# NORTH CAROLINA STATE UNIVERSITY COOPERATIVE TREE IMPROVEMENT PROGRAM

*60th Annual Report | May 2016*

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

## May 2016

Department of Forestry and Environmental Resources

College of Natural Resources

North Carolina State University

Raleigh, NC

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# EXECUTIVE SUMMARY

At age 60 years, the Cooperative Tree Improvement Program continues to provide value to the members, to NC State University, to the forestry and scientific communities, and to the landowners and citizens of the region.

## SELECTION, BREEDING, AND TESTING

Based on conelet and seed counts from 2015, the 4th Cycle is about half completed. With the surge of breeding this year, it is anticipated 2016 will be the last big year of breeding for the 4th Cycle, with 2017 being a "mop up" year for stubborn crosses.

Cooperative members have planted three years of 4th-Cycle tests thus far, resulting in the completed testing of 301, 254, and 267 crosses in the Coastal, Piedmont, and Northern test series, respectively.

After many hours of development, the Breeding Logistics database has been released as part of the TIPRoot database. Cooperative staff and members can more readily manage the complexities of the breeding, testing, and selection program.

Breeding in the Atlantic Coastal Elite population began in 2016. Pollen has been collected from 44 of the 48 ACE selections, so breeding in 2017 should proceed smoothly.

Renovations of the Tree Improvement Labs in Biltmore Hall are complete. Move-in is expected to be in May.

Cooperative members harvested almost 60,000 pounds of loblolly pine seed from orchards in 2015.

## RESEARCH

Age 10 measurements from the Hofmann Forest growth and yield trials were completed this year. Family differences in stand volume and sawtimber potential were large.

Three trials of the Loblolly Adaptation and Mapping Project were established in Texas, Georgia, and Virginia to provide a large number of full-sibling progeny for a very high-resolution genetic linkage map and to establish trees in very different environments to test for genetic effects on adaptability of loblolly pine to drought and cold.

Cold damage was assessed in the Loblolly Pine Biomass Genetics/Cropping Study in Butner, NC. Coastal and Piedmont provenances were significantly different for stem lean, foliage burn, and stem breakage. The odds of severe lean were 64% higher for Coastal families than for Piedmont families. The odds of foliage burn were 62% higher for Coastal families than for those families originating from the Piedmont.

Computer simulation has been used to estimate genetic value of individual progeny and the effects of inbreeding in long-term selection programs.

Development of SNP markers and haplotypes for fingerprinting is progressing. Two sets of candidate SNPs, the first of 144 loci and the second of 151 loci, have been tested as possible markers for fingerprinting purposes.

For the pollination bag study, the only bag types that were marginally significantly different from each other for the 2015 June conelet counts were Lawson and the OP treatments. The overall survival was much higher in 2015 across all bag types when compared to the 2014 experiment.

Third-Cycle pollen mix tests are being analyzed in detail to assess heritabilities and genetic correlations in the Coastal and Piedmont tests. Heritabilities for height and volume were higher than in the 2nd-Cycle diallel trials probably due to better experimental design.

We received a new USDA-NIFA grant to develop genotyping platforms for loblolly pine and sugar pine. The goal of the project is to discover informative single nucleotide polymorphism markers for genomic research and breeding.

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

Three companies joined the Cooperative as Contributing Members, but the merger of Weyerhaeuser and Plum Creek means a loss of one Full Member. There are now 10 Full Members, 20 Contributing Members, and 3 Research Associate Members. Graduate students' contributions to the program continue to be critical. We continue to teach short courses and workshops to members and colleagues at NCSU and around the world. Graham Ford and Chris Ball left the staff for other positions, and Trevor Walker took over as Tree Improvement Manager. We are searching for a new Analyst/Database Manager.

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# 60 YEARS OF TREE IMPROVEMENT AT NC STATE

## *A MESSAGE FROM THE DIRECTOR*

Yep, I did the arithmetic – the Cooperative started in 1956, so 2016 is 60 years. As I do each year, I struggle with what to write in the introduction to the Annual Report. As I have been told, "Reflections" are incredibly tedious, are almost always boring, and are usually written by old fogeys who don't have anything of value to say…OK, I get the hint. BUT, I do think it's worthwhile to describe our past, so that the path forward may be a bit clearer for our future leaders in tree improvement and forestry.

Rather than go back and summarize and reflect on all 60 years, I thought it might be more interesting to talk about the successes and struggles since our Golden Anniversary in 2006. Let's start where I left off 10 years ago in the 50th Annual Report:

> *Despite the upheaval in land ownership, Cooperative membership changes, floods, fires, storms, and pestilence, the Cooperative Tree Improvement Program remains strong and its members remain committed to our mission:*

> ### *To Economically Increase Forest Productivity Through Genetic Manipulation of Forest Tree Populations*

> *Good times and bad will come, but optimism prevails as we start the next 50 years of genetic resource development to help produce a reliable, ecologically sustainable, and economically affordable supply of wood.*

So, we are 20% into "the next 50 years"- how are we doing? In my humble opinion, I think we are doing extremely well. Genetic gains are at an all-time high, and landowners are reaping the benefits by planting more and more acres of seedlings with outstanding genetic qualities. Our 4th-Cycle breeding and testing program is moving along at a record pace. We have 33 members of the Cooperative with 10 Full Members, 20 Contributing Members, and 3 Research Associate Members, and each member's financial and in-kind contribution is critical to the success of the program. Support from the University remains strong, and we are recognized as significant contributors to the Land Grant Mission of NC State University. Our graduate students continue to do vital research and are a mainstay of our scholarly efforts. With our education and outreach to Cooperative members, landowners, the science community, forestry, and the general public, we emphasize the critical role tree improvement and forestry has to the economic, social, and ecological wellbeing of the region.

So here are some highlights of the last 10 years:

In 2006, the 12 companies and 4 state agencies who were the full members of the Cooperative had made the decision that we should consolidate breeding activities as much as possible at a "breeding center". With corporate mergers and divestiture of land by the vertically integrated forest products companies proceeding at a torrid pace, we were losing breeding capacity. While the concept was in place, resources were not available. In August 2007, at the "Crossnore Summit" a strategic plan was adopted that reshaped the Cooperative. The most significant decision by the members was to allow us to "test the waters" and invite non-traditional companies and agencies to become Contributing Members of the Cooperative. The Contributing Membership was designed for companies, organizations, or individuals who own or manage forest land, or for nurseries operating in the southern US that desire information about the genetic value of loblolly pine. Contributing Membership was open to landowners, consultants, nursery owners, and other entities or individuals who want to contribute to the mission of the Cooperative but do not want to own germplasm. They receive breeding values and performance information about all Cooperative selections that are planted in their operating territory, but they do not have access to germplasm. Our pledge was that the funds

from these new members would be used primarily to support and rebuild the capacity to conduct an aggressive and efficient breeding program.

The success of the Contributing Membership surpassed our wildest expectations. We currently have 20 of these members (see page 34), and their contributions have allowed us to not only do breeding at the Arrowhead Breeding Center in Cochran, GA and conduct large clonal testing efforts (e.g. the Atlantic Coastal Elite and Northern Elite Populations), but we also use these funds to support graduate student research and other aspects of our research and breeding programs.

To better serve our membership and to facilitate managing the Cooperative's vast amount of data, we invested substantial time and financial resources over the last five years to develop the online database TIPRoot, which stands for Tree Improvement Program Rapid online output tool. Members now have easy access to all of the breeding values, pedigree information, and **PRS**™ (**P**erformance **R**ating **S**ystem) values for 5361 open-pollinated families and 8584 full-sib families. **PRS**™ Spec Sheets can be generated for these families to easily convey their genetic worth to landowners and foresters. This year, the Breeding Logistics component was added to TIPRoot to help staff and members manage the breeding, testing, and selection phases of the program.



Full, Contributing, and Research Associate Members 1956-2016

*Membership in the Cooperative had plunged to 14 in 2007. The Contributing Membership that started in 2008 and the Research Associate Membership have revitalized the Cooperative and have allowed us to continue aggressive breeding, research, teaching, and outreach programs.*

Breeding for the 4th Cycle started in 2012. The decision to jump from the third cycle to the fourth was due to a long list of reasons (see p. 2-6 in the 57th Annual Report, 2013). For the 4th Cycle, the MateSelect algorithm developed by Kinghorn (2011) for animal breeding was used to design matings among selections. The algorithm allowed us to model various scenarios to optimize genetic gain while putting constraints on relatedness. After comparing these modeling outputs to the subline system planned in the 3rd-Cycle breeding, we decided to immediately commence the 4th Cycle. Crosses made for the 3rd Cycle were incorporated into the new plan, and a total of 800 crosses for the Coastal Plain, 500 for the Piedmont, and 300 for the Northern region were planned. These crosses were designed to maintain genetic variation for long-term gain and diversity, as well as deliver significant value to landowners in the near term with a subset of elite crosses.

Scholarship in the program is at an all-time high. Since 2006, Cooperative staff and students have published 70 refereed journal articles, two book chapters, and 17 popular/proceedings articles. Over the last 10 years, we received 13 competitive grants, bringing in over $3.1 million dollars of federal and state competitive grants that enhance and complement our program. The success of the breeding program and the magnitude of field trials are used to leverage additional grant support.

Unprecedented advances in DNA sequencing technology since 2008 have us poised to develop genomic selection in the coming years. Genomic selection requires repeatable, reliable, and cost-effective genotyping platforms. A recent USDA grant lead by Cooperative scientists aims to discover thousands of markers and design SNP arrays for genomic selection. The Cooperative has also been developing a special population (Atlantic Coastal Elite), which has the potential to validate the predictive ability of markers across generations. Support from Cooperative members and from granting agencies allows us to pursue innovative research that may have profound changes on the way breeding is conducted and genetic gain is realized in the future.
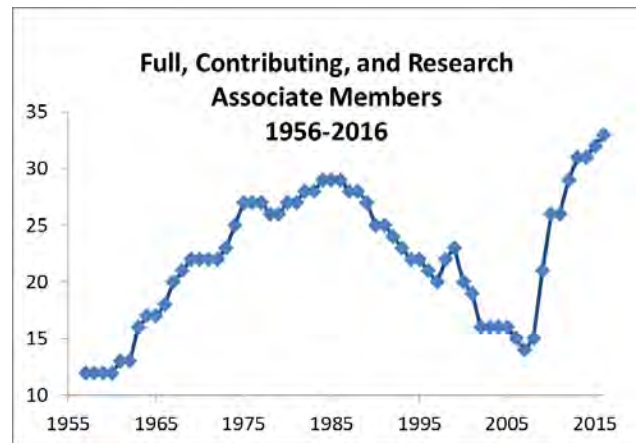
Education and training of graduate and undergraduate students is a high priority for the Cooperative faculty. We continue to offer one undergraduate and three graduate courses in forest genetics/tree improvement. According to a recent survey among US universities with forestry departments, we are the only institution teaching these kind of courses. In addition to the 6 undergraduates currently working for the Cooperative and learning tree improvement skills and techniques, we have 8 graduate students (5 PhD, 3 MS) working on critical research for the Cooperative. Since 2006, 10 PhD and 12 MS students have graduated from our program. All of these graduates are currently employed in genetics and/or forestry positions and will be the future leaders of tree improvement and natural resource management.

Of course with every success story, there are challenges and hardships along the way. While we have gained membership and support, the Cooperative has lost some significant members over the last 10 years. CellFor, Inc., International Paper Company, Joshua Land Management, L.L.C., MeadWestvaco Corporation, Plum Creek Timber Company, Smurfit - Stone Container Corporation, and Temple-Inland Inc. were all full members in 2006. International Paper and MeadWestvaco became ArborGen, and Plum Creek recently merged with Weyerhaeuser, but the other companies were lost from the Cooperative. The most difficult part for me is to witness the impact these losses have on the lives of so many close friends and colleagues. Careers have been disrupted, and many outstanding foresters and tree breeders no longer work with us.

Dr. Bruce Zobel passed away February 5, 2011. Bruce started the Cooperative in 1956, and his legacy with NC State and other forestry programs continues today and will continue on for generations to come. No tree breeder has had a greater influence on forests and forestry, but Bruce's greatest contribution was probably the students he taught and mentored. Many of his 100+ graduate students hold positions of substantial responsibility in universities, governments, and industries throughout the world. The true mark of excellence in teaching is the ability to teach thinking and independent reasoning; Bruce was unsurpassed in this capacity. We still miss him, and we thank him for all he did for us.

And finally, one of the biggest challenges the last 10 years have been all the personnel changes in Raleigh. The list of comings and goings is too long to summarize here, but it seems that when outstanding people work for the Cooperative, other organizations and companies want to hire them. It's rewarding to watch young people have outstanding careers in tree improvement and natural resource management, but it does take its toll on those left to manage the Cooperative. Fortunately, we have maintained an outstanding group of faculty, staff, and students who are dedicated to making the Cooperative Tree Improvement Program a special organization.

What will the next decade bring to Tree Improvement? Let's talk again in 10 years.



*The Arrowhead Breeding Center in Cochran, GA has been a huge change for the Cooperative. All forward selections for the 4th-Cycle program have been topgrafted into the New Breeding Orchard, and breeding is progressing very well. We thank the Georgia Forestry Commission for their support and partnership with us at Arrowhead.*

*Steve McKeand*

*May 2016*

# SELECTION, BREEDING, AND TESTING

## Fourth Cycle Breeding and Testing Progress

### Breeding

The 2016 breeding season was the fourth year of breeding 4th-Cycle crosses, and is on track to be the most productive! This season was the biggest yet for Cooperative breeding at the Arrowhead Breeding Center, where staff put up 312 bags and made 168 unique crosses. Breeding numbers are still coming in as the season winds down, but all signs point to this being an excellent year for making pollinations. Progress through 2015 is reported in Figure 1. By the end of the 2016 season, it is projected more than 75% of the breeding will be completed for all regions.
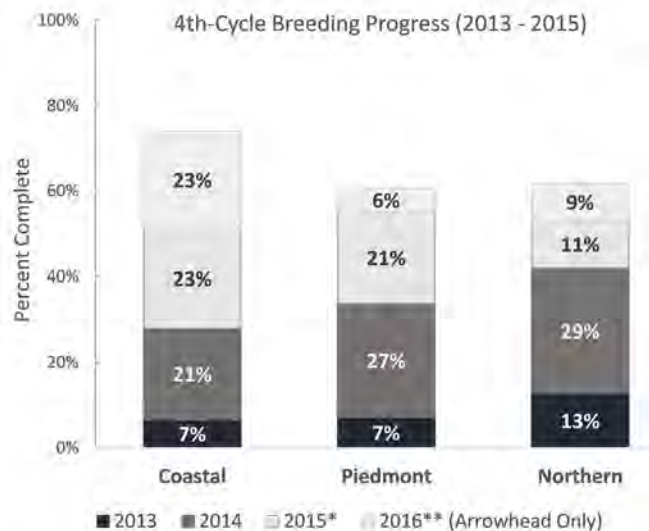


*Figure 1. Progress towards completing the 4th-Cycle breeding by region, as a percent of number of planned crosses (about 600 Coastal, 350 Piedmont, and 180 Northern crosses). Numbers in 2013 and 2014 only include crosses where an adequate amount of seed was harvested (> 90, enough to completely test within one region). *2015 numbers are based on total number of crosses completed. **2016 numbers are based only on breeding done by Tree Improvement staff at Arrowhead (does not include 2016 breeding done by members).*

While great strides have been made towards completing the 4th-Cycle breeding, they have not been without difficulties. Table 1 shows the crossing success rate, calculated as the proportion of crosses made during a breeding year that produced an adequate

*Table 1. Cross success rate by breeding year for 4th-Cycle breeding. A successful cross is defined as having enough seed to complete testing in one region (> 90), assuming adequate germination rates. To fully test a cross, enough seed is needed for all regions where the cross will be considered for testing.*

| Breeding Year | # Crosses Bred | # Crosses Yielding Enough Seed for Testing | Success Rate |
|---|---|---|---|
| 2013 | 126 | 86 | 68.3% |
| 2014 | 330 | 218 | 66.1% |
| 2015 | 308 | | |

amount of seed for sufficient testing. For the first two years of 4th-Cycle breeding, the average success rate was 67%. The best strategy for dealing with the cross success rate is to do conelet counts in the fall/winter to decide if continued breeding is necessary. Tree Improvement staff will re-attempt a cross if fewer than 6 conelets are surviving.

Another setback to the 4th-Cycle breeding timeline was a fire at one of the Cooperative member's facility that resulted in a complete loss of their 2014 breeding. Fortunately, no one was seriously injured, but the setback to the breeding program was significant.

It is anticipated that 2016 will be the last big year of breeding for the 4th Cycle, with 2017 being a "mop up" year for stubborn crosses. Part of the rapid progression of the breeding plan is the increased efficiencies in the testing design, which doesn't necessitate balance and the associated over-breeding for "insurance" seed. However, the biggest contributor to the success has been the effort and cooperation among our members!

### Testing

The 4th-Cycle testing effort has been the Cooperative's most aggressive and hopefully the most efficient series of tests yet. The "rolling front" testing strategy permits testing of crosses as soon as seeds are available from the breeding effort. This is possible because crosses are tested in multiple years using a staggered approach; the

tests that are established each year are comprised of approximately 50% new crosses and 50% crosses that were tested in the previous year (Figure 2). The target number of replicates for each cross is 60, which is achieved after two years of testing when planting 30 reps per year.
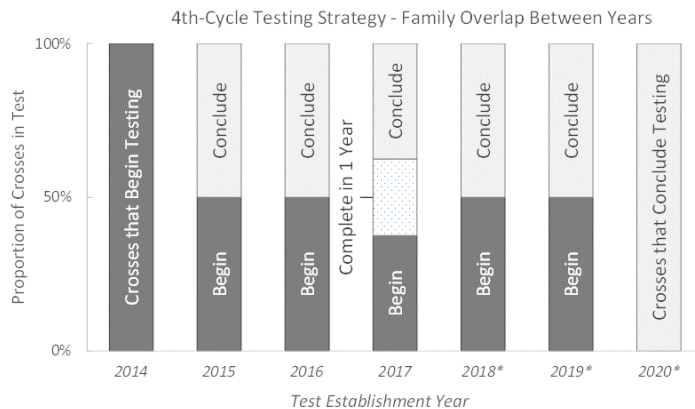


*Figure 2.* Schematic of "rolling front" testing strategy for the 4th-Cycle testing. *Note that tests designed for 2018 and later depend on the success of crosses being completed in the breeding program.*

However, if we continued testing 30 reps per year, the timeline for completing testing for all 4th-Cycle crosses would be 2023. **The Cooperative members have expressed the desire to speed up the testing plan and hasten the timeline; this need has been addressed in the 2017 test design.** The factors that influence the completion timeline of the field tests are 1) number of reps established each year and 2) the number of families tested each year. In response to the former, the Cooperative members committed to doubling up on the number of reps planted each year. To address the latter, the 2017 test design includes more crosses than any of the previous years. Before you start groaning about bigger reps… listen up! We increased the number of families without increasing the rep size. In fact, *the rep sizes are smaller*! How did we do it? By planning *two test series per region* in the 2017 tests (Figure 3). In this approach, crosses are assigned to one of the two series, with a handful of crosses appearing in both series to fortify comparisons between the series. Those families appearing in both series will have their testing completed in one year, accelerating the testing timeline. This approach allows the testing of more families without increasing rep
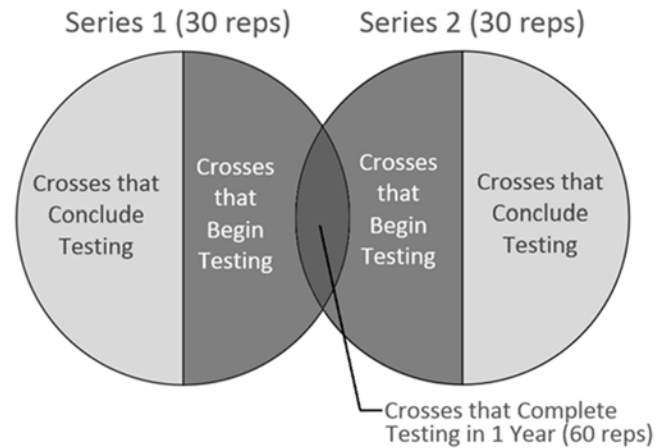


*Figure 3.* *Design of the 2017 progeny tests. By implementing two test series, more families can be tested without increasing the rep size. A portion of the families are planted in both series to foster comparisons between series; these families will have their testing completed in one year instead of two, further accelerating the timeline.*

sizes and while maintaining connectivity with tests from other years.

Cooperative members have planted three years of 4th-Cycle tests (Figure 4), resulting in the completed testing of 301, 254, and 267 crosses in the Coastal, Piedmont, and Northern series, respectively.

Other features of the 4th-Cycle testing strategy include:

- Comparison of families across testing regions (see "regional testing overlap" illustrated in Figure 3 of the 2015 Annual Report).

- Increased analytical power through optimized seedling coordinates within a rep to create incomplete blocks among planting rows and columns (see *Progeny Testing* section on page 5 of the 2013 Annual Report).

Implementation of this advanced testing protocol has been a challenging endeavor, but Cooperative members have established high quality tests with excellent attention to detail. It's hard to believe the first 4th-Cycle tests will be assessed next year!
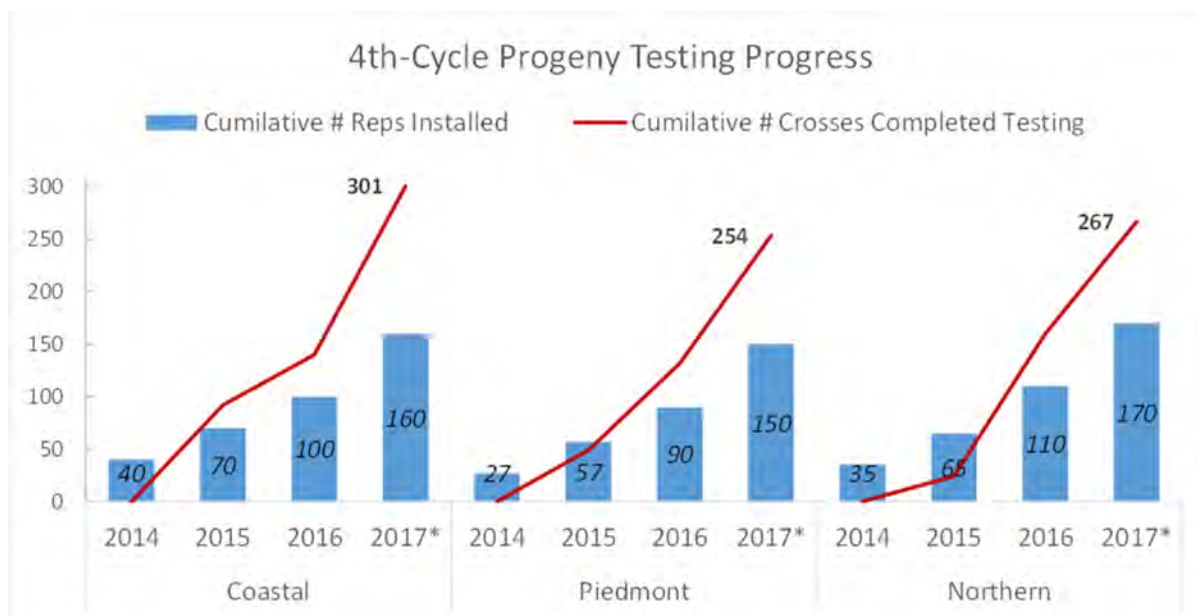
*Figure 4. Progress towards completing the 4th-Cycle testing effort. *2017 tests are being sown this spring and will be grown this summer and be planted in field trials this upcoming winter.*



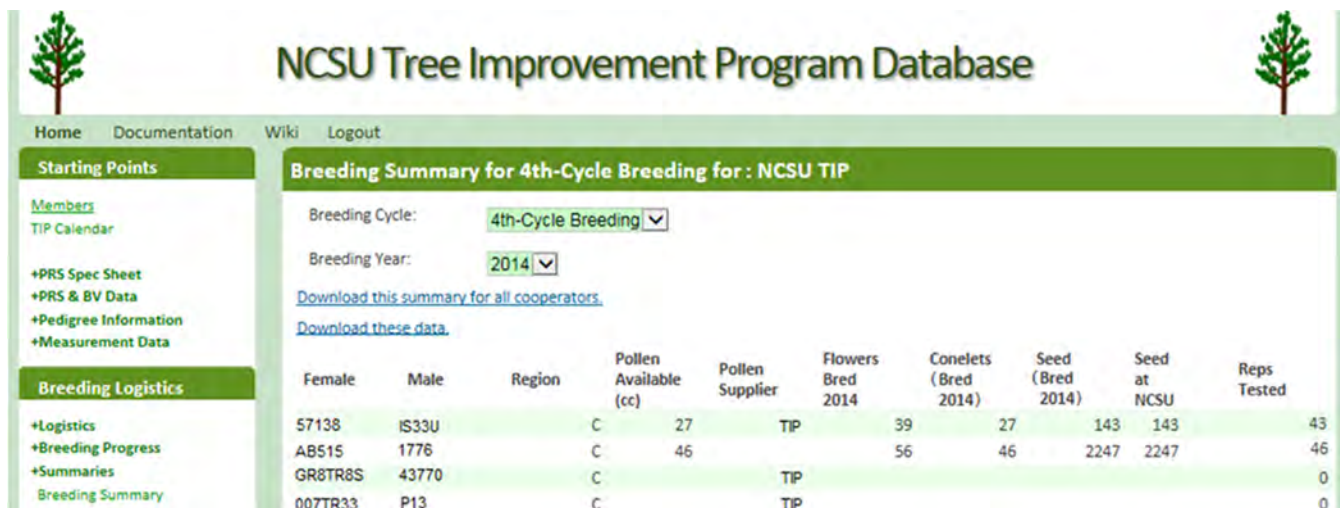*Seedling row-column coordinates are optimized in the 4<sup>th</sup>-Cycle test design, and require careful attention and verification of planting locations in the field, as was done in this test established by the Virginia Department of Forestry.*

# TIPRoot Database Updates[1]

## Breeding Logistics Released on TIPRoot for Full Members

After many hours of development, the Breeding Logistics database has been released as part of the TIPRoot database! All things related to the Cooperative's breeding effort can be found on Breeding Logistics, including breeding/pollen assignments and tracking of progress towards those assignments. The release was implemented in three steps: first, the *Logistics* section was released, followed by the *Breeding Progress* section, and finally the *Summaries* section. The *Summaries* section pulls all of the information into one place to help you decide which crosses to breed in the upcoming breeding season!



*Figure 1.* *The Breeding Summary query on the Breeding Logistics section pulls together all of the information necessary to determine if breeding has been completed for each of the crosses assigned to a member.*

## Arrowhead Database is Operational on TIPRoot for Tree Improvement Staff

Another exciting development on TIPRoot is the Arrowhead database. This database facilitates implementation of the breeding plan by Cooperative's staff, and also tracks long-term trends in breeding operations. The database is designed to merge the resources available at an orchard (trees, topgrafts, flower counts, pollen inventory) with the breeding plan housed in Breeding Logistics. The staff utilized the Arrowhead database for the first time this breeding season with excellent results; more crosses were bred by staff than any other season in the 4th Cycle. After this spring, it's hard to imagine how we will bred trees without it!



*Figure 2. Arrowhead database pages include all aspects of implementing a breeding plan.*

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[1]We love acronyms in the Tree Improvement Program. TIPRoot stands for Tree Improvement Program Rapid online output tool and is the name for our online database.

## Atlantic Coastal Elite Population Breeding Begins at the Arrowhead Breeding Center

In the spring of 2014, selections from the Atlantic Coastal Elite (ACE) Population were topgrafted into the New Breeding Orchard at the Arrowhead Breeding Center in Cochran, Georgia. These 48 selections were identified from 51 crosses of the ACE Population as having a superior rank for volume, stem straightness and forking (for ACE background information refer to the 58th Annual Report, pages 11-12).

The ACE selections are an integral part of the 4th-Cycle Coastal breeding plan, and comprise more than 80 of the planned crosses. The ACE Population is also ideal for genomic selection research and development, because of its population size (only 21 parents) and structure. As part of the original ACE Population, 2362 clones from 51 crosses will be genotyped with SNP markers developed by the ongoing USDA-NIFA grant (2016-67013-24469) to develop marker-trait associations. The objective is to predict volume, stem straightness, fusiform rust disease resistance, and stem forking characteristics of young seedlings prior to testing in the field. Crosses among the 48 new ACE selections will be used to validate the genomic selection models.

2016 was the first year of flower production for the ACE selections. Luckily, pollen was available for collection in 2015 from a handful of the ACE selections. This pollen was used to produce the first ACE crosses during the 2016 breeding season.

In addition to breeding flowers in the New Breeding Orchard, flowers were also bred in the Coastal Breeding Ramet Orchard (ramet orchard in short). The ramet orchard was planted in 2010 using the 8th ramet of the 2362 clones. The intent was to have at least 1 ramet of each ACE clone planted at Arrowhead Breeding Center so that when selections were made at age 6, the trees would already be producing flowers. The idea was to eliminate the waiting period for topgrafts to produce sufficient flowers for breeding. Although the plan was altered slightly by making selections in ACE tests using age 4 data, the idea behind the ramet orchard worked! This year, we were able to begin breeding and collecting pollen from these ACE selections in the ramet orchard.

In addition to collecting pollen from the topgrafts and ramets in the ramet orchard, we also collected pollen from the Georgia Forestry Commission ACE test that is planted at the Arrowhead Breeding Center. From this 6-year-old trial, we were able to collect pollen from 20 of the ACE selections. Collecting pollen from forward selections in future tests could be used to accelerate breeding in the 5th Cycle, especially if competing trees surrounding the selection are removed.

The idea of speeding up breeding using a cloned progeny test was deemed a success. The ability to collect pollen from the ramets in the ramet orchard this year was of great importance, primarily because the majority of topgrafts are not yet producing adequate amounts of pollen. In summary, we were able to collect pollen from 44 of the 48 ACE selections from the above sources. Since pollen is typically a major limitation for breeding with young selections of loblolly pine, we were delighted to be able to collect pollen for nearly all of the selections. With this ACE pollen in hand and high hopes of flowering on all ACE selections in spring 2017, ACE breeding is heating up!



*Austin Heine putting up the first pollination bag this spring for ACE breeding at the Arrowhead Breeding Center.*

# SEED AND CONE YIELDS

Cooperative members harvested almost 60,000 pounds of loblolly pine seed from operational orchards in 2015 (Table 1). This amount was down from 2014, which was a big seed harvest year. Average yield of seed per bushel changed little compared to 2013, with the exception of the Northern yields, which had a considerable drop in pounds of seed per bushel. The decrease in seed harvest occurred in all provenances and generations, except for Coastal 2.0/2.5 orchards,

which saw a modest bump in production. The decline in production for Piedmont and Northern orchards are most likely due to the surplus harvest from 2014.

At approximately 12,000 seedlings produced per pound of seed, last fall's harvest can result in almost 700,000,000 seedlings being grown this year. That is enough to plant approximately 1,400,000 acres of pine plantations!

*Table 1.* Fall 2015 cone and seed yields compared with the figures from 2014 harvest

| Provenance / Generation | Bushels of Cones | | Pounds of Seed | | Pounds per Bushel | |
|---|---|---|---|---|---|---|
| | 2015 | 2014 | 2015 | 2014 | 2015 | 2014 |
| Coastal 2.0/2.5 | 20,522 | 15,282 | 28,442 | 22,066 | 1.39 | 1.44 |
| Coastal 3.0 | 7,388 | 9,511 | 9,560 | 11,474 | 1.29 | 1.21 |
| Piedmont 2.0 | 7,862 | 12,598 | 10,288 | 18,418 | 1.31 | 1.46 |
| Piedmont 3.0 | 4,020 | 11,101 | 5,750 | 14,893 | 1.43 | 1.34 |
| Northern 2.0 | 750 | 1,319 | 501 | 1,511 | 0.67 | 1.15 |
| Northern 3.0 | 2,868 | 2,966 | 3,477 | 3,175 | 1.21 | 1.07 |
| *Totals* | **43,409** | **52,777** | **58,018** | **71,537** | **1.22** | **1.28** |

## Lab Renovations

In April 2015, we were made aware that the Tree Improvement laboratories, used for teaching and research, were approved for renovation under the Chancellor's Faculty Excellence Program. Tree Improvement faculty and staff met with designers and engineers starting in May of 2013 to discuss plans to update the labs in Biltmore Hall. Over the next 18 months, we put forth ideas, room layouts, and infrastructure that would be more conducive to the current research and teaching needs. As blueprints and floorplans were drawn, the project came to life, but it still needed to be funded! With support from the Department and College, the proposals and drawings were sent to Chancellor and University for approval. Much to our pleasure, the lab renovations on the first floor of Biltmore Hall were selected to be funded for fiscal year 2016 by the Chancellor's Faculty Excellence Program and the College of Natural Resources.

Packing up the equipment, tools, and supplies that accumulate in a lab with a 60-year history of research was no small task. In July 2015, four rooms of chemicals, tools, equipment, and freezers were packed up and moved to a temporary lab near Centennial Campus. It was painful, and the freezers have since forgiven us, but we unpacked what we needed and got back to work. Demolition started late August and was scheduled to last 8 months, and it appears the project is on schedule and should be complete this summer!

The new lab spaces are simply unrecognizable. Where there were once 4 segmented rooms, there are now two large open lab spaces that better accommodate the various research tasks under the umbrella of the Tree Improvement Program. The space now includes windows and plentiful counter space for research equipment and student work areas. We are eager to

move back in this summer and have our labs close to the office again. We are thrilled with the results and want to thank the University and the College of Natural Resources for the support!
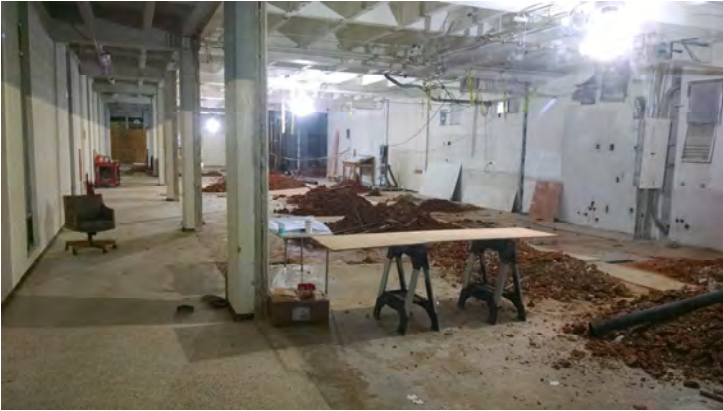








*Clockwise from top right, Austin Heine happily pointing to the new labs. Tree Improvement Staff admiring the views now including windows. Tree Improvement Lab includes mobile work spaces along with a specialized "Dirty" room with a snorkel to contain airborne particulates from seed and pollen processing. The new molecular lab also includes flexible bench space with lots of storage. There is also a soundproof room for grinding equipment and centrifuges. Demolition began in September 2015. We have come a long way from no walls and dirt mounds!*

# RESEARCH

## Age 10 Measurements on Hofmann Forest Genetics Growth and Yield Trials

Over the winter of 2015 and 2016, Tree Improvement staff and students, as well as students from the University of Georgia measured two genetics growth and yield block-plot trials on the Hofmann Forest that are now age 10 years.



*NCSU and UGA students take a break from measuring the GST to pose for a picture!*



**Figure 1.** *Layout of block-plots for the Genetics x Spacing x Thinning (GST) study and the Clonal/FS/OP Demonstration Growth & Yield Trial at the Hofmann Forest, NC.*

The Genetics x Spacing x Thinning study (GST) is over 70 acres in size, containing block-plots for 10 genetic entries with varying degrees of genetic uniformity (clones, full-sibs, half-sibs, and orchard mix). Each genotype is planted at two spacings (narrow and wide) under a thin and no thin scenario, and there are three replicates (Figure 1). The 2006 Hofmann Forest Clonal/FS/OP Demonstration Growth & Yield Trial does not have the spacing or thinning treatments, but contains more genetic entries. Both of these studies seek to address growth and yield differences among loblolly pine genotypes. The Clonal/FS/OP Trial also serves as a demonstration area to display the value of genetics to landowners, foresters, students, and visitors.

While data from the GST study are still in analysis, 10-year results are available from the Clonal/FS/OP Genetics Demo/Trial. Differences in total volume yield existed among genotypes (p value < 0.0001), both between and within levels of genetic uniformity (Figure 2). For example, full-sibs tended to produce

more volume than open-pollinated (OP) families, but there were particular OP families with very high volume production (e.g. OP2 vs FS4, Figure 2). Differences in stem quality traits were observed, such that some stands had a higher yield of trees with sawtimber potential even though their total production was less (e.g. FS4 vs OP2, Figure 2). These observations underscore the need to "know what genetics you're planting"!

Practically all differences in yield on the block-plots could be explained by average tree size ($R^2 = 0.93$). In general, tree survival was high due to very low rust incidence and the short amount of time since crown closure.

Such findings emphasize the necessity of stand level assessments, and the Hofmann Forest is an excellent asset for conducting such trials due to the large contiguous area available. As analysis begins on the GST data, we will gain further insight on "if and how" planting density and thinning interact with genotype – so stay tuned!

Yield at Age 10 on Clonal/FS/OP Genetics Demo/Growth & Yield Trial
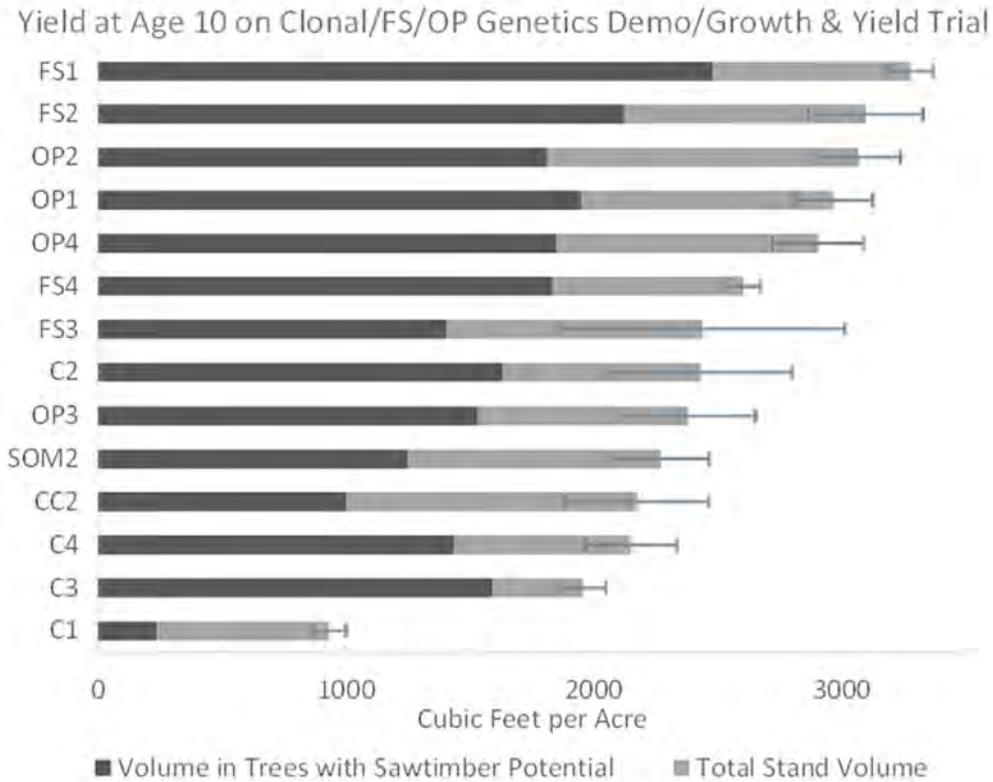
*Figure 2. Mean yields by genotype and product potential calculated from age 10 year measurements on the Clonal/ FS/OP Demonstration Growth & Yield Trial at the Hofmann Forest, NC. Family codes read as follows: FS = full-sib; OP = open-pollinated or half-sib; C = clone; SOM = seed orchard mix; CC2 = NC Coastal non-improved checklot.*

The demonstration portion of the Hofmann Forest Clonal/FS/OP Trial provides an invaluable resource for communicating the value of tree improvement. Upon arrival to the site, visitors immediately see stark difference among genotypes. The trial was designed such that the buffer trees adjacent to the road are also 10-tree family row plots that serve as a demonstration area.

Striking differences among geno-types are easy to observe on the demonstration plots at the Hoffman Forest Clonal/FS/OP Genetics Demonstration Growth & Yield Trial. This photo shows the Unimproved Checklot from the North Carolina Coastal Plain (CC2, center row) growing adjacent to a particularly fast growing open-pollinated family (right).

# Loblolly Adaptation and Mapping Project—Overview and Study Plan Outline

Preparations have begun for a project called the Loblolly Adaptation and Mapping Project (LAMP) at NC State. This project has five objectives, described in more detail later, but the preparations now underway involve growing and planting seedlings in field trials. These seedlings are all full-sibling progeny of a single two-parent cross, and are being planted at three sites – one in east Texas near the western limit of the natural range of loblolly pine, one in central Georgia in the heart of the range, and one in Virginia near the northern limit of the range. Each site will have 1156 seedlings planted, for a total of 3468 plants. The goal of this planting effort is first to provide a large number of full-sibling progeny for a very high-resolution genetic linkage map, and second to establish trees of the same genetic background in very different growing environments to test for genetic effects on adaptability of loblolly pine to drought (in Texas) and cold (in Virginia). The two parents of the cross are 20-1010, a Virginia loblolly pine selection that is the reference genotype used for sequencing and assembly of the draft loblolly pine genome sequence (see Neale et al. 2014 in the list of publications), and a well-tested South Carolina selection from the Coastal Plain. South Carolina was chosen as the source of the other parent to ensure there would be differences between the two parents in terms of growth rate, form, and adaptation to cold.

The overall goal of LAMP is to create a genomic toolbox for loblolly pine breeding and population management by adding value to the loblolly pine genome sequence assembly. The project has five objectives to help reach that goal. The first is to establish a biological resource for high-resolution genetic linkage mapping and analysis of the genetic basis of adaptation; this is the population of over 3400 full-sibling progeny established in field sites this year. The second is to use new DNA sequencing and analytical techniques to increase the size of DNA sequence scaffolds in the 20-1010 genome assembly to the point where at least half of the scaffolds are large enough to be assigned to a genetic linkage map in the correct order and orientation. The third is to use high-throughput genotyping methods to obtain genotype data for the set of full-sib progeny and use those data for genetic linkage mapping of genome assembly scaffolds. The fourth is to identify expressed genes within 20-1010 and assign those genes to the correct positions in the genetic linkage map and the genome sequence assembly. The fifth is to identify regions of genomic DNA that are associated with controlling gene expression, and to test genetic variants in those regions for associations with phenotypic variation across the pine population.

The availability of a draft genome sequence assembly of the Virginia loblolly pine selection 20-1010 means that research into the genetic basis of differences in adaptation in loblolly pine can now begin to move into the genomic era. Preliminary association genetics studies have demonstrated the possibility of detecting genetic variants related to adaptive traits in specific genes, but scaling from preliminary studies to whole-genome analysis requires creating new experimental resources, and overcoming specific technical obstacles. Additional improvement of the 20-1010 genome assembly is needed, along with a better understanding of how similar or different the genomes of other individual pine trees may be to the genome of 20-1010.

The v1.01 loblolly pine genome sequence assembly is highly fragmented, in that it consists of 14.4 million separate pieces, or scaffolds. In addition, the total number and locations of functional genes remain to be determined, and very little is known of the regulatory mechanisms that control gene expression. A v2.0 assembly should be released by mid-2016, and it is expected to be somewhat more "contiguous", that is, to have fewer and larger scaffolds, but it is unlikely to represent a finished product in terms of utility for either genomic research or breeding.

The long-term goal, for breeding applications, is to connect all the DNA sequence scaffolds from the genome sequence assembly to chromosomes in the pine genetic linkage map, and to annotate the locations of functional genes within those scaffolds, so that

[1] Remington D.L., R.W Whetten, B.H. Liu, and D.M. O'Malley. 1999. Construction of an AFLP genetic map with nearly complete genome coverage in *Pinus taeda*. Theor. Appl. Genet. 98 (8):1279-92.

segregation of phenotypic differences within and among families can be associated with specific genes in the genome. High-throughput genotyping technologies can collect data efficiently on specific genetic marker loci, but organizing and analyzing those data will be most efficient if they can be placed into the context of a more contiguous and better-annotated genome assembly. The term "functional genes" is used here to mean both the coding sequence that is used to produce a gene product and the regulatory sequences in DNA near the coding sequence that control when, where, and how much of the gene product is produced. A substantial amount of data exist about potential coding sequences in the pine genome, but virtually nothing is known about potential regulatory sequences that control gene expression.

Anchoring DNA sequences to a genetic linkage map requires analysis of segregation in a large number of offspring. One key biological resource, then, is a set of progeny in which genetic linkage analysis can be used to place DNA sequence assembly scaffolds in the correct order and orientation with respect to each other to form a chromosome-scale linkage group. The PineRefSeq project that conducted the genome sequencing and assembly used a total of about 1000 progeny from two different families to conduct genetic linkage

analysis and to date has reported map locations for a few thousand SNP loci. This provides an order for DNA sequence scaffolds on the linkage map but does not show the correct orientation in most cases. The largest scaffold in the v1.01 genome assembly is 8.8 million DNA basepairs, or less than 0.04% of the 23-billion-basepair length of the pine genome. The length of the pine genetic linkage map is estimated at 1500 cM, so a uniform distribution of recombination in the genome would mean that 1 cM (equivalent to about 1% recombination per generation) corresponds to over 15 million basepairs of DNA, and genetic markers at opposite ends of the largest scaffold would be less than 0.6 cM apart on the genetic linkage map. This is close to the lower limit of resolution for linkage mapping in a set of 1000 progeny, so it is likely that only the very largest scaffolds in the v1.01 assembly can be correctly oriented on a genetic linkage map with respect to other markers on the same chromosome.

Preliminary work to date on LAMP has been supported by the Cooperative Tree Improvement Program, but additional support will be essential in completing all the project objectives. Federal competitive grants and collaborative efforts with other organizations are options to be pursued in seeking additional support to help achieve LAMP objectives.



*LAMP seedlings at about 2 months. Seedlings were later planted in field trials in Texas, Georgia, and Virginia.*

# Loblolly Pine Biomass Genetics/Cropping Study (Butner, NC)

In winter of 2012, twenty of the Cooperative's fastest-growing families, each with varying potential for bioenergy feedstock, were planted on a Piedmont site at the North Carolina Department of Agriculture & Consumer Services Umstead Farm in Butner, NC. Ten families from each of two seed sources (Coastal and Piedmont regions) were selected based on volume productivity. Five of the ten families from each region were selected based on volume production only, and the other five were selected for high sawtimber quality and good volume productivity.

The first objective of the study is to investigate the possibility of substantially increasing the amount of biomass produced by taking advantage of genetic differences that exist in loblolly pine. The second objective is to demonstrate to forest landowners that planting loblolly pine as a dedicated energy crop will provide options both for early harvest for biomass and/or longer rotations for sawtimber production. The final objective, which is the primary focus of this report, was to evaluate the risk/reward of planting fast-growing Coastal families in an area where they are less adapted to the climate than Piedmont families.

In the winter of 2015, when trees were three year old, total height, stem straightness, and presence of fusiform rust galls, forking, and ramicorn branches were assessed. As expected, most Coastal families were substantially taller than Piedmont families, but they were also more prone to forking (see p. 24 in the 2015 Annual Report). Soon after these measurements, the site experienced two severe ice/snow storms and extreme cold temperatures (low of 9°F, -13°C) providing an excellent opportunity to measure cold damage differences among seed sources. A re-measurement was done the following May to assess foliage burn, stem breakage, and lean (see images on opposite page).

Generalized linear mixed models were used to analyze the factors causing stem breakage. Provenances (Coastal and Piedmont) were significantly different for stem lean, foliage burn, and stem breakage. The odds of severe lean were 64% higher for Coastal families than for Piedmont families (p-value < 0.0001). The odds of foliage burn were 62% higher for Coastal

families than for those families originating from the Piedmont (p-value < 0.0001).

The most significant factor influencing stem breakage was tree height prior to the storms; taller trees had a higher probability of stem breakage (Figure 1). Even after accounting for height, provenance still had a significant effect on the probability of stem breakage (p-value 0.0017). For trees of the same height, Coastal families still had a higher probability of stem breakage, with the difference between provenances increasing with taller trees. The overall mean incidence of stem breakage was approximately 4% higher in Coastal families vs. Piedmont families when holding height constant.
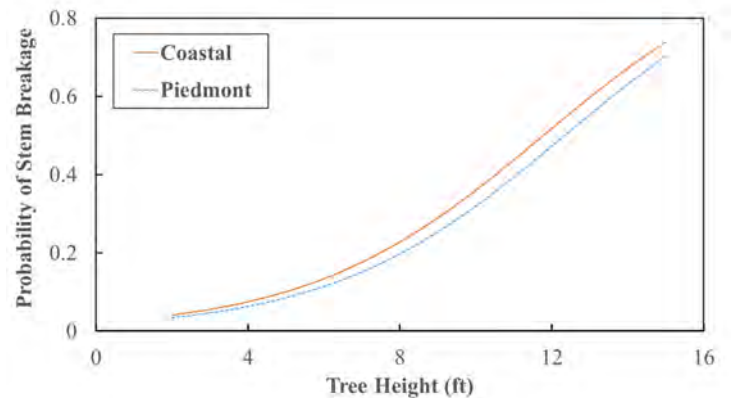


*Figure 1. Stem breakage probability by tree height by provenance, observed after the winter of 2014-2015 at the Loblolly Pine Biomass Genetics/Cropping Study in Butner, NC.*

These findings raise important questions: why did Coastal families have a higher stem breakage incidence than Piedmont families, even after accounting for tree height? The divergence in stem breakage rates between the provenances may be due to differences in crown structure, branch size and angle, stem bending strength, or needle characteristics between the two provenances. We are considering assessing crown structure, foliage length, wood properties, and branch size after the fifth growing season to better understand the underlying causes that contribute to regional differences.

*Tree on the left depicts what was a score = 4 for stem breakage. Stem on the right depicts a score = 2 for foliage burn.*

## Computer Simulation of Breeding Strategies and Inbreeding Depression in Loblolly Pine[1]

The main objectives of this study are to create a computer simulation method which can: 1) estimate total genetic value of individual progeny from simulated crosses, 2) simulate effects of inbreeding depression in progeny after multiple generations of a given breeding strategy, and 3) evaluate potential mating and selection strategies that could be used to mitigate the risk of inbreeding depression in the long term. While this simulation can be applied to multiple breeding programs, it was created for the purpose of evaluating loblolly pine breeding strategies.

The goal of most tree improvement programs is to develop future generations of genetically improved production populations that will result in more wood on less land in less time. Given the significant amount of genetic variation in loblolly pine, substantial genetic gain can be made in traits such as height, diameter, and

fusiform rust resistance that will meet this objective. Tree improvement programs face a trade-off in the rate at which genetic gain can be obtained by having to balance selection intensity with genetic similarity among future prospective selections. This trade-off occurs because mating related individuals will lead to inbreeding and a likely reduction in the average performance progeny (inbreeding depression), leading to reduced economic value.

Managing inbreeding depression within the Cooperative Tree Improvement Program 4th-Cycle breeding population is currently done using the differential evolution algorithm. The algorithm uses the pedigree to put constraints on the relatedness (inbreeding) among selections to be mated, while maximizing genetic gain in designing new crosses.[2] If not managed, the degree of inbreeding can cause

reduction in growth and reduced genetic gain. The ability to assess potential long-term risk of inbreeding depression in progeny performance would be beneficial in developing future selection strategies, and the assessment can most easily be accomplished by utilizing a computer simulation program. Simulation can generate any number of progeny from an array of breeding strategies without bearing the costs of actual breeding and progeny testing. Additionally, using simulation allows for testing of hypotheses for multiple generations, which is not practical with field experiments.

A recently published paper attempted to assess the performance of seven tree breeding strategies under conditions of inbreeding depression.[3] The authors concluded that most cross-breeding and selfing strategies resulted in fixation of unfavorable alleles, so the effectiveness of purging deleterious alleles is low. While this may hold true, the assumptions used to simulate the genetic architecture of founder populations, as well as the types of breeding strategies utilized in their paper, are not realistic or appropriate for evaluating loblolly pine. Additionally, with the constant reduction in cost of obtaining genotypic information, the possibility of evaluating other methods for screening future selections to manage inbreeding depression may also be explored. Some of these other methods which take advantage of genomic information have been investigated in livestock breeding programs.

To test the power of genomic information to manage or possibly purge inbreeding depression, we have created a simulator which more accurately depicts the true underlying genetic structure of loblolly pine and allows assessment of the reliability of using molecular markers in selection strategies. The simulator accepts a multitude of user inputs, including but not limited to the numbers of chromosomes, parents, causative loci that affect traits, and marker loci with no effect on traits. Other user inputs include the allele frequencies at all loci, the magnitude of allelic effects at causative loci, the level of dominance, the trait heritability, and the mating design for each generation. All of these parameters may be adjusted by the user in order to generate a starting population and subsequent generations of progeny that reflect a given situation. The simulator begins by creating a genetic linkage

map with the desired number of chromosomes, using a user-specified genetic map size, and then simulates a parental population. Each chromosome in the simulated genome can have several causative loci that affect a given trait positively or negatively, as well as markers that can be used as predictors to make future selections for breeding. Each simulated parent will have randomly-sampled alleles at the causative loci and at the marker loci, and phenotypes are simulated based on a user-specified combination of additive and dominant genetic effects plus environmental noise.

*Progress & Future Work*

The paper published earlier this year evaluated seven different breeding strategies under conditions of inbreeding depression.[3] Assumptions made in their simulations included 100 unlinked loci, all with equally small additive effects on trait phenotype, and a trait heritability of 0.3. Breeding strategies were assessed using three different allele-frequency distributions: intermediate-frequency, U-shaped, and a major-effect/minor-effect locus scenario. Given their underlying assumptions and our simulator, we could successfully reproduce the results reported in their paper, but some of their assumptions are biologically unrealistic. For example, published estimates of the length of the loblolly pine genetic linkage map range between 1400 and 1900 centiMorgans, so it is unrealistic to assume that 100 causative loci are completely unlinked. Using our simulator, we estimated genetic gain for four of the seven breeding strategies analyzed in their paper over 20 generations under the conditions of additive, partial dominance, or complete dominance, using a biologically-realistic U-shaped allele frequency distribution (Figure 1). These results show that given more realistic assumptions, there is a decrease in the expectation of genetic gain compared to the results reported in Figure 3D of the recent paper.[3] This difference can be accounted for by the fact that loci that are near one another, or linked, do not undergo recombination as often as unlinked loci, and alleles on the same chromosomes are often inherited together in progeny.

[1] *This is a summary of Adam Festa's graduate research .*
[2] *Kinghorn, BP (2011) Genetics Selection Evolution.*
[3] *Wu et al. (2016) G3 (Bethesda) 6(3):529-40*
[4] *Ford et al. (2015) For Sci 61: 579-85*

We have also implemented a mating design similar to that used in the recently published Tree Improvement inbreeding study[4] to depict three scenarios in which our simulator is set up to mimic effects of inbreeding depression on growth and quality traits in loblolly pine (Figure 2). The three traits shown (straightness, height, volume) at four inbreeding coefficient levels (0, 0.125, .25, .5) were recreated using varying amounts of dominance and SNP effects which gave rise to the same types of patterns previously observed in field measurements.

Moving forward, we plan to incorporate analysis of marker genotypes into the simulation procedure. Different strategies for breeding, testing, and selection will be compared to evaluate the relative ability of more traditional methods and marker-based methods to manage inbreeding depression over multiple generations in loblolly pine breeding populations.
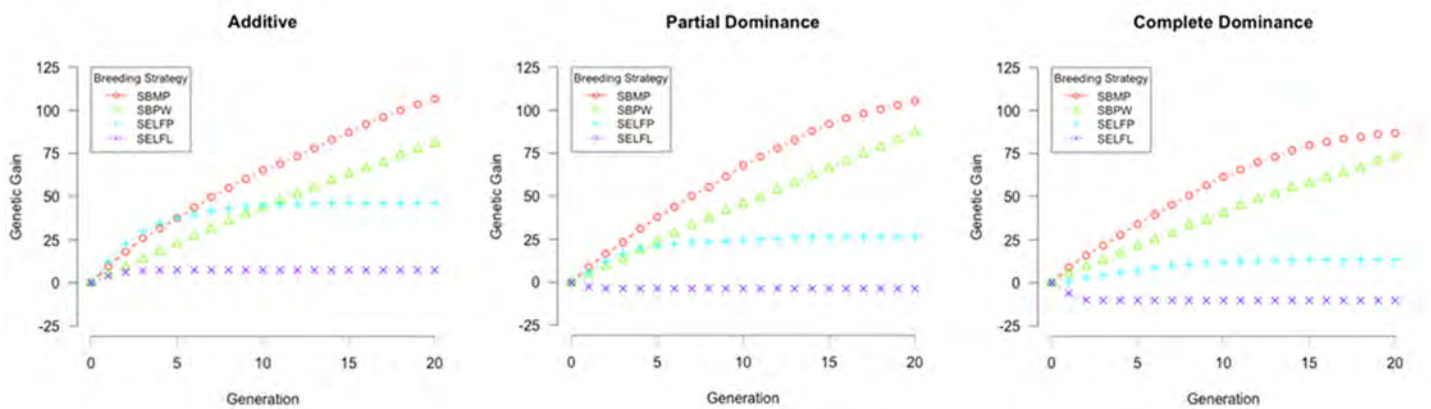


*Figure 1. Estimated genetic gains using four different breeding strategies with varying levels of dominance over 20 generations. The breeding strategies were: Single Base Population with Mass selection (SBPM); Single Breeding Population Within family selection (SBPW); Selfing within Lines (SELFL); Selfing combined with mass selection in single Population (SELFP).*
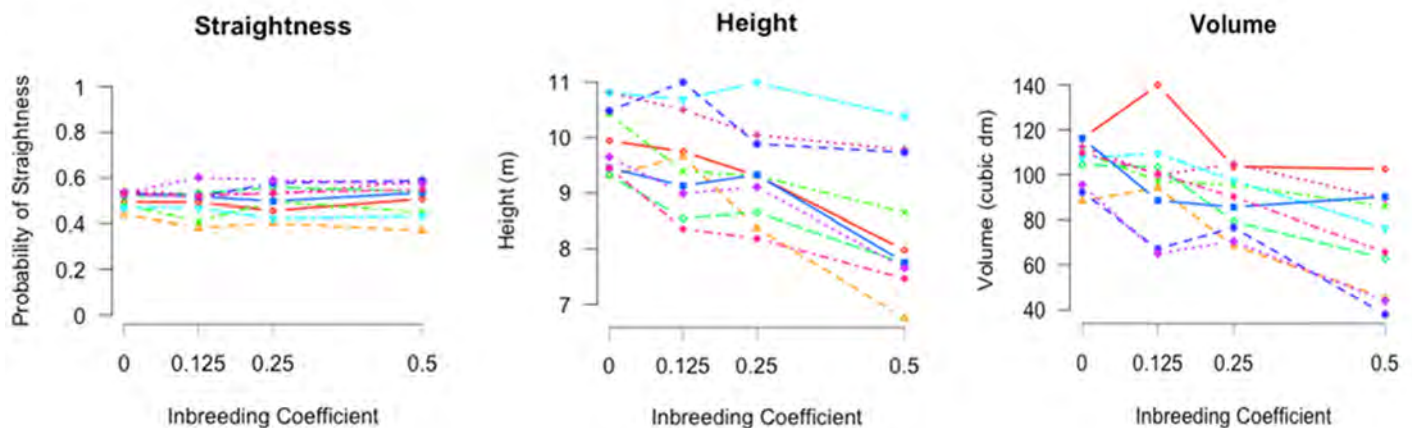


*Figure 2. Simulated family mean genetic value predictions of Straightness, Height, and Volume for 10 parents in an inbreeding study design similar to that reported by Ford et al. (2015).*

# Fingerprinting the 4th–Cycle Breeding Population—Progress Report

Funding was approved at the May 2015 Advisory Committee Meeting for a project to develop a DNA fingerprinting assay, then use it to generate and store DNA fingerprints of all the trees in the 4th-Cycle breeding population, plus all known ancestors based on the recorded pedigree. A "DNA fingerprint" can be broadly defined as a set of DNA-sequence-based genetic markers that provide enough information to uniquely identify a specific individual, just as human fingerprints are said to be unique identifiers of individual people. An additional benefit of DNA fingerprints is that in addition to uniquely identifying individuals, they can under some circumstances also be used to confirm parentage in controlled crosses, or to identify parents of individual offspring from an open-pollinated or mixed pollen seedlot if fingerprints of the candidate parents are also available.

A brief overview of some background information may be useful in setting the stage for discussion of the fingerprinting project. The goal of the Cooperative Tree Improvement Program fingerprinting project is to develop a panel of genetic markers with sufficient information content (genetic variability) to allow both unique identification of individual genotypes and identification of the true parents from among a group of candidates. The key measure of genetic variability is allele frequency, because that determines the proportion of individuals in the population that are likely to have the same genotype at each individual marker. The highest level of genetic variation in the population occurs at a frequency of 0.5 for both the major and minor allele (Figure 1). At 0.5 allele frequency, we expect the highest number of individuals of different genotypes at a single marker locus. The number of markers required to uniquely identify a single individual depends on the number of other individuals from which it is to be distinguished, and the allele frequencies at the markers using in the fingerprinting assay. For a set of markers that all have major allele frequencies of 0.9, about 80% of the individuals in the population are expected to be homozygous for the major allele at any single marker. The proportion homozygous for the major allele at all of N markers is $0.8^N$. The most likely genotype at any given marker depends on the allele frequencies for that
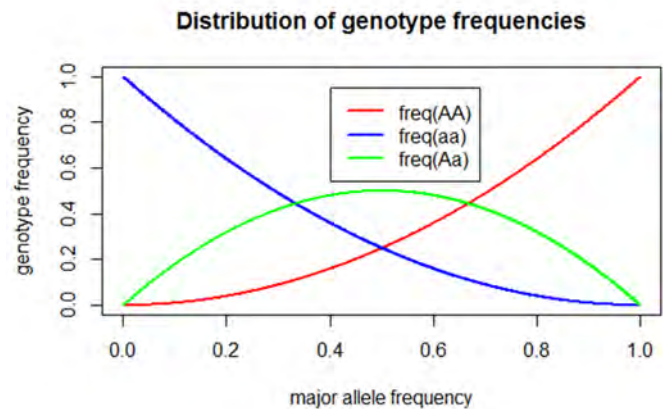


**Figure 1.** *A plot showing the expected frequencies of three possible genotypes in a population as a function of the frequency of the major allele, denoted A. When the A allele is rare, the frequency of individuals homozygous for the minor allele (genotype aa) is high (blue line), and when the A allele is frequent in the population, the frequency of individuals homozygous for the major allele (genotype AA) is high (red line). The point at which there are the greatest number of individuals with different genotypes is when the minor and major allele frequencies are both 0.5, because the frequency of heterozygous individuals (genotype Aa) is highest at that point (green line).*

marker; for marker loci with allele frequencies between 0.33 and 0.67, the heterozygous genotype is most likely, while for markers with major allele frequencies above 0.67, the most likely genotype is homozygous for the major allele.

The true distribution of allele frequencies in the 4th-Cycle breeding population is unknown, but we can estimate it based on previous analyses of other pine populations. Laura Townsend's MS project collected genotype data from the Plantation Selection Seed Source Study, which represents offspring from 420 trees sampled from across the range of loblolly pine east of the Mississippi River. This dataset provides a reasonable basis for estimating the likely distribution of allele frequencies in other pine populations. Analysis of Laura's data shows that the observed allele frequencies fit a beta distribution with shape parameters equal to about 0.4 (Figure 2). This gives a relatively high proportion of allele frequencies less than 0.33 or greater than 0.67; about 80% of all

marker loci have allele frequencies in the range where the most likely genotype in the population is the homozygous state. Another product of the analysis of the Plantation Selection Seed Source Study genotype data is a table of candidate single-nucleotide polymorphism (SNP) marker loci, filtered to retain only those that meet specific criteria. This table contains over 160,000 candidate marker loci, along with the estimated allele frequency in the population, the two nucleotide alleles, and 50 bases of DNA sequence on either side of the candidate SNP position. These markers are available for design of SNP assays for cooperators to use in fingerprinting or parentage analysis of selections within their breeding or deployment populations.
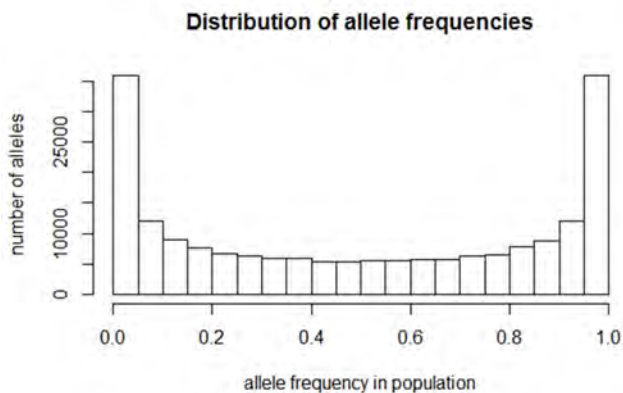


*Figure 2. A simulated allele frequency distribution using a beta distribution with shape parameters of 0.4, as estimated from observations in the Plantation Selection Seed Source Study dataset genotyped by Laura Townsend. In this distribution, the "minor alleles" are those with frequency less than 0.5. About 35.8% of the minor alleles occur at frequencies below 5% in the population, while 22.5% occur at frequencies between 30% and 50%. Less than 20% of the allele frequencies fall between 0.33 and 0.67, which is the range in which the heterozygous genotype is the most frequent in the population.*

The probability that a single individual will have a particular genotype at a single marker is equal to the frequency of that genotype in the population; the probability that two individuals will have exactly the same genotype at a single marker is the square of the frequency of that genotype in the population. For example, at a marker with a major allele frequency of 0.9, the frequency in the population of individuals

homozygous for the major allele is expected to be 0.81, so the probability of a randomly-chosen individual having the AA genotype is 0.81. The combined probability of two independent events both occurring is the product of their independent probabilities, so the chance of randomly selecting two individuals that both have the AA genotype is 0.81 x 0.81, or 65.6%. If a second marker locus (independent from the first) is included, the chance that the two individuals will share the AA genotype at both markers is 0.656 x 0.656, or 43%. This kind of calculation can be expanded to include estimation of the expected number of individuals with shared genotypes in a population of 1500 (about the expected number of trees plus ancestors in the 4th-Cycle breeding population). The expected number of individuals with shared genotypes, in turn, can be used to calculate the probability that all individuals will have unique genotypes, which is the desired outcome, for different numbers of marker loci (Figure 3). The probability of success (every individual has a unique DNA fingerprint of marker genotypes) is highest when allele frequencies are intermediate, and lowest at the extremes of very high and very low allele frequency.

The progress made to date toward the goal of fingerprinting the 4<sup>th</sup>-Cycle breeding population has been in the areas of collecting foliage samples, extracting DNA, and testing candidate SNP loci for suitability as fingerprinting markers. Foliage samples of over 2300 clones in the Atlantic Coastal Elite clonal test in Wayne County, Georgia were collected in May 2015, and samples from 1500 grafts of 237 different selections in the Arrowhead Breeding Center were collected in October 2015. DNA has been isolated from all those foliage samples, and is ready for fingerprinting. Additional foliage samples will need to be collected in 2016 from selections that are not available at Arrowhead, so a complete set of selections and ancestors is available for the 4<sup>th</sup>-Cycle breeding population.

Two sets of candidate SNPs, the first of 144 loci and the second of 151 loci, have been tested as possible markers for fingerprinting purposes. The first set of 144 loci were chosen from data obtained from the Plantation Selection Seed Source Study genotyping experiments, and were selected to have multiple SNPs

in close proximity in order to maximize the information content of the genotyping experiments. All those candidate loci were identified as likely to be present in a single copy per haploid genome based on the annotation of the version 1.01 loblolly pine genome, but the results of the fingerprinting assay indicate that in fact, only about 50 of the 144 loci are likely to be useful for fingerprinting. The others either did not yield data at all, or yielded more than two haplotypes from a diploid DNA sample, indicating that the locus is present in multiple copies per haploid genome. Such multi-copy loci can still be useful for verifying clonal identity, but are not well-suited for parentage analysis, because it is not clear how the marker genotypes should segregate from parents to progeny. The second set of 151 markers were chosen from loci that were assayed successfully by previous USDA-funded genotyping projects (ADEPT2 and CTGN), and 94 of the 151 markers in this set behave as single-copy markers likely to be suitable for both clonal identity verification and for parentage analysis. The DNA sequences of all 295 loci, along with the information regarding which markers seem likely to be useful for parentage analysis, will be made available through the TIPRoot database or the treeimprovement.org website.
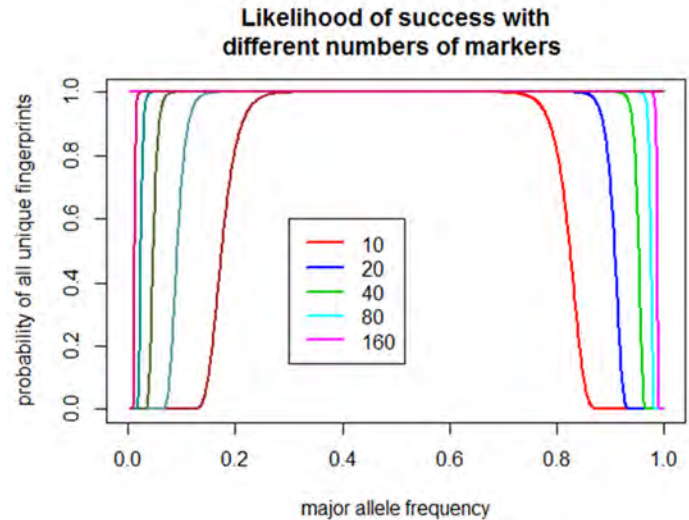


**Figure 3.** *The distribution of probabilities of successful fingerprinting (no individuals with identical genotypes across all marker loci) for different numbers of markers (10, 20, 40, 80, or 160 loci), across the range of allele frequencies. The probability of success is calculated assuming the same genotype (either all loci are homozygous for the major allele, or all are homozygous for the minor allele) and the same allele frequency for all markers. These are not realistic assumptions, but they serve to show that the probability of success is lowest at extremes of the allele frequency distribution, and highest for intermediate levels of allele frequencies. The bright colors shown in the legend correspond to the probabilities of success when all genotypes are homozygous for the major allele; the darker colors correspond to the probabilities of success when all genotypes are homozygous for the minor allele.*

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

During the spring of 2014, members of the Cooperative participated in a study that compared prototypes of four PBS International pollination bags to the industry standard Lawson pollination bag. In addition to these 5 bags, two other treatments were added to this study - open-pollinated flowers and a Lawson bag including a wire for support.

Based on findings from the early assessment of the success of some bag types from the 2014 study (see p. 23 of the 2015 Annual Report), a second round of testing began in the spring of 2015. The 2015 study

design was the same as the previous year's study with a few exceptions. The biggest change was that four new bag prototypes were tested in addition to a modified bag type B from the previous year. These bags were designed to provide greater rigidity and smoothness to prevent damage to flowers inside the bags.

The same approach from the earlier study was used to assess the performance of the bags in the 2015 version of the study. This early assessment was to examine flower survival at time of bag removal and after the

period of "June drop". Preliminary analysis of these data found that differences in bag type for June flower survival were not as pronounced as in the 2014 version of study, partly due to high survival across all treatments. Slight differences between bag types in mean flowers survival at June drop can be seen in Figure 1.

In summary, the June drop data for the 2015 version of the study had a lot of noise compared to the 2014 version of the study. Based on the 2015 June drop results, the only bag types that were marginally significantly different from each other were Lawson and the open pollinated (OP) treatments (F test Pr<0.10). What is clear is that the overall survival was much higher in 2015 across all bag types when compared to the 2014 experiment. In the 2014 version, two groups seemed to emerge; PBS bags A, B, OP, and Lawson wire with high conelet survival, and PBS bags C, D, and the Lawson with lower conelet survival. We did not observe such a distinction among treatments for June flower survival in the most recent 2015 experiment.

To quantify success and utility of each bag, cone survival and number of seed and seed efficiency per bag is currently being calculated from the cones collected in the fall of 2015 from the 2014 pollination study. Staff, graduate students, and undergraduate students have been hard at work helping to process this large number of cones (Figure 1, and image below). As the processing of the cones from the 2014 version of the study is finalized, we will soon know whether June drop data correlates with an increase in cone survival, and if the bags from the 2014 study led to a difference in seed efficiency or seed yield. Stay tuned!
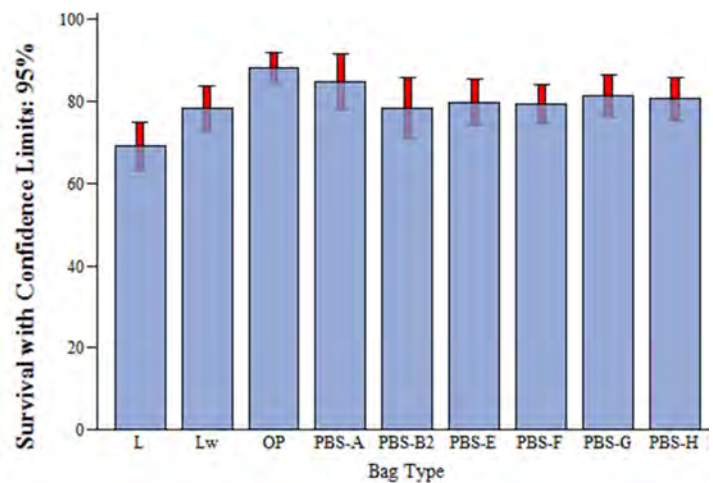


*Figure 1. Overall mean percent survival in June 2015 for each bag type. Overlapping confidence intervals suggest that bag types are not significantly different from each other. L and OP were the only treatments significantly different from each other (adjusted p value < 0.0297).*



*For the PBS Bag Study from the 2014 pollination season, there are over 2000 cones for seed yields and for cone analysis. Shown are 180 bags of the cones for one of the nine cooperators waiting to have seeds extracted for seed yields in the Tree Improvement Lab at NC State University.*

# Alternative Approaches to Estimating Breeding Values Within Family[1]

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

Currently, advanced-generation selections of loblolly pine for breeding are made on the basis of an index of the family mean and the individual performance within family, where phenotypically superior individuals are selected from top performing families. The selected individuals must be progeny-tested to obtain reliable BV estimates before they can be used in the next cycle of breeding. This requirement limits the size of breeding populations, and therefore limits the selection intensity that can be applied.

In order to estimate the BV of a tree, linear mixed models of phenotype use pedigree data to estimate genetic covariance among a set of families from a mating design. It is reasonable to propose that genetic variation among family means can be partially accounted for by differences in gene structure, and partially by differences in gene regulation. Published work in maize has described the use of pair-wise comparisons of gene expression levels among inbred parents of full-sib F1 hybrid families to identify a set of genes that are differentially-expressed in at least one pair-wise comparison. Gene expression data was then used to create covariance matrices, either using the quantitative differences or simple binary comparisons. Genetic differences among F1 hybrid progeny in field performance were accurately predicted using statistical models trained with a subset of the data in a cross-validation study.

An alternative approach would be to use a covariance matrix based on family-mean levels of all gene expression values from sequencing DNA copies of messenger RNA, whether differentially expressed or not, as a measure of genetic covariance in gene regulation. This approach uses no information about differentially-expressed genes, but is a simpler approach to capturing similarities as well as differences among families. The sequencing approach will also provide a means to collect information on genetic covariance among families in gene structure as well as gene regulation patterns. Utilizing covariance structures based on genetic variation in gene expression in linear mixed models instead of, or in addition to the standard numerator relationship matrix, may provide a higher prediction accuracy of BVs. New software tools allow incorporation of multiple covariance matrices in linear mixed models, with variable weights on each matrix.

To test this hypothesis, we chose a total of 62 different parent trees of loblolly pine from a wide geographic distribution, with existing progeny field test data available from multiple sites. Seed lots (54 half-sib and 8 full-sib families) from these parents were grown in two different batches in a greenhouse, and pooled seedlings were harvested at age 3 months for RNA extraction, cDNA library preparation, and high-throughput sequencing. The sequencing results were used to assess reproducibility of family-mean gene expression patterns and the extent of differential gene expression. The data on gene structural variation and gene expression levels from these seedlings are currently being used to create covariance matrices reflecting genetic variation in coding sequences on one hand, and variation in gene regulatory networks on the other. Cross-validation models using these covariance matrices, as well as a standard numerator relationship matrix were used to test the hypothesis that phenotypic variation can be accurately modeled by covariance of these classes of genetic variation.

In summary, this is a pilot study, with the objective of assessing the relative contribution of separate covariance matrices modeling gene structure and gene expression in cross-validation studies to predict progeny performance from crosses among a small number of parents. While it is unlikely that this study will provide definitive evidence that using gene expression differences will result in better estimates of parental breeding value, there are several practical questions that will be addressed. (1) Can we obtain

reasonably reproducible results from triplicate samples of seedlings from open-pollinated, polymix, and controlled pollinated families with respect to estimating family mean levels of gene expression for a set of parents? (2) Can we identify methods for combining those family mean estimates of gene expression levels into covariance estimates for pair-wise-combinations of parents that show utility in cross-validation studies for modeling phenotypic variation? (3) Do covariance matrices based on coding sequence SNP variation, gene expression level variation, or pedigree-based estimates of allele sharing have independent value for modeling phenotypic variation, or are they redundant so that one approach has the same information present in the other two? Overall, the resulting analyses should provide insight into the value of using RNA expression patterns as another screening effort in selecting individuals as parents for future breeding populations.

*Progress and Results*

RNA Sequencing data from the first batch of seedlings was received in summer 2015. Preliminary analysis using R packages Random Forest and Weighted Gene Coexpression Network Analysis resulted in three correlation matrices which included "top" genes selected to be associated with a given trait. Utilizing Omic Kriging, a 10-fold cross validation was conducted with the three correlation matrices and resulted in a $r^2$ value of 0.59 (Figure 1A). However, there was concern that the high $r^2$ prediction accuracy was biased upwards due to utilizing phenotypes of the first batch in order to identify associated genes and then subsequently using those transcripts to predict parental BVs in a cross validation.

A second batch of seedlings was grown in fall 2015 to provide additional samples for use as a test and training set from the total data. Additionally, if this approach were to be utilized in a breeding program, it would be imperative that results are consistent from RNA-seq data obtained any given year and provide the same relative predictive information. The second batch of samples were submitted for sequencing in fall 2015, and data were received in early January 2016. The same three correlation matrices that were found to provide a high prediction accuracy of BVs in Batch 1, were utilized to predict parental BV's of seedlings grown in Batch 2. (Figure 1B)

Given the low predictive level of the second batch, we are currently exploring alternative methods in order to identify genes associated with the trait which do not involve the use of phenotypes. A few examples of these alternative analyses include constructing gene networks or identifying differentially-expressed genes. Comparing gene expression levels within a single family to the average of all other families detects differentially-expressed genes in numbers ranging from 14 to 12,633 genes per family.

While gene expression data obtained from RNA-seq encompasses differences between family gene regulation and/or structure, another type of difference that may be observed are those within coding sequences, such as SNPs. Utilizing freebayes software, a haplotype based variant detector, we identified SNPs from each family and built genomic relationship matrices. These matrices provide estimates of similarity among families based on differences in coding sequences that they may have in common, instead of based on similarity utilizing only resulting gene expression estimates. The relationship matrices will be incorporated into future predictions of parental BV's to assess their value when compared to, or in addition to, matrices obtained using gene expression values.
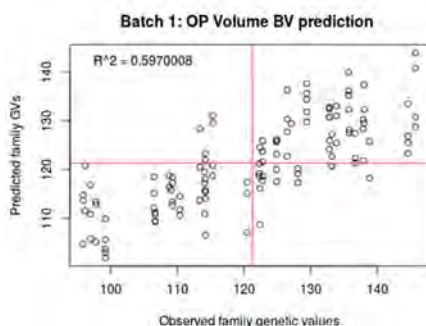


*Figure 1A (left). Prediction of 119 OP biological replicates from Batch 1 using 3 correlation matrices obtained from RF and WGCNA.*

*Figure 1B (right). Prediction of 48 OP biological replicates in Batch 2 using the same 3 correlation matrices as in Batch 1.*
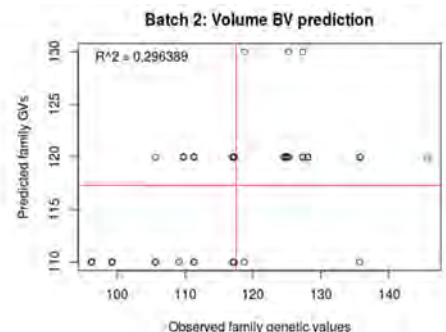
# Genetic Analyses of 3rd–Cycle Pollen Mix Tests[1]

In the third breeding cycle, the Tree Improvement Program deployed a large number of pollen mix (PMX) trials of loblolly pine. The primary objective of these trials was to determine breeding values of parents from the Coastal, Piedmont, and Northern breeding populations. Families within each region were tested at many sites over a wide geographic area to provide accurate estimates of breeding values.

The first step to quantifying genetic gain is to estimate the degree to which parental contribution affects the performance of progeny. For the PMX trials, breeding values have been estimated and are available to Cooperative members in the *PRS*™ database accessible on the TIPRoot database. The objective of this study was to use data from the PMX trials to 1) produce estimates of heritability at each site and within each series, 2) estimate genetic correlation among traits for each test series, 3) determine if test site attributes affect the expression of genetic variation, and 4) test for significance of genotype-by-environment (GxE) interactions and examine possible explanations for GxE.

Data from four Coastal and three Piedmont test series were analyzed, totaling 34 test sites in the Coastal region and 17 sites in the Piedmont series. Each of these series contained between 68 and 91 genetic entries in Coastal series and between 64 and 75 entries in Piedmont series. The genetic entries were comprised of crosses between selections and a pollen mix of 20 specific males representing average of parents from the region. The 3rd-Cycle checklot, a previous cycle's checklot, and a few well characterized common families were included for test connection and estimation of gain.

Height, diameter at breast height (DBH), straightness score, stem sweep, and presence of fusiform rust, forks, and ramicorns were assessed in each test between ages 4 and 7. Straightness score was assessed on a 1-to-6 scale, where 1 denotes the straightest trees. The experimental design was a single-tree plot randomized complete block design at each site and a nested incomplete block design on a series basis. Each site and series was fit with a linear mixed-model for height, volume, and straightness.

This analysis focused on the height, volume, and straightness measurements. For each of these traits, individual-tree narrow-sense heritability was calculated for each site and test series, and genetic correlations were estimated between the traits on an individual-tree basis. The impact of site means on expression of genetic variation was evaluated using Pearson correlation between the site mean for each trait and the heritability for the trait at each site. The presence of genotype-by-environment interaction (GxE) was evaluated for each series using the likelihood ratio test (LRT) and the Type-B genetic correlation.

Height means ranged from 4.9 to 8.4 m, and volume means ranged from 20.1 to 54.6 dm$^3$ across test series. Heritabilities for each trait were relatively consistent across test series (Table 1). The heritabilities for height tended to be greater than those for volume, which tended to be greater than the straightness heritabilities. The heritabilities for individual test sites varied more than at the series level and ranged from 0.05 to 0.72 for height, 0.10 to 0.70 for volume, and 0.05 to 0.43 for straightness.

Heritability estimates of height and volume for series were mostly similar to estimates in previous studies. For reference, the 2nd-Cycle testing effort summary reported average heritabilities of 0.19 and 0.16 for height and volume, respectively (McKeand et al. 2006). The overall trend suggests that heritabilities were slightly higher for these 3rd-Cycle tests, likely due to improved site selection for homogeneity and improvements in experimental design, testing strategy, and test site maintenance.

Genetic correlations were strongly positive between height and volume, ranging from 0.76 to 0.82 across the series (Table 1). Moderate to weak negative correlations were observed between height and straightness and volume and straightness. Because straighter trees receive lower straightness scores, a negative relationship is favorable. The weak correlations imply that selection for height or volume will not influence straightness.

[1]*This is a summary of part of Andrew Sims's M.S. research.*

No correlation appeared to exist between the test site heritability and the site mean for growth traits; Pearson correlation of 0.06 for height and <0.01 for volume (Figure 1). These results imply that site productivity does not affect the heritability, at least on the range of sites where these PMX tests were established. Differences in heritabilities among sites is most likely attributable to environmental heterogeneity; poorly maintained sites, high mortality, or differences in environment within sites will deflate estimates of heritability.

GxE, while significant in all but two series for one trait, has not yet been fully analyzed. Type-B genetic correlations (Table 2) suggest that GxE, while significant, was generally not important for height and straightness. For volume, the Type-B genetic correlations were consistently lower. More investigation into the causes for the differences in GxE for these traits is underway.

**Table 1.** *Genetic correlations between traits by series. Negative correlations with straightness are favorable, because straighter trees receive a lower straightness score.*

| Series | (Height, Volume) | (Height, Straightness) | (Volume, Straightness) |
|--------|------------------|------------------------|------------------------|
| CPMX1 | 0.82 | -0.13 | -0.08 |
| CPMX2 | 0.78 | -0.13 | -0.09 |
| CPMX3 | 0.76 | -0.19 | -0.12 |
| CPMX4 | 0.82 | -0.13 | -0.07 |
| PPMX1 | 0.78 | -0.09 | -0.04 |
| PPMX2 | 0.78 | -0.04 | 0.03 |
| PPMX3 | 0.84 | -0.12 | -0.06 |
| Range | (0.76, 0.82) | (-0.19, -0.04) | (-0.12, 0.03) |

**Table 2.** *Type-B genetic correlations by trait for each test series with the standard errors in parentheses. Values less than 0.67 suggest that GxE interaction can be important.*

| Series | Height | Volume | Straightness |
|--------|--------|--------|--------------|
| CPMX1 | 0.71 (0.052) | 0.58 (0.062) | 0.57 (0.067) |
| CPMX2 | 0.63 (0.069) | 0.41 (0.076) | 0.70 (0.069) |
| CPMX3 | 0.69 (0.060) | 0.57 (0.068) | 0.76 (0.079) |
| CPMX4 | 0.61 (0.073) | 0.52 (0.070) | 0.91 (0.092) |
| PPMX1 | 0.56 (0.084) | 0.51 (0.073) | 0.70 (0.093) |
| PPMX2 | 0.84 (0.047) | 0.63 (0.062) | 0.69 (0.085) |
| PPMX3 | 0.76 (0.098) | 0.53 (0.135) | 0.89 (0.232) |
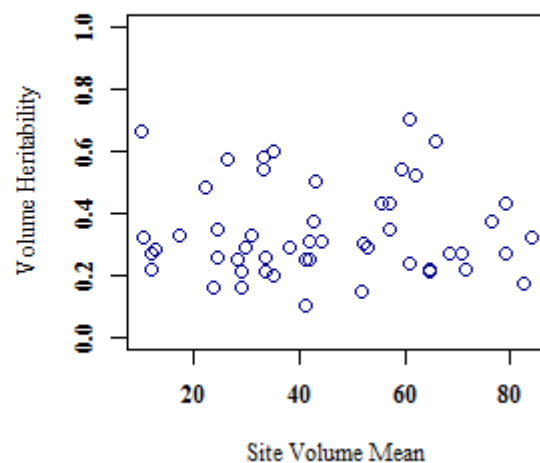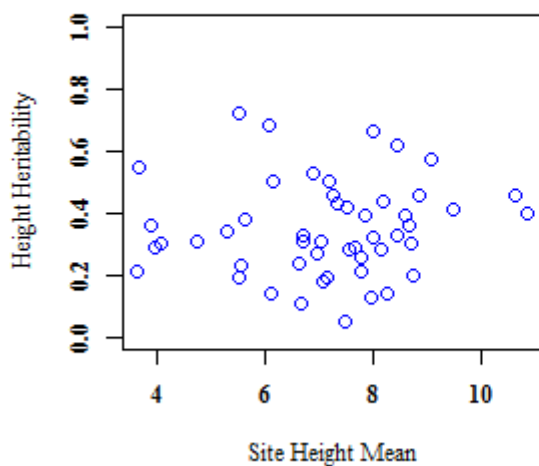


**Figure 1.** *Scatterplots of test site mean and narrow-sense individual-tree heritability for height and volume for 51 PMX tests in the Piedmont and Coastal Plain. The lack of a trend suggests that the expression of genetic variation (heritability) for a site does not depend on the site mean.*

# Update on USDA Funded Projects—PINEMAP and IBSS

The USDA-funded Pine Integrated Network: Education, Mitigation, and Adaptation Project (PINEMAP) has been underway since March of 2011, with a comprehensive set of objectives in six major areas of research, outreach, education, and extension activities. The five-year project period ended in February 2016, and the project is now in a final one-year no-cost extension phase, wrapping up some specific projects aimed at adding additional value to the research and extension outputs delivered to date by the project. The primary deliverable extension product of the PINEMAP project is a web-based Decision Support System (DSS), available to the public at http://pinemapdss.org. This system allows professional foresters, landowners, and land managers to explore possible future scenarios in loblolly pine management and production, using a three-map layout and time series graphing tools to visualize climate projections from downscaled global climate models and regional ecological models. This product includes a suite of tools designed to aid in assessing climate risks and opportunities, including projections of possible changes in typical seasonal conditions, occurrences of extreme minimum temperature at a variety of different

thresholds, and likely shifts in dynamic hardiness zones. Such changes in hardiness zones have the potential to create new markets for cold-tolerant seedlings, and may also change seed source ranges of planting stock for greater productivity. Each tool summarizes guidance from a set of 20 downscaled global climate models and includes a historical perspective for that spread of possible future outcomes. The DSS interface is fully interactive, allowing users to explore both large-scale and local projections for two emissions scenarios and four future time periods. Additional tools currently under development include regional forest productivity model outputs, such as projections of gross and net primary productivity, net ecosystem productivity, merchantable volume, carbon above ground, and water stress, under alternative future climate scenarios.

The USDA-funded Integrated Biomass Supply Systems (IBSS) project also began in 2011, and is now in its fifth year. The Cooperative Tree Improvement Program received funding from the IBSS project in the first four years for research on pine productivity and wood quality characteristics, but is not funded in the fifth (current) year of the IBSS project.

# Grants

*Towards Genomic Selection in Forest Trees*

Fikret Isik received a new USDA-NIFA grant (Award # 2016-67013-24469) in October 2015 to develop genotyping platforms for loblolly pine and sugar pine. The project started in October 2015 and will end in 2019. The Co-PIs in the project are Drs. Jill Wegrzyn (University of Connecticut), Andrew Eckert (Virginia Commonwealth University), Richard Sniezko (USDA Forest Service, Dorena Genetic Resource Center), and Juan Acosta (Camcore, North Carolina State University).

The goal of the project "Towards Genomic Selection in Forest Trees" is to discover up to 100,000 informative *single nucleotide polymorphism* (SNP) markers in loblolly pine and sugar pine for genomic research and breeding. Publicly funded research projects (e.g. CTGN, PINEMAP, PineRefSeq) have

produced vast genomic resources for loblolly pine, and this new project will use DNA and RNA sequence data from these projects. Over 20 million candidate SNP markers will be screened using a bioinformatics pipeline (comparative analysis, validation, annotation) and high-performance computing. Informative SNP markers will be organized into the TreeGenes database for community access. In the second year of the project, the project team will take the lead to establish a consortium to design SNP arrays and genotype a large number of trees (~15,000).

The SNP arrays are reliable and repeatable genotyping platforms, which is needed to promote novel research and development projects (genomic selection, high density genetic maps, marker-aided selection for disease resistance) in pine. Genomic selection has the potential to produce a paradigm shift in tree breeding,

and the new technology will likely reduce the breeding cycles by half by reducing expensive and time-consuming progeny testing. The disease and insect resistance of trees could be predicted when they are small seedlings, or trees with desired wood quality could be selected without the need to grow them in the field for many years.

New Grant

Fikret Isik, is the principal investigator on a USDA-National Institute of Food and Agriculture grant, "Towards Genomic Breeding in Forest Trees", $370,000. 11/01/2015-10/31/2019

Continuing Grants

Steve McKeand, Fikret Isik, and Ross Whetten on a North Carolina Department of Agriculture, Loblolly Pine Biomass Cropping Study: Years 3-5. $81,165. 1/2016-12/2017.

Ross Whetten, Steve McKeand, and Fikret Isik. Subcontract with the University of Florida, USDA Coordinated Agricultural Project (CAP) "Integrating research, education, and extension for enhancing southern pine climate change mitigation and adaptation". Tree Improvement Program portion: $867,665. 3/2011 to 2/2017.

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

# ASSOCIATED ACTIVITIES

## Meetings, Workshops, and Short Courses

The annual Tree Improvement Contact meeting decided to "Head 'em up, move 'em out!" and go West with a visit to the Pacific Northwest in August 2015. The group was hosted by many folks including Dr. Keith Jayawickrama, Director of the Northwest Tree Improvement Cooperative and representatives from the Bureau of Land Management, GreenWood Resources, Hampton Tree Farms, Oregon St. University, Weyerhaeuser Co., and the US Forest Service. The group hit the road each day to see how forest management and tree improvement activities are executed with several different tree species in this unique environment. Visits included travel to seed orchards and trials in Oregon, field sites at Mt. St. Helens, forestry operations along the Pacific Coast, and research sites along the Columbia River. We can't thank our hosts enough for the experience and the insight into to tree improvement in another economically important area for forestry in the US.



*Contact Meeting attendees (pictured right) gather for a picture in front of old growth Douglas-fir at the Wind River Experimental Forest in Washington.*

Our faculty members are also in high demand to teach courses all over the world. Steve helped teach two short courses this past year, one in Chile at the University of Concepción (Curso Internacional de Genética y Biotecnología Forestal 2015 led by Drs. Sofía Valenzuela and Claudio Balocchi) and another in China at Nanjing Forestry University in November 2015 on Genetics and Tree Improvement. Fikret also taught a short course in China at Nanjing Forestry University in April 2016 on quantitative genetics and data analysis methods.



*Both short courses with the students from Nanjing Forestry University over the past year. Dr. Steve McKeand was there in November 2015 (left) and Dr. Fikret Isik in April 2016 (right).*

The Arrowhead Breeding Center has become a facility where our staff can really show off the breeding and testing activities of the Cooperative. This year we hosted several groups during the breeding season, some who are familiar with tree improvement activities and others who wanted to learn more. In February 2016, we hosted a group of industry researchers from J.D. Irving, Ltd. in Canada (Greg Adams, Shona Millican, and Jason Killam), as well as a group of forest genetics professors from South China Agricultural University in Guangzhou, China (Drs. Shaowei Huang, Tianyi Liu, and Chunxin Liu). In March 2016, Steve, staff, and students also helped host a Georgia Forestry Association workshop that brought landowners to the Breeding Center to demonstrate the tree improvement activities carried out by the Georgia Forestry Commission and the Cooperative members. We focused on the research and development investment made at Arrowhead and throughout the Cooperative to bring landowners the best possible genetics. We also had the chance to host some of the NCSU College of Natural Resources administrators, faculty, and public relations representatives for a day. Our objectives for bringing these visitors to the Breeding Center was to educate them about our research, create opportunities for collaboration, and ensure their support for future funding and resources. Great PR never hurts! Now you may be wondering, didn't you folks already have enough to do during the breeding season? The answers is *absolutely*, but there is never a better time to exhibit the investments (people, time, resources) made by the Cooperative



*Top row: Brian Smith, Jodi Forestter, Marian McCord, Steve McKeand. Bottom row: Tom Gower, Sarah Corica, Fikret Isik, at Arrowhead Seed Orchard, Cochran, GA*

*Georgia Forestry Commission hosted a Georgia Forestry Association landowner workshop held at the arrowhead Breeding Center. This was right in the middle of breeding season so landowners really got a good look at the research and development that goes into the seedlings they purchase to regenerate their land.*

Tree Improvement staff also participated in these extension activities for landowners and foresters:

*Economic and Ecological Impacts of Tree Improvement in the Southern US*, Presentation to Southern Group of State Foresters Meeting, San Antonio, TX, June 24, 2015

*Pine Seedling Selection and Planting in the SC Piedmont*, Presentation to Greenville County Forestry and Wildlife Society, Greenville, SC, July 24, 2015

*Tree Improvement and Forest Productivity / Value in the South Carolina Piedmont*, Presentation to Tree Farm Group, Edgefield, SC, July 25, 2015

*Tennessee Division of Forestry and the NCSU Cooperative Tree Improvement Program*, Presentation to Tennessee Division of Forestry, Foresters Meeting, Pikeville, TN, August 20, 2015

*Forest Genetics & Seedling Productivity*, Presentation to Southern Forest Nursery Management Cooperative Short Course, Auburn AL, September 9, 2015

*Right Tree Right Site*, Presentation to forester and landowners. Symposium on Forest Health Impacts from Climate Change, Raleigh, NC, February 9, 2016

*Tree Improvement & Forest Productivity in the Southeast US*, Presentation to foresters at the SAF Sandhills Chapter, and Central Carolina Forestry Club Meeting, Southern Pines, NC, February 9, 2016

*Pine Genetics*, NCDA BRI Research/Industry Update Meeting, Raleigh, NC, March 17, 2016

*Tree Improvement Research at the Hofmann Forest*, Presentation to NCSU faculty and staff and to landowners, Jacksonville, NC– June 8 and July 13, 2015 and to Southern Lumber Manufacturer's Association, April 6, 2016

## Staff

We have had yet another year of changes in the staff at the Tree Improvement Program. They say change can be good…but we certainly miss these folks and their contributions!

After almost two years on the Cooperative staff as Tree Improvement Manager, Graham Ford left for a position with Plum Creek, in Brunswick, GA. He was a great asset and is still continuing his PhD program with the group. With Graham's departure we welcomed Trevor Walker in his place. As a new graduate student, Trevor had worked very closely with Graham during the time period that we were without a Data Analyst. Trevor really got to know the ins and outs of the breeding program, and he took right to the duties at hand while also juggling a PhD program with the group. We like over-achievers!

And again, just as we were thinking all was well and finally getting into a familiar groove, history repeated itself. In March 2016 we lost Chris Ball, who had been a recent addition to the staff as Analyst and Database Manager. Chris did a great job understanding what it is we do and helped translate that back into the database. However, the calling of a job opportunity from the Research Triangle Park was too strong, so we are currently searching for a new Analyst/ Database Manager.

We also said goodbye to our post doc, Yusuf Kurt, who had been working on the PINEMAP project. Yusuf worked with Ross Whetten and developed high-throughput and efficient DNA extraction protocols for forest trees, an important step for genomic research. Yusuf also worked with the collection and preparation of tissue samples from Tree Improvement's grafts at the Arrowhead Breeding Center to perform fingerprinting/pedigree analysis. He returned to Turkey in January after a 2-year post- doctoral research position at NCSU, one year with John Frampton and Christmas Trees Genetics and one year with our group.

On a brighter note we also have a new face helping out with administrative duties, April Meeks. She is assisting the staff while doing her PhD research. She became experienced in administrative work with the Forest Productivity Cooperative, so she is very familiar with the Cooperative environment. She has been a huge help, and we are thrilled to have her on the team!



*Tree Improvement Staff: Center picture from left to right, J.B. Jett, Austin Heine, Ross Whetten, Steve McKeand, Trevor Walker, and Fikret Isik were all in the office while April Meeks (left) and Tori Brooks (right) were out doing real work.*

## Visitors

This year we have had 2 scholarly visitors, one short term and one long term. David Kainer (pictured top right) came to us from sunny Australia and the Australian National University in Canberra. He had a three-month sabbatical with us starting in August 2015 and returned home in November in 2015. David worked with Fikret Isik on genomic approaches to selection for essential oil yield in *Eucalyptus polybractea*, an undomesticated species of interest for oil production. Essential oils are used in cosmetics, pharmaceuticals, aromatherapy, and solvents. He is also quite the artist, and left us a cool drawing detailing pine bark as a parting gift for the office!

Our other visitor is Ricardo Duran (pictured bottom right), a PhD student with the Biotechnology Center at the University of Concepción in Chile, who started his visit with the Tree Improvement Program in mid August 2015. Ricardo has worked with Fikret Isik to apply genomic selection methods in *Eucalyptus globulus*, an important tree species for plantations in Chile. He also followed some graduate courses and seminars during his stay. Ricardo plans to go back to Chile in late June 2016 to finish his PhD program. He has put in many hours over the last 9 months but has managed to get out and travel, and we have enjoyed having him in the office.

As always, we have enjoyed hosting folks over the years and hope to continue the trend. We wish these folks the very best in their educational and career goals.

## Teaching

Each year the faculty associated with the Cooperative teach a wide range of courses at both the graduate and undergraduate level. In the spring, Steve McKeand and Ross Whetten teach FOR 411 (Forest Tree Genetics and Biology), our undergraduate tree improvement course, and graduate level FOR 725 (Forest Genetics). Steve, with the assistance of Austin Heine, also taught FOR 727 (Tree Improvement Research Techniques) in spring 2016 – see the cover photo of this report for what fun the students had in his class! Each spring semester, Ross also teaches FOR 350 (Professional Development III: Ethical Dilemmas in Natural Resource Management), a discussion-based class on ethical perspectives and issues in natural resource management, and BIT 815 (Analysis of Deep Sequencing Data Analysis), a hands-on introduction to analysis of high-throughput DNA sequencing data in the Linux command-line environment. Fikret Isik rounds out the spring semester with his NR554 course, Introduction to Data Analysis in Natural Resources. The course covered general statistical procedures using SAS and R programing. He will be teaching Advanced Quantitative Genetics and Breeding (FOR/CS/ANS 726) this fall. This course is offered in even years and attracts students from crop science, animal science, and bioinformatics in addition to forestry.

*From left to right; Adam Festa, Andrew Sims, April Meeks, Austin Heine, Graham Ford, Leo Arnoux, Serenia Larrison, and Trevor Walker*

## Graduate Students

Over the summer Tree Improvement students Greg Albert, Laura Townsend, and Alfredo Farjat successfully defended their research and graduated. We also welcomed both a Masters and 2 PhD students this year – Serenia Larrison, April Meeks, and Leo Arnoux. Below is the list of current students and their research:

**Adam Festa, PhD candidate** – Adam continues his research titled, "Modeling methods for prediction of genetic value and breeding efficiency in tree improvement". He successfully passed prelims and expects a summer 2016 finish.

**Andrew Sims, MS** – Andrew, who started as an undergraduate worker from statistics and got hooked, is finishing up his research "Genetic parameter estimates from $3^{rd}$-Cycle pollen mix progeny tests in loblolly pine". He is set to defend this summer.

**April Meeks, PhD** – April came to us after getting her MS with the Forest Productivity Cooperative. She is currently swamped with classes and assisting in the Tree Improvement office, so she hopes to zero in on a project over the  summer.

**Austin Heine, MS** – Austin took the lead on the PBS Study and decided the data analysis would make an excellent MS project. His research is titled "Comparison of Pollination Bags for Mass Production of Control Cross Seeds in Loblolly Pine".

**Graham Ford, PhD candidate** – Graham took a position with Plum Creek, now Weyerhaeuser in August of 2015, but is committed to finishing his PhD research in 2016. His research is titled "Analysis of genetic variation within and among eastern and western provenances of loblolly pine".

**Léo Arnoux, PhD** – Léo is another treasure find from Fikret's sabbatical in France. He came to us in August 2015, and his project is titled "Developing genomic resources for genomic selection in pines".

**Serenia Larrison, MS** – Serenia is a graduate forester from the University of Georgia and joined us in the fall after completing two internships with Cooperative members Rayonier and Weyerhaeuser. Her project is titled "Soil compaction in loblolly pine seed orchards and the impacts on tree health".

**Trevor Walker, PhD** – Trevor started in January 2015 and transferred over to the Tree Improvement staff side as Tree Improvement Manager in August 2015. He has had a pretty full year with working and classes, but hopes to focus on a project in quantitative genetics.

## Membership in the NCSU Cooperative Tree Improvement Program

2015-16 has been a very interesting year for membership and certainly in keeping with the spirit of *change*. We added three new Contributing Members with the addition of Larson & McGowan, Blanton's Longleaf Container Nursery, and Four Rivers Land & Timber Company. We said goodbye to a Research Associate Member this year, The American Chestnut Foundation. However, the biggest change was a move that we have not seen in a few years: one full member company bought another. We officially said goodbye to Plum Creek Timberlands in February 2016 as the merger with Weyerhaeuser was finalized. As always, we value the members' support of our research and breeding activities and look forward to continuing collaboration with our Cooperative partners listed below.

### Full Members

ArborGen, Inc.

Georgia Forestry Commission

Hancock Timber Resources Group

International Forest Company

North Carolina Forest Service

Rayonier, Incorporated

Tennessee Division of Forestry

Virginia Department of Forestry

The Westervelt Company, Inc.

Weyerhaeuser Company

### Research Associate Members

Arauco - Bioforest, S.A.

PBS International

USDA Forest Service

### Contributing Members

American Forest Management

Blanton's Longleaf Container Nursery

Campbell Global, LLC

Charles Ingram Lumber Co.

Dougherty & Dougherty Forestry Services, Inc.

F&W Forestry Services, Inc.

Four Rivers Land & Timber Company, LLC

Jordan Lumber & Supply Company

Larson and McGowin, Inc.

Meeks Farms & Nursery, Inc.

Milliken Forestry Company

Molpus Woodlands Group, LLC

NC State Natural Resource Foundation, 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 (2013-2016)

From our inception in 1956, there have been over 925 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

## 2016

Čepl J., D. Holá, J. Stejskal, J. Korecký, M. Kočová, Z. Lhotáková, I. Tomášková, M. Palovská, O. Rothová, R.W. Whetten, J. Kaňák, J. Albrechtová, and M. Lstibůrek. 2016. Genetic variability and heritability of chlorophyll *a* fluorescence parameters in Scots pine (*Pinus sylvestris* L.). Tree Physiology (in press).

Isik, F., J. Bartholomé, A. Farjat, E. Chancerel, A. Raffin, L. Sanchez, C. Plomion, L. Bouffier. 2016. Genomic selection in maritime pine. Plant Science 242:108-119 doi:10.1016/j.plantsci.2015.08.006.

Kurt, Y., J. Frampton, F. Isik, C. Landgren, and G. Chastagner. 2016. Variation in needle and cone characteristics and seed germination ability of *Abies bornuelleriana* and *Abies equi-trojani* populations from Turkey. Turkish Journal of Agriculture and Forestry 40:169-176.

Plomion, C., J. Bartholomé, I. Lesur, C. Boury, I. Rodríguez-Quilón, H. Lagraulet, F. Ehrenmann et al. 2016. High-density SNP assay development for genetic analysis in maritime pine (*Pinus pinaster*). Molecular Ecology Resources 16 (2): 574-587.

## 2015

Amerson H.V., C. D., T.L. Kubisiak, E.G. Kuhlman, and S.A. Garcia. 2015. Identification of nine pathotype-specific genes conferring resistance to fusiform rust in loblolly pine (*Pinus taeda* L.). Forests 6(8): 2739-2761. doi:10.3390/f6082739

Egbäck, S., B.P. Bullock, F. Isik, and S. McKeand. 2015. Height-diameter relationships for different genetic planting stock of loblolly pine at age six. For. Sci. 61(3): 424-428. http://dx.doi.org/10.5849/forsci.14-015

Farjat, A.E., F. Isik, B.J. Reich, R.W. Whetten, and S.E. McKeand. 2015. Modeling climate change effects on the height growth of loblolly pine. For. Sci. 61 (4, 5): 703-715. http://dx.doi.org/10.5849/forsci.14-075

Ford, G.A., S.E. McKeand, J.B. Jett, and F. Isik. 2015. Effects of inbreeding on growth and quality traits in loblolly pine. For. Sci. 61(3): 579-585. http://dx.doi.org/10.5849/forsci.13-185

Isik, F., Kumar, S., Martínez-García, P. J., Iwata, H., & Yamamoto, T. 2015. Chapter three-Acceleration of forest and fruit tree domestication by genomic selection. Advances in Botanical Research 74: 93-124.

Kim, T.J., B.P. Bullock, and S.E. McKeand. 2015. Spatial autocorrelation among different levels of genetic control and spacings in loblolly pine. For. Sci. 61(3): 438-444. http://dx.doi.org/10.5849/forsci.14-034

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

Qi Z.C., Y. Yu, X. Liu, A. Pais, T. Ranney, R.W. Whetten, and Q.Y Xiang. 2015. Phylogenomics of *Fothergilla* (Hamamelidaceae) by RAD-tag based GBS: insights into species origin and effects of software pipelines. Journal of Systematics and Evolution 53(5): 432-477.

Wood, E.R., B.P. Bullock, F. Isik, and S.E. McKeand. 2015. Variation in stem taper and growth traits in a clonal trial of loblolly pine. For. Sci. 61(1): 76-82. http://dx.doi.org/10.5849/forsci.12-068

Zapata-Valenzuela, J., F. Ogut, A. Kegley, W.P. Cumbie, F. Isik, B. Li, and S.E. McKeand. 2015. Seedling evaluation of Atlantic Coastal and Piedmont sources of *Pinus taeda* L. and their hybrids for cold hardiness. For. Sci. 61(1): 169-175. http://dx.doi.org/10.5849/forsci.12-610

## 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. Chian. 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 26(3): 876-893. 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 45(3): 379-401. DOI 10.1007/s11056-014-9422-z.

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

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

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

Ögüt, F., C. Maltecca, R.W. Whetten, S.E. McKeand, and F. Isik. 2014. Genetic analysis of diallel progeny test data using factor analytic linear mixed models. For. Sci. 60(1): 119-127.

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

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

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

## 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.

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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.

# SEEING FORESTRY FROM NEW HEIGHTS!



*Mount St. Helens was spectacular for our 2015 Cooperative Tree Improvement Contact meeting. A portion of the forests destroyed during the 1980 earthquake and volcanic eruption were owned by Weyerhaeuser Company. Within months of the disaster, Weyerhaeuser began planting 18 million seedlings, by hand. Some of those stands were being harvested while we were visiting.*

*Once again we would like to thank our colleagues at the Bureau of Land Management, GreenWood Resources, Hampton Tree Farms, Oregon St. University, Weyerhaeuser Co., and the US Forest Service. They made this a fantastic experience for their flatlander friends from the South.*

**Front Cover:** We take teaching to a whole new level in Professor McKeand's Forestry 727 class – Tree Improvement Research Techniques. Students spent their spring break getting hands-on experience at the Arrowhead Breeding Center with experts like Chuck Little (front center). Presentations and videos are great, but when we train future tree improvement leaders, we aim high!

**Pictured Above**: Sea of bags for mass production of control cross seed. Over 1 million pollination bags were bred this spring throughout the South. Operational production of full-sib crosses of loblolly pine continues to increase each year.

**Pictured Below:** This demonstration plot at the Hofmann Forest has a lot to show. The 10-tree row plot in the center is non-improved loblolly pine from Coastal North Carolina; the family to the right is an excellent open-pollinated family from the third-cycle. We can always show the numbers when it comes to genetics (see page 11 inside for experimental results), but these trees speak for themselves!