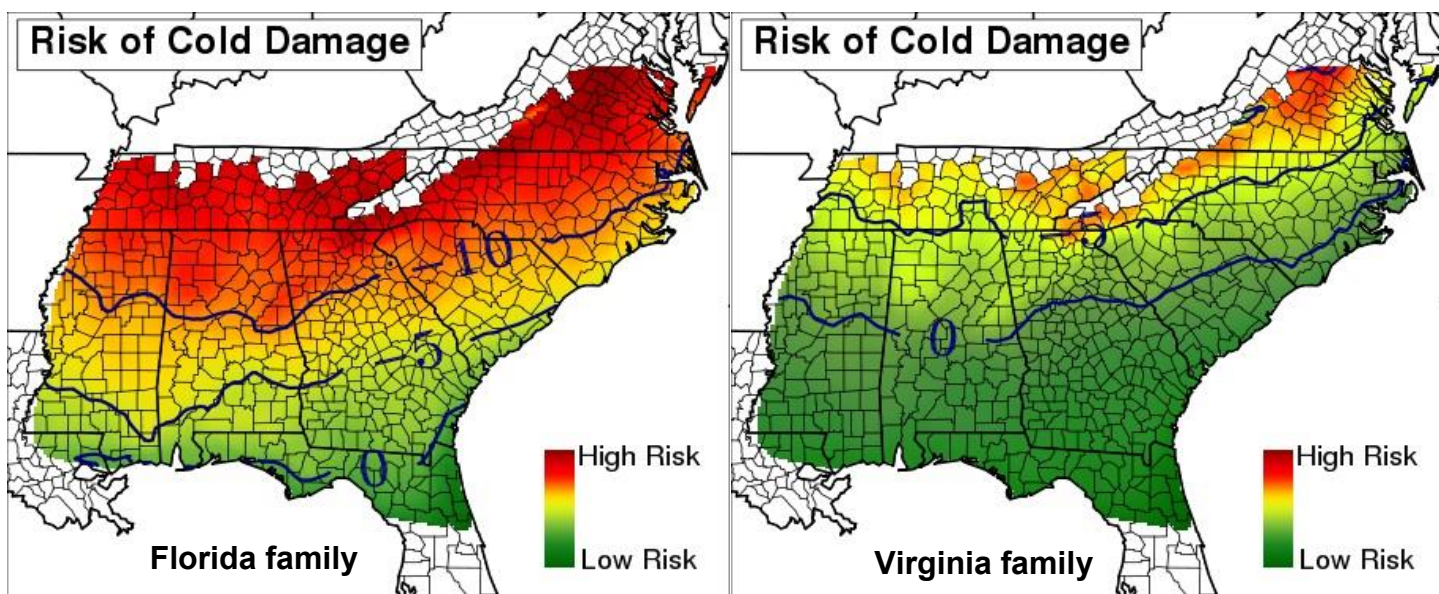
In addition to the database, users of **TIPRoot** are also allowed to create their own **PRS**<sup>TM</sup> Spec Sheets. NCSUCTIP has been creating **PRS**<sup>TM</sup> Spec Sheets for Cooperators for many years, but now Cooperators are able to generate these sheets on their own and within a matter of seconds. **PRS**<sup>TM</sup> Spec Sheets conveniently summarize the **PRS**<sup>TM</sup> scores and cold tolerance adaptability (Figure 1) for a family or a group of families. The **PRS**<sup>TM</sup> Spec Sheet is great for marketing genetics for our cooperative members, allowing a cooperator to provide their customers an easy-to-use summary for selecting the appropriate families to deploy.

There is much more that we are looking to build into **TIPRoot**. **TIPRoot** is being designed as a data archiving tool and will contain the majority, if not all, the data related to NCSUCTIP’s breeding and testing efforts. One of the projects currently on our list of developments is creating a system for tracking the breeding

efforts during early spring. Using this system, Cooperative members would have the critical information they need from NCSUCTIP in order to conduct their breeding efforts related to the 4<sup>th</sup> Cycle.

We are very excited to bring our Cooperative members these additional tools through the **TIPRoot**, and always welcome their feedback and suggestions on how we can improve these tools.

**Figure 1.** Examples of new maps generated in the database for the **PRS<sup>TM</sup>** Spec Sheets. The left map shows the risk of cold damage for a family that comes from Florida; the map on the right shows the risk of cold damage for a family that comes from Virginia (both would be from open-pollinated seed from orchards located in the Southern Atlantic Coastal Plain). Planting in the green shaded areas on the map up to 5°F colder (south of -5° line) has minimal risk of cold damage. Planting in areas that are 5-10°F colder than the origin (between -5° and -10° lines) will increase the risk of cold damage. Areas that are more than 10°F colder than the origin are too cold, and planting is not advised (north of -10° line)<sup>1</sup>.



<sup>1</sup>These adaptability guidelines were developed by the USDA Forest Service (Schmidting 2001), *Southern Pine Seed Sources*, available at: [http://www.srs.fs.usda.gov/pubs/gtr/gtr\\_srs044.pdf](http://www.srs.fs.usda.gov/pubs/gtr/gtr_srs044.pdf)

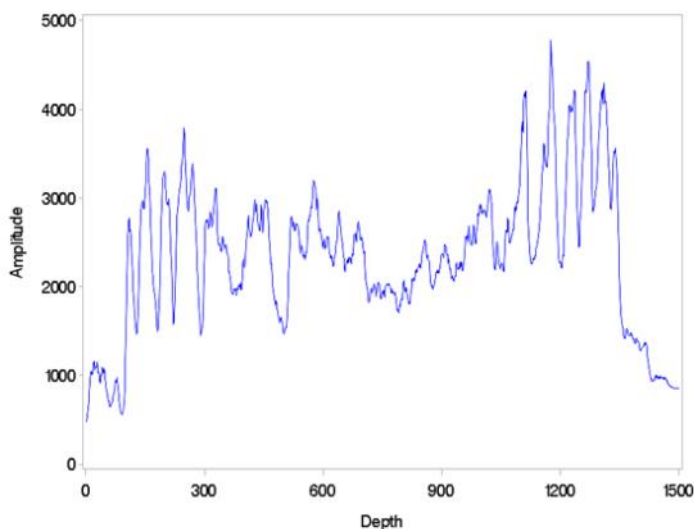
## Speaking of risk of cold damage...

Much of the Southeast U.S. experienced an unusually cold winter. Raleigh got several inches of snow and ice over multiple periods. Due to some unfortunate turbulent weather in the fall, our protective cover blew off the pad shortly after it was installed, leaving the Northern Elite hedges and cuttings to the mercy of Mother Nature. While we were not trying to screen for cold tolerance we found many of the hedges and cuttings did not take the freezing too kindly. Luckily several did make it, and for those that did not, we had back up trees/families in reserve for such an event. New cuttings will be propagated in the coming year.



## Analysis of wood properties in Coastal Polymix 1 Test Series

Wood strength and density are among the most important properties related to the value of wood products. However, traditional sampling procedures for these metrics inherently require time-consuming, expensive lab work which have been major impediments to incorporating these traits into tree breeding programs. Fortunately, there are now efficient and rapid procedures to sample these wood properties (e.g. Eckard et al. 2010 *Forest Science*, 56(3), 233-241; Li et al. 2007 *Canadian Journal of Forest Research*, 37(10), 1886-1893). The Fakopp TreeSonic measures acoustic stress-wave velocity which has a strong genetic correlation with bending strength. The IML Resistograph is a micro-drilling tool that measures drilling resistance across the cross-sectional profile of a tree (Figure 1). The drilling resistance, measured in amplitude, was strongly correlated with wood specific gravity (Isik and Li 2003 *Canadian Journal of Forest Research*, 33(12), 2426-2435). Use of these non-destructive techniques have been promising for reducing time and cost of selecting genotypes with superior wood properties. We recently evaluated the new IML Resi PD400 for determining specific gravity and to determine family breeding values for acoustic stress-wave velocity, based on the Fakopp TreeSonic. We wanted to verify the value of using this new equipment. Two test sites were selected from the Coastal



**Figure 1.** Cross-sectional display of Resistograph micro-drill resistance (amplitude)

Polymix1 test series. The ArborGen site was near Lumberton, NC, and the Plum Creek site was near Pembroke, GA. A total of 70 half-sib families were tested in 20 replications at each of these two sites. Test sites were established in 2005. Growth measurements were collected at age 6 and 7. Wood property assessments were taken at age 7 and 8.

Acoustic stress-wave velocity was collected with a Fakopp TreeSonic. The average time of flight for three measurements per tree was taken and converted to velocity ( $\text{km}^2/\text{s}^2$ ). All trees were drilled with the Resistograph from bark to bark at breast height (4.5 feet from ground level). A SAS macro was used to calculate an average resistance amplitude per tree. To correlate the amplitude values with specific gravity values, 12mm, bark-to-bark cores were taken at breast height with a gas-powered drill, and volumetric specific gravity was determined.

The following linear mixed model was used to analyze height (HT), volume (VOL), stress-wave velocity (SWS), Resistograph amplitude (Amp), and specific gravity (SG).

$$[1] \quad y_{ijkl} = \mu + S_i + B(S)_{j(i)} + F_k + FS_{ki} + E_{ijkl}$$

where,  $\mu$  is the overall mean,  $S_i$  is the fixed  $i$ th site effect,  $B(S)_{j(i)}$  is the random  $j$ th block effect within the  $i$ th site,  $F_k$  is the random general combining ability of the  $k$ th family,  $FS_{ki}$  is the random interaction between the  $k$ th family and  $i$ th site,  $E_{ijkl}$  is the random error term.

Narrow-sense and family-mean heritabilities for each trait indicate that all the traits were under moderate to strong genetic control (Table 1). Narrow-sense heritabilities were encouraging, ranging from 0.18 for amplitude to 0.68 for specific gravity. Half-sib family mean heritabilities ranged from 0.56 (amplitude) to 0.87 (specific gravity). Since our emphasis is on identifying superior families for bending strength and wood specific gravity, the relatively strong family-mean heritabilities indicate that family selection for these traits will be effective.

Family breeding values were obtained for each trait after running the linear model. Using a bivariate model

**Table 1.** Narrow-sense heritability,  $h_i^2$ , and half-sib family mean heritability,  $h_{HS}^2$ , are given for wood properties traits and growth traits.

Trait	$h_i^2$	$h_{HS}^2$
Stress-Wave Velocity	0.29	0.70
Amplitude	0.18	0.56
Specific Gravity	0.68	0.87
Height	0.35	0.69
Volume	0.25	0.61

in ASReml, attempts were made to calculate genetic correlations for all pairs of traits. Due to model convergence issues, genetic correlations only between SG-Amp and SWS-Amp were calculated with bivariate models (Table 2). Therefore, correlations for other traits are based on family breeding values.

**Table 2.** Correlation among breeding values for each pair of traits\*.

	<u>Amp</u>	<u>SWS</u>	<u>SG</u>	<u>HT</u>
<u>SWS</u>	0.26			
<u>SG</u>	0.90	0.38		
<u>HT</u>	0.49	0.14	0.17	
<u>VOL</u>	0.34	-0.13	-0.07	0.82

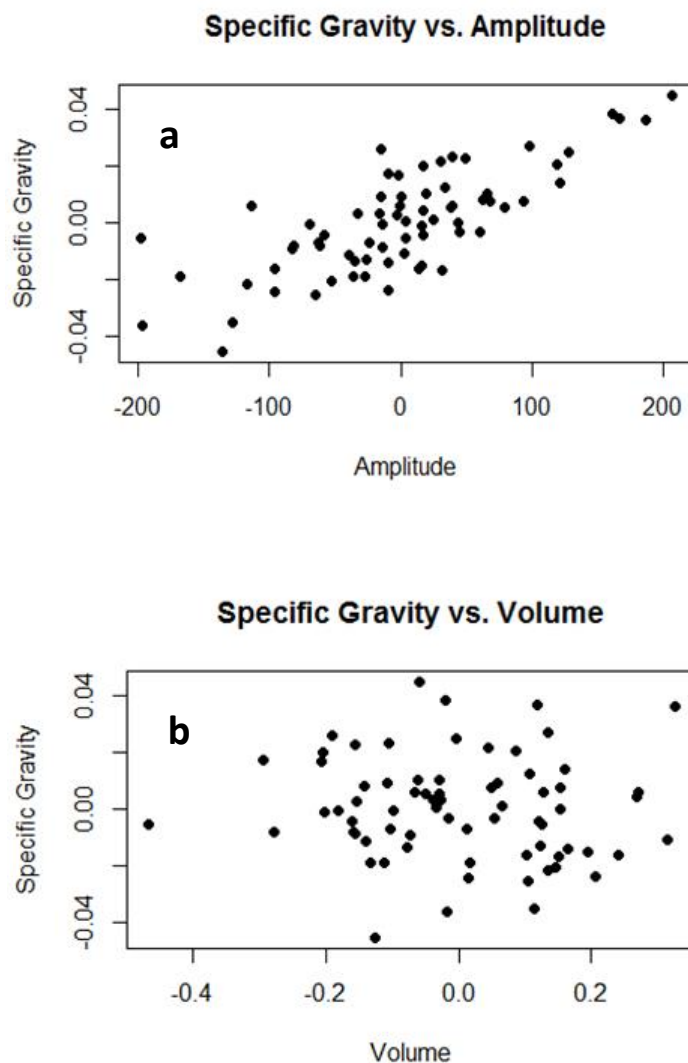
\* Amp= amplitude, SWS= stress-wave velocity, SG=specific gravity, HT=height, VOL=volume

There is a strong, positive genetic correlation (0.90) between specific gravity and Resistograph amplitude. This result is illustrated in Figure 2a, and it is consistent with previous studies for this device.

In addition to the original research objective, this analysis confirms that wood properties have little to no correlation with growth traits in our loblolly pine populations. Family breeding values for stress wave velocity and specific gravity had very weak correlation with family breeding values of height and volume (Table 2). The weak correlation between specific gravity and volume is illustrated in Figure 2b.

With the results of this study, the Cooperative has implemented plans to assess wood properties in the Cooperative's progeny trials. Sampling in Coastal PMX

Series 2, 3, and 4 and Piedmont PMX Series 1 trials has been underway this spring. These data will be integral in identifying families with superior wood properties. This will provide landowners and forest managers with yet another tool during the decision-making process of what genetics to deploy on their lands.



**Figure 2.** Correlation of family breeding values between Specific Gravity-Amplitude (a) and Specific Gravity-Volume (b). As expected the correlation of SG-Amp has a strong, positive relationship, while there is no significant correlation between SG-VOL.

## Clonal Selections from Atlantic Coastal Elite (ACE) Population

The Cooperative's elite breeding populations are intensively managed subsets of the mainline population selected for short-term genetic gain for each of the three geographic regions (Northern, Coastal, and Piedmont). In order to increase the gain from the Atlantic Coastal Elite (ACE) Population, a clonal testing effort of the progeny trials was conducted. Since the same genotypes were replicated across test sites, an improvement in within-family selection is expected.

The initial genetic material consisted of 76 crosses produced from 24 elite parents of the Atlantic Coastal Population of loblolly pine. Since fusiform rust resistance is a critical trait for most of the Coastal Plain region, seedlings were first screened at the USDA Resistance Screening Center (RSC) in Asheville, NC. A broad-base field inoculum that covered the expected range of eventual deployment for the genotypes was used. After 6 months, seedlings were culled based on the presence of rust galls with the assumption that a seedling with a "no gall" response at the RSC would be more likely to be resistant in the field than seedlings with galls. Through this culling, genotypes that may be susceptible in the field to rust were eliminated, and the remaining seedlings were used. Based on RSC results, 51 out of the initial 76 crosses were selected for progeny testing in field trials. Individual genotypes within each full-sib family were hedged and clonally multiplied using rooted cuttings. A total of 2362 clones were used for the experiment (see details in the 2008, 2009, and 2010 Annual Reports).

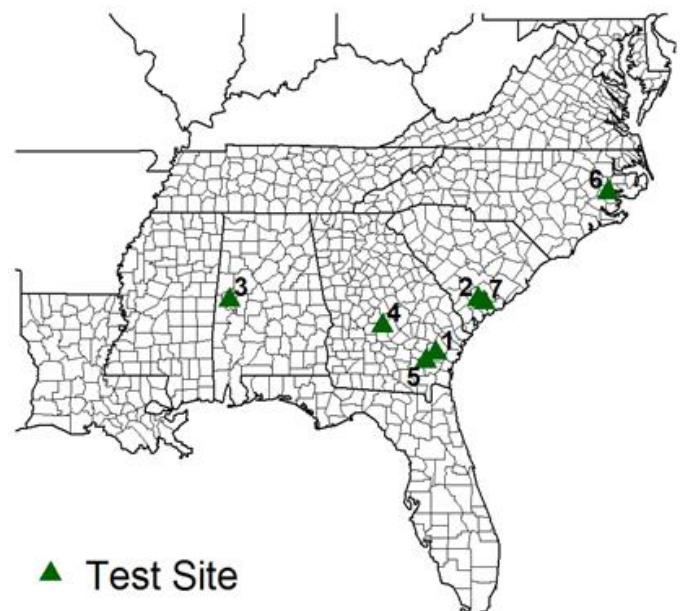
For the field trials, 8 test sites were originally established across the southeastern United States; data from 7 of these sites were available for the analysis (Figure 1 - Table 1). The design of the experiment was an incomplete block design, where each row and column is an incomplete block, and each test is a replication. In order to compare the performance of the selected crosses, seedlings from 7 coastal common families were used as checks. These check families were the same families used in the mainline testing program and were randomly distributed throughout each test site. Measurements (height, diameter at breast height, straightness score, fusiform rust incidence, forking, ramicorn branching, and survival) were taken at age 4 years (one test was age 3). In total, over 15,000 trees

were measured. The overall summary statistics at age 4 years (Table 2) show that the rust frequency across test sites was only around 2%; the culling process at the RSC worked.

The breeding values (BV) for each of the measured traits of the clones, the clone-mean repeatability, and the narrow-sense heritability at each test site were estimated using the appropriate linear mixed effects model. In order to rank the clones, an index that weights volume, straightness, and forking and ramicorn branching (called VSF index) breeding values was constructed. The weights of the VSF index were set to 0.5 for volume, 0.35 for straightness, and 0.15 for forking and ramicorn branching. The seven crosses with the lowest index value were eliminated since they

**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	Peirce County, GA
6	Weyerhaeuser	Beaufort County, NC
7	ArborGen	Colleton, SC



**Figure 1.** ACE test site locations across the southeastern United States.

**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.

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 (ft <sup>3</sup> )	0.36	13,157	0.03	1.7	0.24
FORK/RAM freq	0.24	13,219	0	1	0.42

ranked below most of the checklot families used as a relative measure of performance. Furthermore, another seven crosses were eliminated because of poor straightness and/or forking breeding values. Many of the resulting top crosses were half-sibs or somehow related to one another, thus to avoid inbreeding, the

number of selections was limited to one for most of the 37 crosses. A total of 48 selections were made from the ACE population, and all were topgrafted into the New Breeding Orchard at the Arrowhead Breeding Center. These selections will be an integral part of the 4<sup>th</sup>-cycle breeding for the Coastal region.

## SEED AND CONE YIELDS

Cooperative members harvested a little more than 30,000 pounds of seed from operational orchards in 2013 (Table 1). This is a 24% increase in seed yield from the harvest in 2012. Average yield of seed per bushel decreased modestly relative to 2012. Nearly all provenances and generations had an increase in seed collected. Only Coastal 3<sup>rd</sup>-generation and Northern

2<sup>nd</sup>-generation orchards showed a decline in seed produced.

At approximately 12,000 seedlings produced per pound of seed, last Fall's harvest will result in over 363,000,000 seedlings being grown this year! That is enough to plant approximately 800,000 acres of pine plantations this year alone.

**Table 1.** Fall 2013 cone and seed yields compared with the figures from 2012 harvest

Provenance / Generation	Bushels of Cones		Pounds of Seed		Pounds per Bushel	
	2013	2012	2013	2012	2013	2012
Coastal 2.0/2.5	7139	5132	9054	7339	1.27	1.43
Coastal 3.0	6851	7782	8107	10631	1.18	1.37
Piedmont 2.0	4224	1509	5479	2314	1.30	1.53
Piedmont 3.0	4567	2241	5446	3007	1.19	1.34
Northern 2.0	550	494	482	715	0.88	1.45
Northern 3.0	1685	409	1730	425	1.03	1.04
<b>Totals</b>	<b>25,016</b>	<b>17,567</b>	<b>30,297</b>	<b>24,431</b>	<b>1.14</b>	<b>1.36</b>



## RESEARCH

### Genomic analysis of loblolly pine adaptability to climate conditions<sup>1</sup>

The projected increase in severe weather events due to climate change has the potential to affect the productivity of pine plantation stands and impact the forest industry. Loblolly pine has a very diverse gene pool and is adapted to various environments, and is thus a good candidate to breed for adaptation to climate change. The PINEMAP project, funded by USDA in 2010, proposed to accomplish this by testing for associations of individual genetic marker loci with growth and quality characteristics using climate factors as covariates. Existing high-throughput genotyping methods for pine are too expensive to allow routine application in breeding populations. Over the past three years, we have worked with other PINEMAP investigators to develop a cost effective, easily implemented, and high-throughput genotyping method which we are now using to test the hypothesis that genomic regions controlling climate adaption can be identified.

The genetic marker system will be used to collect genotype information from trees at a Plantation Selection Seed Source Study (PSSSS) site. The PSSSS consists of 140 pollen mix families planted in 20 test locations representing much of the loblolly pine range. Phenotypic data from measurements at ages 4 and 8 years are available to be used together with genotypes from the genetic marker analysis.

A method known as genotyping-by-sequencing (GBS), previously described for maize and barley by Elshire et al. (PLoS ONE 6: e19379, 2011) and for wheat and oats by Poland et al. (PLoS ONE 7: e32252, 2012) was adapted for use with pine. This method is carried out in 96-well format, and employs methylation-sensitive restriction enzymes to enrich for gene regions in the pine genome. To reduce cost, the method uses “barcoded” adapters and an index primer set to allow combining individual samples in a single sequencing lane while maintaining individual sample identities. DNA sequencing was carried out using Illumina HiSeq2000. The HiSeq2000 is the current platform of choice due to the number of reads and the read quality obtained.

#### Progress and Results

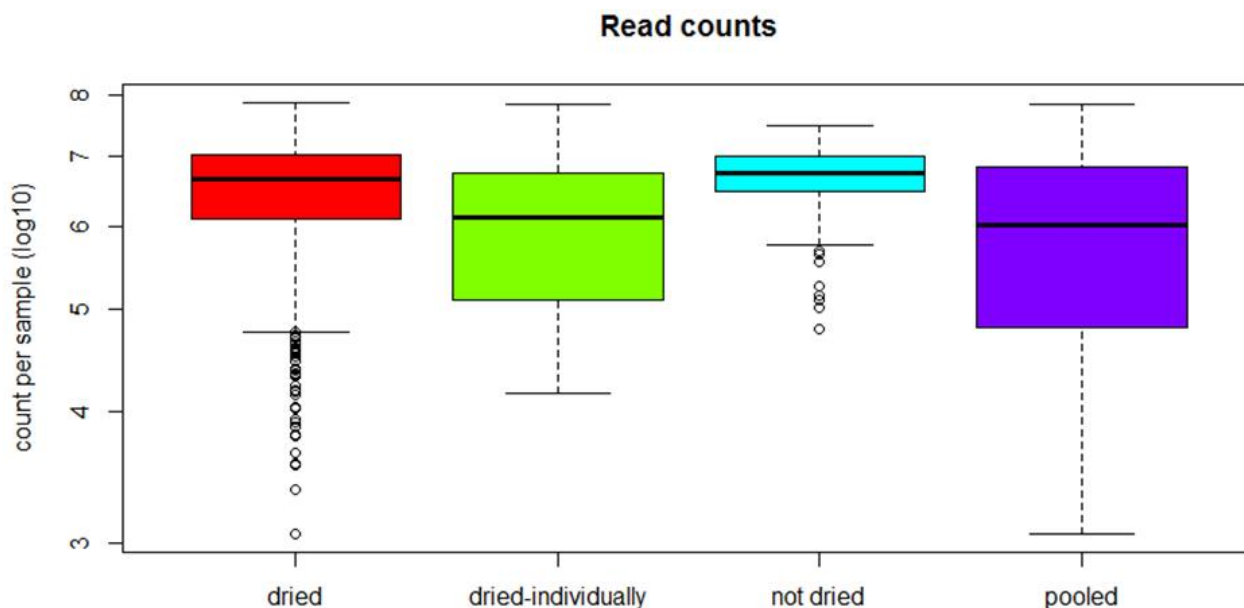
Tissue samples were collected as 3/4-inch bark disks from 1600 trees in the PSSSS site in Screven County, GA, in October 2012. The phloem tissue was peeled from the underside of each bark disk in the field, and stored at ambient temperature in a barcoded vial containing an anti-oxidant preservative solution until DNA was extracted using a procedure adapted from Ivanova et al. (2008, PI MolBiol Rep 26:186). Methods for grinding tissue samples in 96-well format have been optimized for the phloem tissue samples, and over 3600 DNA extractions have been completed. The yield of DNA is somewhat variable from these samples, and the amount of phloem tissue obtained from a bark disk is inconsistent, so we have incorporated a 96-well assay for DNA quantitation into the sample-processing workflow. Of the 1600 samples collected, 1471 had DNA yields high enough to be sequenced. Due to these variable yields in DNA/sample, four different DNA concentration strategies were used in the library preparation process. The estimated cost of consumable supplies and reagents for DNA extraction and library preparation was about \$1.50 per sample. All samples were submitted for sequencing in the summer of 2013, and data analysis has begun.

Of the sequenced reads returned, there appears to be significant differences in the distribution of the number of reads when compared to the four different DNA concentration strategies used (Figure 1), as well as the barcode adapters used for each individual (Figure 2). Using the STACKS pipeline software, we were able to detect unique polymorphic SNP counts in ~94.3% of the samples sequenced, with an average of ~5562 SNPs per sample. This value is lower than the 8000-10,000 unique genetic loci per sample that was expected based off of the results of a smaller preliminary sequencing run performed in the summer of 2012, which involved a single loblolly pine parent tree and a set of 90 haplotype megagametophytes from seeds of that parent. This lower number of unique polymorphic SNPs is mostly likely due to the large amount of repet-

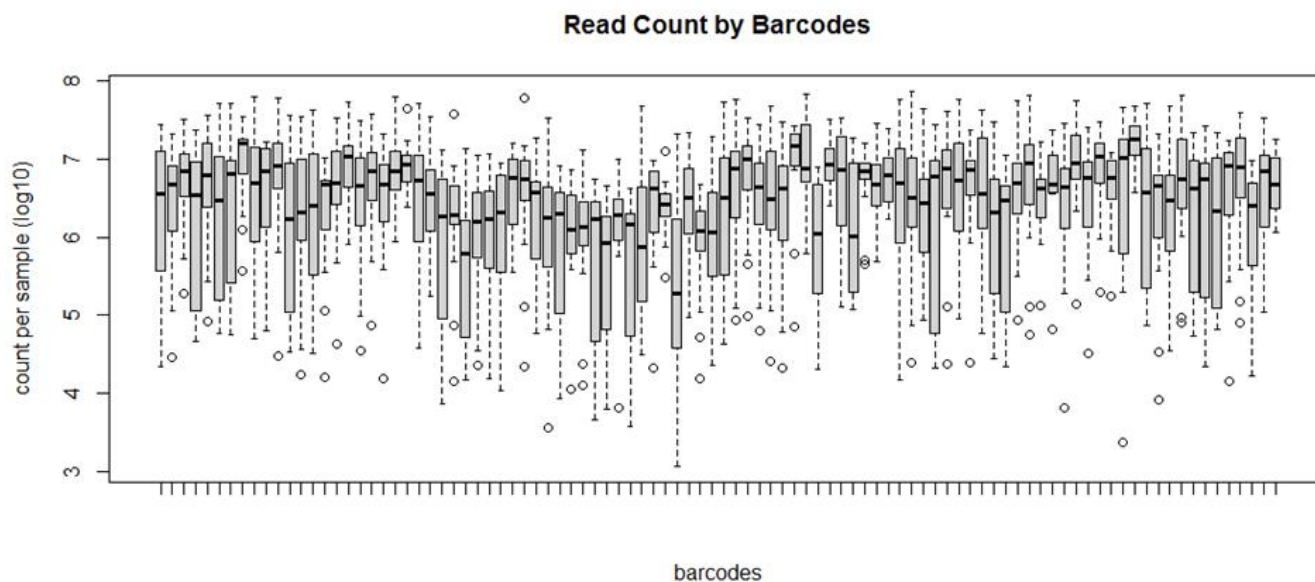
<sup>1</sup>This is a summary of Laura Townsend's graduate research working with the PINEMAP project, [www.pinemap.org](http://www.pinemap.org)

itive DNA found in loblolly's genome (approximately 82%). Further analysis is underway using the newly published loblolly pine genome to perform genotype calling of the PSSSS data set. We will use this information to make a relationship matrix to determine the full-sib and half-sib relationships for the previously unknown pollen parents. By combining the kinship matrix and the phenotypic data we can use BLUP anal-

ysis to derive estimates of the proportion of phenotypic variance associated with a proportion of certain haplotypes. We will also be able to look at how associations vary among the 7 different seed sources along with climate data. These determined haplotypes can provide a good idea of the full allelic diversity of loblolly pine, and can potentially be used for screening of future offspring in breeding.



**Figure 1.** Distribution of reads per individual by DNA concentration strategies used. Four distinct strategies were used when preparing the DNA libraries for sequencing. Not dried samples had high enough concentrations to be used directly; dried were concentrated in a large scale (192 samples) speed vacuum; dried-individually were concentrated down individually in a single tube speed vacuum; and pooled were samples with DNA yields so low that higher volumes of them were added while pooling plates together.



**Figure 2.** Distribution of reads by 96-barcodes. Each barcode was assigned between 13-16 individuals each, and the plot shows the range in the number of reads per individual for each barcode.

## Modeling methods for predictions of genetic value and efficiency in tree improvement<sup>1</sup>

One of the challenges facing any tree improvement program is the time that it takes to make genetic gain. A major obstacle is that selections from progeny tests must be made before the typical rotation age in order to achieve genetic and economic gain faster. Another factor is the risk of inbreeding depression due to high selection intensity as well as multiple within family selections for future generations. The computer program Pedigree Viewer MateSelect option has been used to determine which selections, with respect to progeny performance and amount of relatedness, should be used in the next generation. However, the ability to assess breeding strategies for multiple generations and the risk of inbreeding depression in progeny performance would be beneficial in looking at long-term selection strategies.

The ability to use statistical models to correlate the accuracy of selection-age phenotype with rotation age phenotype has allowed progeny selection at early ages. The current selection age for the next generation in breeding strategies is 4 to 5 years. If selections could be made on progeny tests at 6 months, genetic gain would occur more than twice as fast as it does now, and could result in a larger yearly economic gain for

“A transcriptome represents that small percentage of the genetic code that is transcribed into RNA molecules — estimated to be less than 5% of the genome in humans... The proportion of transcribed sequences that are non-protein-coding appears to be greater in more complex organisms. In addition, each gene may produce more than one variant of mRNA because of alternative splicing, RNA editing, or alternative transcription initiation and termination sites. Therefore, the transcriptome captures a level of complexity that the simple genome sequence does not...”

Quote from: Adams, J. (2008) Transcriptome: connecting the genome to gene function. *Nature Education* 1(1):195

landowners. This may be achievable with the use of a computer analysis that incorporates transcriptome data (see attached box for definition) of progeny at 6 months to help predict the phenotype or genetic value of a given open-pollinated family or full-sib cross.

Transcriptome data has recently been shown to correlate with phenotype in several species, and work is planned in loblolly pine to test if the same phenomenon exists. The hypothesis to explain why transcriptome data provide better phenotypic predictions than just using DNA sequence variation, is that the transcriptome data include quantitative variation in the levels of RNA transcripts produced from the DNA, and this quantitative information includes previous interactions among DNA sequence variants. Current selections for breeding populations from progeny tests within the Tree Improvement Program are made based on individual-tree phenotype, which can be greatly affected by the environment, along with pedigree data. The true breeding value of each selection must be confirmed by progeny testing, which requires several years and substantial investment of time, labor, and land area to complete. Evaluating selections on the basis of transcriptome data collected from seedlings a few months after germination would allow us to eliminate some individuals from future progeny testing, provided that the transcriptome data can be shown to predict field performance in pine as well as it does in model organisms such as maize, mice, and fruit flies.

The goal of this project is to create a computer simulation program that can simulate multiple generations of a given breeding strategy utilizing marker and transcriptome data of parents to predict: 1) progeny genetic values (phenotype) and 2) potential long-term risk of inbreeding depression. The first step in accomplishing these goals is to re-create dominance, recessive, and epistasis effects to simulate inbreeding depression in progeny after multiple generations. This is done by adding user-defined factors such as the dominance coefficient and the effect of each individual genetic vari-

<sup>1</sup> This is a summary of Adam Festa's graduate research which is partially supported PINEMAP [www.pinemap.org](http://www.pinemap.org)

ant on phenotype. Both of these factors are then used in the calculation of the total genetic value of progeny. The simulator program also creates genetic marker data at single-nucleotide polymorphisms, or SNPs, to allow testing of analytical methods that use genotype data to model phenotypic variation. This part of the project is complete; the simulator program can now produce multiple generations of parents and progeny that display the typical sorts of additive and non-additive genetic variation observed in real pine breeding populations, including inbreeding depression.

The next step is incorporating the ability to simulate transcriptome data of simulated individual trees, and use the transcriptome data to simulate the phenotypes and total genetic value of offspring. The transcript levels for each individual will be simulated based on the specific genetic variants present in the individual, just as real genetic variants interact in complex ways to give rise to the transcript levels observed in real transcriptome datasets. Effects of individual genetic variants can be combined in many ways, ranging from simple additive interactions to complex positive or negative epistasis or multiplicative effects, to simulate the level of each transcript. The overall value of an individual's transcriptome (created by the sum of all transcript values for an individual), would provide the basis for simulation of the phenotype. Random noise will be included to reduce the heritability of the phenotypes simulated from transcript effects, and this will also be a user-defined parameter. Once the simulator is completed, the user should be able to define all parameters in the genetic architecture of a trait and choose a breeding strategy, then simulate multiple generations to see phenotypes and genetic values of future progeny.

In its current form, the simulation program can calculate the total genetic value of selections from each generation as well as the total genetic value of each progeny produced from a cross. In addition, it can be used to simulate a trait phenotype for each individual in the cross by utilizing the underlying genotypes at genetic loci that control the trait. The genetic value is based on the genotype, while the phenotype incorporates random variation to reduce trait heritability to the user-specified level. User-defined parameters include the number of loci that affect the trait, the number of SNP

marker loci, the mating design, the dominance coefficient of each SNP locus that affects the phenotype, the size of the individual SNP effect on phenotype, and the trait heritability. Values of these parameters have been identified that produce inbreeding depression in successive generations of simulated offspring, with a range of variation and magnitude of effect similar to those observed in the TIP inbreeding study, as previously reported by Graham Ford in his M.S. thesis.

Multiple breeding strategies can be simulated within one simulation run by changing the individuals to be crossed after each generation. A simulation can use a different mating design in every generation of the simulation, or the same design in the first generation and each successive generation. This will allow tree breeders to simulate how their current or planned breeding strategy may affect potential long-term inbreeding depression and progeny performance.

The outcome of this study may assist in the way future selections are made for breeding populations within the Tree Improvement Program. Multiple options for use of transcriptome data in breeding programs can be imagined; the most cost-effective and time-efficient will be determined in the course of future research. One possibility is that using transcriptome data will provide a way to make genetic gain faster, if the accuracy of models that predict genetic value from transcriptome data is high enough. Another option, if the accuracy is not sufficient to allow direct selection based only on transcriptome data, is to increase the selection intensity by screening many families using transcriptome data, then field-testing a smaller number of families that show the highest potential for genetic gain. Increasing genetic gain per unit time, whether through completing the testing faster or by increasing selection intensity, would allow higher-value planting stock to be produced sooner and therefore also result in faster economic return on the investment made in tree improvement.

## Broad-based testing compared to local testing of loblolly pine families for resistance to fusiform rust<sup>1</sup>

Fusiform rust disease caused by the fungus *Cronartium quercuum f.sp. fusiforme*, causes swollen regions called galls on stems or branches of pines. Galls produce cankers which can result in mortality on young trees as well as deform older trees, reducing growth and weakening the stems until breakage in windstorms becomes likely. Fusiform rust is commonly recognized as the most economically important disease of loblolly pine in the southern United States, and breeding loblolly pine with resistance to fusiform rust is a central focus of southern pine tree improvement programs. Substantial genetic variation in expression of host resistance to fusiform rust is present in loblolly pine, and use of resistant planting stock is the only feasible means of controlling the disease in plantations.

Identification and deployment of pine families with enhanced resistance to fusiform rust across a broad range of sites are critical for the full benefit of breeding programs to be realized. Testing of loblolly pine's resistance to fusiform rust is done either by field testing or by controlled inoculations. The USDA Forest Service's Resistance Screening Center (RSC) in Asheville, NC evaluates seedlings for resistance to fusiform rust. The RSC provides results in much less time than is possible in field progeny tests, but there is some discrepancy between field testing results and results from the RSC. Isik et al. 2008 (Can. J. For. Res. 38: 2687-2696.) found that loblolly families that were resistant in field tests are not always resistant in controlled inoculations. This brings up questions such as: Is the RSC too stringent in their assessments of resistance? Are genotype by inoculum interactions occurring? These are essential questions to answer, because we rely on accuracy of local field tests.

The vast majority of the Cooperative's breeding values come from local tests in narrow geographic regions (e.g. the diallel tests from the 2<sup>nd</sup>-generation testing program). If genotype by inoculum interactions are common, using results from local field tests could be a problem for testing fusiform rust resistance for families being deployed across a wide range of sites. The Plantation Selection Seed Source Study (PSSSS) trials offer a unique opportunity to compare broadly-tested

versus narrowly-tested breeding values for families tested in both the diallel and PSSSS trials.

The PSSSS aimed to gain information on loblolly families from various geographic sources planted in different regions. The PSSSS tested 140+ pollen mix families throughout the eastern portion of loblolly pine's native range. Single-tree-plots were established as randomized complete blocks replicated 24 times at each site. There were 14 sites used in the PSSSS with 12 sites from the Coastal Plain and 2 sites from the South Carolina Piedmont. The current study used 56 coastal families that were also tested in the diallel trials (locally tested). This gives us the ability to compare rust breeding values that are based on broad geographic testing to those same families that were locally tested in the diallel trials.

In the diallel tests, these 56 families were tested along with many other families in 31 different test series throughout the Coastal region. Each series consisted of 4 tests in a local geographic region. At each test, a full-sib family was represented by 36 seedlings, and a half-sib family by 180 seedlings (i.e. each half-sib family is composed of 5 full-sib families). The test series had a wide spread of rust incidence with a mean rust incidence of 0.42, a minimum of 0.17, and a maximum of 0.74. The 14 PSSSS test sites also had a balanced spread of rust incidence with a mean rust incidence of 0.50 across the trials (Table 1).

Breeding values for the 56 families in both the PSSSS and the plantation selection diallels were calculated using ASReml. Regression analysis was performed to determine if there was a correlation between the breeding values of locally and broadly tested families. The regression between the diallels and PSSSS breeding values had an  $R^2$  of 0.66 (Figure 1), suggesting that the diallel breeding values are reasonably good predictors of the PSSSS breeding values.

---

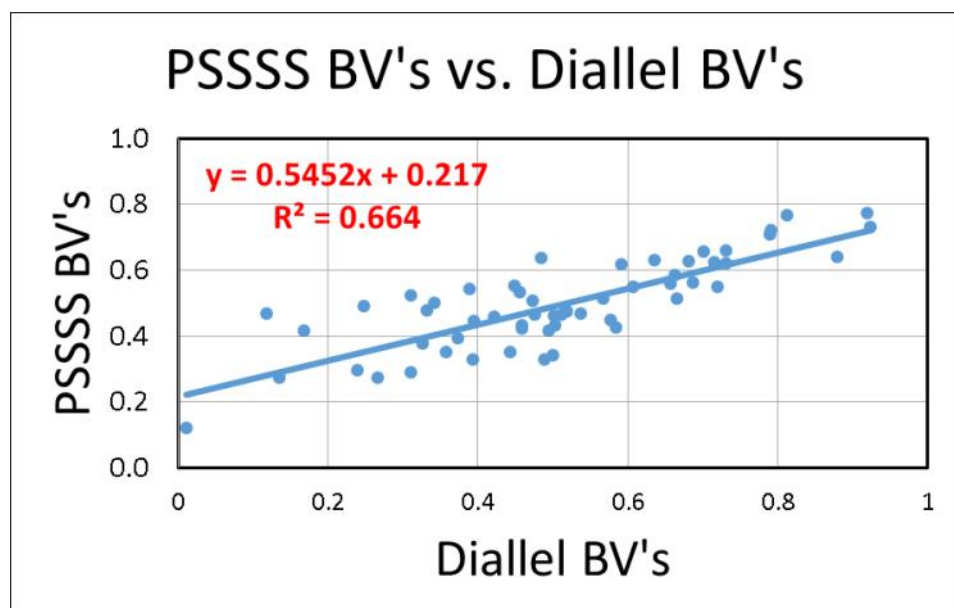
<sup>1</sup> A summary of Jesse Spitzer's graduate research.

Local testing for rust resistance appears to be a reliable predictor for rust resistance across a broad range of sites. Locally-derived rust breeding values generally hold true across a broad range of sites.

**Table 1.** Summary Statistics for 14 PSSSS Tests

Site	Rust Incidence	Height (m)	DBH (cm)	Volume (dm <sup>3</sup> )	STRT
Northampton, NC	0.83	7.9	15.2	56.9	3.51
Laurens, SC	0.44	8.9	14.2	52.4	3.45
Newberry, SC	0.68	9.2	16.5	75.0	3.51
Robeson, NC	0.45	10.4	16.3	80.1	3.50
Autauga, AL	0.68	8.8	15.5	61.2	3.44
Williamsburg, SC	0.42	7.7	12.2	36.2	3.30
Randolph, GA	0.61	7.0	11.4	28.0	3.07
Escambia 1, AL	0.08	10.5	13.7	60.6	3.44
Escambia 2, AL	0.27	10.6	16.5	84.4	3.30
Screven, GA	0.54	8.9	14.5	55.8	3.43
Berkeley, SC	0.69	11.9	17.3	103.4	3.76
Tattnall, GA	0.48	12.3	16.3	96.6	3.55
Bradford, FL	0.62	8.4	11.2	33.7	3.55
Ware, GA	0.21	8.0	10.4	26.6	3.38

**Figure 1.** Regression of broad-based (PSSSS) breeding values and locally-based (Diallel) breeding values gives an  $R^2$  of 0.66.



## Modeling climate change effects on the growth of loblolly pine families across the southeastern U.S.<sup>1</sup>

The establishment and analysis of provenance tests for investigating the genetic variation among forest trees has a long tradition in forestry. Provenance tests consist of replicated experiments, comparing trees grown at several locations that were collected from many geographic regions of a species distribution. Such tests can also provide meaningful information for assessing the response of genotypes to environmental change.

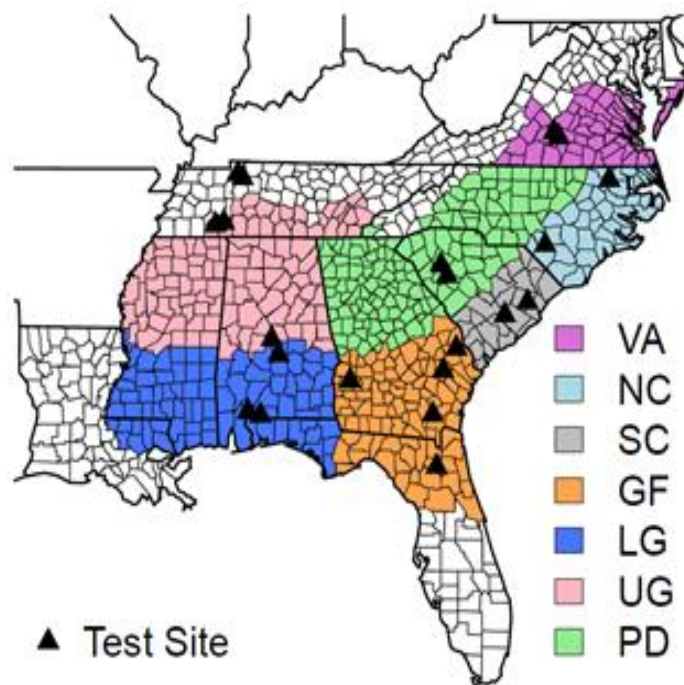
In the mid 1990s, the Plantation Selection Seed Source Study (PSSSS) was initiated by the North Carolina State University Cooperative Tree Improvement Program. The purpose of the PSSSS was two-fold for seed sources: first, identify the performance of loblolly pine seed sources for multiple traits, and second, define and explain any trends of genotype by environment (G×E) interactions. The plantation selections were initially selected from pine plantations based on superiority of phenotypic traits such as height, stem form, and disease resistance. Pollen mix seedlings from 140 of these selections were planted at 25 test locations throughout the southeastern United States. Height, diameter, stem straightness, and fusiform rust incidence were measured at tree ages 4 and 8 years. Better growth of Coastal sources compared to inland or northern sources has been reported in several studies, and the PSSSS was not an exception. Seed sources from the lower Atlantic Coastal regions (SC and GF - Figure 1) outperformed the seed sources from Virginia, the Gulf Coast, and inland regions.

Previous studies and modeling efforts have focused on the characteristics and response behavior of the seed sources and provenances, but genetic differences among families within the same seed source exist and cannot be ignored for making informed deployment decisions. Empirical evidence indicates that families from the same seed source can have very different responses to environmental factors.

In this work, we first propose a formal method of statistical inference for testing whether the relative performance across test sites of two different families differ significantly from each other. Then we propose a statistical model to predict the relative performance of

different families under a given climate scenario using climate variables as predictors. To adjust for differences in site index and overall performance, family performance is expressed relative to locally adapted non-improved checklots at each site. The model was developed and tested using data from the Plantation Selection Seed Source Study.

The local checklots (Table 1) were assumed to be adapted to the local climate of each test site and, therefore, can serve as a baseline or reference to measure genetic gain in growth traits relative to other non-local families. It is important to emphasize that the checklots are different for each region. The observed 8-year height was the chosen trait to assess growth performance because it is a heritable trait, and because it is



**Figure 1.** The geographic regions of loblolly pine seed sources and test site locations for the Plantation Selection Seed Source Study (PSSSS). The seven geographic regions are sketched: Virginia (VA), North Carolina Coastal Plain (NC), South Carolina Coastal Plain (SC), Georgia-Florida Coastal Plain (GF), Lower Gulf Coastal Plain (LG), Upper Gulf Coastal Plain (UG), and Piedmont (PD).

<sup>1</sup>This is a summary of Alfredo Farjat's PhD research with PINEMAP, [www.pinemap.org](http://www.pinemap.org)

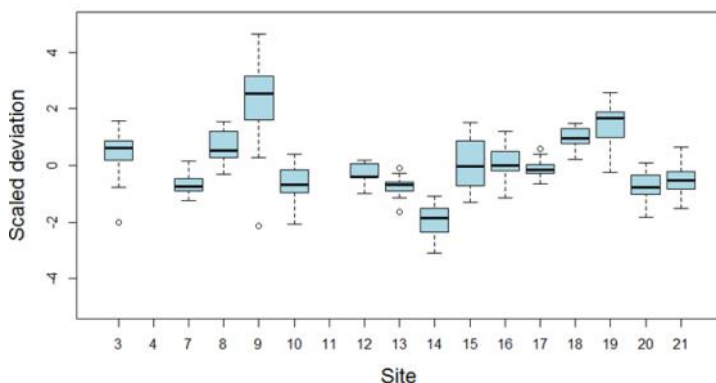
**Table 1.** Sample mean, standard deviation, and number of observations of local genetically non-improved checklots from each geographic region of loblolly pine.

Region	Checklot	Mean	Standard Dev.	N
1	CC1	24.80	3.65	351
2	CC2	30.08	4.59	130
3	CC3	32.64	5.81	205
4	CC4	29.73	8.01	351
5	CC5	32.08	4.48	211
6	CC6	30.22	4.32	260
7	CC7	27.52	3.55	159

very likely to be subject to natural selection. To quantify the observed  $l$ -th deviation  $Z_{ikl}$  of a specific family, say  $k$ , planted at the  $i$ -th site, the following quantity was defined:

$$Z_{ikl} = \frac{X_{ikl} - \bar{X}_{LF_{j(i)}}}{S_{LF_{j(i)}}}$$

where  $X_{ikl}$  is the  $l$ -th 8-year height observation of family  $k$  at site  $i$ ;  $\bar{X}_{LF_{j(i)}}$  denotes the sample mean height of the local non-improved checklot from region  $j$ , which includes the corresponding  $i$ -th site, and  $S_{LF_{j(i)}}$  is the estimated standard deviation of the local non-improved checklot from region  $j$  associated with the  $i$ -th site. The deviations  $Z_{ikl}$  are scaled by the standard deviation to account for the presence of non-constant variance across the local families of the different geographic regions. These scaled deviations are a measure of the deviations of a particular family from the local genetically non-improved seedlings gauged in standard deviation units (i.e. Figure 2). The climatic variables of the test sites (predictors of relative performance) were estimated using the PRISM (Parameter-elevation Regressions



**Figure 2.** Box-plot of the scaled deviations of family #119 from local checklots as a function of test site.

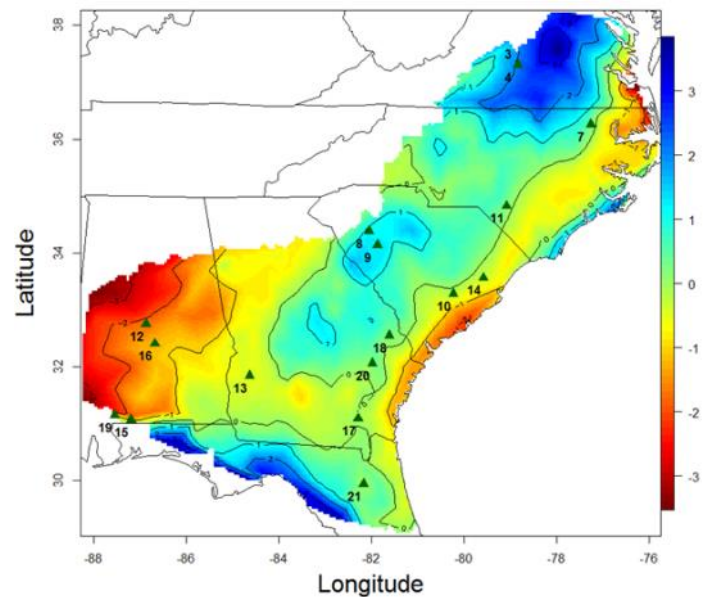
on Independent Slopes Model) climate mapping system (PRISM Climate Group, Oregon State University).

Multiple linear regressions were used to model the scaled deviations of families as a function of the climate variables of the test sites. The general form of the model is given by:

$$Z_{ikl} = \beta_0 + \beta_1 TMIN_i + \beta_2 PPT_i + \beta_3 TMAX_i + \beta_4 (TMIN \times PPT)_i + \beta_5 (TMIN \times TMAX)_i + \beta_6 (PPT \times TMAX)_i + E_{ikl}$$

where  $Z_{ikl}$  is the  $l$ -th scaled deviation of the  $k$ -th family planted at the  $i$ -th site;  $\beta_m$  for  $m = 0, \dots, 6$  are the regression coefficients associated to the explanatory variables of the model;  $TMIN_i$  is the yearly average minimum temperature of the  $i$ -th site;  $PPT_i$  the yearly average precipitation of the  $i$ -th site;  $TMAX_i$  is the yearly average maximum temperature of the  $i$ -th site;  $(TMIN \times PPT)_i$ ,  $(TMIN \times TMAX)_i$ ,  $(PPT \times TMAX)_i$  are the cross products of the climate variables associated with the  $i$ -th site; and  $E_{ikl} \sim N(0, \sigma^2)$  is a random error term that follows a normal distribution with zero mean and constant variance  $\sigma^2$ .

To illustrate the proposed model, the 8-year scaled deviations from local checklots of family #119 (from VA)



**Figure 3.** Predicted 8-year scaled deviations from local genetically non-improved checklots across the Southeast for family #119 (from VA) under current climate conditions. The numbered test site locations are indicated with green triangles. This family is predicted to perform better than local checklots (blue and green) in the northern regions under the simulated climate conditions.



were estimated across the Southeast (Figure 3). Since the model uses climate variables as predictors (Table 2), 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. Under this scenario, family #119 is predicted to perform better than local families (blue and green areas) in the northern regions. This family also exhibits a superior relative performance in inland South Carolina. It is worth pointing out that the same set of variables used for predicting the relative performance of family # 119 (Table 2) explained between 40% to 60% of the total variation for most of the 140 loblolly pine families in the PSSSS trials.

**Table 2.** Statistical model for 8-year relative performance of pine family # 119. The regression coefficient estimates of the climatic variables are listed along with associated standard errors, *t* statistics, and *p* values. *TMIN*: minimum temperature; *TMAX*: maximum temperature; *PPT*: precipitation. For family # 119, the model explained about 55% of the observed total variation.

The statistical model can be used as a quantitative tool to model the effect of climatic variables on the relative performance of specific loblolly pine families. Furthermore, the model could also be used to identify superior performing families, and to estimate their growth potential for a given planting site under a given future climate. The ability to predict relative family performance, and the capacity of identifying the most productive and resilient families, will equip land owners and managers with the information they need to make informed decisions about how best to balance risk and potential productivity on specific sites, as well as increasing the resilience of plantations to climate change.

Climate Variable	Estimate	Std. Error	Pr(>   t  )
<b>Intercept</b>	0.05	0.04	0.246
<b>TMIN</b>	-32.6	3.8	< 0.001
<b>PPT</b>	70.3	13.6	< 0.001
<b>TMAX</b>	16.9	3.0	< 0.001
<b>TMIN×PPT</b>	18.1	1.2	< 0.001
<b>TMIN×TMAX</b>	13.1	3.4	< 0.001
<b>PPT×TMAX</b>	-75.6	14.4	< 0.001



*Pictured left: PSSSS trial owned by Plum Creek. The trial is age 18 and located in Oliver, GA*

## Acknowledgments

This study is part of the PINEMAP Project, a Coordinated Agricultural Project funded by the USDA (Grant # 2011-68002-30185). Field trials were funded by members of the North Carolina State University Cooperative Tree Improvement Program.

## Grants

We continue the work on all of the research initiatives that were mostly sponsored in 2011. Below is the list of current projects funded:

### Continuing Grants

Ross Whetten, Steve McKeand, 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, Modeling of Cellulose, Hemicellulose and Lignin-Carbohydrate Complex Formation and Regulation to Understand Plant Cell Wall Structure. TIP Portion: \$20,223. 9/2011-8/2014.

Steve McKeand, Bronson Bullock, Ross Whetten, and Fikret Isik. Biofuels Center of North Carolina. “Loblolly Pine Biomass Genetics/Cropping Study”. \$148,419. 7/2011 to 9/2013. (Note that this grant came to an end one year earlier than planned. The Biofuels Center was shut down in October 2013.)

## ASSOCIATED ACTIVITIES

### Meetings, Workshops, and Short Courses

Dr. Ross Whetten taught a six-day course at the Wuhan Botanical Garden, Wuhan China, on August 3-8, 2013. There were 35 students who learned about laboratory techniques and data analysis methods for high-throughput genotyping. Additionally, both Ross and Fikret taught a three-day workshop in Copenhagen, Denmark Sept 17 - 19, 2013. With a total of 16 students, the workshop focused on genetic analysis of marker data for predictions of breeding values and analysis of sequence data.



*Pictured left: Ross Whetten's 6-day course in Wuhan China, Pictured Right: 3 day workshop in Copenhagen, Denmark*



*Above: Group photo of attendees to the TIP Contact Meeting and short course field tour.*

The annual TIP Contact Meeting was in Birmingham, AL with Hancock Forest Management hosting the field tour. We held a TIP short course before the Contact Meeting to get the next cycle of breeding off to a good start. The short course helped get everyone up to date on the 4<sup>th</sup>-Cycle plans and remind everyone about the techniques and complexities of running an effective tree improvement program. As with any good contact meeting, we did have an adventure planned for everyone. This time it was an elevator to the top of the Vulcan statue with a killer city view of Birmingham. Everyone made it up and down and got to enjoy the remaining exhibits at the Vulcan Museum on the statue and history of Birmingham. This was definitely one of our coolest meeting outings yet.

International Forest Company hosted a grafting workshop in early February 2014 in Moultrie, GA with the masters of grafting, TIP'S very own J.B. Jett and Steve McKeand. The workshop was timely for the attendees - right before the grafting season. We hope all the folks learned a lot or enjoyed the refresher course and found themselves prepared for the grafting season in their own orchards.

Even though Fikret is on sabbatical with INRA scientists in France, he still managed to continue his workshop schedule. In March 2014, he taught a workshop on Genomic Selection in Forest Tree Breeding in Howick, South Africa. With an invitation from Sappi, a major forestry company in South Africa, he taught a workshop on genomic selection in forest trees. The course covered theory as well as hands-on data analysis. Nine participants from two major forest companies (Sappi and Mondi), Professor Zander Myburg from the University of Pretoria and his research associates attended the workshop. Fikret was also invited to give a talk on genomic selection in forest trees at the 10<sup>th</sup> South Africa Plant Breeding Conference at the Maria Moroka Nature Reserve, Thaba 'Nchu, South Africa.



*Above: Workshop attendees in Pretoria, South Africa.*



As always, we made our appearance at NC Arbor Day and NCSU Earth Day celebrations here in Raleigh, NC. We always dress in the TIP uniform, spread the word about Tree Improvement, and give out baby pine trees. We come with tools of the trade like bags full of cones, pollination bags, bottles of pollen and pollinators, and lots of trees. Since the weather was cooler this spring, we were able to take cut branches with female flowers and have folks try their hand at pollinations. The young ones particularly enjoyed the demonstration and hopefully took a little more knowledge about the Tree Improvement Program along with their baby trees :)!

*Left: Steve McKeand demonstrating a pollination at the Arbor Day Celebration at the NC Farmers Market in Raleigh, NC.*

## Staff

Put on your seatbelts everyone; this past year has been like riding a rollercoaster! We will start from July 2013 with the addition of the new data analyst, Jarrod Morrice. He has jumped in head first and bravely tackled the database making huge strides. He also has become intimately involved in all TIP data, so he can serve members and TIP staff alike. Jarrod has been a wonderful addition to the TIP family.

In October 2013, we lost Josh Steiger, who is still in Tree Improvement but is now with Rayonier. He and his wife are now Floridians and have welcomed a new baby girl in March 2014. After an open search, we found Josh's replacement just down the hall. Graham Ford completed his masters with the Tree Improvement Cooperative in 2013 and is also pursuing his PhD. Graham has been "drinking from the fire hose" with the management of the 4<sup>th</sup>-Cycle breeding and testing and has done a wonderful job keeping up with all the complexities of this new effort.

January 2014 was a big month, Fikret left on sabbatical for 6 months in Bordeaux, France, and Jadie Andrews took a position in timber procurement in central NC. This position puts Jadie back on the family farm, which he was helping manage from a distance, so we are thrilled for him. As for Fikret, he likes to send us teasers about how fat and happy he is on the French diet. We are thinking about purchasing a pair of running shoes to help him sweat out all that wine he has in his system when he returns in August.

This year we had two big happenings. First, Dr. Ross Whetten was promoted from Associate to Full Professor. We are of course super proud of him and are lucky to have him as part of the team. Dr. Steven E. McKeand was awarded the Outstanding Extension Service Award and was inducted into the Academy of Outstanding Faculty Engaged in Extension. The Academy of Outstanding Faculty Engaged in Extension (AOFEE) is the only academy of its kind in the nation. The AOFEE is charged to be active in the promotion and recognition of excellence in extension and outreach at NC State University and elsewhere. AOFEE is active in stimulating and developing faculty and staff to address critical social problems and/or opportunities that require creative, interdisciplinary solutions or collaborations. We must say we are very proud of our fearless leader :)!

After another open search in late April, we filled the position of Operations Manager with Austin Heine. Austin has worked with the group for several years, first as an undergraduate and then continued on as a masters graduate student. He is just getting his feet wet, and we are excited to have him join the TIP crew. Steve and Tori have declared that no one else can leave TIP unless it is for retirement, and NCSU HR department is looking forward to Tori no longer breaking their system every few months :)!

*The TIP staff retreat at the Pungo Palace (Steve's happy place). Pictured are both current and past staff from left to right:*

*Jarrold Morrice, J.B. Jett, Jadie Andrews, Tori Brooks, Josh Steiger, Graham Ford, Ross Whetten, Fikret Isik, and Steve McKeand*



## Visitors

Due to the changes in staff and with Fikret being out of the country we have not had too many folks visiting the office, but we did somehow score our own “resident anthropologist”. Kevin Burke is a PhD student from the University of Pennsylvania and joined us in January 2014. He has been thrown into all meetings, breeding, field and lab work, and took Steve’s and Ross’s Tree Improvement courses. So, you may still be wondering...what is an anthropology student doing in Tree Improvement? Don’t worry, we asked him the same thing. He just happens to think the folks in the tree improvement community are pretty interesting. He is studying up on Dr. Bruce Zobel’s archives and tracking the evolution of tree improvement and the domestication of loblolly pine. He carries his small digital recorder everywhere, so needless to say, we are a little worried about what he is transcribing; let’s just say he spent a week down at Arrowhead...



## Teaching

Each year, the faculty associated with the Cooperative teach a wide range of courses at both the graduate and undergraduate level. Steve McKeand and Ross Whetten teach FOR 411 (Forest Tree Genetics and Biology), our undergraduate tree improvement course, and FOR 725 (Forest Genetics). Steve also taught FOR 727 (Tree Improvement Techniques) this spring. Students in FOR 727 were able to spend a week at Arrowhead doing hands on selection, grafting, and operational breeding work. Ross also teaches FOR 350, Professional Development III: Ethical Dilemmas in Natural Resource Management, and BIT 815, Deep Sequencing Data Analysis.



*Above left: FOR 727 class learning how to take core samples from director and FOR 727 professor Steve McKeand. Students use the cores for an associated class lab project.*



*Above right: FOR 411 and FOR 725 on a class field trip to North Carolina Forest Service at Goldsboro, NC.*

## Graduate Students

Tree Improvement student David Barker defended his dissertation in December 2013 and received his PhD degree in May 2014. He has been working with Rayonier since January 2013 and holds the position of Seed Orchard Specialist in Manor, GA. We also welcomed a couple of new students; all students are listed below:



**Greg Albert, MS** - Greg joined us in spring of 2012 and will be working at SETRES2. His research is, "Developing a high throughput ring-by-ring density and percent latewood assessment for tree breeders".

**Alfredo Farjat, PhD** - Alfredo is continuing his efforts on the PINEMAP project titled: "The effect of climatic variables on pine growth". Alfredo's research has been instrumental in enhancing the cold hardiness map for **PRS™**.



**Adam Festa, MS** – Adam was an undergraduate in Biology here at NCSU and decided he was interested in Forestry. His research is titled: "Modeling methods for prediction of genetic value and efficiency in Tree Improvement."

**Austin Heine, MS** - Austin was an undergraduate student worker for several years and decided to stay on for a masters. His research is titled "Impact of Improved Loblolly Pine Seedlings in the Southeastern United States since the Inception of the NCSU Tree Improvement Cooperative."



**Jesse Spitzer, MS** – Jesse's research is titled "Broad-Based Testing Compared to Local Testing of Families of Loblolly Pine for Resistance to Fusiform Rust". He should be completed by summer 2014.

**Laura Townsend, MS** - Laura's research is titled "Genomic analysis of loblolly pine adaptability to climate conditions". She is busily analyzing genetic sequence data and is expected to be completed by spring 2015.



## Membership in the NCSU Cooperative Tree Improvement Program

We keep the annual theme of change in effect when it comes to membership as well. We said goodbye to one group and welcomed another when it came to Research Associate Members. The Biofuels Center of NC was dissolved by the NC Legislature in October of 2013 and PBS International joined in 2014. PBS International is a major producer of pollination bags and is supporting our breeding research. As always, we appreciate the support of our research and breeding, testing, and selection 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  
 Dougherty & Dougherty Forestry Services, Inc.  
 F&W Forestry Services, Inc.  
 Jordan Lumber & Supply Company  
 Milliken Forestry Company, Inc.  
 Molpus Woodlands Group, LLC  
 North Carolina Natural Resources Foundation  
 Pacolet Milliken Enterprises, Inc.  
 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 (2010-2013)

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>

### 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 21<sup>st</sup> 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
- 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.
- 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. *Forest Science*. (in press).
- 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.* (in press).

### 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, L.J., 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.



The staff, graduate students, and undergraduate students were treated to a tour of Jordan Lumber & Supply Company this past December. It is always great to get out and see our member organizations in action, and it was an excellent learning opportunity for our students as well. Thanks for hosting us Chip!

This year has been a whirlwind. We have lost staff, gained staff, and forged ahead with the 4<sup>th</sup>-Cycle breeding and testing after catching our breath from last year's start.

*Front Cover: New Breeding Orchard at the GFC–NCSU Arrowhead Breeding Center with tops full of 4<sup>th</sup>-Cycle grafts.*

*Back: (clockwise) Chuck Little looking for Spring because it was late and made for a long breeding season in the Southeast US; Early morning breeding means great lighting; Steve McKeand showing Graham Ford and Austin Heine how to select 4<sup>th</sup>-Cycle winners.*

