NORTH CAROLINA STATE UNIVERSITY
COOPERATIVE TREE IMPROVEMENT PROGRAM

61st Annual Report | May 2017

DEPARTMENT OF FORESTRY & ENVIRONMENTAL RESOURCES

COLLEGE OF NATURAL RESOURCES

NC STATE UNIVERSITY

TREE
IMPROVEMENT
PROGRAM
NC STATE UNIVERSITY

61st Annual Report

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Department of Forestry and Environmental Resources

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EXECUTIVE SUMMARY

At age 61 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

More than 90% of the 4th-Cycle crosses in the Coastal population have been completed. The Piedmont and Northern crossing should be 80% complete when we receive all the data from the 2017 breeding. Breeding for the Atlantic Coastal Elite (ACE) Genomic Selection population started this year with 67 crosses made among 69 clonal selections.

The final 4th-Cycle tests should be planted in 2020. The first 4th-Cycle tests will be measured in winter 2017-18. By spring of 2019, we will have complete information for about 150 crosses, then another 150 crosses in 2020, and about 250 crosses per year beginning in 2021 thanks to the acceleration of the testing program starting in 2017.

There were 74 new selections in the 2017 Coastal Big BLUP and 113 in the Piedmont Big BLUP.

Using 2nd-Cycle rust incidence from 845 diallel tests, hazard maps for fusiform rust were developed.

Cooperative members harvested over 60,000 pounds of loblolly pine seed from orchards in 2016.

RESEARCH

Based on 5-year data from the Loblolly Pine Biomass Genetics/Cropping Study at Butner, NC, Coastal families had 42 ft^3/acre more volume than Piedmont families, despite having more cold damage two years earlier.

In a study of wide crosses of loblolly pine planted in AR, MS, and GA, E x E crosses had consistently greater growth than pure western crosses (W x W). The inter-provenance crosses (E x W and W x E) height and volume were more similar to pure eastern crosses. Family rankings were very similar in all test locations.

Using computer simulation, marker-based methods were more efficient than pedigree-based methods at producing genetic gain over multiple generations.
SNP genotypes from genes expressed in 2-month-old seedlings explained 53% of the variation in stem volume at age 6 years for 56 parents in field tests.

PBS International pollination bags with greater rigidity and smoothness prevented damage to strobili and resulted in higher seed yields compared to the industry standard paper bags in two separate studies.

Soil compaction in loblolly pine seed orchards was assessed using a soil penetrometer. The percentage of “usable soil” ranged from an average of only 7% to 18% in the traffic rows for the five orchards sampled but was 18% to 45% in the sample positions near the tree where traffic was minimal.

Survival of about 50% at the Texas site in the Loblolly Adaptation and Mapping Project (LAMP) study will allow for Genomic analyses to determine genetic effects on drought adaptability.

Resistograph amplitude (surrogate measure for wood density) and TreeSonic acoustic velocity (surrogate for wood stiffness/ bending strength) are available to Cooperative members for 270 Coastal families.

Differences among genotypes for stand mean diameter were considerable in the pre-thinned stands and persisted after thinning in the Genetics Demo trial at the Hofmann Forest. Differences in defect rates were large in the pre-thinned stands but were reduced considerably by thinning, except in the non-improved checklot.

In the Atlantic Coastal Elite trials, the clone-mean h^2 for growth traits at age six (0.55 for height) was much greater than the individual-tree h^2 observed in seedling progeny testing (0.2 to 0.3 in third-cycle polymix tests).

In a long-term progeny test of longleaf pine, there was significant variation among families for growth traits and survival at ages 3, 7, 17, 30, and 40 years. Family genetic values for height were strongly correlated (r = 0.81) between ages 17 and 40 years indicating that selection at 17 years would predict performance at age 40.

For DNA fingerprinting, comparison of the sequences of amplicons that yielded useful SNP data with DNA sequences previously placed on a consensus genetic linkage map of loblolly pine suggests that the detected markers provide reasonably complete genome coverage.

ASSOCIATED ACTIVITIES, GRADUATE STUDENTS, COOPERATIVE STAFF, MEMBERSHIP

Three companies joined the Cooperative as Contributing Members, and 2 companies and 1 agency joined as Research Associate Members. There are now 10 Full, 22 Contributing, and 6 Research Associate Members. Graduate and undergraduate 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. Daniel Genung joined us as Analyst / Database Manager. We are particularly proud of Fikret Isik and his coauthors, Jim Holland and Chris Maltecca for the publication of their new book, Genetic Data Analysis for Plant and Animal Breeding.

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61 YEARS OF TREE IMPROVEMENT AT NC STATE

A MESSAGE FROM THE DIRECTOR

Juggling Chainsaws

Tree breeding is a science and art combining many disciplines including silviculture, quantitative genetics, statistics, plant physiology, reproductive biology, plant pathology, entomology, molecular biology, genomics, and *chainsaw juggling*. The last discipline may be the most critical skill that a good tree breeder has in his or her bag of tricks. The Cooperative staff does an incredible job of planning and organizing the plethora of activities involved in a typical tree breeding cycle: breeding strategies are developed; crossing schemes are planned and implemented; cones are collected and seeds processed; progeny trials are planned with experimental designs that optimize the efficient use of resources; data are collected, analyzed, and interpreted; and so on and so forth.

Then out of the blue, someone (probably a gremlin with a sick sense of humor) tosses us a running chainsaw and says "catch". This past breeding season was a great example of staff, students, and faculty being well prepared and ready to implement the breeding plans that had been in preparation for months. The new Breeding Logistics database in our **TIPRoot** database was up and running, helping to simplify much of the decision making. We were poised and ready to roll… then the warmest mid-January through February in recent memory hit. When Chuck Little called on Valentine's Day and said "*get yor A_ _ down here*", we knew that female strobili and pollen were developing much quicker than even us old-timers expected. We knew the breeding season would be early, but Chuck had already been bagging loblolly pine flowers since February 12 … YIKES!

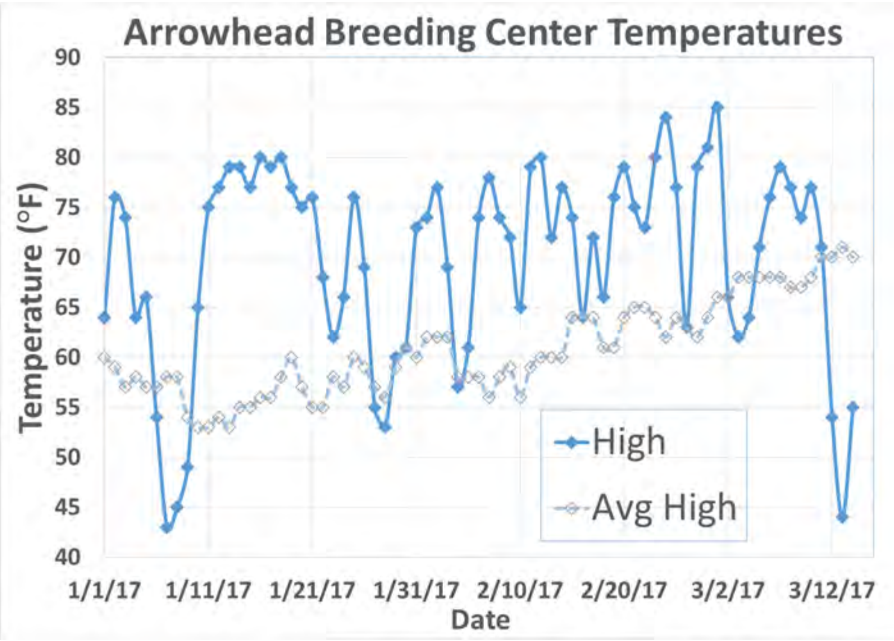
Our dedicated staff dropped most everything in Raleigh, loaded up, and headed to Arrowhead. Austin was the "winner" this year spending 2½ weeks at *The Chalet at the Arrowhead*. Trevor was there just short of 2 weeks, and Fikret and Daniel did yeoman's service for over a week. Even yours truly put in some time up in the aerial lifts this year. This was a record year for breeding at Arrowhead with 202 crosses being made. We initiated breeding for Fikret's genomic selection population and just about wrapped up the 4th-Cycle breeding for all three regions; we collected pollen from 66 selections for future genomic selection breeding and any last-minute crosses for the 4th Cycle; topgrafts of a few critical selections were made; and we also initiated plans with the Georgia Forestry Commission staff to develop more breeding orchards at Arrowhead for the 5th-cycle selections that will be topgrafted in 2019… less than two years from now… Oh My!

Fortunately, April and our ever-reliable graduate and undergraduate students were in Raleigh keeping the rest of the program moving forward. The original plans to get all the 4th-Cycle seed pulled from the freezer and sent off to Cooperative members by the end of February went down the tube. Fortunately, April and crew were able to get all the seed to the growers by early-mid March in plenty of time for seeds to be stratified and sown in containers. We also had 4th-Cycle progeny test seedlings to organize and deliver to our Northern cooperators. And of course, a severe freeze hit Raleigh the weekend before the seedlings were to be delivered. Unfortunately, the warm weather had the seedlings actively growing, but fortunately, Austin and the crew got the seedlings covered, and they came through just fine and were delivered on time and in great condition to cooperators. All of this was after the breeding values for the Coastal and Piedmont populations were calculated and made available to Cooperative members in January.

By golly, I think we actually completed all the critical tasks on time, and disasters were avoided one more time. All of this because of the dedication and effort of our staff, students, and faculty. I say this all the time... there is no more hard-working, dedicated, intellectually gifted group of tree breeders any place on earth! If juggling chainsaws was an Olympic sport, we would field a team of gold medal winners!

Steve McKeand, May 2017

I did find out that the Guinness World Record for the most chainsaw juggling catches is 94 (www.guinnessworldrecords.com/world-records/most-chainsaw-juggling-catches). And, I am not sure if Austin or Trevor is the star of this video: https://www.youtube.com/watch?v=OoJW-_OeFtw



Left: *Temperatures were well above average this winter and pushed the breeding ahead by about 3 weeks at Arrowhead.*

Pictured below: Center: *The weeks of planning for 4th-Cycle and Genomic Selection breeding continued in the field. Trevor Walker and Fikret Isik adjusted breeding plans based on availability of flowers in the Breeding Ramet Orchard (BRO).* ***Upper left:*** *Graduate students Nasir Shalizi, April Meeks, and Jessie Maynor spent hours in the lab pulling 4th-Cycle seeds, preparing for sowing in the spring.* ***Upper right:*** *Our new database manager, Daniel Genung, realized that breeding pine trees was easier than breeding pigs (previously in animal genetics). Daniel and Fikret spent time bagging crosses for 4th-Cycle and Genomic Selection Breeding.* ***Bottom left:*** *Daniel Genung, Rhodes Kelly, and April Meeks organized 4th-Cycle tests for Northern cooperators.* ***Bottom right:*** *Austin Heine spent 2+ weeks up in the sky… what a vacation!*

SELECTION, BREEDING, AND TESTING

Fourth Cycle Breeding and Testing Progress

Fourth-Cycle Breeding Update*: Are we there yet?*

The 2017 breeding season marks the fifth year of breeding for the 4th Cycle. Progress towards completing the planned crosses has been steady (Figure 1), with about 220 new crosses attempted each year and an average success rate of 75%. Success is defined as harvesting at least 90 sound seed, the minimum amount needed to reliably test a cross in its native region. More than 90% of the crosses in the Coastal population have been completed. The Piedmont and Northern populations are expected to achieve 80% of the crosses attempted when we receive all the data from the 2017 breeding season.

The experience by TIP staff at Arrowhead and "word on the street" among Cooperative members suggest

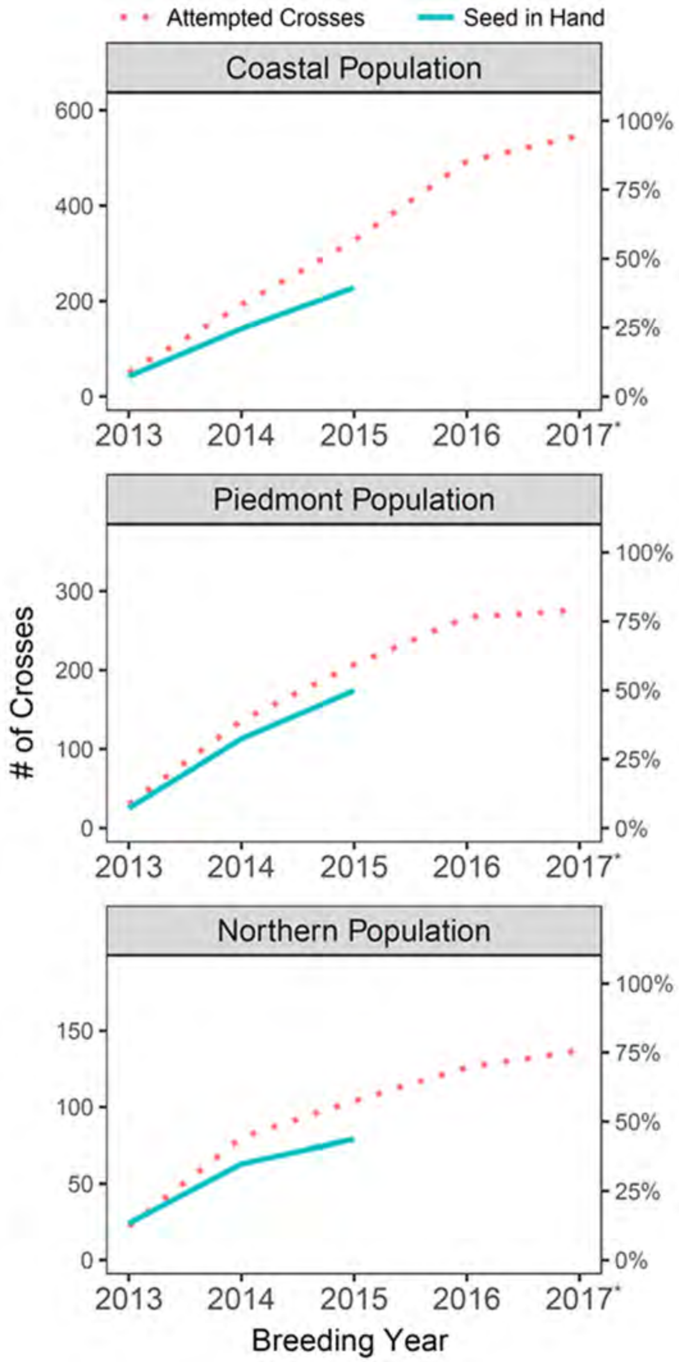


Figure 1 (left). *The Cooperative's progress towards completing the 4th-Cycle breeding plan by population. Crosses where adequate seed has been harvested (90 seed, the minimum required to reliably test a cross in its native region) are labeled as "seed in hand" (solid line on graphs). Nearly 100% of the Coastal crosses have had breeding work performed. The Piedmont and Northern populations are expected to reach 80% for 2017 (dotted line on graphs). The gap between attempted crosses and completed crosses represents breeding failures (e.g. poor flower survival, pollen viability, or insect damage). Note that 2017 numbers only include work done by TIP staff at Arrowhead; data for work done by members were not available at time of printing.*

Pictured above: *TIP staff enjoying a morning pollen party during the 2017 breeding season at the Arrowhead Breeding Center. More than 140 unique pollens were used, requiring a substantial amount of preparation each morning. Left to Right: Chuck Little, Trevor Walker, Fikret Isik, Steve McKeand, and Daniel Genung (Austin Heine was behind the camera).*

that 2017 was a tough year for controlled pollinations. The unusually early spring (Figure 2) caught many off guard, reducing the time available for managers to develop tactical breeding plans and transfer pollen. Additionally, poor flowering was reported for orchards affected by the severe drought in late summer and fall 2016. Temperature dips in early March slowed flower development and prolonged the time before bags could be removed, and nothing good happens inside those bags when left on for a long time. To top it off, a late freeze in mid-March severely damaged flowers in some orchards.

The amount of breeding work performed by TIP staff has grown considerably since 4th-Cycle breeding began in 2013. Most of the breeding effort has been with the ACE selections that were top-grafted in 2014. The 2017 season marked the busiest, with just over 500 bags installed. Use of the Arrowhead database in **TIPRoot** to efficiently plan and implement breeding work has allowed TIP staff to best use the limited time available for pollinations. A good group of folks working at Arrowhead also goes a long way note one of the many early morning pollen parties at The Chalet!

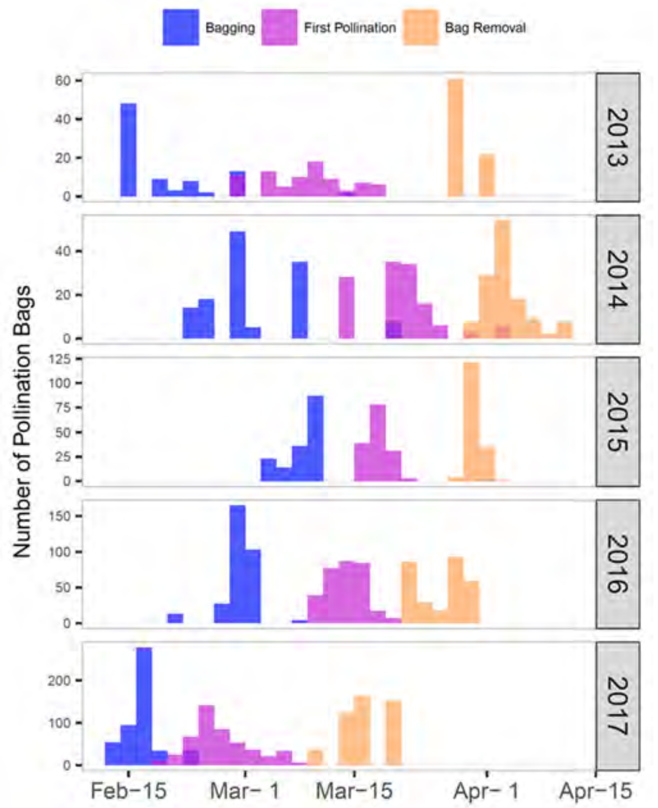


Figure 2. *Number of pollination bags by day of installation, first pollination, and when the bags were removed by TIP staff at Arrowhead Breeding Center for each year of 4th-Cycle breeding. The number of pollination bags installed has grown considerably each year as the ACE selections have started to flower. This past breeding season (2017) was the earliest, with the majority of pollinations occurring about two weeks earlier than the previous years.*

At the 2016 Contact Meeting, TIP staff briefly presented a plan for the efficient collection of flower phenology data by clone. We tested the plan at Arrowhead this spring and found that it required minimal extra time to record the phenology data, yet provided a much more objective way to schedule pollinations. Additionally, these data can be used to characterize clones for flowering phenology as "early", "middle", or "late" (Figure 3). Using heat sums/degree days to predict flower development is an informative tool that has been under-utilized by many of us in the Cooperative.

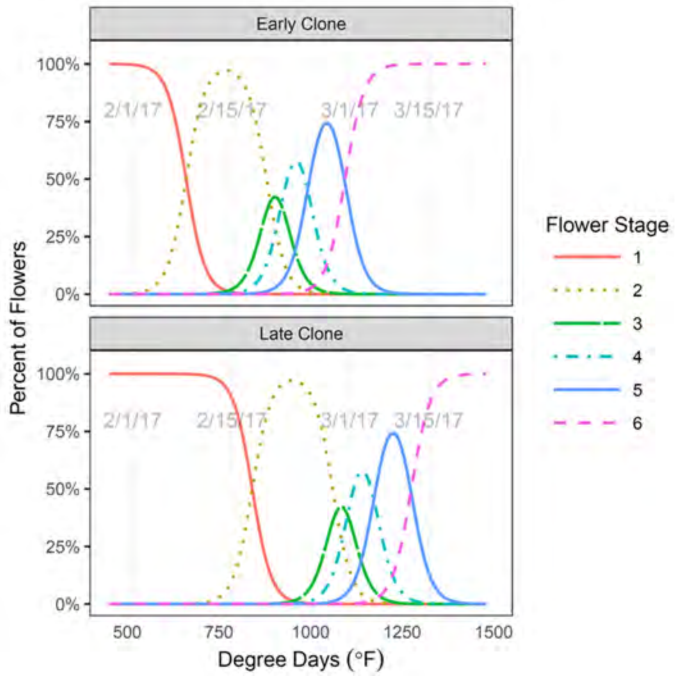


Figure 3. *Predicted flower development based on phenology data collected for clones bred in 2017 by TIP staff at the Arrowhead Breeding Center. Model predictions are shown for an early clone and a late clone. We found that flower phenology data required minimal extra time to record and provided an objective way to schedule pollinations. Degree days were calculated based on Boyer (Journal of Forestry, Vol. 76, No. 1, 1978). However the starting date for heat sums was changed to January 1 to account for the early spring.*

Progeny Testing: *Putting the pedal to the metal!*

The Cooperative's adoption of a "rolling-front" progeny testing strategy in the 4th-Cycle has allowed new crosses to be tested faster than ever before. In this strategy, crosses are put into tests as soon as they are available from the breeding effort, with enough seed reserved for testing the following year to allow comparisons with crosses that have yet to be harvested (illustrated in Figure 1). Ideally, the proportion of crosses beginning and concluding testing would be equal across years, but issues such as limited seed and test failures (e.g. due to drought) add variability, although the connections are ultimately very strong. Starting with the 2017 tests, the Cooperative made a commitment to double up on the testing effort by installing twice as many reps per year and implementing a two-series test design. This allowed the testing of more crosses in a shorter time period, especially because testing of some crosses is completed in one year (Figure 1).

Another feature of the 4th-Cycle testing plan is the "regional overlap" that will permit comparisons of families from different populations (Figure 2). Coastal families are routinely planted in regions outside of the Coastal Plain, hence the need for comparisons. Coastal tests only contain Coastal families, while Piedmont and Northern tests contain a target of 40% from outside the region. Coastal families planted in the Northern and Piedmont regions are identified based on adaptability using the average annual minimum temperature of the selection origin. Deviations from the 40% target occurred when there was not enough seed from adapted crosses, such as the number of Coastal crosses planted in Northern tests. The large number of crosses from multiple regions in each test will allow direct comparison of breeding values for parents from different genetic source regions.

At the current rate of testing (Table 1), the target date for establishing the final 4th-Cycle tests is 2020. The

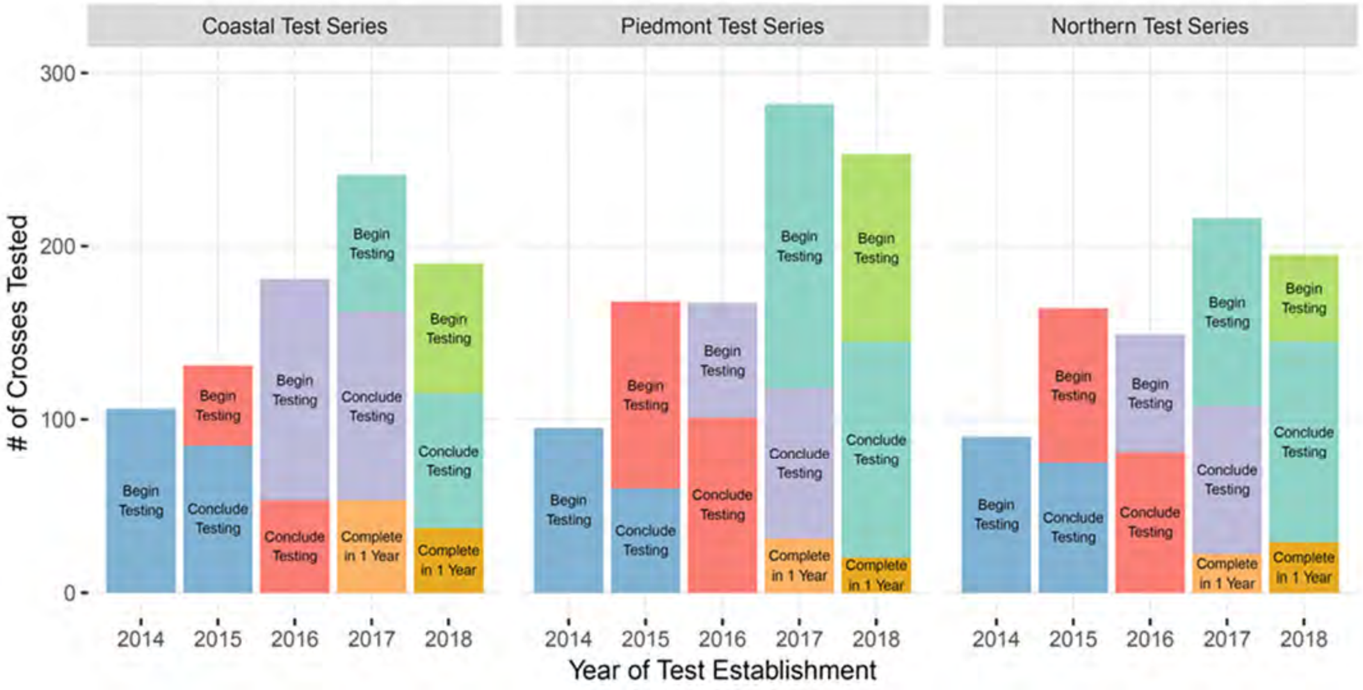


Figure 1. *The 4th-Cycle testing plan uses a "rolling front" strategy so that crosses can be tested as soon as seeds are available. This approach requires staggering of the years in which a cross is tested to facilitate overlapping families for genetic connections across test series. The actual number of crosses tested are shown in this figure by year of establishment and test series. The same colors on the bars from one year to the next year within a region indicate when groups of families were first tested and when testing was completed for those same families. Note that 2017 tests were planted this past winter, and the seed for 2018 tests have been sown this spring.*

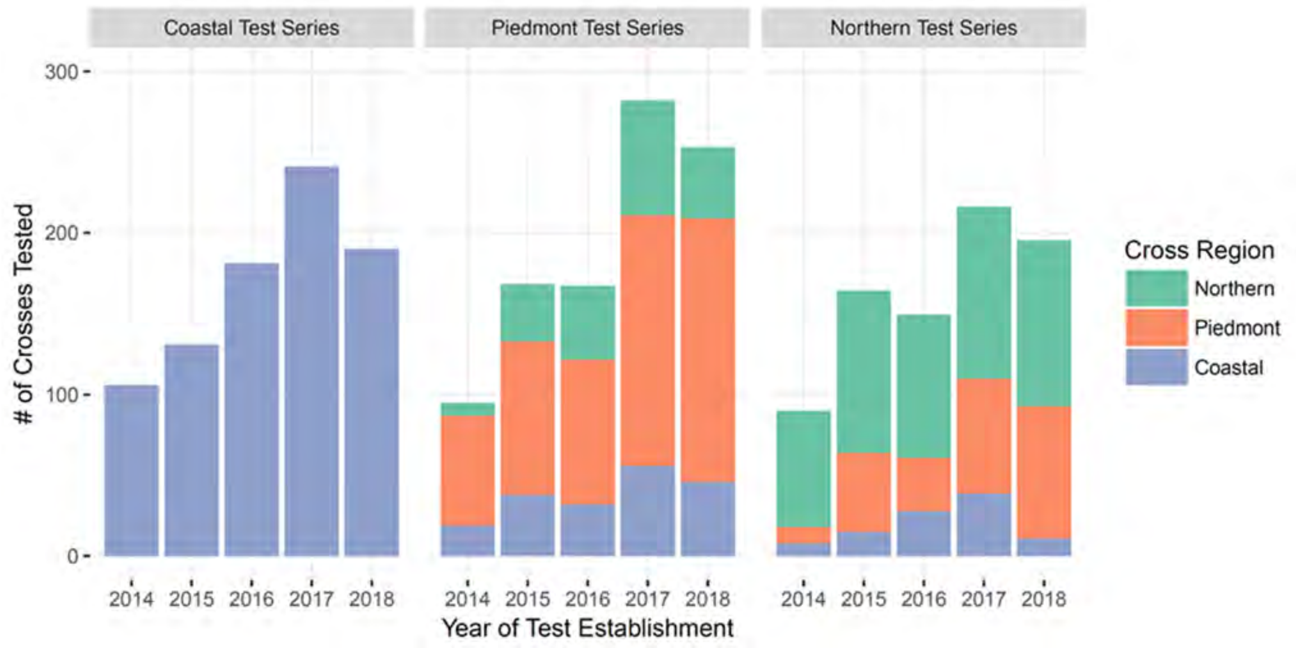


Figure 2. *Testing crosses outside their breeding region is another feature of the 4th-Cycle testing plan that will permit comparisons of family performance across all regions. Coastal tests only contain Coastal families, while Piedmont and Northern tests contain a target of 40% from outside the region.*

first 4th-Cycle tests are scheduled for assessment this upcoming winter. By spring of 2019, we will have complete information for about 150 crosses, then another 150 crosses in 2020, and about 250 crosses per year beginning in 2021 thanks to the acceleration of the testing program starting with tests in 2017.

Only three 3rd-Cycle progeny tests have yet to be measured, and they are scheduled for measurement by 2019 (Figure 3). The 4th-Cycle tests are planned for measurement from 2018 to 2024. At this rate, 5th-Cycle test establishment is right around the corner!

Table 1. *Fourth-Cycle testing progress by test series and year established. The testing effort doubled in 2017 to reduce the target date of final test establishment to 2020. Note: 2017 tests were planted this past winter, and seed for 2018 tests were sown this spring, so these number of reps planted are a projection (orange color text).*

4th-Cycle Test Population	**Year Established**	**# of Reps. Planted**	**Cumulative # of Reps. Successful at Age 1**	**Cumulative # of Crosses Complete (Native Population)**	**Cumulative Percent Complete**	**Planned Measurement Year**
Coastal	2014	40	40	0	0%	2018
	2015	30	70	94	12%	2019
	2016	30	100	138	17%	2020
	2017	60	160	294	37%	2021
	2018	60	220	405	51%	2022
Piedmont	2014	27	22	0	0%	2018
	2015	30	47	26	5%	2019
	2016	32	74	71	14%	2020
	2017	60	134	151	30%	2021
	2018	60	194	240	49%	2022
Northern	2014	35	30	0	0%	2018
	2015	30	40	31	10%	2019
	2016	45	70	70	12%	2020
	2017	54	130	129	43%	2021
	2018	46	176	174	58%	2022

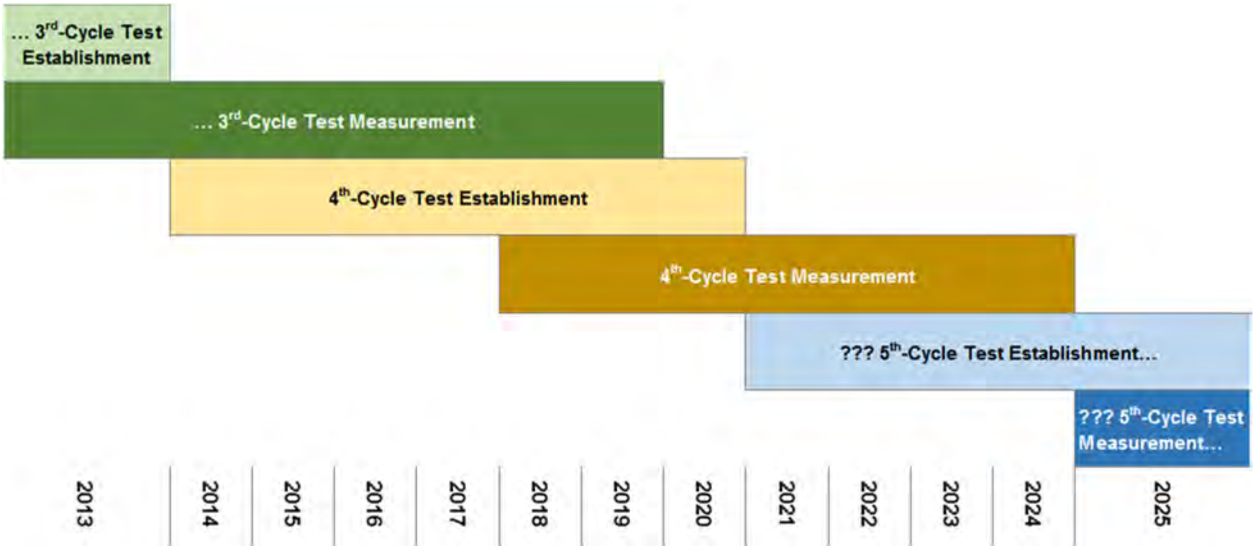


Figure 3. *The final test measurements for the 3rd-Cycle will wrap up in 2018 and 2019. The first 4th-Cycle tests will be measured in 2018 and are planned to continue through 2024. Progeny testing in the fourth cycle is expected to wrap up over a 10-year period. When will the first 5th-Cycle tests be established?*

Sometimes it rains... and sometimes it floods!

On October 8, 2016, the southeast US had an unwelcomed visitor named Hurricane Matthew. Some seed orchards were more fortunate than others. Matthew dumped over 16” of rain in much of eastern North Carolina. The NC Forest Service Claridge Nursery and Seed Orchard in Goldsboro, NC was inundated for several days. The orchards recovered and most of the cones were harvested, but essentially the entire bareroot nursery crop was lost. Photo courtesy of James West, NC Forest Service.

Big BLUP and ***PRS***™ Updates

In 2016, Big BLUP analyses were run for the Coastal and Piedmont breeding populations. The resulting ***PRS***™ versions were released on our TIPRoot database in March 2017, the first Big BLUP analysis since 2014.

New data for the Coastal Big BLUP came from the addition of tests and updated measurements (age 6 vs. age 3) for tests in the CPMX4, CPMX5, and CPMX6 series. Overall, there were 671,590 data points analyzed from 579 tests (up from 571 tests in 2014). There were 74 new selections in the 2017 Coastal Big BLUP, and nine of them showed in the top 95th percentile for volume, rust, and straightness (Figures 1 and 2). Very few selections experienced significant rank changes. The correlations between breeding values for versions 2014 and 2017 Coastal were r > 0.99.

New data from the Piedmont Big BLUP came from the addition of tests in the PPMX3 and PPMX4 series. There were 545,727 observations from 508 tests (up from 504 in 2014). There were 113 new selections in the 2017 Piedmont Big BLUP, 12 of which performed in the top 95th percentile for volume (Figure 1). The correlations between 2014 and 2017 breeding value the Piedmont versions were r > 0.99.

There was not enough new data to justify analysis for the Northern region. Based on 4th Cycle test establishment, there will be sufficient data to run a Northern Big BLUP in 2018.

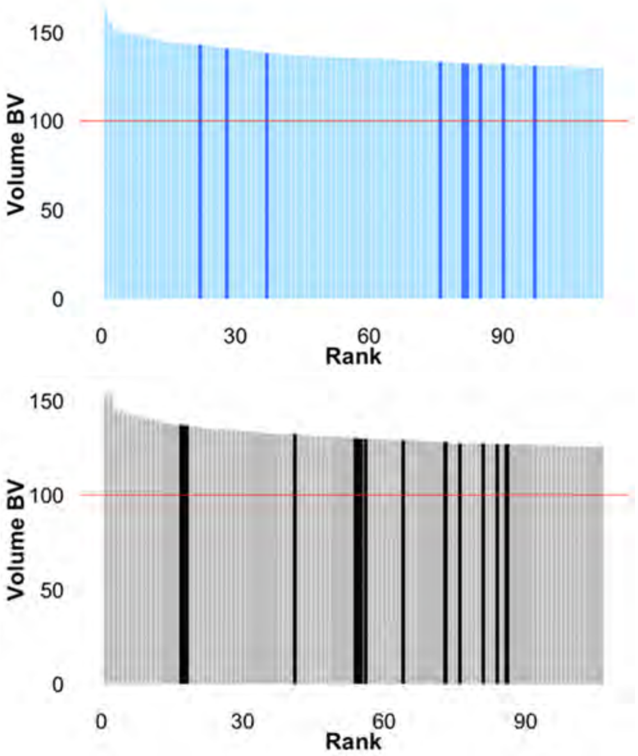


Figure 1. *Top selections for the 2017 Coastal (blue) and Piedmont (grey)* ***PRS***™ *Versions for volume breeding values. New selections are shown in darker colors, and the red line represents the population mean.*

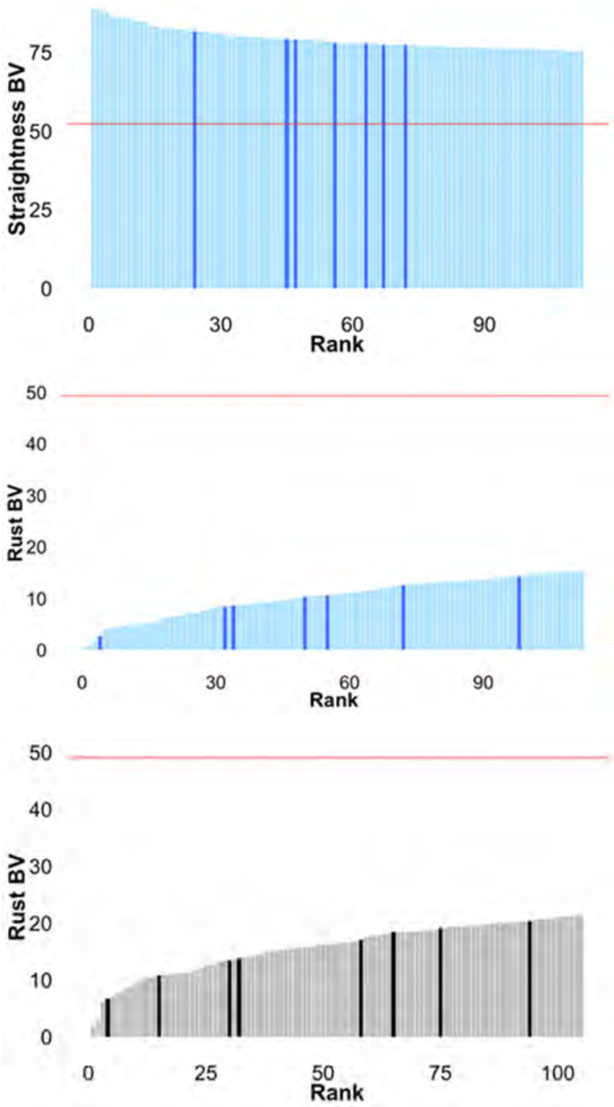


Figure 2. *Top selections for the 2017 Coastal (blue) and Piedmont (grey)* ***PRS***™ *Versions for straightness and rust breeding values. New selections are shown in darker colors, and the red line represents the population mean for breeding values. No new selections placed in the top 5% for straightness in the Piedmont.*

Breeding for Genomic Selection

Breeding within the Atlantic Coastal Elite (ACE) population is ideal to test and verify genomic selection in loblolly pine due to the small number of founders (21) and closed, multiple generation pedigree structure in the population (Figure 1). The founders were mated in the early 2000's to produce 76 crosses. The full-sib progeny were challenged with fusiform rust inocula at the US Forest Service Resistance Screening Center in Asheville, NC in 2007. Based on the results from the screening center, 13 susceptible crosses were removed from the population, leaving 53 crosses for progeny testing. From each cross, an average of 46 full-sib progeny (2362 in total) were cloned in 2007 using rooted cutting methods. A genetically identical copy of each clone was planted at eight field locations in 2010 and 2011 using an incomplete block row-column design.

Six-year data were collected in 2016, and an animal model was used to predict breeding values of clones. A selection index with 0.6 weight on growth and 0.2 on both stem straightness and stem forking was constructed for each clone. A total of 204 candidate clones out of ~2300 were available for breeding.

A breeding plan was developed using the MateSelect tool of the Pedigree Viewer software. A modified MateSelect input file was run using a target degree of 25. In the mating, the maximum allowable inbreeding coefficient was 0.025, and the maximum matings per individual was 3 crosses to maintain diversity. The second generation of ACE breeding was carried out in February 2017 using 69 unique clones from 34 first-generation crosses out of the original 53 tested (Figure 2). A total of 67 new crosses were made with a target number of seed per cross of 300 to allow for genotyping samples and potentially for hedges for clonal testing.

Plans for coming years

About 200 seedling progeny of each cross will be challenged with fusiform rust inocula in the spring of 2019 at the US Forest Service Resistance Screening Center in Asheville, NC. About 40 gall-free progeny

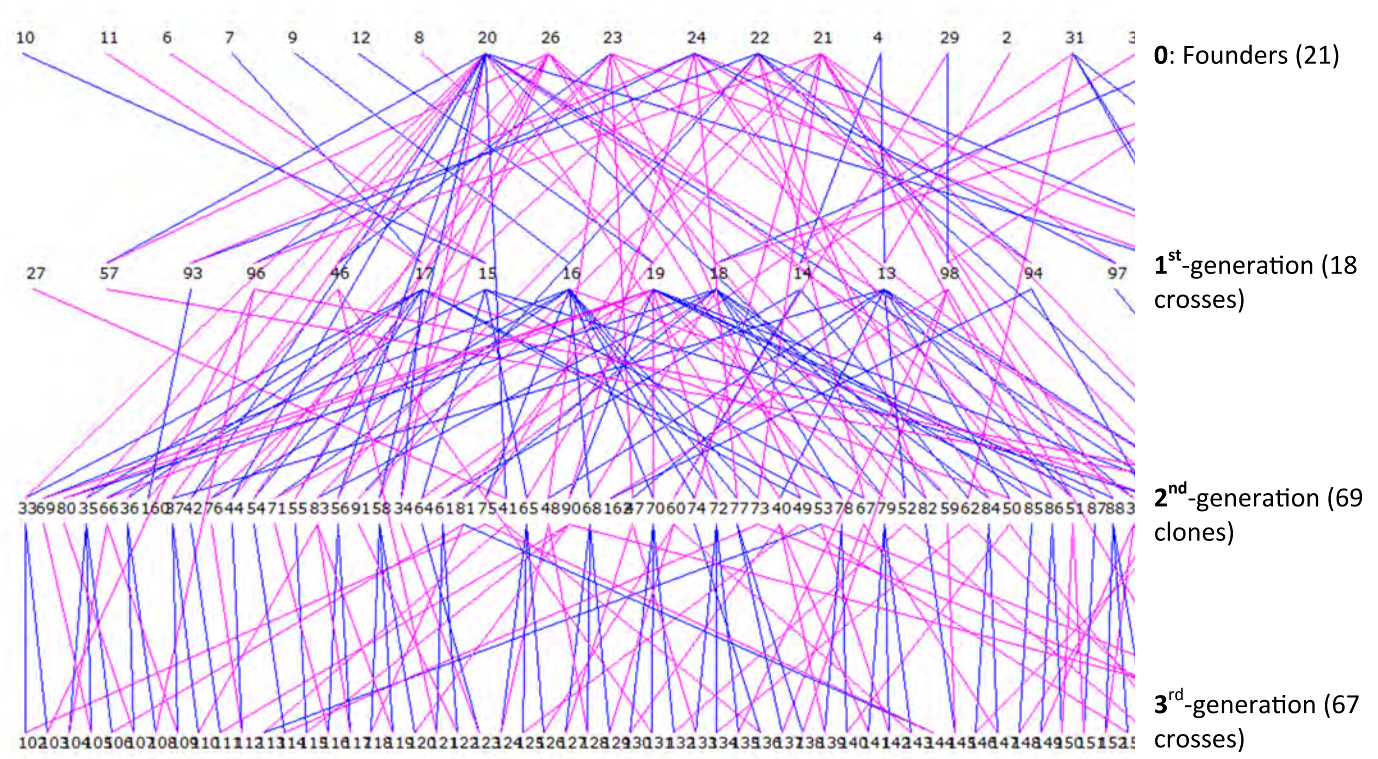


***Figure 1**. Atlantic Coastal Elite (ACE) breeding population pedigree. The top row indicates the founder population (21 individuals). The second row shows crosses originated from the founders. The third row is the 69 clones selected to breed, and the last row is 67 crosses bred in 2017.*

of each cross will be hedged in late 2019 for cloned progeny testing. Not all the hedges typically survive, or they will not have enough rooted cuttings, so the target is to have ~20 cloned progeny per cross in field trials (a total of 1200 clones). Each clone will be represented by 12 ramets (1200 x 12 = 14,000 plants) in field trials established in winter of 2021. The remaining disease-free seedling progeny of each cross will be planted in replicated field trials in the winter of 2020. The new generation of the ACE population (67 crosses) will be represented by both cloned and seedling progeny tests.

Genotyping and validation of genomic selection: The 21 founders, ~2300 cloned progeny in the first generation, and the 1200 cloned progeny (no phenotype) will be genotyped using a medium-density Pine SNP array currently under development as part of the NIFA project (Award #: 2016-67013-24469). Genomic breeding values of 1200 cloned progeny will be predicted when they are in the seedling stage (2019) using marker-trait associations from the training population (21 founders and 2300 clones). Ramets of 1200 cloned progeny will be planted in replicated field trials in December 2021 and phenotyped at age four. A correlation between genomic estimated breeding values and breeding values of 1200 clones from field trials will be calculated to validate the predictive ability of markers (2026).

The results from this study should show the efficiency of genomic selection by examining the linkage disequilibrium between markers and putative genes after the population goes through a mating cycle and chromosome recombination. More details can be found at *Isik, F. (2014). Genomic selection in forest tree breeding: the concept and an outlook to the future. New forests, 45, 379-401.*

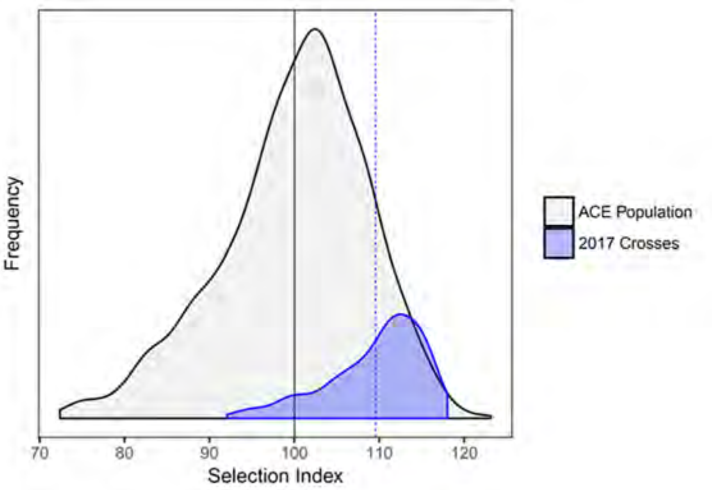


***Figure 2**. The distribution of selection index scores for all 2362 clones in the Atlantic Coastal Elite (ACE) clonal population and for the new 67 crosses bred in 2017. The black vertical line represents the mean of the 2362 clones. The vertical dashed blue line is the expected mean of the 67 new crosses. Expected gain from the new crosses is 10% higher than the overall population mean.*

***Pictured right**: We were even able to get Dr. Isik to the field this spring to pollinate new ACE crosses at the Arrowhead Breeding Center. Fikret appears to be **very intent** on getting this breeding done so that the genomic selection work will continue on schedule.*

Fusiform Rust Hazard Mapping with Progeny Test Data

Progeny test measurements are critical for identifying the best families and selecting the best genetics to advance breeding populations, but they are also useful for other, unanticipated questions. The incidence of fusiform rust (the most economically important disease of southern pines in the United States) is a routine measurement in the Cooperative's loblolly pine progeny tests. Another routine component of the Cooperative's testing is the inclusion of trees from local, non-improved seed as checklots, primarily for estimating the gains from tree improvement. Using the measurements and test site locations from the extensive 2nd-Cycle progeny tests, we mapped the incidence of fusiform rust for the local checklot to create a version of a rust hazard map (Figure 1). The map shows declining rust incidence in the more inland and northern regions of the testing range and is consistent with the maps that have previously been published using plots established in operational plantations[1]. These checklots in 2nd-Cycle progeny tests are appropriate for evaluating rust hazard, because they receive the same silviculture as intensively managed plantations but have no genetic improvement for rust or any other traits.

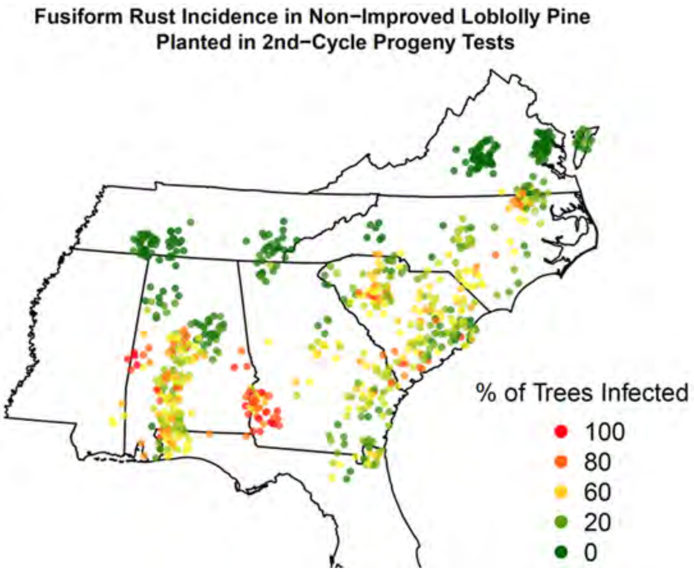


Figure 1. *Fusiform rust incidence in the local checklot (seed collected from non-improved, wild stands) in the Cooperative's 2nd-Cycle progeny tests was mapped based on county location information to create a hazard map. There were 845 tests measured at age six years from 1990 to 2002. Estimating rust hazard with progeny test checklots is advantageous because they receive the same silviculture as intensively managed plantations but have no genetic improvement. Note that test site locations had to be jittered slightly to avoid overlapping points.*

Since progeny tests tend to be clustered on the land bases of Cooperative members and are not uniformly distributed across the region, they are not ideal for fine-detail mapping of rust hazard gradients. However, these historic test data turned out to be incredibly valuable to illustrate serious problems in the most recently published rust hazard maps. These results are in stark contrast to those published in 2015 in the Journal of Forestry[2], which indicated that the current rust hazard in Virginia was as severe as the Upper Coastal Plain and the Piedmont of Georgia and South Carolina. The assumptions made in the Journal of Forestry article were inappropriate for the plantations sampled. The authors assumed that low rust incidence in a plantation was due to the planting of rust resistant seedlings. In the northern and inland regions of the US South, selected families are no more resistant to fusiform rust than the local, wild, non-improved seedlings. Low incidence in these northern and inland regions for the local non-improved checklots indicate that the risk of infection for fusiform rust is very low.

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[1] *Squillace, A.E. 1976. Geographic patterns of fusiform rust infection in loblolly and slash pine plantations. USDA For. Serv., Res. Note SE- 232, Southeastern Forest Experiment Station, Asheville, NC.*

Starkey, D.A., Anderson, R.L., Young, C.H., Cost, N.D., Vissage, J.S., May, D.M. and Yockey, E.K. 1997. Monitoring incidence of fusiform rust in the south and change over time. US Department of Agriculture, Forest Service, Southern Region Forest Health Protection: Atlanta, GA, USA, 4.

[2] *Randolph, K.C., Cowling, E.B., Starkey, D.A., 2015. Long-term changes in fusiform rust incidence in the southeastern United States. Journal of Forestry 113: 381–392.*

SEED AND CONE YIELDS

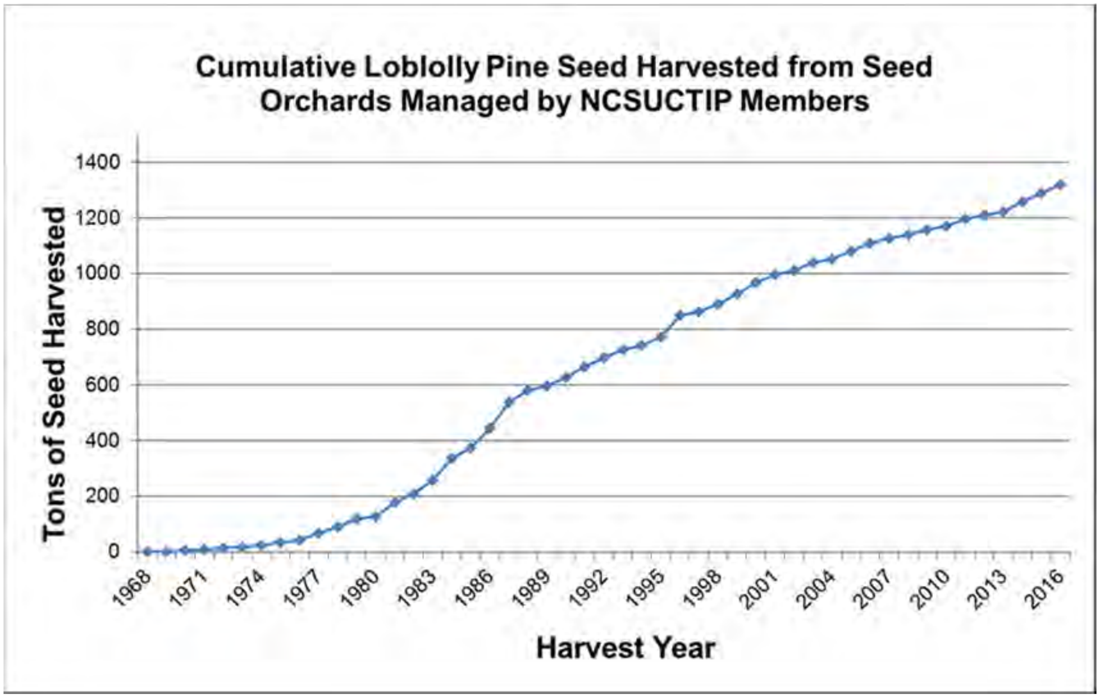
Cooperative members harvested a little more than 60,000 pounds of loblolly pine seed from operational orchards in 2016 (Table 1). This amount was up from 2015, which was also a big seed harvest year. Average yield of seed per bushel changed little compared to 2015, with the exception of the Northern yields, which had a considerable bump in pounds of seed per bushel.

At approximately 12,000 seedlings produced per pound of seed, last fall's harvest has the potential to produce almost 700,000,000 seedlings this year. That is enough to plant approximately 1,400,000 acres of pine plantations at 500 trees per acre!

Table 1. *Fall 2016 cone and seed yields compared with the figures from 2015 harvest*

Provenance / Generation	Bushels of Cones		Pounds of Seed		Pounds of Seed per Bushel	
	2016	2015	2016	2015	2016	2015
Coastal 2.0/2.5	11,872	20,522	17,468	28,442	1.47	1.39
Coastal 3.0	14,938	7,388	22,255	9,560	1.49	1.29
Piedmont 2.0	2,913	7,862	4,664	10,288	1.60	1.31
Piedmont 3.0	7,889	4,020	10,037	5,750	1.27	1.43
Northern 2.0	106	750	109	501	1.02	0.67
Northern 3.0	6,290	2,868	6,814	3,477	1.08	1.21
Totals	***44,008***	***43,409***	***61,347***	***58,018***	***1.32***	***1.22***

From 1968 to 2016, over 2.6 million pounds of improved seed have been produced by Cooperative members. At 12,000 seedlings per pound, this is enough seed to grow over 31 billion improved seedlings. We look forward to greater production from advanced generation seed orchards in the near future!



RESEARCH

Loblolly Biomass Genetics/Cropping Study- Five Year Summary[1]

The Loblolly Pine Biomass Genetics/Cropping Study was planted in the spring of 2012 and is now in its 6th growing season. The main purpose of this study is to evaluate 20 of the Cooperative's top volume producers for their potential to be grown for biomass and/or sawtimber production. The families were planted at a high density, 6' x 7' spacing or 1037 stems per acre, to assess any genetic differences that might exist when these families are planted using a biomass management regime. Additional objectives for this study were previously described in the 2016 Annual Report (pg. 15).

Five-year measurements were completed in December of 2016 and included height, diameter at breast height (DBH), and presence of rust, forking, and ramicorn branches. Using year 5 data, we calculated volume per acre for each family. For volume yield, the difference between provenances was large, on average ~42 ft^3 per acre more for Coastal families compared to Piedmont families (Figure 1). Coastal families were significantly larger on average despite the fact that they had a higher incidence of crown damage than Piedmont families due to two winter storms at age 3 (see 2016 Annual Report for details). Even with this damage (striped bars in Figure 1), Coastal families still had a higher non-damaged volume yield (solid bars in Figure 1) than those from the Piedmont. Another finding is that even though the families selected for this study were all top volume producers, there were considerable differences among families within a provenance.

For a landowner, these findings demonstrate the value of planting high volume producing families, but also the potential loss in volume for different families given a major winter storm event. Year-five data also show that even with the storm damage, the risk of planting higher-risk Coastal material in the Piedmont can be rewarding, depending on the family. Knowing this information allows landowners to evaluate their options and the variation that exists when selecting families for biomass production.

The Butner study is an on-going experiment that will be maintained through rotation. Harvesting/thinning

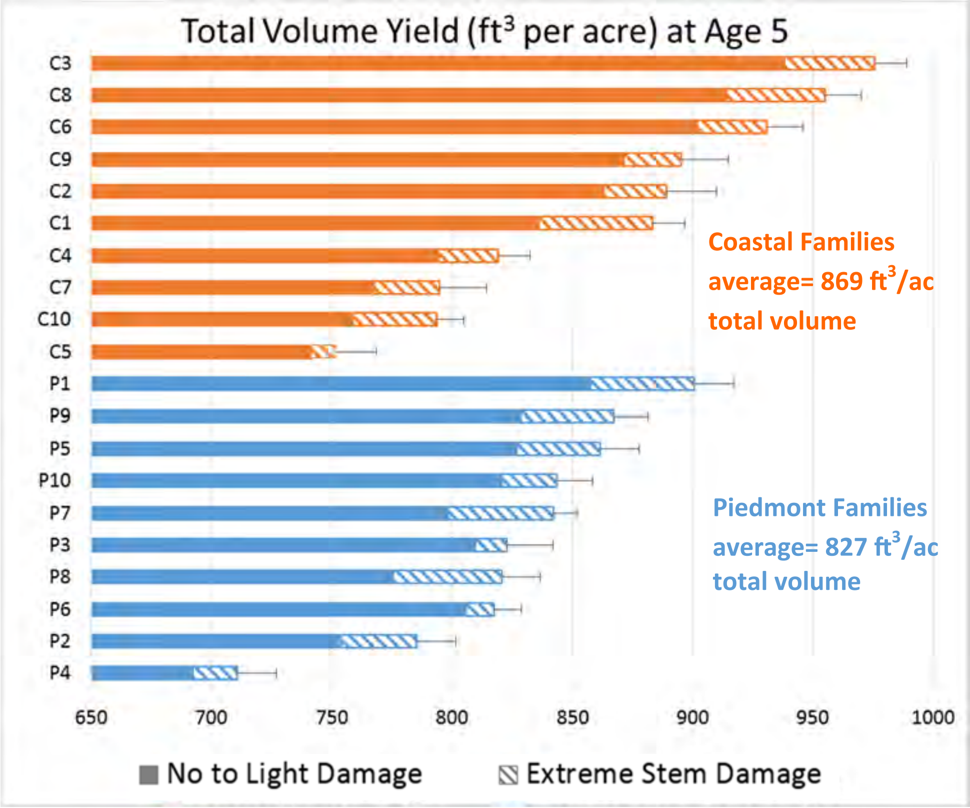


Figure 1. *Total volume per acre family means (with standard error bars) for trees with and without severe storm damage. Orange bars represent Coastal families, and blue bars represent Piedmont families.*

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

Additional work completed at the Butner Biomass Genetics/Cropping Study this past year was a flight using drone technology to capture high resolution images of the site (Reps 1-3 shown above). We plan to use this imagery to help quantify crown size and structure for each family to see if any relationships exist between these traits and the stem breakage that occurred from the ice and snow storms at year 3. Also, we are going to compare year 5 height measurements to the drone imagery to see how accurately individual tree heights can be measured using this new technology.

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[1] *Initial support from the Biofuels Center of North Carolina and continued support from the NCDA & CS Bioenergy Research Initiative Grant Program have made this study possible.*

Performance and Stability of Inter- and Intra-Provenance Crosses of Loblolly Pine[1]

Coordinated efforts of region-wide seed source testing of loblolly pine began in the 1950s. As results of broad-scale and locally-specific seed source studies were published, forest industry deployment protocols were adjusted, and research continued to explain the geographically-dependent variation in growth, disease resistance, and adaptability. As the patterns of natural variation were described, seed sources were moved to realize operational gains. Operational successes in long-distance movement of Atlantic Coastal germplasm to lands in Arkansas/Oklahoma were reported in the 1980s and early 2000s.

Plum Creek Timber Company (now Weyerhaeuser Company) established an internal research trial to investigate suitability of available genetic material for deployment on lands in southern Arkansas and northern Louisiana. Testing prior to operationally establishing plantations was initiated to determine if full-sib families of Atlantic Coastal origin could be reliably deployed in the western South. Crosses among 27 eastern and 15 western loblolly selections were used to generate test progeny. Eastern parents were bred with other eastern parents (crosses denoted E x E) and as well as western parents (E x W). Western parents were crossed with other western parents (W x W), as well as eastern parents (W x E). At test establishment, out of 146 crosses (including the checklots) 51 were ExE, 14 were WxW, 49 were ExW, and 32 were WxE crosses. Crosses were broadly tested at three locations in the East (tests E1, E2, and E3) and three locations in Arkansas (W1, W2, and W3) (Figure 1). Tests were established in 2006 and 2007, and each test was measured after six growing seasons in the field.

E x E crosses had consistently greater growth than pure western crosses (W x W). The inter-provenance crosses (E x W and W x E) height and volume were more similar to pure eastern crosses (Figure 2). Narrow sense heritability of volume was moderately low, ranging from 0.15 (Test W3) to 0.20 (Test E2). Full-sib family mean heritability for volume were variable among tests, ranging from 0.33 (Test E1) to 0.53 (Test E2).

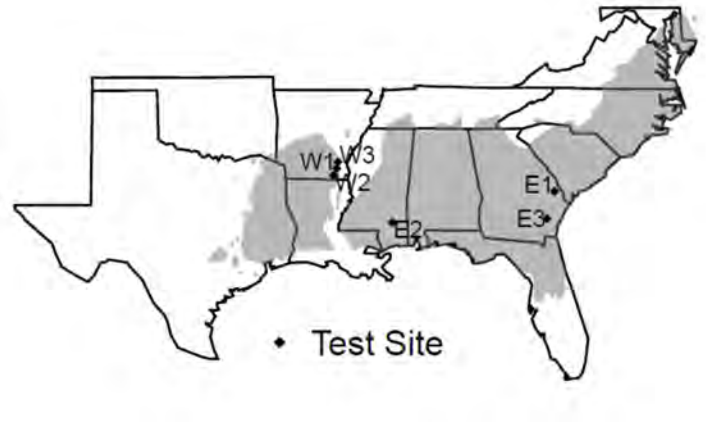

***Figure 1.** Locations of East-West Diallel test sites, with loblolly pine natural range shaded gray.*

To determine if test region had an effect on the relative performance for the different cross types, test sites were grouped based upon their test location. Additive genetic effects were strongly correlated (r = 0.96) between the East and West test regions as were dominance genetic effects (r = 0.83). The strong correlation of genetic values across the eastern and western test regions was true for all cross types in the trial (i.e. within- and among-provenance, Figure 3). The only manner the four cross types differed was in average genetic value, with crosses involving parents from the East having the greater volume than pure W x W crosses). Concurrence of test results between the East and West test regions in this trial demonstrates that minimal genotype-environment interaction is to be expected of loblolly pine full-sib families moved from the east to southern Arkansas.

While these results are interesting, the trials were limited to some degree. The three sites in Arkansas are located in the southern and eastern portion of the state (Figure 1), so the conclusions of planting full-sib families involving eastern germplasm are only applicable to that general area of the western South. Additionally, long-term adaptability is best assessed for at least one-half of a rotation age or about 12 to 15 years for loblolly pine. Therefore, these trials should be maintained to at least one-half rotation age. And

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[1] *This is a summary of Graham Ford's PhD research thesis.*

finally, more tests should be installed with these cross types, from a broader genetic sample, over a wider range of edaphic and climatic factors to delineate the limits of performance stability.

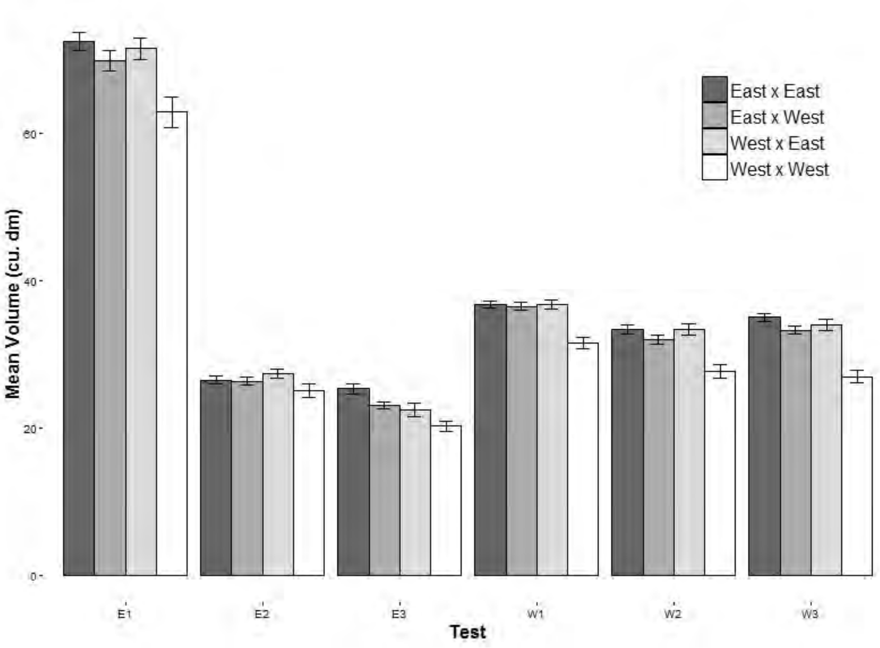

Figure 2. *Mean volume (cubic dm) and standard errors for each of four loblolly pine cross types at each test location.*

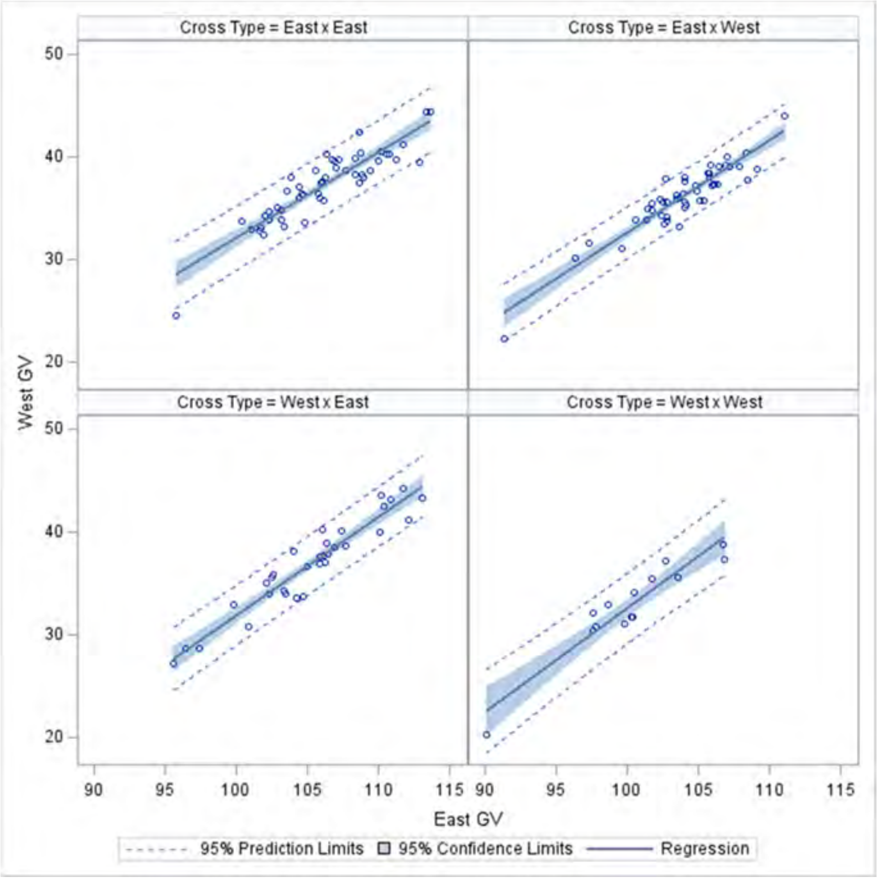

Figure 3. *Loblolly pine full-sib family genetic values estimated in eastern tests (East GV) are strongly correlated with genetic values from western tests (West GV) for each of four cross types.*

Computer Simulation of Breeding Strategies and Inbreeding Depression in Loblolly Pine- An Update[1]

This is an update of the work that was presented in the 2016 Tree Improvement Annual Report (p. 16-18). 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.

Founder populations were established with a genetic architecture based on current knowledge of the loblolly pine genome. Breeding strategies assessed were built to model the current 4th-cycle breeding strategy by applying a co-ancestry threshold to crosses made each cycle while attempting to maximize genetic gain through emphasizing crosses among the best individuals. The selection strategy applied in all cases first used family mean pedigree-based BLUP values for among family selection. The number of families selected from each generation was the same as the founder starting population. For within-family selection, a single selection was made by using either estimated BLUPs with a relationship matrix composed from shared markers (AGA) or a pedigree based relationship matrix (AAA).

Founder populations of 280 unrelated parents were simulated with an 1800 cM genetic map and included 12 chromosomes, 2,500 quantitative trait loci (QTL) affecting the phenotype, and 120 evenly spaced fully heterozygous markers. Rare, fully-recessive deleterious alleles were assigned to 1960 out of the 2500 QTL as a means of modeling inbreeding depression, as described in the 2016 Tree Improvement Annual Report. The remaining 540 QTL were modeled to have equal additive effects on phenotype. Phenotypes for the initial founder parents, as well as progeny from each generation, were simulated to have an individual-tree heritability of 0.3. To determine the initial genetic value of founder parents, they were first mated in an open-pollinated mating design, crossing each parent to all others. Parental estimated breeding values were then determined from phenotypic mean values of progeny from a given parent.

For all simulation runs an assortative mating scheme and co-ancestry threshold was applied at each of the 10 generations, which results in more crosses among the best individuals and fewer crosses among the lower ranking individuals. This strategy was applied in the simulator by partitioning the founder parents into 4 groups by their rank in estimated breeding value. Individuals in each group were determined as follows:

Group 1: 76th percentile to highest-value individual,
Group 2: 51th to 75th percentile,
Group 3: 26th to 50th percentile, and
Group 4: lowest-value to 25th percentile rank.

Each individual within Group 1 was mated a single time to another individual within Group 1, two times to individuals within Group 2, and one time to an individual within Group 3. Individuals in Group 2 were mated once each with selections from Groups 3 and 4, in addition to the crosses made to Group 1 selections. No crosses were made among the lowest-ranked selections in Groups 3 and 4.

The co-ancestry threshold applied at each generation was implemented by using a pedigree-based relationship matrix to identify potential crosses which fall below a threshold of co-ancestry. This co-ancestry threshold was applied only to crosses made within Group 1 as well as crosses between Groups 1 and 2. Restricting co-ancestry in this way ensures that the best crosses can still be made in a given generation. In practice, this also assumes that a breeder is more likely to be cautious about mating good individuals that have a high co-ancestry as opposed to poor individuals.

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[1] *This work is part of the dissertation research conducted by Adam Festa, a PhD student with the Tree Improvement Program.*

While the long-term objective is to run the model with all 280 parents, as well as reduced population subsets, this modeling exercise is still in progress, so here we present results for 2 subsets which include the top-ranked 140 or 64 founder parents, selected based on results of the simulated open-pollinated testing.

Overall, across both founder population sizes and selection strategies, there was a general trend for purging deleterious alleles and increasing genetic gain over ten generations. Utilizing an assortative mating design and making more crosses than selections seems to be an effective method to potentially expose rare deleterious alleles and remove them from the population. Among the two different selection approaches, multiple general trends can be seen using pedigree-based (ABLUP) versus marker-based (GBLUP) relationships for within-family selection, regardless of population size. Utilizing GBLUP for within-family selection resulted in a faster reduction of deleterious alleles as well as a higher rate of genetic gain (Figures 1 and 2). Genetic variance from ABLUP for within-family selection is highly variable, whereas when using GBLUP, there is a general decreasing trend over time. (Figure 3).

Future work with this project will include conducting multiple simulation runs using the full set of 280 parents and additional testing on the effect of using GBLUP for within-family selection with varying number of markers and allele frequencies. Additional prospective work could involve incorporating other population parameters, such as mutation rate, which may help to more accurately depict long-term response of populations to selection. Finally, the current version of the simulator makes selections from each generation based on breeding/phenotypic values simulated of a single trait, so opportunity exists to enhance progeny selection based on multiple traits or a selection index.

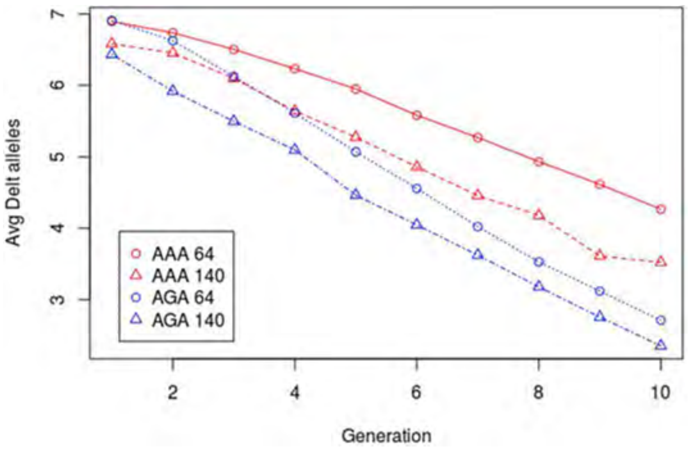

Figure 1. *Purging deleterious recessive alleles over time. Pedigree-based (in red) and marker-based (in blue) models are both effective in removing recessive deleterious alleles from the population over ten generations. Larger population sizes (dotted line) are more efficient than smaller sizes (solid lines)*.*

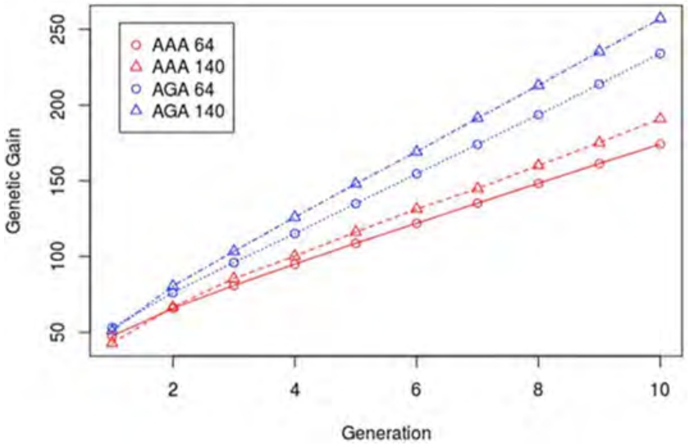

Figure 2. *Genetic gain over time. Marker-based methods (blue lines) are more efficient than pedigree-based methods (red lines) at producing genetic gain over ten generations, and larger populations (dotted lines) are more efficient than smaller populations (solid lines)*.*

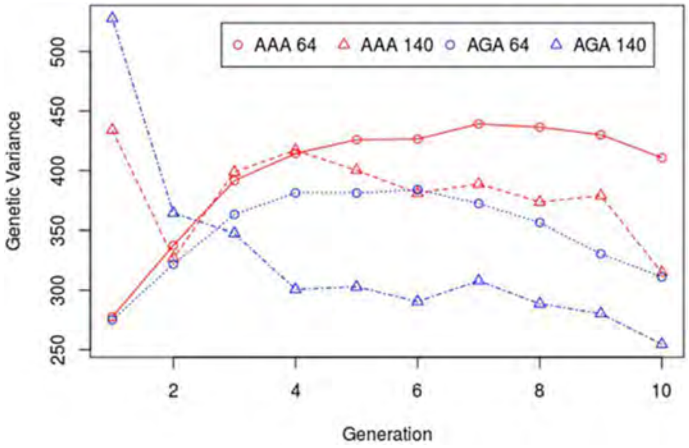

Figure 3. *Genetic variance over time. For clarity, no error bars are shown, but the variation is much greater in the pedigree-based models (red lines) than in the marker-based models (blue lines)*.*

**In the legends, AAA symbolizes the pedigree-based (ABLUP) methods and AGA symbolizes marker-based (GBLUP) methods. The numbers are the population sizes.*

Estimating Breeding Values Using RNA Expression Patterns - An Update[1]

This is an update of the pilot study that was summarized in the 2016 Tree Improvement Annual Report, p. 23-24. The objective is to assess the relative predictive ability of covariance matrices based on gene structure, gene expression, and SNP markers for tree stem volume in a small population of loblolly pine (56 families). In the study, there are several practical questions that will be addressed. (1) Can we obtain reasonably reproducible results from triplicate samples of seedlings to estimate family mean levels gene expression? (2) Can we use family mean estimates of gene expression levels as covariance estimates for modeling phenotypic variation? (3) Do covariance matrices based on pedigree, coding sequence SNP variation, or gene expression level variation have independent value for modeling phenotypic variation, or are they redundant? Overall, the resulting analyses provide an insight into the value of using RNA expression patterns as another screening effort in selecting individuals as parents for future breeding populations.

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. Several alternative approaches are possible; the simplest is to use a genetic covariance matrix based on family-mean levels of all gene expression values to estimate similarities and differences among families. More complex options could be first identifying a subset of genes considered informative; these may be selected on the basis of differential gene expression among families, or on other criteria. New software tools allow incorporation of multiple covariance matrices in linear mixed models for prediction, with variable weights on each matrix. Using covariance matrices based on variation in gene expression, or a relationship matrix based on SNP variants detected in expressed genes, along with (or in place of) the standard numerator relationship matrix may provide higher prediction accuracy of breeding values.

To test this hypothesis, we chose a total of 56 different parent trees of loblolly pine from a wide geographic distribution, with existing progeny field test data available from multiple sites. Seedlings from these parents were grown in two different batches in a greenhouse, and pooled seedlings were harvested at 2 months of age 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 were 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 different classes of genetic variation.

Evaluation of the consistency and quality of RNA-seq data across technical and biological replicates was conducted using a correlation analysis of read counts per transcript, estimated from alignment of trimmed reads (Figure 1). Correlations of technical replicate count data were assessed prior to normalization (A in x-axis), and correlations of biological replicate count data were assessed post-normalization (B in x-axis). The difference in the distributions of pre-normalized (A in x-axis) vs. post-normalized (B in x-axis) gene expression correlations among replicates indicate that the mixed model accounting for lane, index, and pedigree successfully adjusted for systematic variation in sequencing. Correlations of gene expression estimates among biological replicates with full-sib and open-pollinated families were also high (C and D in x-axis in Figure 1), indicating that separate samples from the same families captured the same information on gene expression patterns. Repeatability across years was assessed for five families that were grown and sampled in each of two years, by examining correlations of biological replicates between years (Figure 1, E in x-axis). Family-mean values of gene expression were calculated as the arithmetic mean of biological replicates within family, and pair-wise correlations of these family mean values were also high (Figure 1, F in x-axis).

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[1] *This work is part of the dissertation research conducted by Adam Festa, a PhD student*

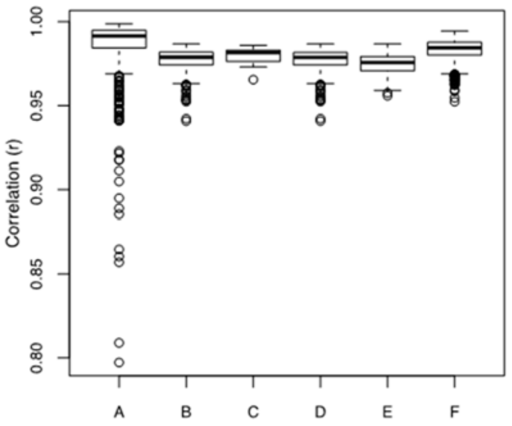

***Figure 1**. Correlations among samples at different stages of analysis. The x-axis labels: (A)* Correlations of *technical replicates within family prior to normalization, (B) Post-normalization correlations of biological replicates within families,* (C) *post-normalization correlations among biological replicates within full-sib families, (D) post-normalization correlations among biological replicates within open-pollinated families, (E) correlation of biological replicates of families which were present in both batches, (F) pairwise correlations*

The overall high correlation within these sample subsets suggests that there is high reproducibility among RNA expression obtained from grouped biological replicates groups of 2-month-old seedlings. The reproducibility of biological replicate seedlings, either between or within batches, is encouraging as it suggests that each family has a set of expression levels which can be reproduced. However, it is somewhat surprising that even transcript expression levels of families which are unrelated are also high. Given the uniformity of the environment in which the seedlings were grown, this observation suggests there is a robust similarity in transcript expression or underlying regulatory interactions which are responsible for seedling growth within the first two months regardless of genetic background.

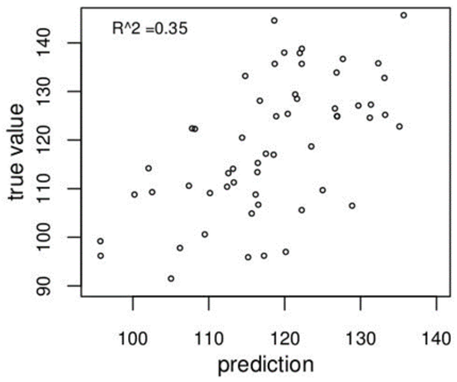

***Figure 2.** Predicted value for stem volume at age 6 years based on gene expression levels in 2-month-old seedlings is plotted against true genetic values (y-axis) based on field test data. The predictions explain over one-third of the variation in the true genetic values.*

The predictive power of models for tree volume based on covariance in gene expression levels were tested by conducting a leave-one-out cross-validation method. The best result came from using a subset of genes; this model explained 35% of the variation (R^2 = 0.35) in volume (Figure 2). A covariance matrix based on SNPs in expressed genes was used to predict breeding values for each of the 56 families. Again, a leave-one-out cross-validation method was used, and a subset of informative SNPs was identified by regressing individual SNP markers on tree volume. Significant SNPs (q-value 0.005) associated with volume across all 55 regressions were then used for prediction on the 56th family left out. The validation model explained 53% of the variation (R^2 = 0.35) in volume breeding value (Figure 3).

In conclusion, it is possible to obtain reproducible RNA transcript data across batches and between biological replicates of families from 2-month-old seedlings. Additionally, we have identified a prospective method and set of coding region SNPs that give reasonable prediction accuracy for volume breeding values. A validation test is currently underway with a new set of families to test the predictive accuracy of this modeling approach beyond the initial set of 56 parents.

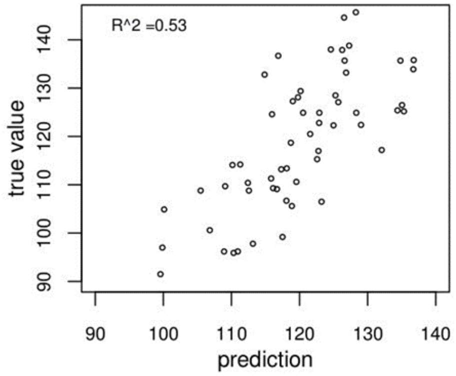

***Figure 3.** Predicted values for stem volume at age 6 years based on SNP genotypes from genes expressed in 2-month-old seedlings, plotted against true genetic values (y-axis) based on field tests. The SNP genotypes explain over half the variation in the true genetic value for this set of 56 parents.*

PBS Pollination Bag Study Update[1]

The Pollination Bag Study began in 2014 and has now shifted into a project with installations across multiple years and with multiple objectives. The original project began when members of the Cooperative installed four bag prototypes from PBS International along with the industry standard Lawson pollination bag with and without a support wire. The purpose of testing these prototypes was to see if new pollination bags produced by PBS International (www.pbsinternational.com/) would increase cone survival and seed yield.

Based on the early assessments (see p. 23 of the 2015 Annual Report), a second round of testing began in the spring of 2015. The 2014 installation suggested bags with greater rigidity and smoothness prevented damage to strobili inside of the bags. The mean female strobili survival in June (“June drop”) from the 2015 installation clearly showed no significant differences between bag types (with the exception being Lawson and the open-pollinated control) as was seen in the 2014 June drop data (2016 Annual Report pg. 22).

Cones were harvested from the 2014 and 2015 installations in the fall of 2015 and 2016, respectively. Survival of female strobili from time of bagging to the point of final harvest was analyzed for both years of installation. Probability of survival for each year of installation at time of June drop and at final harvest was modeled by bag type. The relative survival for the different bag types at time of final harvest was nearly identical to that for June drop (Figure 1).

From the 2014 installation, seed yield per cone was not significantly different for bag type, with mean seed yields per cone ranging between 77-81 seed per cone for all bag types (OP was the one exception with 114 seed per cone). From cone analysis for the 2014 installation (Figure 2), bag type was not significant for seed potential. Mean seed potential ranged from 165 seed per cone with Lawson to 170 seed per cone for bag type A (OP had 174 seed per cone). While the seed potential did not vary much, there were some interesting results for the classification of ovules inside of each cone for each bag type (Figure 2). Filled seed (green bar) was highest for OP cones. Bag type A had the highest number of filled seed and highest seed efficiency (not shown) of any bag type, although it was not significantly different from any of the bags for either trait.

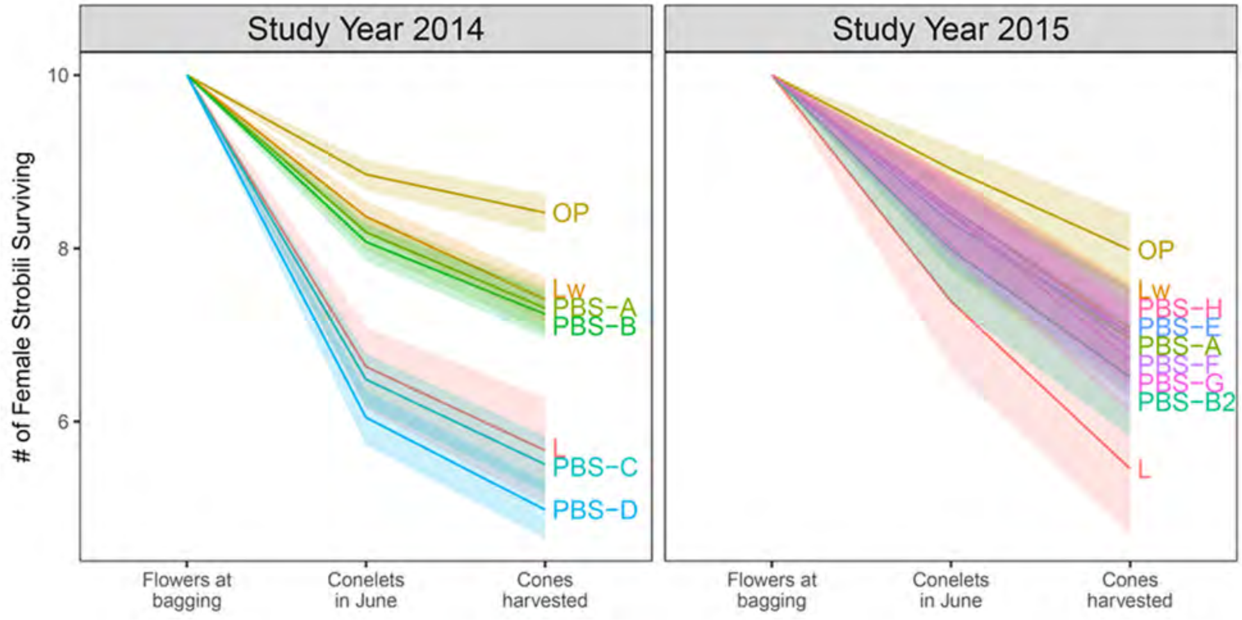

Figure 1. *Estimated survival at June drop and cone harvest starting with 10 flowers (10 being the average number of flowers bagged across all orchards). Shaded bands indicate standard error of estimates. The survival in June of year 1 was closely associated with cone survival at harvest.*

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[1] *This is a summary of Austin Heine’s graduate research .*

Seed yields and cone analysis are still being conducted for the 2015 installation.

In the spring of 2017, another round of prototype testing began which included two new prototypes (PBS A2 and I2) along with the original PBS-A, a Lawson bag with wire support, and the open-pollinated control. Additionally, a time and motion study was performed by several cooperators to quantify the time and effort of using PBS pollination bags compared to Lawson pollination bags.

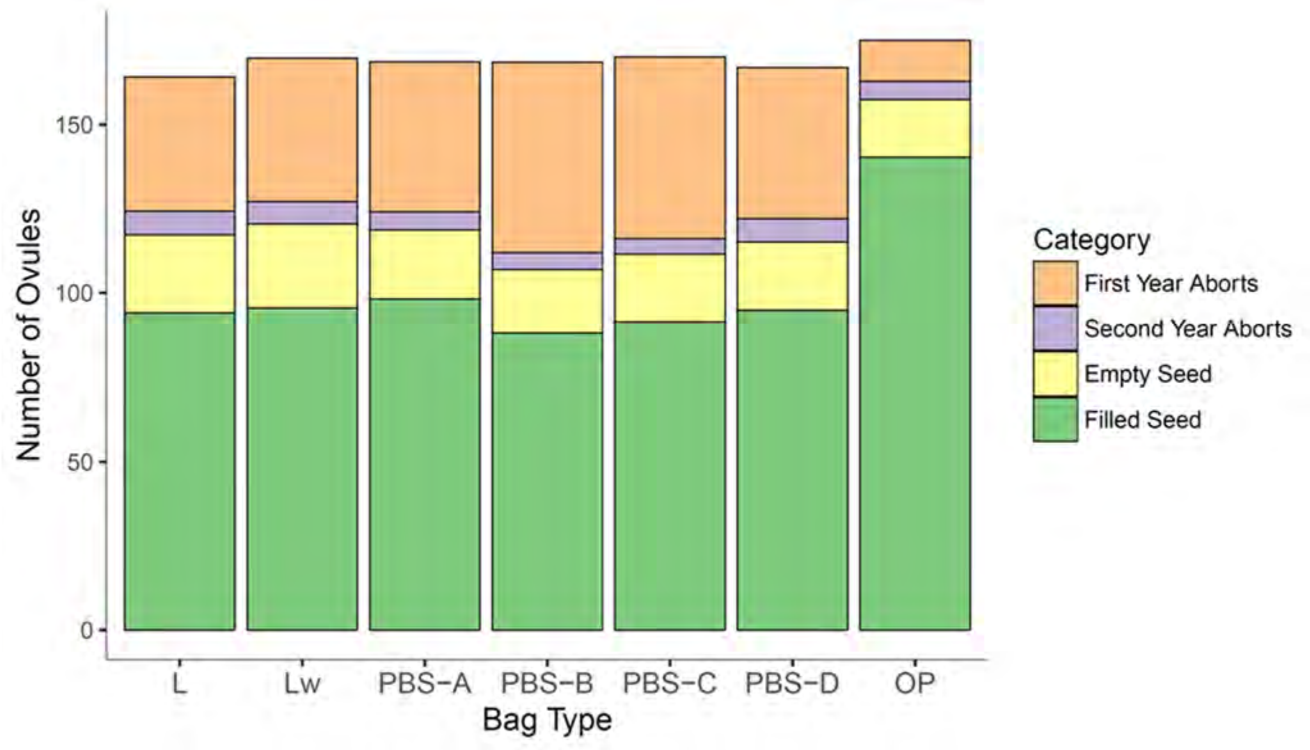

Figure 2. *Cone analysis by bag type from the 2014 installation of the PBS study. The four categories of ovules are shown as their proportional representation of the average seed potential found for each bag type.*

Soil Compaction in Loblolly Pine Seed Orchards[1]

The high genetic and economic value of genetically improved full-sib loblolly pine seedlings has increased the incentive for seed orchard managers to produce large quantities of control-cross seed. Mass production of controlled crosses requires the use of heavy aerial lifts in seed orchards, often when the soil is wet, and this increased traffic increases soil compaction which could have negative impacts on tree health and cone yields.

A study to assess soil compaction in seed orchards began in the fall of 2015. A soil penetrometer was used to measure soil strength[2] which is an indirect measurement of soil compaction. The penetrometer was equipped with a cone tip and was pushed through the soil profile and recorded the pressure required to penetrate the soil. Penetrometers are designed to mimic the resistance a root will experience as it grows through the soil profile.

The study included five seed orchards, owned and managed by Cooperative members (Table 1). Orchard E was subsoiled in 2014, while the other four had no soil remediation. Five soil strength readings were taken around each tree using a penetrometer. Ten soil cores were also collected in each orchard to determine moisture content and bulk density, a direct measurement of soil compaction. Moisture content has a large impact on soil strength and was used to understand the accuracy of the penetrometer measurements.

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[1] This summary is part of Serennia Larrison O'Berry's graduate research. [2] Soil strength is defined as the amount of force required to move or rearrange soil particles. It is affected by three main factors; moisture content, soil texture, and bulk density.

***Table 1**. Orchard descriptions including, soil type, drainage class, water table depth, and age. Note: Orchard C has two major soil types in the measurement area, and the percentage of each soil type is illustrated.*

Orchard	Soil Type	Drainage Class	Depth to Water Table (cm)	Age
A	Tifton Loamy Sand	Well Drained	100-140	14
B	Tifton Loamy Sand	Well Drained	100-140	34
C	Lynchburg Loamy Sand (60% of sample)	Somewhat Poorly Drained	45-76	15
C	Plummer Sand (40% of sample)	Poorly Drained	0-30	15
D	Chipley Loamy Fine Sand	Moderately Well Drained	45-91	31
E	Greenville Sandy Loam	Well Drained	>200	29

Penetrometer readings were taken at sample positions within the tree row on each side of the tree at 3.5 meters (between trees where equipment use was unlikely). Readings were also taken on one side of each tree within the traffic row at 2, 3.5, and 5 meters where most equipment use is likely (see Figure 1 for sample positions). Readings were taken by pushing the penetrometer probe into the ground, and a measure of the force required to push the probe through the soil profile was recorded every 2.5 centimeters (1 in). The penetrometer model used in this study measured to a maximum of 1000 PSI (pounds per square inch). Due to the limitations of the penetrometer, samples were taken to a depth of 30.5 cm (12 in), or when the soil strength reached 1000 PSI. Soil compaction patterns, soil strength, and the amount of usable soil for root function were assessed using the penetrometer readings.

In the four orchards that were not subsoiled, the mean soil strength at 2 meters within the traffic row was significantly different ($\alpha=0.05$) from the mean soil strength at 3.5 and 5 meters within the traffic row (Figure 2). There was not a significant difference between the mean soil strength at 3.5 and 5 meters within the traffic row indicating that aerial lift use within the traffic row is having similar impacts 3.5 and 5 meters away from the tree. However, at 2 meters from the tree, equipment use appears to be having a lower impact on soil strength. Since equipment use had less of an impact on the 2-meter sample position within the traffic row, it should be more typical of the native, non-disturbed soil.

For orchard E that was subsoiled just prior to the soil sampling, the mean soil strength at 5 meters from the sample tree in the traffic row was very different from the other orchards. The impact from subsoiling to reduce soil compaction was obvious since the soil strength measurements were much lower at the 3.5- and 5-meter traffic positions when compared to the other orchards (Figure 2).

Moisture content, as measured with the bulk density samples, was not ideal when samples were taken, therefore comparing the within traffic row 3.5- and 5-meter sample position to the 2-meter position (which is relatively non-disturbed) is a better way of assessing the soil compaction. In all five orchards, the mean soil strength at the 2-meter position is substantially lower

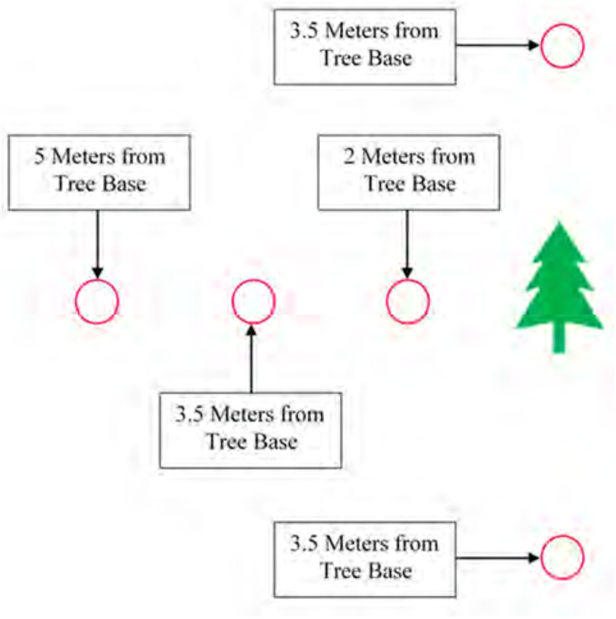


***Figure 1.** Example of penetrometer sample positions around an orchard tree. Three samples were taken at 2, 3.5, and 5 meters from the tree within traffic rows, and two samples were taken on both sides of the tree at 3.5 meters within the tree row. In this report, the focus is on the samples within the traffic row.*

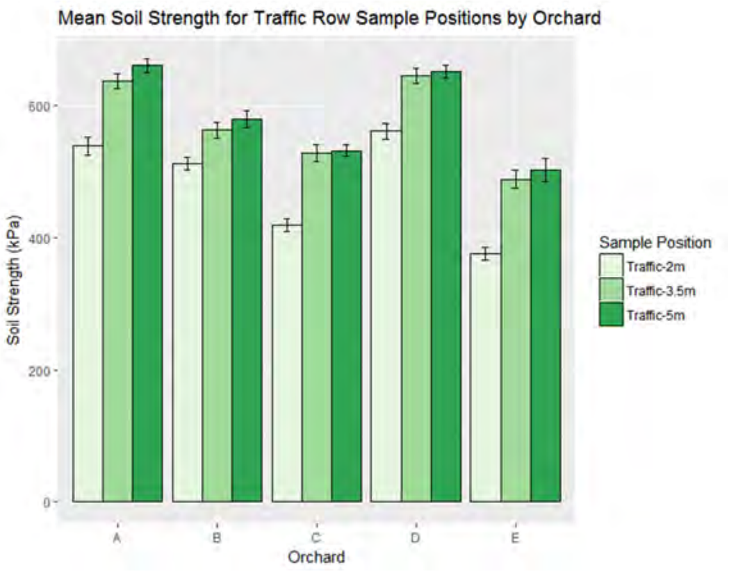


Figure 2. *Means with standard error bars for soil strength (surrogate for soil compaction) for soil profiles to 30.5 cm (12 in) depth within the traffic row positions of 2, 3.5, and 5 meters away from the tree. Root growth is substantially restricted above values of 400 PSI. Soil compaction is much higher at the 3.5- and 5-meter sample points where aerial lifts and other heavy equipment typically operate. In Orchard E, the soil strength at the 5-meter sampling position is much lower than in most other orchards, because Orchard E was subsoiled the year prior to taking measurements.*

than the 3.5- and 5-meter positions (Figure 2). High soil strength in the middle of the traffic row and the large difference between the 2-meter position and the 3.5- and 5- meter positions suggests equipment use is having detrimental impacts on soil structure.

Many agricultural studies have shown root growth restrictions when soil strength reaches 360 PSI. A mean depth to the restrictive soil condition (the depth in the profile when soil strength exceeded 400 PSI) was found for each sample position within the traffic row in orchards that were not subsoiled. This is considered the mean maximum depth. At the 2-meter position within the traffic row, the maximum depth reached before a soil strength of 400 PSI was encountered, was deeper than the 3.5- and 5-meter positions in all four of these orchards. Again, comparing each position to the 2-meter position can be useful in understanding how much impact heavy equipment is having on soil strength. In most orchards, the maximum depth to reach 400 PSI at 3.5 and 5 meters from the tree was roughly half the depth at 2 meters (Figure 3). This depth illustrates how little soil is available for the roots to grow and properly function out in the traffic rows.

"Total possible usable soil" available for each tree was calculated based on the volume of a cylinder around each tree. The radius was the distance of the sampling point from the trees, and the depth was 30.5 cm (12 in), the maximum depth possible using the penetrometer. This volume was divided by 4 to represent only the quadrant sampled. The 400 PSI threshold was used to assess the amount of "actual usable soil" around each tree (Table 2). For each tree in the study, three usable soil volumes were calculated for the within traffic row samples. The distance from the tree (2, 3.5, or 5 meters) was used as the radius, and the depth when 400 PSI was reached was used as the depth of the cylinder. Based on the possible soil volume and usable soil volume, a percentage of usable soil volume was calculated for positions within the traffic row. In all the orchards, the percentage of usable soil was low. Orchard C had the highest percentage of usable soil volume at 23.5% and orchard A had the lowest at 9.7% (Table 2). The higher percentage of usable soil in Orchard C is most likely due to the young age of the orchard (15 years, so relatively few years of heavy equipment use) and the high sand content of the soil. Sandier soils tend to be better drained, which helps minimize compaction in orchards where traffic is high.

The results of this study show that soil compaction has become a problem in these orchards. While direct impacts of compaction on tree health and cone yields are still being assessed, based on agricultural studies, the soil strength seen in these orchards is likely to be problematic for root growth and nutrient uptake and ultimately tree health and cone production. This study should help tree improvement staff design and manage future seed orchards to minimize the negative impact of heavy equipment on soils.

[3] *During the study, cone yields per tree were counted in the fall of 2015. In the fall of 2016, crown density was determined on each tree using UrbanCrowns (https://www.srs.fs.usda.gov/pubs/39430), a software developed by U.S. Forest Service scientists. A crown density and color score was also given to each tree. The relationship between soil strength and tree health measurements and cone yield will be reported in Serenia's thesis.*

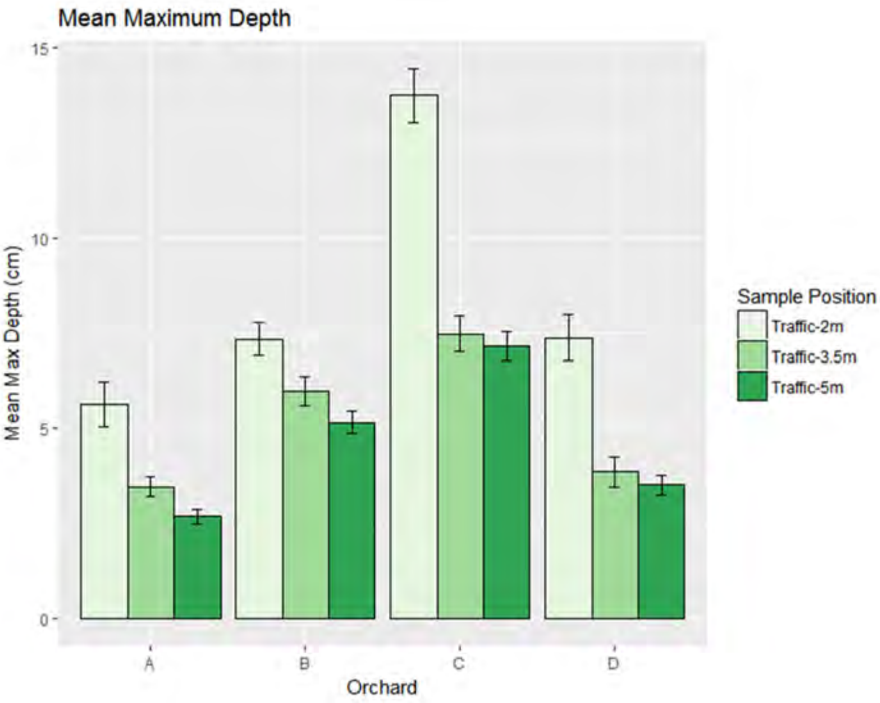


Figure 3. *Means with standard error bars for maximum penetrometer depth to the restrictive soil condition (the depth in the profile when soil strength exceeded 400 PSI) for each soil sample position (2, 3.5, and 5 meters from the tree) within the traffic row for the four orchards that were not subsoiled. In each of these orchards, the depth or amount of soil with lower soil strength (e.g. less compacted) was substantially reduced in the traffic area 3.5 and 5 meters out from the tree. For example, in the highly compacted Orchard A, at 5 meters from the tree, average soil strength was > 400 PSI at only 2.5 cm (1 inch) below the soil surface.*

Table 2. *Total possible volume of soil* in a quadrant around each sample tree in the five orchards studied. The actual volume*$^+$ *(and percentage) of usable soil is defined as the depth of the soil sample before soil strength of 400 PSI was reached. The 400 PSI value was used as the threshold where root growth is substantially restricted. The high level of soil compaction is best demonstrated at Orchard A where less than 20% of the soil volume is at a soil strength considered to be usable or not severely restrictive for root growth at all three sample points around the tree.*

Within Traffic Row Sample Position	**Total Possible Volume* (m^3)**	**Actual Usable Soil Volume (m^3) for Each Orchard$^+$**				
Usable Volume		**A**	**B**	**C**	**D**	**E**
2-meter (m^3)	0.96	0.18 (18.4%)	0.23 (24.0%)	0.43 (44.9%)	0.23 (24.2%)	0.25 (25.9%)
3.5-meter (m^3)	1.98	0.23 (11.4%)	0.39 (19.5%)	0.48 (24.4%)	0.25 (12.7%)	0.21 (10.6%)
5-meter (m^3)	4.01	0.27 (6.7%)	0.52 (12.9%)	0.72 (17.9%)	0.35 (8.8%)	0.30 (7.4%)
Total	**6.95**	**0.68**	**1.14**	**1.63**	**0.83**	**0.76**
Total %		***9.7%***	***16.3%***	***23.5%***	***12.0%***	***10.8%***

** The total possible soil volume was determined for each sample tree and soil sample position based on the volume of a cylinder around each tree: $V=pr^2d$ where r is the radius of the cylinder (r = 2, 3.5, or 5 m depending on the sample position), and d is the depth of the cylinder (d = 0.305 m, the maximum depth in meters of any sample taken). This depth was used to calculate the volume of possible soil, because it is the highest volume of soil that could have possibly been measured. The inner volumes were subtracted from the 3.5 m and 5 m sample volumes. Each total volume was divided by four to represent each quadrant of the soil that was sampled.*

$^+$ *The actual volume of soil was calculated in a similar way, except that the depth of the soil where soil strength >400 PSI was used as the depth of the cylinder.*

Loblolly Adaptation and Mapping Project (LAMP) Update

In the spring of 2016, three installations of full-sib progeny from a single cross with two very different parents (coastal-fast growing and northern-cold hardy parent) were planted across the natural range of loblolly pine. One installation was planted in the western limit of the range in east Texas, one in central Georgia, and one in the northern portion of the range in Virginia. Before planting, foliage samples were collected from each full-sib seedling and labeled appropriately. Each seedling was identified and mapped by this same label when planted at the given site.

This project has five objectives, as described in the 2016 Annual Report (p. 13-14). One of the major objectives of these plantings is to provide a large number of progeny for a high-resolution genetic linkage map. Another major objective is 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). Drought adaptability is the only objective for which data are available at present.

This past summer and fall, much of the range of loblolly pine experienced a severe drought (Figure 1). Over the summer and fall, the Texas site was assessed periodically to monitor seedling survival. As expected, the site faced extreme heat and drought over this period of time, and the survival reflected these extremes (Figure 2). By mid-September, about half of the seedlings planted at the Texas site had died. This survival of about 50% is not surprising, and should be optimal for analysis of this binary trait to estimate any genetic effects on survival.

Survival checks at the other two locations show very high survival (>98%). We are interested to note that mortality was low this past winter at the Virginia location, given the hard freezes experienced at the site. Cold tolerance and drought tolerance show related mechanisms in some species, and one objective of this test is to determine if there is any connection between these traits in loblolly pine. Genetic linkage analysis of the loci associated with survival under dry conditions at the Texas site will allow comparison with loci associated with survival at the cold Virginia site, provided that mortality at the Virginia site is high enough to allow a robust analysis of the data.

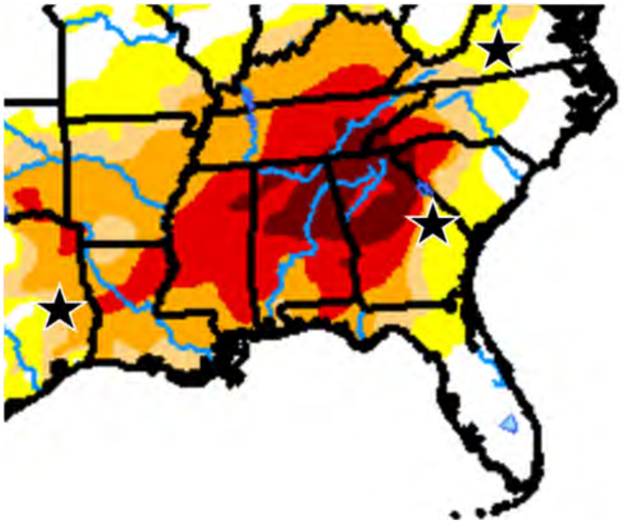


Figure 1. *U.S. Drought Map November 22, 2016 depicting much of the range of loblolly pine suffering from exceptional drought. Black stars indicate the three test site locations. Image courtesy of the National Drought Mitigation Center http://droughtmonitor.unl.edu/*

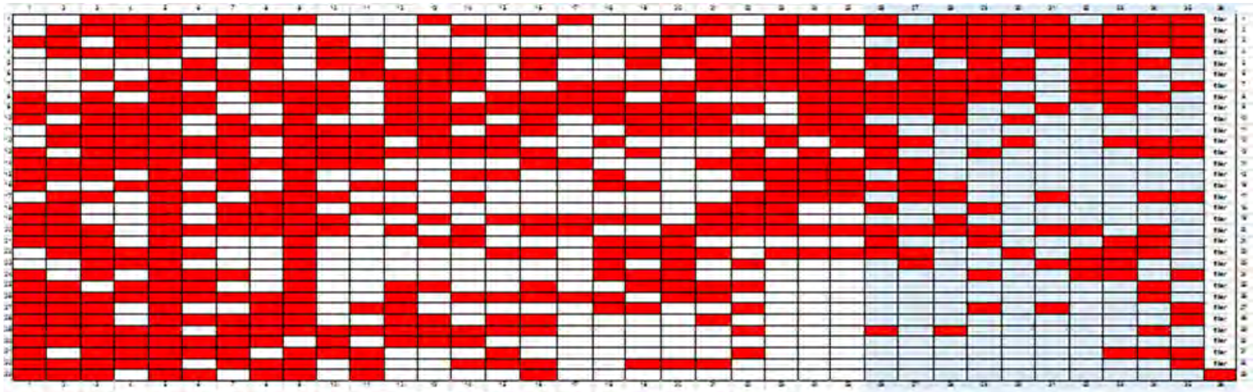


Figure 2. *Map of survival for the Texas test site. Red cells represent trees that had died, white cells are living trees. As shown, survival was 48.5% as of 9/12/16.*

Wood Quality Update

Wood density and bending strength are among the most important properties determining the quality of wood products. However, the traditional sampling methods for these traits are labor intensive and require time-consuming laboratory work, which has historically been a major impediment to including wood quality into tree breeding programs. Fortunately, there are now tools that can rapidly assess standing trees to indirectly measure these wood properties (see Isik and Li. 2003 *Canadian Journal of Forest Research*, 33, 2426-2435). In 2013, the Cooperative began wide-scale use of two rapid assessment technologies, the drill resistance method (using a tool called the Resistograph) and the acoustic velocity method (using a tool called TreeSonic). The Resistograph measures the amplitude required to drill a fine needle at a constant speed through wood and is a surrogate measure for wood density (Picture 1, Figure 1). The TreeSonic measures the velocity of a sound wave through the wood as a surrogate measure for wood stiffness/bending strength (Figure 2). Since 2013, the Cooperative has assessed 1028 half-sib families from sixteen 3rd-Cycle loblolly pine progeny tests for wood quality. Trees were assessed around age eight to identify how their juvenile wood (corewood) properties are controlled by genetics.

Picture 1. *The Resistograph tool is a surrogate for wood density assessment and measures the resistance (in amplitude) to drill a fine needle at a constant speed through wood. With an expert operator like April Meeks, about 80 trees can be assessed in an hour.*

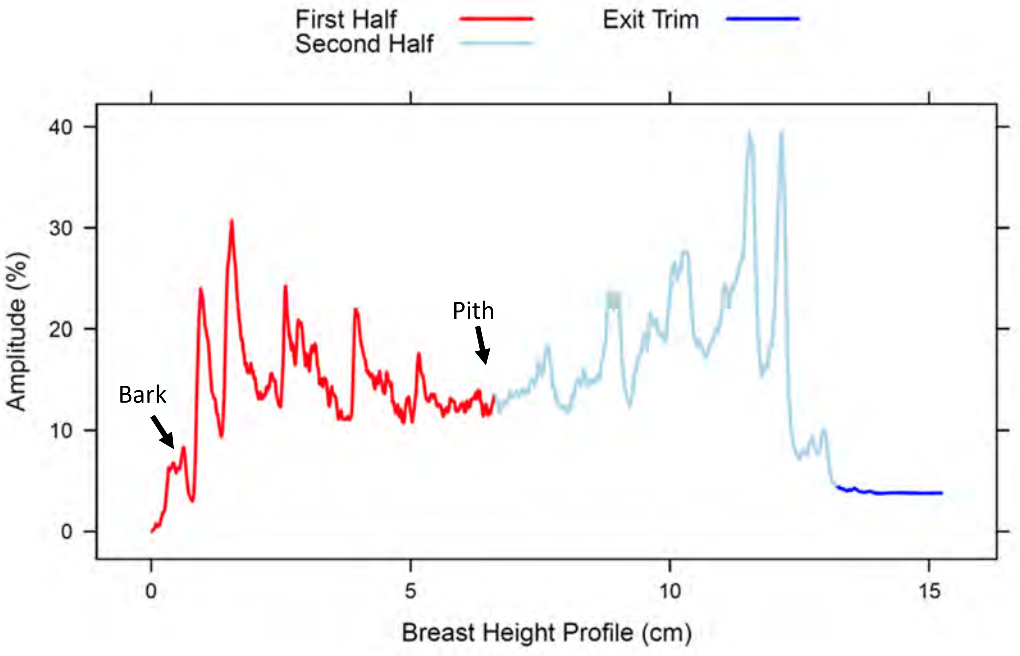


Figure 1. *A typical Resistograph measurement, showing the drill resistance (a proxy for wood density) throughout the profile of the tree. The annual rings are easy to spot due to the higher density of latewood. An algorithm was used to segregate the profile into three parts and drop the "exit path trim" before analysis.*

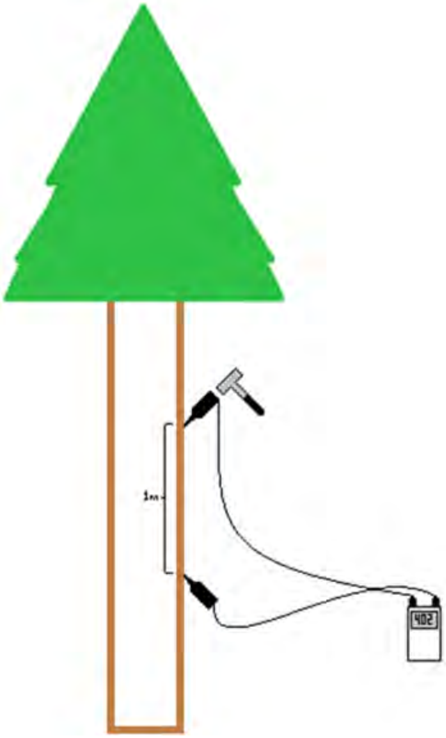


Figure 2. *Diagram of the TreeSonic tool used to measure acoustic velocity as a surrogate for wood stiffness. Two probes are placed 1 meter apart near breast height of the tree, and the top probe is tapped with a small hammer. The device measures the time (in microseconds) for the signal to travel the 1 meter distance. A faster reading indicates that the wood has a higher bending strength/stiffness. Around 120 trees can be assessed in an hour.*

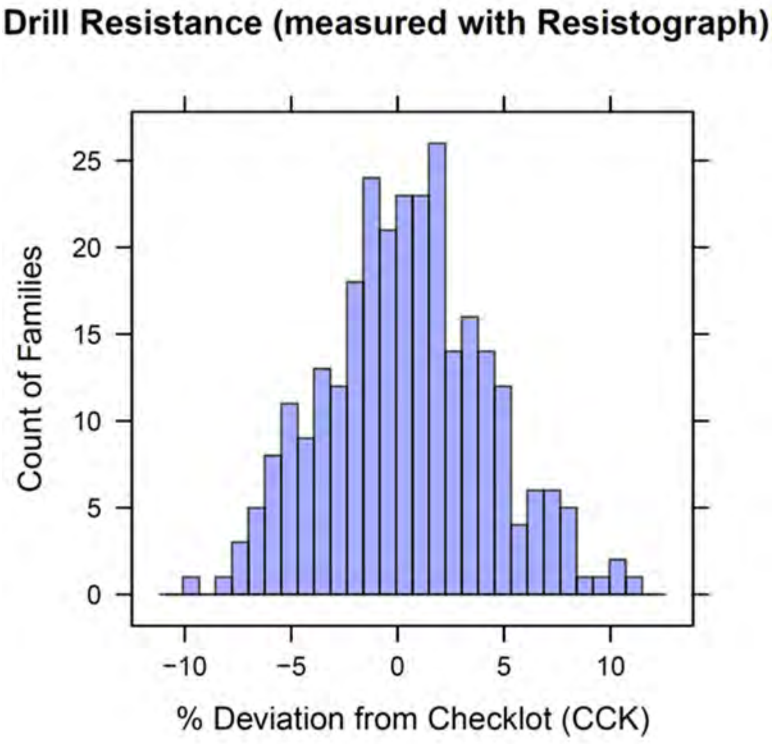


Figure 3. *Distribution of breeding values for drill resistance (a proxy for wood density) around the checklot (CCK) for 270 parents from the Coastal population. About half of the families were better than the checklot. These results suggest that some families can have as much as 10% denser juvenile wood than the population mean.*

Results from the Coastal population (270 families in eight progeny tests) are now available. For drill resistance (Resistograph), a considerable amount of genetic variation was found. Individual-tree heritability was 0.28 and family-mean heritability was 0.67. The Coastal checklot (CCK) ranked very near the middle (Figure 3). The breeding values indicate that there are families with as much as 10% denser corewood than the average. No relationship was found between the volume breeding values and the drill resistance breeding values. In fact, the family with the best drill resistance was also the second fastest grower (Figure 4). These results suggest that wood density and volume growth are independent for this population of loblolly pine, and that no inadvertent selection for poorer wood density has occurred while selecting for volume.

The acoustic velocity (TreeSonic) values also showed a large amount of genetic variation. Individual-tree heritability was 0.28 and family-mean heritability was 0.70. The checklot (CCK) also ranked very near the middle (Figure 5), suggesting that no selection (intentional or unintentional) for this trait has occurred. The breeding values indicate that many families have 10% or greater stiffer wood than average. One family had an unusually high acoustic velocity, and also tended to be very tall considering its diameter (a tall, skinny family, Figure 6). This finding suggests that families that tend to be very tall relative to their girth require stiffer wood.

The **TIPRoot** database has been expanded to provide this information to our members. The results from the Coastal population are available now, and TIP staff are currently analyzing the Piedmont (six progeny tests) and Northern (two progeny tests) populations to make them available soon.

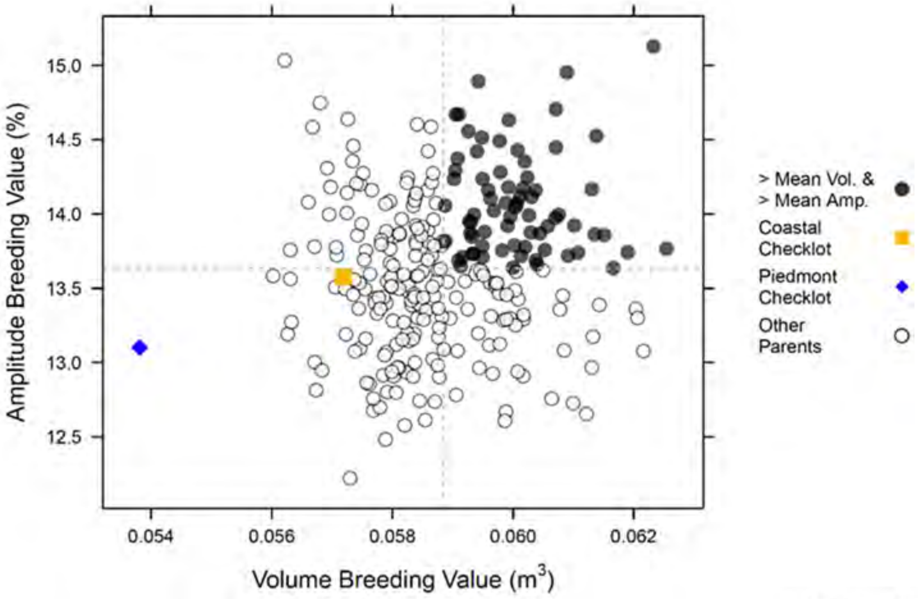
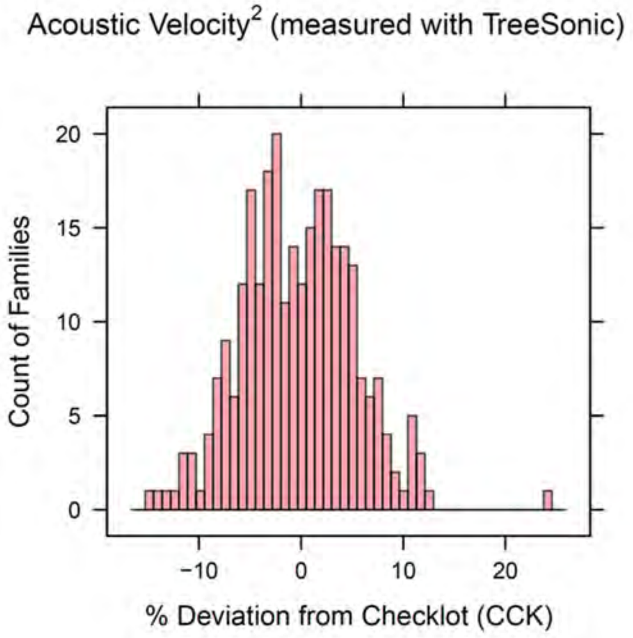


Figure 4. *(left) Volume (productivity) breeding values and drill-resistance breeding values were not correlated (genetic correlation = 0.10, not significant), suggesting that these two traits are independent in the Coastal population of loblolly pine. Additionally, the native checklot (CCK) was very near the average, implying that no inadvertent selection for poorer wood density has occurred while selecting for volume growth. A quarter of the families were above average for both traits (filed circles), and the best family for wood density also was the second fastest grower.*

Figure 5. *(right) Distribution of breeding values for acoustic velocity, a surrogate for wood stiffness/bending strength, around the checklot (CCK) for 270 parents from the Coastal population. The checklot (CCK) ranked very near the middle. The values suggest that many families can have 10% or greater stiffer wood than average. One parent had a very large breeding value that was about 25% higher than the checklot.*



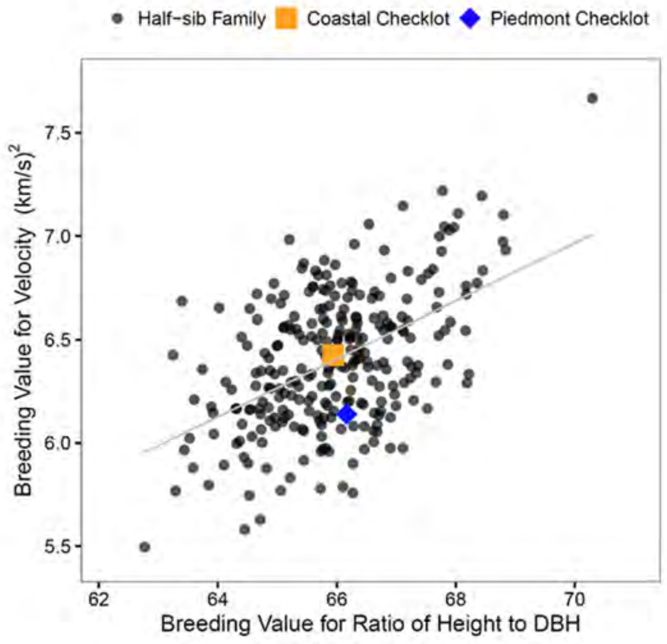


Figure 6. *(left) Relationship between acoustic velocity breeding values (surrogate for wood stiffness) and tree slenderness BV (measured as the ratio of height to dbh). The genetic correlation was 0.60. The family with the highest acoustic velocity tended to be very tall for its diameter, suggesting that skinny trees require stiffer wood.*

Thinning Plan for the Growth and Yield Trials at the Hofmann Forest

There are two growth and yield trials established by the Cooperative at the Hofmann Forest, NC that are now entering their 12th growing season. The Genetics x Spacing x Thinning (GST) study is over 70 acres (28.3 hectares) in size, and contains block-plots for 10 genetic entries with varying degrees of genetic homogeneity (clones, full-siblings, half-siblings, and a seed orchard mix). Each genetic entry was planted at two spacings (a typical 435 trees per acre and a wide 218 trees per acre) under a thin and a no thin scenario. There are three replicates. The other trial, the Genetics Demo, does not have the spacing or thinning treatments, but contains more genetic entries (14 genotypes) planted with three replicates. Both trials have about 90 trees per plot.

Over the winter of 2016-2017, Tree Improvement Program staff and students marked the trees for thinning following an experimental plan for the residual trees. Density differences among the genotypes were great (Figure 1), so a thinning plan that allows for separation of the response due to genotype and the response due to pre-thin stand conditions was necessary. To solve this problem, we implemented the non-thinned counterpart method described by Pienaar (1979), where thinning response is estimated based on the growth of a non-thinned stand with the same age and trees per acre as the residual stand. We used the no-thin, wide spacing plots as the non-thinned counterpart for each genotype. The motivation for using this approach was

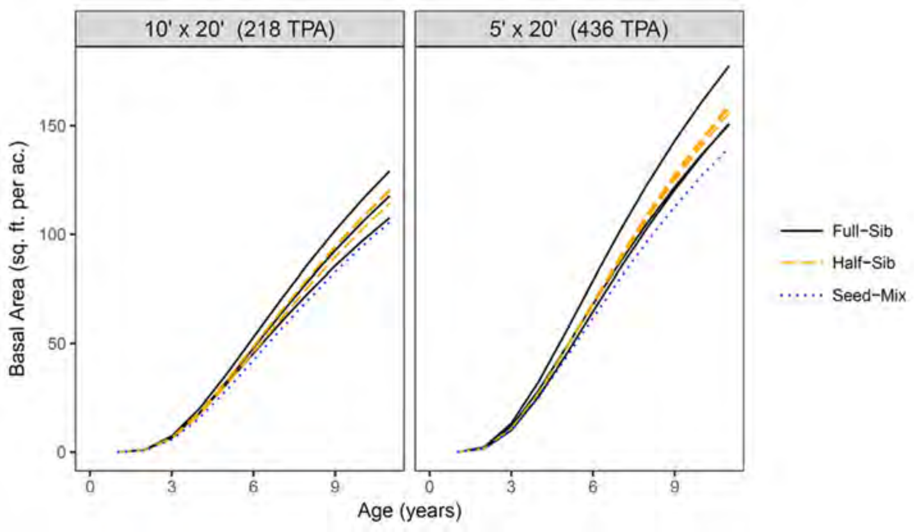


Figure 1. *(left) Large differences in basal area development were observed among loblolly pine genotypes at age 10 on the Genetics x Spacing x Thinning (GST) study at the Hofmann Forest. All families outperformed the seed orchard mix (SOM) at both spacings/planting densities.*

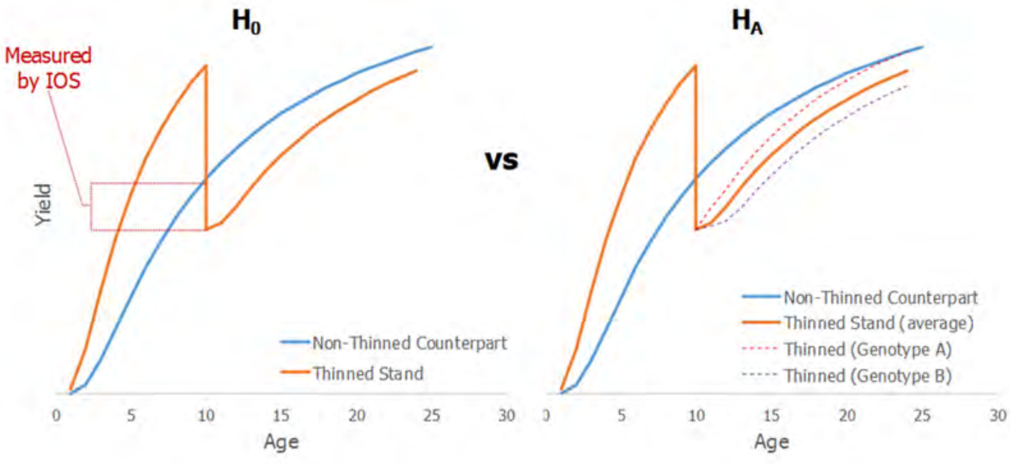
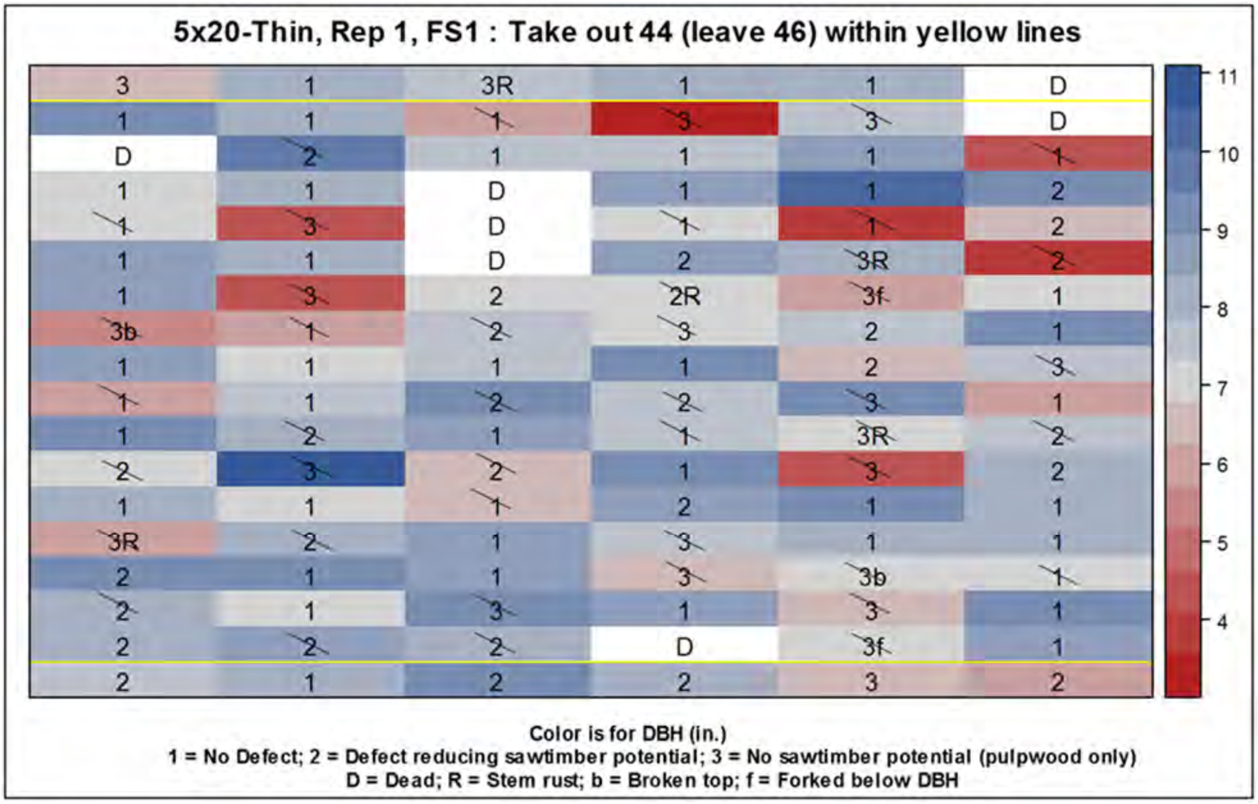


Figure 2. *(right) The objective of the thinning plan for the Genetics x Spacing x Thinning (GST) study was to determing if differences exist in genotype's response to thinning at the same intensity. We are testing the hypotheses* H_0*: genotypes respond the same to thinning, versus* H_A*: thinning response depends on genotype. The plan adapted the non-thinned counterpart method by using the plots in the no-thin, wide spacing treatment as the goal for number of "leave trees" in each family plot. An index of suppression (IOS) is used to account for differences in pre-thin competition among the genotypes.*



5x20-Thin, Rep 1, FS1 : Take out 44 (leave 46) within yellow lines

3	1	3R	1	1	D
1	1	~~1~~	~~3~~	~~3~~	D
D	~~2~~	1	1	1	~~1~~
1	1	D	1	1	2
~~1~~	~~3~~	D	~~1~~	~~1~~	2
1	1	D	2	~~3R~~	~~2~~
1	~~3~~	2	~~2R~~	~~3f~~	1
~~3b~~	~~1~~	~~2~~	~~3~~	2	1
1	1	1	1	2	~~3~~
~~1~~	1	~~2~~	~~2~~	~~3~~	1
1	~~2~~	1	~~1~~	~~3R~~	~~2~~
~~2~~	~~3~~	~~2~~	1	~~3~~	2
1	1	~~1~~	2	1	1
~~3R~~	~~2~~	1	~~3~~	1	1
2	1	1	~~3~~	~~3b~~	~~1~~
~~2~~	1	~~3~~	1	~~3~~	1
2	~~2~~	~~2~~	D	~~3f~~	1
2	1	2	2	3	2

Color is for DBH (in.)
1 = No Defect; 2 = Defect reducing sawtimber potential; 3 = No sawtimber potential (pulpwood only)
D = Dead; R = Stem rust; b = Broken top; f = Forked below DBH

Figure 3. *Example of thinning from below on paper (scratches indicate tree removal) for one of the family plots using data from the age 10 (winter 2015-2016) assessments of The Genetics x Spacing x Thinning (GST) study. Cell colors represent tree DBH (blue is big, red is small) and the numbers within each cell represent product potential (1=sawtimber potential, 3=pulpwood only). A thin from below aims to leave bigger trees with the best product potential. The target residual TPA comes from the corresponding family plot in the 10 x 20 No-Thin treatment in the same replicate (the non-thinned counterpart).*

to estimate differences among the genotype's response to thinning at the same amount of pre-thin competition (Figure 2). The amount of competition before the thin was measured with an index of suppression (IOS), calculated as the ratio of the harvested basal area to the basal area of the non-thinned counterpart (see the IOS label in Figure 2).

Marking the thin was a bit of a challenge, because we wanted to leave the best trees (thin from below) at the most uniform residual spacing as possible. A nice feature of the planting spacing (20' beds) was that take-out rows were not needed. To guide us in the field, the thin was first done "on paper" using the age 10 measurements (Figure 3). With the plan in hand, TIP staff and grad students made the official calls on what to leave and what to take in the woods (see picture).

Pictured right: *Tree Improvement graduate student, Nasir Shalizi, marking a tree for removal in the GST study. Thinning was done "from below", removing the smaller and defective trees while maintaining a uniform spacing for residual trees.*

The other trial, the Genetics Demo, does not include thinning and spacing treatment plots, so we employed a less elaborate plan and simply thinned down to half of the planting density. This provided us with a unique chance to compare pre- and post-thinned stands to gain insight on how different genetics are "cleaned up" by thinning. The mean diameter after thinning was improved across the board, but differences among genotypes persisted in the residual stands (Figure 4). Defect rates varied considerably among genotypes prior to thinning, but the differences were reduced in the residual stands, with only the local, non-improved checklot (CC2) having significantly poorer defect rates post-thinning.

As described in the 2016 Annual report, full-sibling families did better overall, but did not always outperform half-siblings. Rather, differences among genotypes were dependent upon their parents. For example, FS2 and OP2 both had the same mother tree and produced the largest mean diameter. Similarly, FS1 had the lowest defect rates, and was a cross between the parents of the two open-pollinated families with the lowest defect rates (OP1 and OP4). These results emphasize the need for landowners to know the specific families that they are planting.

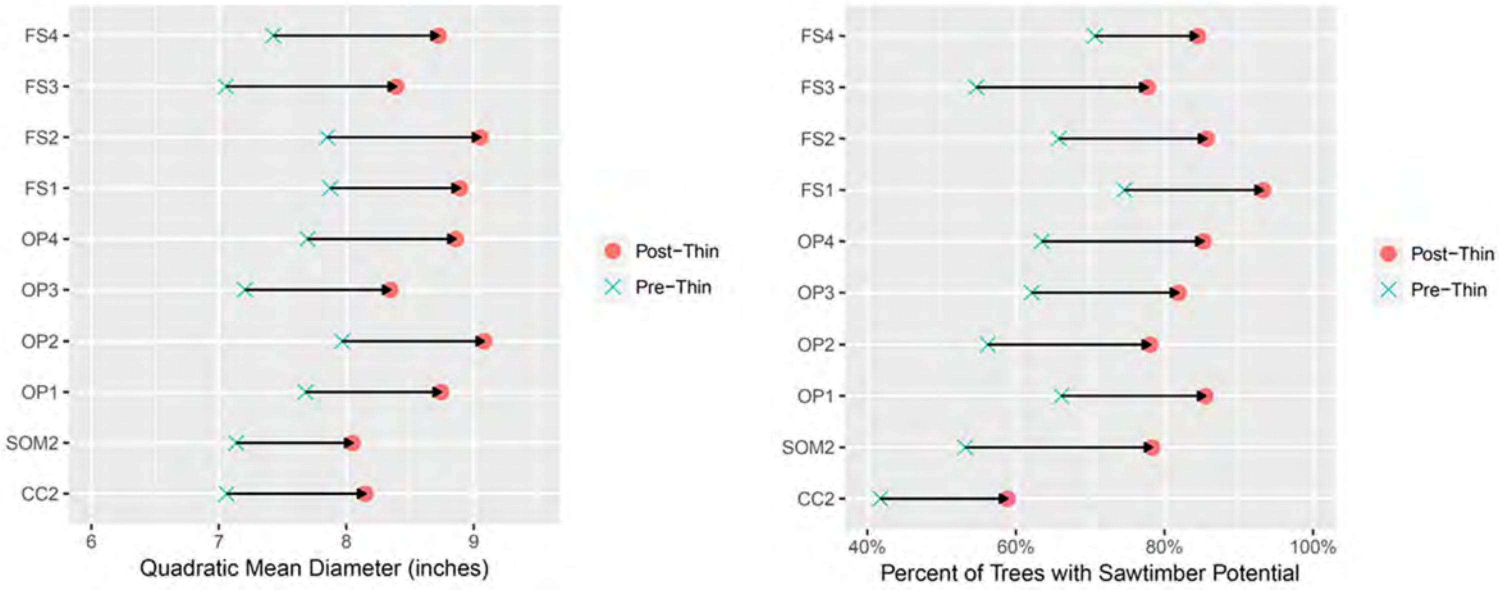


Figure 4. *Differences among genotypes for stand mean diameter (left plot) were considerable in the pre-thinned stands and persisted after thinning in the Hofmann Genetics Demo. Differences in defect rates (right plot) were large in the pre-thinned stands, but were reduced considerably by thinning, with only the local, non-improved checklot (CC2) having significantly fewer trees with sawtimber potential. Family differences were also observed: the full-sibling family with the lowest rates of defect (FS1) was a cross between the female parents of OP1 and OP4, which had the lowest rates of defect among half-sibling families.*

Thin trees were marked in blue for removal in the Genetics x Thinning x Spacing (GST) study. Thinning was done "from below", removing the smaller and defective trees while maintaining a uniform spacing for residual trees.

Loblolly Pine Atlantic Coastal Elite (ACE) Cloned Population- Six Year Results[1]

The ACE population was started with crossing of 24 elite parents of loblolly pine in the early 2000s to provide elite selections for Cooperative members. Seedling progeny from each of the 76 crosses were challenged with fusiform rust bulked inocula at the USDA Forest Service Resistance Screening Center (RSC) in Asheville, NC in 2007. For each of 53 crosses that were selected based on RSC results, about 46 full-sib seedling progeny without rust galls were cloned via rooted cutting techniques and were planted at eight locations in the southeastern US. An incomplete block row-column design was used in the field, and each clone had one ramet per location. Tree height, diameter at breast height, stem straightness (1 to 6, where 1 is the straightest), and the incidence of fusiform rust disease (yes or no), and stem forking (yes or no) were assessed at age six years. Age four height results were previously summarized in the 2015 Annual Report.

As expected, the test sites differed for growth (Table 1), but the most compelling story in the data is fusiform rust incidence. The overall rust incidence was 2% at age four and only 5.4% at age six years (Figure 1). The incidence of rust was similar at six sites for the two assessments but increased at test sites 2 and 7 (Figure 1). Selection of seedling progeny for rust resistance at the RSC has been tremendously successful. This is a large reduction for fusiform rust incidence in typical loblolly pine plantations in the region where rust incidence on average is about 30% from the Cooperative progeny tests. Since overall rust incidence was low, no further analysis was performed.

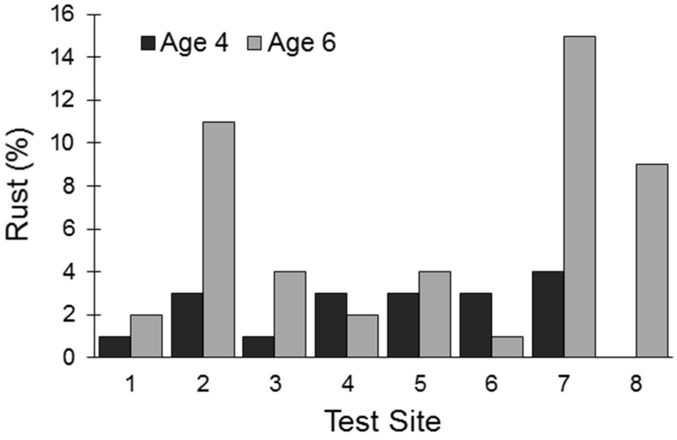


Figure 1. *Mean percent fusiform rust observed at ages four and six at test sites. Site 8 was not assessed at age 4 because trees were too small. Rust incidence was similar for two assessments (age 4 and 6) at five sites but increased substantially at sites 2 and 7.*

Mean forking across all sites was 38%, and the proportion of forking ranged from 14-58% across sites (Table 1). Mean forking incidence observed in the population was higher than the overall incidence we typically observe in seedling progeny tests (~20%).

Individual-tree models were fit to partition the observed phenotypic variance into genetic and environmental effects. A compound symmetry genetic variance structure (common correlation between pairs of sites and uniform variance at sites) was fit. The

Table 1. *Test means and overall means for the eight ACE trials. Tests 7 and 8 were measured at age five years; others were measured at age six.*

Test site	**Height (ft)**	**Volume (ft^3)**	**Rust (%)**	**Forking (%)**
1-Plum Creek	22.9	1.13	2	14
2-CellFor	28.3	2.51	11	31
3-Westervelt	29.8	2.62	4	51
4-GA Forestry Commission	24.0	1.67	2	49
5-Rayonier	21.6	1.57	4	44
6-Weyerhaeuser	25.3	2.15	1	58
7-ArborGen	22.2	1.37	14	20
8-NC Forest Service	15.6	0.69	9	51
Overall	**24.4**	**1.79**	**5.4**	**38**

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[1] This is a brief summary of Nasir Shalizi's PhD research .

residual variance-covariance structure was block diagonal (separate residual variance for each site). For stem forking, a common correlation between sites and different additive genetic variances for each site was the best fit. The clone mean heritability for height, volume, and straightness was calculated using the following equation (Isik et al. 2017. In press: Genetic Data Analysis for Plant and Animal Breeding. Springer International, 1st edition, 204p).

$$H_{\bar{c}}^2 = \frac{r_g\hat{\sigma}_{gi}^2}{\frac{\hat{\sigma}_{gi}^2}{s} + \frac{(s-1)r_g\hat{\sigma}_{ide}^2}{s} + \frac{(s-1)r_g\hat{\sigma}_{gi}^2}{s} + \frac{1}{s^2}\sum_{i=1}^{s}\frac{\sigma_{\varepsilon i}^2}{r}}$$

Where, r_g is the additive genetic correlation between sites, $\hat{\sigma}_{gi}^2$ is additive genetic variance, $\hat{\sigma}_{ide}^2$ is non-additive genetic variance, s is the number of sites, $\sigma_{\varepsilon i}^2$ is the sum of the residual variances, and r is number of trees per clone per site.

Additive genetic variance for all continuous traits was three to five times higher than for non-additive genetic variance (Table 2). The average additive genetic correlation between individual sites was 0.7 for both height and volume, 0.9 for forking, and 0.8 for straightness indicating some modest GxE for growth traits. Low correlation between pairs of sites for growth is partially contributed by the growth differences between sites (scale effect). Sites seven and eight were established one year later than others, and site eight suffered from the side effect of herbicide application in neighboring fields. As a result, the growth and survival were much lower than other trials.

Trait	σ^2_A/σ^2_{NA}	h^2_c *age* 4	h^2_c *age* 6
Height	3.40	0.47	0.55
Volume	2.95	0.36	0.51
Forking	-	0.65	0.43
Straightness	5.26	0.61	0.59

Table 2. *Ratios of additive and non-additive genetic variances* $(\sigma^2_A/\sigma^2_{NA})$ *and clone mean heritability* h^2_c *for height, volume, stem forking, and straightness measured at ages four and six. Non-additive genetic variance for stem forking at age six was zero. Rust incidence was not analyzed due to very low overall incidence in the data.*

The clone-mean heritability for growth traits at age six (0.55 for height) is considerably greater than the individual-tree heritability observed in seedling progeny testing (0.2 to 0.3 in third-cycle polymix tests). The clone-mean heritability estimates at age six for height and volume increased compared to age four (Table 1), likely due to a reduction in the environmental "noise" caused by the rooted cutting propagation effect. Straightness clone mean heritability did not change however, and the heritability of forking decreased from 0.65 to 0.43. The differences in heritability estimates between the two assessment ages may also be influenced by adding data from test site eight. With the higher clone-mean heritabilities in these trials, it is clear that clonal progeny testing can capture greater genetic gain than seedling progeny testing, but it does take two more years to produce rooted cuttings.

The influence of the family structure in the ACE trial is demonstrated by the large mid-parent effect on clone breeding values. For demonstration purposes, an overlaid histogram of clone breeding values belonging to two families is presented in Figure 2. It is clear that family 5 has many more superior clones (higher breeding values) than family 91. When making selection based on animal models, breeders should be careful about selecting clones based on clone means only, because the best clones usually have common

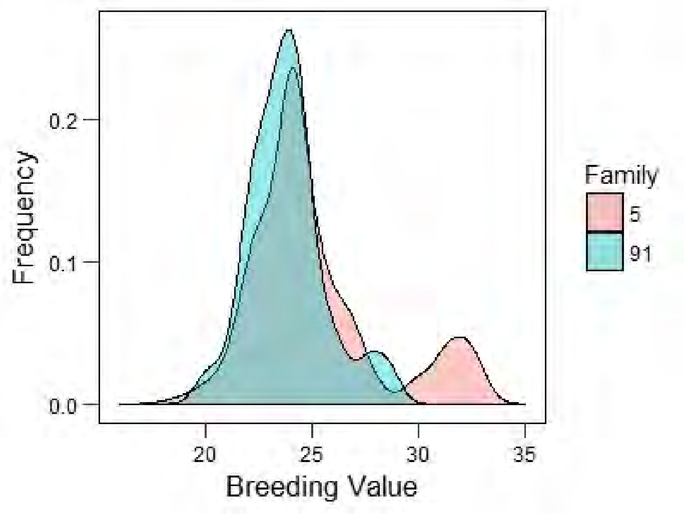

Figure 2. *Distribution of height breeding values for clones from two families measured at age six years suggesting strong family effect on clone breeding values. Family 5 has many more superior clones (higher breeding values) than family 91.*

Heritability and Variation in Growth and Survival in a Long-term Longleaf Pine Diallel Test[1]

A longleaf pine (*Pinus palustris* Mill.) progeny trial, initiated from a 13-parent diallel, was established by the US Forest Service on the Harrison Experimental Forest, near Saucier, MS in 1960. All possible crosses among 13 parent trees, including selfs were produced, and the experiment was planted as a randomized complete block design with 8 blocks. Crosses were represented by eight-tree row plots. Growth and survival data were collected for all trees at ages 3, 7, 17, 30, and 40 years. The objective was to partition observed phenotypic variance and estimate heritabilities for growth and survival of longleaf pine. The long-term nature of this project is unique for longleaf pine and enables inference into the effectiveness of early-age selection for later-age performance.

There was significant variation among full-sib families for growth traits and survival at ages 3, 7, 17, 30, and 40 years. Family genetic values for height were most strongly correlated (r = 0.81) between ages 17 and 40 years indicating that selection at 17 years would predict performance at age 40. Family genetic values for survival were strongly correlated between ages 17 and 30 years (r = 0.95), but family survival for all ages was mostly consistent (Figure 1). The variation in growth and survival were under additive genetic control in younger ages, with a trend of increasing importance of dominance variance with age (Table 1). Survival had slightly higher heritability than either height or volume.

Conservation efforts for the longleaf forest cover type continue through planting efforts by landowners and government cost-share programs. Currently, there is little information about growth characteristics, productivity levels, or survival rates in the longleaf

Table 1. *Heritability estimates (individual tree, half-sib family mean, and full-sib family mean) and ratio of dominance to additive genetic variances for longleaf pine height[1], volume, and survival at each measurement age (standard errors are in parentheses).*

Traits	h_i^2	h_{hs}^2	H_{FS}^2	Dom./Add.
Height – Age 3	0.14 (0.06)	0.63 (0.13)	0.79 (0.08)	0.04
Height – 7	0.14 (0.06)	0.50 (0.13)	0.80 (0.06)	0.41
Height – 17	0.06 (0.03)	0.24 (0.12)	0.79 (0.06)	2.06
Height – 40	0.11 (0.05)	0.36 (0.14)	0.82 (0.06)	1.10
Volume – Age 17	0.12 (0.06)	0.30 (0.12)	0.85 (0.04)	1.63
Volume – 40	0.08 (0.05)	0.24 (0.12)	0.86 (0.04)	2.40
Survival – Age 17	0.23 (0.10)	0.52 (0.16)	0.89 (0.05)	0.61
Survival – 30	0.27 (0.11)	0.57 (0.14)	0.90 (0.04)	0.45
Survival – 40	0.14 (0.08)	0.22 (0.12)	0.89 (0.04)	2.88

[1] *Height at age 30 was not analyzed due to small number of observations, N=332.*

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[1] *This is a brief summary of Graham Ford's PhD research with longleaf pine.*

pine sources being deployed in the South. Benchmarking longleaf pine variation is important to ensure that improved selections become the primary contributors to the reforestation pool. Establishment of new longleaf pine forests should be with tested seed sources, to ensure the performance and survival of longleaf plantations meet conservation and forest management goals.

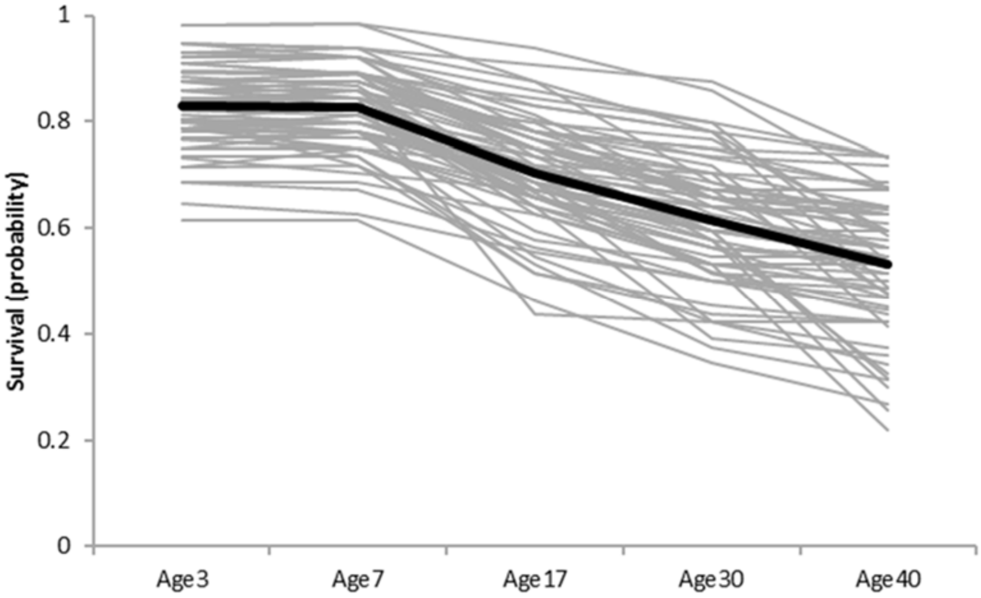


Figure 1. *Survival of 78 full-sib families (gray lines) across ages and the overall survival (thick black line) in the longleaf pine diallel experiment. Overall survival was about 82% at age 3 and dropped to 53% at age 40.*

As part of his PhD research, Graham Ford analyzed the long-term longleaf pine diallel test at the Harrison Experimental Forest near Saucier, MS. The trial is still ongoing; Jim Roberds (US Forest Service, retired) examines the stand at 53 years of age in 2013.

Exploring Genetic Fingerprinting Technology for Tree Breeding Programs

The goal of genetic fingerprinting is to provide genetic marker data to allow validation of clonal identities, identify co-ancestry among individuals of unknown ancestry, and to identify parent-offspring relationships in situations of ambiguous parentage. The ideal fingerprinting technology will be low cost, scalable from a few samples to many thousands of samples, and provide highly-informative genetic marker data. No single existing technology platform meets all these requirements, so experiments to test new technology platforms are justified.

The technology platform tested in 2016 is called multiplex amplicon sequencing, and was carried out with a service provider called Floodlight Genomics in Knoxville, TN. An “amplicon” is a DNA fragment amplified from a specific target segment of DNA or RNA; “multiplex” refers to the fact that hundreds or thousands of such DNA fragments can be analyzed in parallel in a single experiment. This approach is based on cost-effective and high-throughput DNA sequencing methods. The cost of DNA sequencing continues to drop much faster than the cost of competing genotyping technologies such as SNP arrays, so the favorable cost structure of amplicon sequencing is likely to remain an advantage for the foreseeable future. Amplicon sequencing also has a relatively low entry cost; new genetic marker loci can be added to existing marker sets at any time, at a cost of around $10 per marker locus. Competing array-based genotyping technologies often have a significant capital cost for synthesis of the genotyping assays, and can achieve relatively low costs per assay only if that high capital cost is spread over many thousands or millions of assays.

The key issue addressed in experiments in the past year is the information content of the genetic marker data produced. A key difference between amplicon sequencing and array-based genotyping technologies is that an amplicon consists of 50 to 80 bases of DNA, so there are multiple sites at which genetic variation can be detected, while array-based methods assay individual SNP loci. This means that array-based methods must assay many more individual SNP loci in order to produce the same amount of information available from a smaller number of amplicons.

Preliminary experiments to test a set of 295 candidate amplified loci for utility in a fingerprinting assay were described in the TIP Annual Report 60 in 2016. A follow-up experiment with 183 of those 295 candidates was conducted in fall of 2016, using DNAs extracted from breeding selections grafted at the Arrowhead Breeding Center near Macon, GA. A total of 1346 DNA samples were provided to Floodlight Genomics in October 2016, and the data were returned in November 2016. Data analysis to evaluate alternative approaches to utilizing the DNA sequence data in fingerprinting assays suggests that careful filtering of the data will be valuable in removing noise from low-quality sequences and improving the resolution of the genetic information for verification of clonal identities and family relationships. A set of 433 SNPs in 145 of the amplicons were genotyped in 1109 individuals out of the 1346 (82.4% success across samples). Insufficient quantity or quality of DNA is the most likely cause of failure for samples that yielded little or no useful data.

Comparison of the sequences of amplicons that yielded useful SNP data with DNA sequences previously placed on a consensus genetic linkage map of loblolly pine (Westbrook et al, G3 5:1685, 2015) suggests that the detected markers provide reasonably complete genome coverage (Table 1). For purposes of clonal validation, the available markers should be sufficient in most cases, but additional marker loci will be sought to fill in large gaps between markers; at present, all linkage groups are predicted to have intervals between markers greater than 30 cM. A marker set with more uniform coverage of all linkage groups will be useful for low-density genotyping in breeding applications and for other research objectives.

Routine application of fingerprinting by cooperators will require optimization of methods for collecting tissue and identification of service providers willing to carry out DNA extractions and fingerprinting assays on a schedule and at a cost acceptable to individual organizations. Several candidate service providers exist, and experiments to test tissue collection methods and DNA extraction services are being planned.

Table 1. *Numbers of markers predicted to fall on linkage groups (LG) of the consensus loblolly pine linkage map (Westbrook et al, 2015). Multiple SNPs are detected at most locations, so the probability of at least one informative marker in a given region of the linkage map is fairly high. For each linkage group (LG) 1 through 12, the count of amplicons predicted to map to that LG is shown, along with the minimum (min) and maximum (max) positions of amplicons on the LG and the overall length of the linkage group in centiMorgans (cM). The average interval (avg. interval) between markers on each linkage group and the standard deviation of interval size between markers are shown in the two columns on the right side of the table, also in cM.*

Linkage Group	Count	Min	Max	LG Length	Avg. Interval	Standard Deviation
1	12	16	173	185	14.5	20.5
2	9	17	207	222	23.1	22.0
3	15	24	167	187	11.2	10.7
4	9	37	162	186	18.0	15.9
5	20	28	200	216	10.0	10.1
6	8	30	173	194	21.6	21.9
7	7	29	184	193	26.3	29.9
8	12	1	163	190	13.6	19.6
9	17	14	156	172	9.2	12.1
10	7	24	205	211	29.4	22.0
11	6	22	145	147	24.2	15.9
12	10	17	185	203	18.5	26.7

Grants

New Grant

Steve McKeand, Fikret Isik, and Ross Whetten on a North Carolina Department of Agriculture. Loblolly Pine Biomass Cropping Study: Years 5-7. $147,779. 1/2017-12/2019.

Richard Venditti, et al. “Preparing Diverse and Rural Students and Teachers to Meet the Challenges in the Bioenergy and Bioproducts Industry”. TIP portion: $33,588. 2017-2021.

Continuing Grants

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

Steve McKeand, Fikret Isik, and Ross Whetten on a North Carolina Department of Agriculture. Loblolly Pine Biomass Cropping Study: Years 3-5. $81,165. 1/2015-12/2016.

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

ASSOCIATED ACTIVITIES

Meetings, Workshops, and Short Courses

The Cooperative staff held the Seed Orchard Managers meeting in September 2016 in Savannah, GA with associated field tour hosted by Weyerhaeuser. It was very well attended by both Cooperative members and those from outside the Cooperative (pictured right). The focus on seed orchards was a great way to get folks prepared for this year's early breeding season. Attendees, both old timers and newbies, heard from staff and faculty about the latest research and techniques in seed orchard management as well as shared information and personal experiences making it a super useful event for all.

For the annual TIP Contact meeting we decided to beach it and go to Panama City Beach, FL in mid-November 2016. The field tour was hosted by Dr. Patrick Cumbie and Chris Rosier of ArborGen, Inc. The group toured the Bellamy Seed Orchard and looked at field tests of pine, eucalypts, and sweetgum. We dined on a great BBQ lunch and then did something a little different by going underground to tour the Florida Caverns (pictured left) It was a great meeting, and we are looking forward to the next one in Williamsburg, VA in November 2017.

The faculty members associated with the Cooperative were invited to teach courses all over the world. Steve taught a short course in China at Nanjing Forestry University in December 2016 on Genetics and Tree Improvement. Fikret taught a workshop for the South African tree improvement community from July 27 to August 3, 2016 (group pictured right). The workshop was hosted by the Institute of Commercial Forest Research, Sappi and Mondi, Camcore, The Forest Molecular Genetics Research Program at University of Pretoria, and the Forestry Sector Innovation Fund of South Africa. The aim of the workshop was to provide forest scientists a range of training opportunities on quantitative genetics and genomic selection methodology and provided the opportunity for participants to benchmark and network with the international presenters and forest scientists.

Jeff Fields, Chief of Reforestation, Georgia Forestry Commission.

Chuck Little has been responsible for much of the breeding at the Arrowhead Breeding Center.

Education and Outreach are important bonuses of the Arrowhead Breeding Center.

Once again this year, we have seen the value of the Arrowhead Breeding Center to the Cooperative's breeding program. The support from the Georgia Forestry Commission has been strong since we started working there in 2008. Topgrafting and breeding of all forward selections from the 4th Cycle are just about finished, and we have started planning for establishing breeding orchards for the 5th Cycle. In particular, we thank Jeff Fields, Chief of Reforestation, for his efforts to provide the facilities and resources that have moved our program through the 4th Cycle towards the 5th Cycle. And of course, Chuck Little, retired GFC and current NCSUCTIP staff, has been the heart and soul of the Breeding Center since its inception. Thanks to all our friends in the Georgia Forestry Commission and to all Cooperative members for the support you give to us.

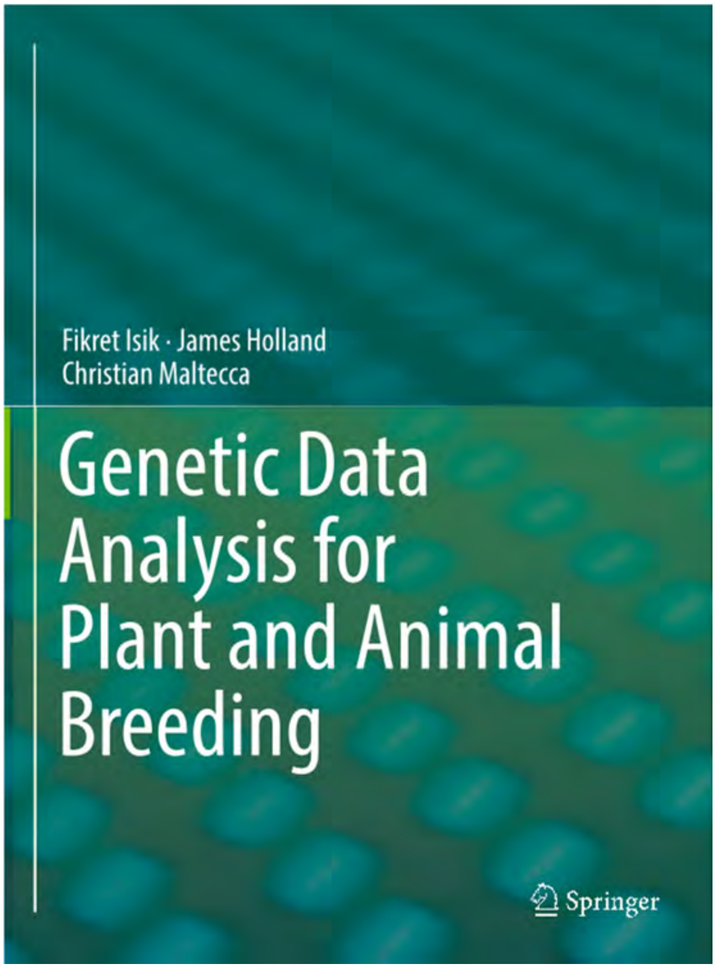
All forward selections for the 4th Cycle have been topgrafted in the New Breeding Orchard at the Arrowhead Breeding Center. Selections from the 5th Cycle will be topgrafted here and in the expanded breeding orchards starting in 2019.

New book to be published:

Genetic Data Analysis for Plant and Animal Breeding

Fikret Isik, James Holland, and Christian Maltecca

North Carolina State University


Fikret Isik · James Holland
Christian Maltecca
Genetic Data Analysis for Plant and Animal Breeding
Springer

Fikret Isik, Jim Holland, and Chris Maltecca had a crazy idea about writing a text book back in 2012. They started collaborating on teaching international workshops on genetic data analysis for breeding and taught the first workshop at NC State in 2011 and the second one at Czech University of Life Sciences in Prague in 2012.

Fikret had resurrected Gene Namkoong's Advanced Quantitative Genetics course (FOR 726) at NC State University in 2012. He invited Dr. Jim Holland (maize geneticist) and Dr. Chris Maltecca (animal geneticist) to co-teach the course and make it an interdepartmental (Forestry, Crop Science, and Animal Science) course. They expanded the workshop notes to course notes and offered the first course in Autumn 2012. They then used the course notes to write a text book.

It took the three authors more than four years to complete the first draft. Changes in software used for genetic data analysis, especially R packages to analyze DNA markers for prediction were changing almost every six months while they were writing the chapters. They had to scrap many chapters already written and start over.

This is a different kind of book than most on the market. It introduces brief theory of the subject, such as genomic relationships derived from markers. Then, it introduces the software with example codes on how to analyze a set of mostly real data. The output from the software is given and interpreted.

This book fills the gap between textbooks on quantitative genetic theory and software manuals that provide details on analytical methods but give little context or perspective on which methods may be most appropriate for a particular application. Accordingly, this book is composed of two sections. Chapters 1 to 8 cover topics of classical phenotypic data analysis for prediction of breeding values in animal and plant breeding programs. Chapters 9 to 12 provide the concept and overall review of available tools for using DNA markers for predictions of genetic merits in breeding populations.

The book is expected to come out in July 2017. Electronic copy and hard copy will be available to purchase at the following link:

http://www.springer.com/us/book/9783319551753

Fikret, Jim, and Chris deserve thanks and praise from all of us in the tree improvement community. Students and scientists are already using the book to help analyze complex data in many different breeding programs. Writing a book is a huge endeavor, and we are all grateful for their work.

***Pictured above**: Attendees posing during the field tour at the seed orchard in Marianna, FL during the 61st Annual Contact Meeting*

TIP staff also participated in these extension activities for forestry community in the last year:

Austin Heine presented at the Southern Lumber Manufacturer Association meeting at Hofmann Forest on April 6, 2016.

Fikret Isik was invited to be the PhD thesis examiner (opponent) and speaker by the Swedish University of Agricultural Sciences, Umea, May 24-27, 2016 for PhD Zhiqiang Chen.

Steve McKeand was an invited speaker at the National Association of Plant Breeders Meeting, August 15-18, 2016.

Steve McKeand was an invited speaker at the IUFRO Regional Congress for Asia and Oceania 2016 in Beijing, China, October 24-27, 2016.

Fikret Isik served on the USDA-NIFA foundation program as a panel member to review research proposals, November 15-17, 2016.

Fikret Isik served on USDA-DoE Plant Feedstocks Genomics for Bioenergy as panel member to review research proposals, April 27, 2017.

Staff

In June of 2016 we added Daniel Genung to our staff to fill the Analyst and Database Manager vacancy. He brings a strong genetics background as well as the database skills needed for the job. He has fit right in the group, and we are glad to have him onboard. If you have not had the pleasure of meeting you can't miss him; he currently holds the title of tallest TIP staff member!

We boasted in the fall about our director, but one more time wouldn't hurt, right? Dr. Steve McKeand was awarded the Governor James E. Holshouser Jr. Award for Excellence in Public Service in December 2016. His accomplishments over the years with the University and the Cooperative were recognized by the UNC system board of Governors. Created in 2007, this award aimed to encourage, identify, recognize, and reward public service by faculty of the 17 institutions of the University of North Carolina System. As Steve would say, "This is quite the feather in the cap", and we couldn't agree more! We are proud of him and his contributions to the Cooperative, the University, the State of North Carolina and beyond.

Pictured above: *Tree Improvement staff and students at Steve McKeand's award celebration. From left to right, back row: Daniel Genung, Austin Heine, Ross Whetten and J.B. Jett. Front Row: Nasir Shalizi, Serenia O'Berry, Fikret Isik, Steve McKeand, April Meeks and Trevor Walker. Not pictured: Tori Brooks.*

Visitors

This year we welcomed Thi "Hai Hong" Nguyen who was here on the Nicholson Fellowship from the Swedish University of Agricultural Sciences (SLU). During her time here in the fall semester, Hong was able to sit in on Fikret's class, Advanced Quantitative Genetics and Breeding, as well as work on research initiatives. We have enjoyed hosting folks each years and hope to continue the trend. We enjoyed her during her time here and we wish Hai Hong the very best in her educational and career goals.

Teaching

The faculty associated with the Cooperative instruct courses at both the graduate and undergraduate level. In the fall semester, Dr. Fikret Isik taught Advanced Quantitative Genetics and Breeding (FOR/CS/ANS 726). This course is offered in even years and attracts students from crop science, animal science, and bioinformatics in addition to forestry. 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), but this year both courses were also made into distance education (DE) classes. April Meeks was the teaching assistant and took the lead preparing the web page, grading homework and being responsive to all of the students, especially the long distance ones. Given the success of the trial run, Steve and Ross (and hopefully April) plan to teach the courses online again next year and will advertise broadly for students to learn about Tree Improvement.

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 was again called into action this spring semester with his course NR554, Introduction to Data Analysis in Natural Resources, which covers general statistical procedures using SAS and R programing.

***Pictured above**: FOR 411 & 725 class field tour at NC Forest Service in Goldsboro, NC.*

From left to right: *Adam Festa, Jessica Maynor, April Meeks, Austin Heine, Serenia O'Berry, Nasir Shalizi and Trevor Walker*

Graduate Students

This was another great year for students who successfully defended their research and graduated. Graham Ford and Andrew Sims both finished in Spring 2017. We also welcomed both a new Masters and PhD student this year; Nasir Shalizi began his PhD program in the fall, and Jessica Maynor began in this spring semester. 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 began a job at Monsanto this April and expects a summer 2017 finish.

April Meeks, PhD – April is working towards a better understanding of the fusiform rust and loblolly pine pathosystem within the Atlantic Coastal Elite population. Her research will focus on the pathogen variation and potential loblolly family by inoculum interactions.

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

Serenia Larrison, MS – Serenia is a graduate forester from the University of Georgia and joined us in fall 2015 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".

Jessie Maynor, MS – Jessie had been an undergraduate student worker with the Cooperative for a couple years and was hooked! She took to the Butner research as an undergraduate and will continue the next phase of research for Bioenergy. Her research is titled "Assessing Improved Loblolly Pine Families for Biomass Potential".

Nasir Shalizi, PhD- Nasir began in Fall of 2016 and had previously been a MS student with Dr. Barry Goldfarb. He jumped right in and has been working on ACE data. His research is titled "Genetic parameter estimates from a large cloned loblolly pine population".

Trevor Walker, PhD – Trevor has been studying applications of quantitative genetics in several loblolly pine traits, including growth and yield, wood quality, and phenology. He has been learning more about genomic tools and plans to apply them to the ACE population for his PhD project.

Undergraduate Army

Our Cooperative simply would not be capable of doing what we do without the help from our outstanding undergraduate students. There has been a tradition here in Tree Improvement to have a steady flow of bright, talented, dedicated, and hardworking students to help with all aspects of our work, and we certainly have some great ones right now. The students help the program by entering data, testing pollen in the lab, pulling seed for testing, measuring wood properties in Alabama when it is 100 degrees or measuring Butner when it was below freezing. These folks are tough and are steam powering this locomotive. The group of students we have learning from our program and helping us right now are as good as they get, and we have no doubt that some of them will be out working for the members of our Cooperative in a few short years! Here are some pictures of our current undergraduates (8 of them) as they are helping with many of the Cooperative activities.

Top from L to R: *Rachel Burris (Sophomore in Forest Management) painting trees at Hofmann for thinning, Doug Dobson (Junior in Forest Management) learning to graft, Dwight Hilton (Sophomore in Forest Management) and Rhodes Kelly (Senior in Ag Business) using the TreeSonic in Goldsboro, NC, and Austin Quate (Sophomore in Forest Management) on a cold morning at the Butner Field Site.*

Bottom from L to R: *Paige Green (Sophomore in Natural Resources) measuring with the TreeSonic at Hancock's PMX test in Alabama with our newest undergraduate student David Wilkinson (just kidding David), Rhodes Kelly and Alex Theodorakis (Junior in Forest Management) filling trays with soil for growing the 2018 Northern 4th-cycle tests, Rachel Powell (Sophomore in Environmental Technology and Management) in the lab drilling cones as part of the cone analysis for the PBS Bag Study.*

Membership in the NCSU Cooperative Tree Improvement Program

This year we have new members both near and far! We added 3 new Contributing Members with the addition of Gelbert, Fullbright & Randolph Forestry Consultants, Forest Investment Associates, and GreenWood Resources. Research Associate Members grew as well this year; we welcomed J.D. Irving, Ltd and New Brunswick Tree Improvement Council, both research groups located in eastern Canada and GenoVerde, Inc. With the sale of the timber deed for the Hofmann Forest in June 2016, the NC State University Natural Resources Foundation left the Cooperative as a Contributing Member. As always, we value the members and their support of our research and breeding activities. Our Cooperative partners are listed below.

Full Members

ArborGen, Inc.
Georgia Forestry Commission
Hancock Timber Resources Group
International Forest Company
North Carolina Forest Service
Rayonier, Inc.
Tennessee Division of Forestry
Virginia Department of Forestry
The Westervelt Company, Inc.
Weyerhaeuser Company

Contributing Members

American Forest Management
Blanton's Longleaf Container Nursery
Campbell Global, LLC
Charles Ingram Lumber Co.
Dougherty & Dougherty Forestry Services
F&W Forestry Services, Inc.
Forest Investment Associates, LLC
Four Rivers Land & Timber Company, LLC
Gelbert, Fullbright & Randolph Forestry Consultants
GreenWood Resources
Jordan Lumber & Supply Company
Larson and McGowin, Inc.
Meeks Farms & Nursery, Inc.
Milliken Forestry Company
Molpus Woodlands Group, LLC
ProFOR Consulting
Resource Management Service, LLC
Scotch Land Management, LLC
Shoeheel Land Management (Z.V. Pate, Inc.)
South Carolina Forestry Commission
Timberland Investment Resources, LLC
White City Nursery, LLC

Research Associate Members

Arauco - Bioforest, S.A.
GenoVerde, Inc.
J.D. Irving, Ltd
New Brunswick Tree Improvement Council
PBS International
USDA Forest Service

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

2017

Farjat, A.E., A.K. Chamblee, F. Isik, R.W. Whetten, and S.E. McKeand. 2017. Variation among loblolly pine seed sources across diverse environments in the southeastern United States. For. Sci. 63(1): 39-48.

Farjat, A.E., B.J. Reich, J. Guinness, R.W. Whetten, S.E. McKeand, and F. Isik 2017. Optimal seed deployment under climate change using spatial models: application to loblolly pine in the southeastern US. Journal of the American Statistical Association (In press) http://dx.doi.org/10.1080/01621459.2017.1292179

Holliday, J.A, S.N. Aitken, J.E.K. Cooke, B. Fady, S.C. Gonzalez-Martinez, M. Heuertz, J.P. Jaramillo-Correa, C. Lexer, M. Staton, R.W. Whetten, C. Plomion. 2017. Advances in ecological genomics in forest trees and applications to genetic resources conservation and breeding. Molecular Ecology 26(3):706-717.

Isik, F., C. Maltecca, and J. Holland. 2017. Genetic data analysis for plant and animal breeding. Springer International. ISBN 978-3-319-55177-7 (in press).

Kohlway, W.H., R.W. Whetten, D.M. Benson & J. Frampton. 2017. Response of Turkish and Trojan fir to *Phytophthora cinnamomi* and *P. cryptogea*, Scandinavian Journal of Forest Research, DOI: 10.1080/02827581.2017.1280076

Pais, A.L., R.W. Whetten, Q-Y. Xiang. 2017. Ecological genomics of local adaptation in *Cornus florida* L. by genotyping by sequencing. Ecology and Evolution 7(1):441-465.

Spitzer, J.E., F. Isik, R.W. Whetten, A.E. Farjat, and S.E. McKeand. 2017. Correspondence of loblolly pine response for fusiform rust disease from local and wide-ranging tests in the southern United States. For. Sci. (In press).

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Bartholomé J, Bink MC, van Heerwaarden J, Chancerel E, Boury C, Lesur I, Isik F, Bouffier L, Plomion C. 2016. Linkage and association mapping for two major traits used in the maritime pine breeding program: height growth and stem straightness. PLoS One. Nov 2;11(11):e0165323.

Bartholome, J., J. Van Heerwaarden, F. Isik, C. Boury, M. Vidal, C. Plomion, and L. Bouffier. 2016. Performance of genomic prediction within and across generations in maritime pine. BMC Genomics, 17.

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Gräns, D., F. Isik, R.C. Purnell, and S. McKeand. 2016. Genetic variation in response to herbicide and fertilization treatments for growth and form traits in loblolly pine. For. Sci. 62(6): 633-640.

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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.

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Our Director, Dr. Steve McKeand, was the proud recipient of the 2016 Governor James E. Holshouser Jr. Award for Excellence in Public Service given by the Board of Governors of the University of North Carolina. We are thrilled he was recognized for his impacts on forestry.

Cover: The 4th-cycle breeding is almost done. We are breaking through the fog and can finally see the light at the Arrowhead Breeding Center at the Georgia Forestry Commission's Arrowhead Seed Orchard.