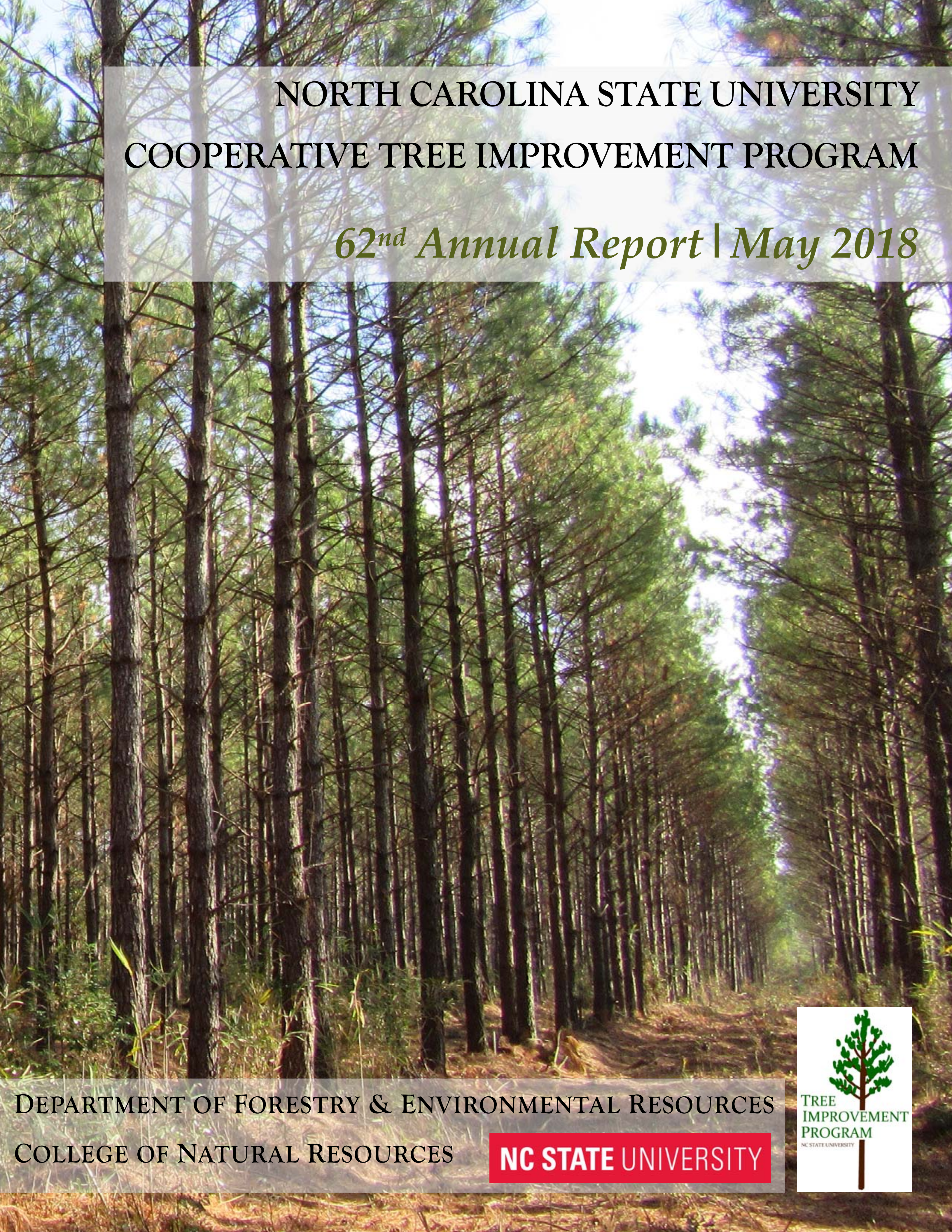

NORTH CAROLINA STATE UNIVERSITY
COOPERATIVE TREE IMPROVEMENT PROGRAM

62nd Annual Report | May 2018

DEPARTMENT OF FORESTRY & ENVIRONMENTAL RESOURCES

COLLEGE OF NATURAL RESOURCES

NC STATE UNIVERSITY

TREE
IMPROVEMENT
PROGRAM
NC STATE UNIVERSITY

62nd Annual Report

May 2018

Department of Forestry and Environmental Resources

College of Natural Resources

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Raleigh, NC

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EXECUTIVE SUMMARY

The 62nd year of the Cooperative Tree Improvement Program will be Steve McKeand's last as Director. The Cooperative 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

Crossing for the 4th-Cycle breeding populations has been completed. Kudos to Cooperative members and to Cooperative staff at the Arrowhead Breeding Center in Cochran, GA for the big push in 2018. Breeding for the Atlantic Coastal Elite (ACE) Genomic Selection population was also completed; 68 crosses will be available for ACE2.

The first of the 4th-Cycle test data was collected in 2018. 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.

To prepare for breeding these future selections, a new breeding orchard was started at the Arrowhead Breeding Center.

New queries were added to the TIPRoot database allowing for estimation of mid-parent breeding values for non-tested crosses and to provide wood quality data. Breeding and testing progress can also be easily queried with new functions.

Cooperative members harvested nearly 70,000 pounds of loblolly pine seed from orchards in 2017, 70% from Cycle 3+ orchards.

RESEARCH

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. Based on a time-motion study, many more of the PBS bags can be installed per day during the pollination season.

Age 4- to 6-year data were analyzed for the Cooperative's longleaf provenance/progeny trial. Although several of the trials had survival problems, there was consistent family performance for height at the better sites.

Based on 6-year data from the Loblolly Pine Biomass Genetics/Cropping Study at Butner, NC, Coastal families had 45 ft^3/acre more volume than Piedmont families but had lower volume in sawtimber trees due to more cold damage two years earlier. Wood stiffness of the Coastal families was significantly higher than the Piedmont families.

Four-year results from the ***PRS***™ Calibration Study were encouraging. Strong correlations between ***PRS***™ scores and observed volume, fusiform rust incidence, and mean height were observed on most sites.

Resistograph amplitude (surrogate measure for wood density) and TreeSonic acoustic velocity (surrogate for wood stiffness/bending strength) have been completed for 280 Coastal, 200 Piedmont, and 67 Northern families.

In the Hofmann Forest growth and yield genetic studies, thinning at age 12 drastically reduced defect rates for open-pollinated families, but the best full-sib families still had significantly more volume in trees with sawtimber potential. Defect rates for the local, non-improved checklot did not benefit as much from the thin as improved genotypes. Clonal genotypes appeared to have less stand uniformity and were characterized by wider height and DBH distributions, while full-sib and open pollinated families had similar stand uniformity.

The Cooperative is moving forward with plans to design a screening array for loblolly pine in 2018. The application array is expected to be available in July 2018. The Cooperative plans to genotype the ACE population to develop genomic selection strategies.

ASSOCIATED ACTIVITIES, GRADUATE STUDENTS, COOPERATIVE STAFF, MEMBERSHIP

We have one new Contributing Member and one new Research Associate Member. Unfortunately, the Tennessee Division of Forestry dropped Cooperative membership. There are now 9 Full, 22 Contributing, and 7 Research Associate Members. Eddie Lauer joined us as Analyst / Database Manager. 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 and host visiting scientists in Raleigh and at Arrowhead.

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62 YEARS OF TREE IMPROVEMENT AT NC STATE

A MESSAGE FROM THE DIRECTOR

Steve's 40 Years of Tree Improvement

So my Granddaughter asked me, "What are you going to do when you retire Grandpa"? I told her, "I'm going to spend more time with you". The look on her face told a significant story; I'm not sure she is very excited about my plans, but I know I am!

Actually, I am not completely retiring. I start phased retirement (½ time position) on July 1 and will be stepping down as Cooperative Director. I officially joined the Cooperative faculty July 1, 1983 when I became an Assistant Professor and TIP Geneticist. Before that, from 1980 to 1983, I was a tree improvement specialist / research assistant with the Tissue Culture Program which was a joint project between Tree Improvement/Forestry and the Botany Department. Two years prior to that, I was a PhD student with the Tree Improvement Cooperative. So I've been hanging around TIP for about 40 years. I'm not sure that sets a record, but I don't think the Guinness folks have a category for that anyway.

Some of the younger foresters have asked about the changes that have occurred in forestry and tree improvement over those 40 years, and there have been many. The most significant change has been in forestland ownership in the South. When I moved to the South, the large vertically integrated forest products companies seemed to have always been here, and I figured they would always be here. Of course, that was an incredibly naïve perspective, but I'm not sure anyone could have foreseen the dramatic changes that would occur. In 1978, there were 26 members of the Cooperative; 23 of them were forest products companies, each with large pulp and paper mills and most with sawmills and some with plywood mills. Today, none of the members are directly involved in pulp and paper manufacturing… what a change!

Our most challenging time for support came in 2007 when we had 14 Members of the Cooperative, and three of those members had announced that they were selling their land and leaving the program. In 2008, we started the Contributing Membership option for forestry companies, organizations, individuals, or nurseries who desired information about the genetic value of loblolly pine. With this significant change, the financial support for tree improvement and forest management in general, changed from a necessary cost supporting the wood supply chain of the vertically integrated companies to more of an investment in forestland to enhance productivity and value to landowners.

Today, our membership stands at 39: 9 Full Members, 22 Contributing Members, and 7 Research Associate Members. I think this is clear testimony to the value that tree improvement brings to forest management and to landowners, and to the quality of the work done here by the incredible faculty, staff, and students associated with the program (more on them in a bit).

The second big change that I witnessed is how the improved genetic material has been deployed across the landscape. In the mid 1970s, foresters began to establish plantations with individual open-pollinated families. Today, over 95% of the loblolly pine plantations are established with either individual open-pollinated families, full-sib families, or clonal varieties. Knowledgeable landowners and foresters recognize the need and value of fully understanding the genetics of the seedlings that they will live with for 25 to 35 years. This profound change led us to develop the ***PRS***™ system to make our genetic information more user-friendly and to provide third-party verification for the genetics being deployed. With many nurseries now providing a full range of genetic options to all landowners, the recognition of the value of our breeding program is at an all-time high.

I think my most significant accomplishment as Director the past 13 years is that I have hired the most skilled, brilliant, competent, and dedicated staff imaginable. These folks have developed and implemented incredible breeding and research programs that will impact forestry and the lives of landowners for decades and centuries to come. My one claim to fame is that I have always been able to hang around people who are a lot smarter than I, and fortunately, I have been able to facilitate that as Director.

As I start my "ride into the sunset" with phased retirement, I know that the Cooperative Tree Improvement Program will continue to be innovative, creative, prosperous, and successful. The staff, students, faculty, and administration here at NC State won't allow the Cooperative to be anything less, and the members, landowners, and stakeholders in the region will demand that the program remains strong. The one thing that has not changed over the past 40 years is the dedication of tree breeders, foresters, and landowners to be true stewards of the forest resources in the South. Our future is in good hands.

And finally, I would be remiss if I did not recognize and thank the most important people in my life, my family. I am blessed to still have my Mom and Dad, Betty and Jerry, who continue to pat me on the back and give me hugs and kisses when they visit. My wife, Lou, has put up with me for 43+ plus years. Her love and support have been incredible; I can only hope that she will continue her tolerance when I'm hanging out with her more in the coming years. My children, Heather, Theresa, Anthony, and Tim, survived growing up with a part-time Dad, but I think my absence was usually a positive (when I was home for a few weeks, I would often hear them ask their Mother, "When is Daddy leaving so we can ___? *fill in the blank*). My added family, Chris, Matt, Emily, and Angela (sometimes known as "the outlaws"), have brought extra joy the past 20 or so years. And of course my beautiful grandkids, Kathryn, Emily, Luke, Drew, Gracie, and Grady keep Lou and me on our toes and young in spirit.

It's been a blast!

Steve McKeand
May 2017

Steve is not sure if all these folks are happy about his decision to retire, but we know that he is looking forward to spending more time with all his family.

SELECTION, BREEDING, AND TESTING

Fourth Cycle Breeding and Testing Progress

Breeding for the Cooperative's 4th-Cycle began in 2013. A mating list was developed using a differential evolution algorithm developed by Kinghorn (2011, Genetics Selection Evolution 43(1):4. https://doi.org/10.1186/1297-9686-43-4) to optimize genetic gain while maintaining a diverse genetic base for the subsequent generation. There was also seed in hand from the tail end of 3rd-Cycle breeding that was rolled over (the substitute crosses). After six breeding seasons, *we are excited to report that the target number of crosses and number of parents have been exceeded for the Coastal population and are 97+% of the targets for the Piedmont and Northern populations* (Figure 1).

There was a big push in 2018 to get the remaining parents bred, and deviations from the original cross list were encouraged to circumvent situations where one parent was not available (such as poor flowering, early flowering, or pollen inventory errors). The genetic gain expected from the original breeding plan compared to the gain projected from the actual crosses bred through 2018 was either surpassed (for the Coastal and Piedmont populations) or very similar (for the Northern population) (Figure 2). The 4th-Cycle breeding has been a tremendous effort by the Cooperative members and by Cooperative staff at the Arrowhead Breeding Center in Cochran, GA. We expect that additional 4th-Cycle breeding will be limited to crosses with poor conelet survival or seed harvest.

Cooperative members were more productive in 2018 compared to spring 2017, which was characterized by an early spring that caught many off guard and a late freeze that damaged tender conelets in some orchards. This was especially true for the Piedmont and Northern populations. Spring 2018 was also the most productive for Cooperative staff at the Arrowhead Breeding Center, with a record number of crosses made (509 pollination bags installed to breed 270 crosses (including ACE2 and other crosses) with 185 different pollen parents).

The Arrowhead database on **TIPRoot** allows Cooperative staff to store information associated with all aspects of breeding, which becomes particularly valuable as data accumulates for clones in the breeding orchard. For

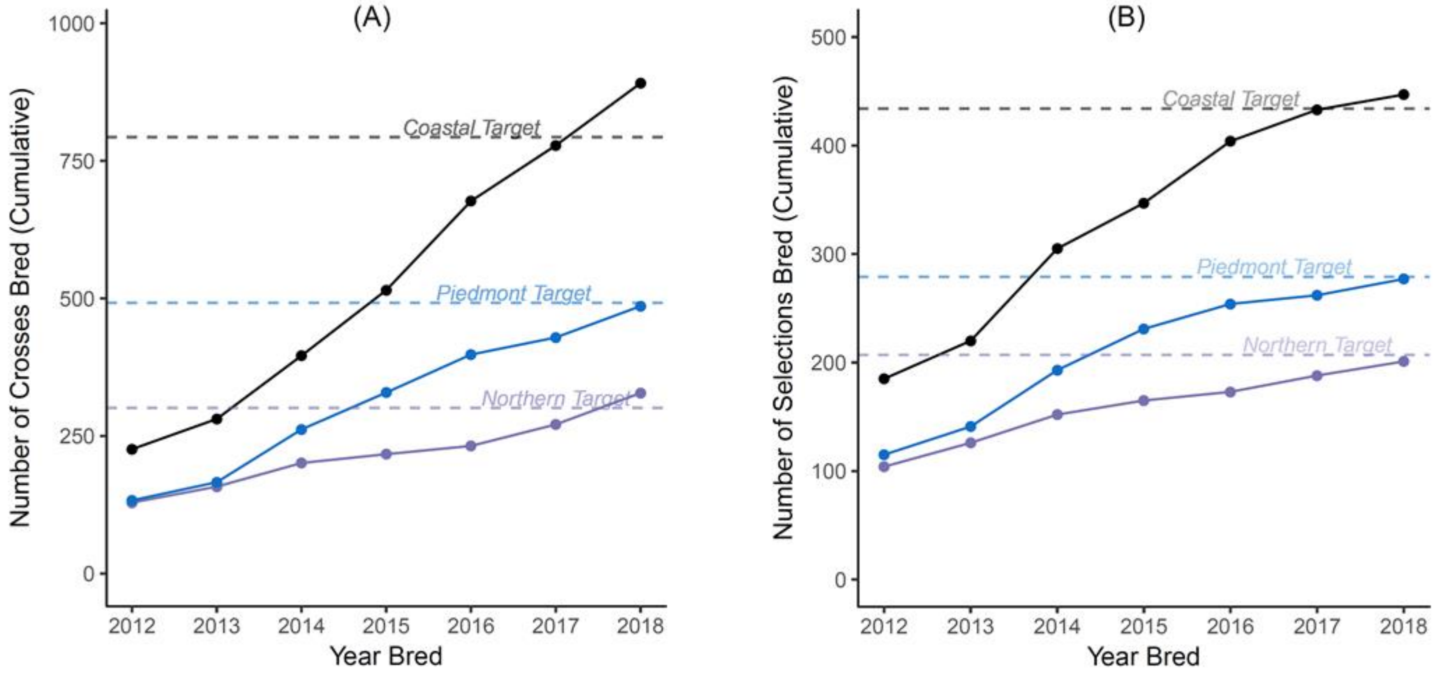


Figure 1. *Cumulative number of unique crosses bred (A) and unique parents bred (B) throughout the 4th Cycle along with targets from the breeding plan. The Coastal targets have been met, and the Piedmont and Northern targets are within 97% of completion. Crosses bred before 2017 were only counted if more than 90 seeds were harvested; crosses bred in 2017 and 2018 were counted if more than 5 flowers were bred. Crosses/parents bred by 2012 represent the Substitutes rolled over from the 3rd-Cycle.*

example, Cooperative staff perform flower counts in early February for the list of clones that are planned for breeding that spring. With observations for six breeding seasons, we can begin to characterize clones as either prolific or poor for flower production, which can be invaluable information for orchard managers. However, not all clones are visited each year, and older grafts tend to be bigger and produce more flowers, therefore, we used a generalized linear mixed effects model to account for the effects of graft age, orchard, and year. The predicted flowers per graft are shown for clones with multiple years of data in Figure 3. The clone at the 50th percentile had a prediction of around 25 flowers per graft at four years after grafting. The poorest ranked clones produced little to no flowers over multiple years, whereas the most productive often had too many flowers to count!

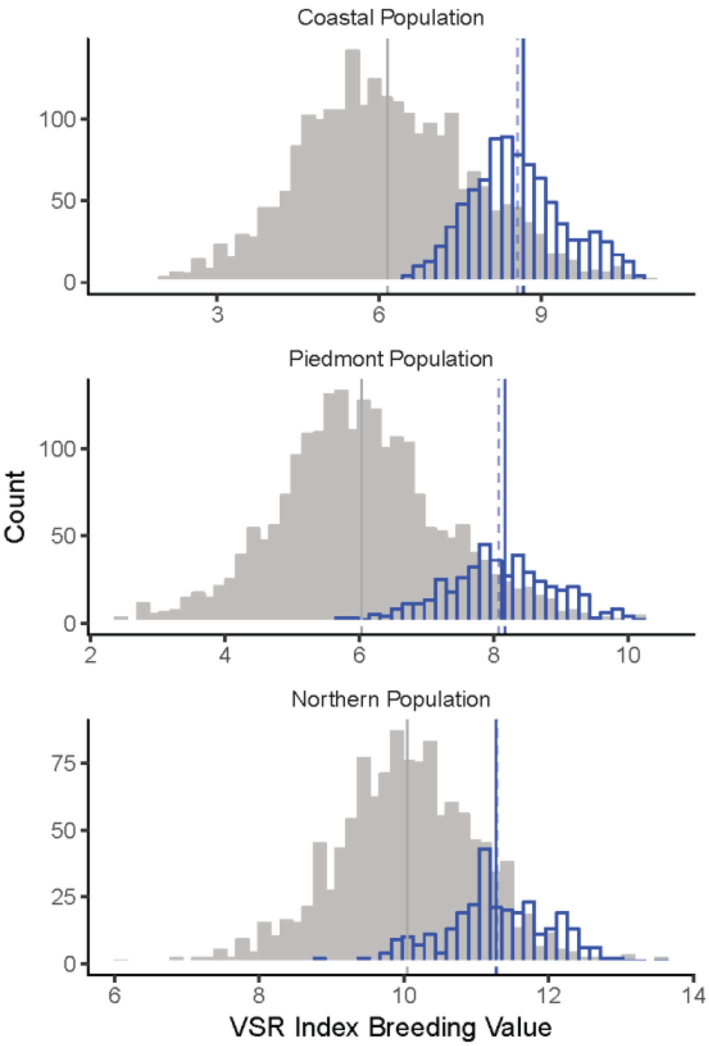
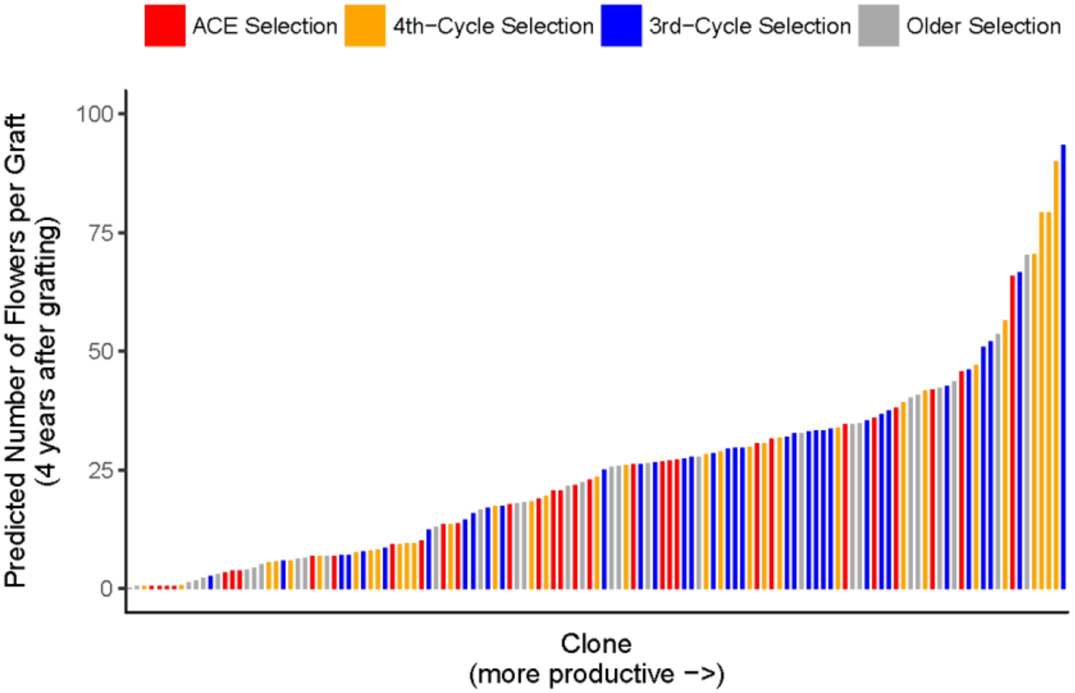


Figure 2. (Left) *Distribution of Volume-Straightness-Rust (VSR) index breeding values for all parents tested in the 2nd and 3rd cycles (gray bars) and the mid-parent values for 4th-Cycle crosses bred through 2018 (blue bars). Solid blue lines are the mean of the bred population, and dashed blue lines are the targets from the MateSelect breeding plan. VSR index breeding values for the Coastal and Piedmont populations were calculated with the following weights: 60% volume, 20% straightness, and 20% rust disease incidence; for the Northern population the weights were 70% volume and 30% straightness due to a lack of rust breeding values.*

Figure 3. (Right) *Flower productivity predictions by clone based on annual flower counts since 2013 at the Arrowhead Breeding Center. A generalized linear mixed effects model was used to account for clone, orchard, and year effects. Predictions are shown for four years after grafting.*



Progeny Testing Update

The 4th-Cycle testing strategy employs several methods to increase testing speed and efficiency. The most notable method is the “rolling-front” design, where crosses that are generated by the breeding program are sown for testing the spring after they are harvested. This results in an increasing pool of selection candidates for the 5th Cycle as the 4th-Cycle testing effort progresses. For example, the first 4th-Cycle tests were planted in 2014, and by 2019 there will be enough measurement data to make selections among 96 crosses for the Coastal population, which represents 12% of the breeding (Table 1). Based on the number of tests installed to date and the number of seed presently being sown for planting in 2019, more than 60% of the crosses are expected to have sufficient measurement data to make selections by 2023 for the Coastal and Piedmont populations and 2024 for the Northern population (Table 1). The remaining selections depend on the success of pollinations made in 2017 and 2018.

Another aspect of the rolling front testing design is the need to connect crosses that are tested in different years. This is being done in the 4th Cycle by staggering the number of replicates for a full-sib family across multiple testing years, with seed beginning testing as soon as it as harvested and continued testing the following year. For example, 85 crosses were tested in the Coastal tests planted in 2014, and of those, 79 continued testing the following year alongside new crosses from the breeding effort (Table 1). This provides very strong connectivity, linking crosses that were first tested early in the 4th Cycle to crosses that will be tested in the final years. Starting in 2017, the Cooperative accelerated the testing program by establishing twice as many replicates per year and completing the testing for some crosses in one year. At this rate, 2021 may be the last year of 4th-Cycle test planting, assuming that the crosses bred in spring 2017 and 2018 are successful.

Another important point in the 4th-Cycle testing design is the inclusion of regional overlap among genotypes in the testing population. To evaluate how parents from the

***Table 1. (Right)** Testing progress by 4th-Cycle population for crosses in their native population through 2019. The 2018 tests have recently been planted, and seed for the 2019 tests are currently being grown in containers. The number of crosses that begin testing is reflective of the breeding success three years prior (e.g. crosses bred in 2016 are field planted in 2019). The number of crosses that have completed testing (more than 40 reps per cross) represents the pool of selection candidates for the 5th Cycle, which is expected to reach more than 60% of the breeding plan by 2019 for all three populations.*

			# of Crosses in Breeding Population			
Testing Population	Year Planted	Measurement Year	Begin Testing	Continue Testing	Completed Testing	% Completed Testing
Coastal	2014	2018	85			
(Cycle4C)	2015	2019	46	79	96	12%
	2016	2020	124	47	144	18%
	2017	2021	106	123	289	36%
	2018	2022	112	78	401	51%
	2019	2023	169	73	511	64%
Piedmont	2014	2018	40			
(Cycle4P)	2015	2019	57	38	23	5%
	2016	2020	39	51	74	15%
	2017	2021	83	52	138	28%
	2018	2022	77	75	214	43%
	2019	2023	71	86	299	61%
Northern	2014	2019	45			
(Cycle4N)	2015	2020	41	33	8	3%
	2016	2021	36	47	21	7%
	2017	2022	44	52	80	27%
	2018	2023	21	56	146	49%
	2019	2024	15	48	196	65%

Coastal breeding population compare to the Piedmont and Northern populations, many Coastal crosses are being tested in those testing populations. The number of crosses tested and planned for testing through 2019 are shown by testing population in Figure 1. For example, of the almost 600 crosses tested/being sown for testing in the Piedmont testing population (Cycle4P), about 100 are of Coastal genetic origin and another 100 of Northern genetic origin. So far, the Cooperative has established 75 tests for the 4th Cycle through 2017 across the southeastern US (Figure 2), which is expected to produce rigorous assessments of genetic values across the region.

Progeny testing is hard work. For tests established through 2016, at least 2 tests in each population had poor survival due to drought, cold damage, pales weevil, or excessive weed competition (Figure 3). These serve as a reminder that to continue making genetic gain, good test site preparation and maintenance are essential. The large majority of tests have better than 90% survival and are expected to yield high-quality genetic estimates.

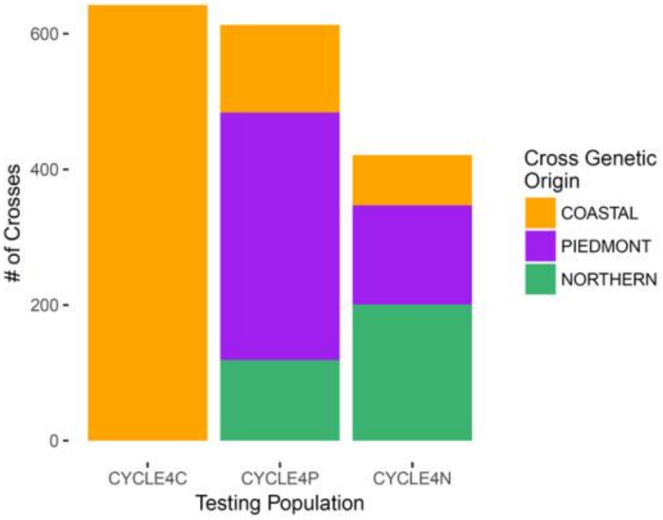


Figure 1. *Regional overlap/seed source movement design of crosses tested and planned for testing through 2019 in the 4th Cycle. The Coastal tests (Cycle4C) only contain genetic material of Coastal origin, while the Piedmont (Cycle4P) and Northern (Cycle4N) tests contain material from all three genetic origins.*

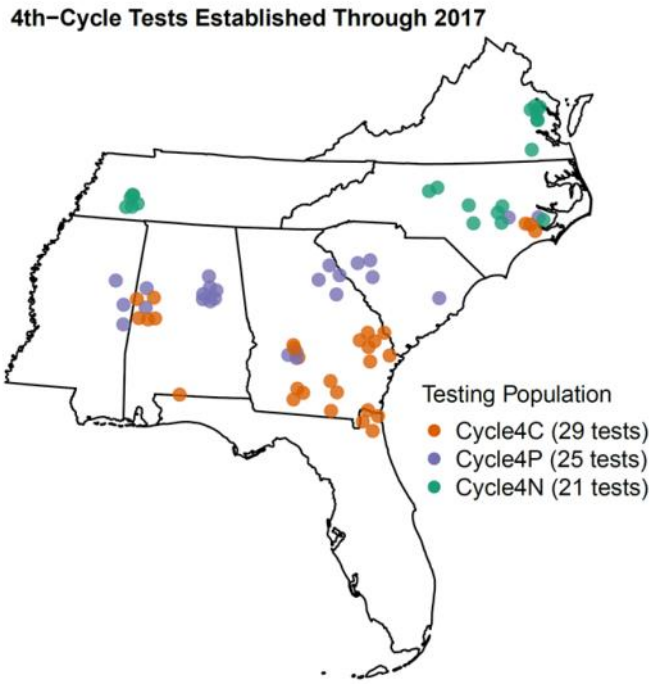
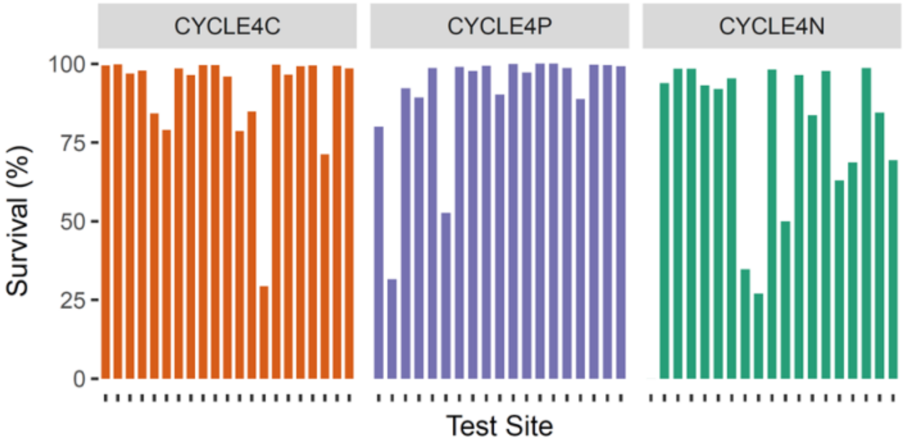


Figure 2. *Approximate locations for 4th-Cycle tests established through 2017.*

The 3rd-Cycle tests have all had base measurements, but a few are planned for wood quality measurement in summer 2018. The first 4th-Cycle test measurements were made this past winter, and are expected to continue until 2024. For each cycle, the Cooperative has improved their progeny testing experimental design, which begs the question "what new features will be included in the 5th-Cycle testing design?".

Figure 3. (Right) *Percent of test trees surviving after one growing season for 4th-Cycle tests established through 2016 for each testing region (Cycle4C = Coastal, Cycle4P = Piedmont, Cycle4N = Northern).*



Planning for the Future at Arrowhead Breeding Center

In the spring of 2017, TIP staff came up with a plan to ensure that we will have adequate rootstock available for topgrafting the selections that will come from 4th-Cycle testing over the next few years. The first step to determine the number of rootstock needed was to predict the number of selections that will be made from 4th-Cycle tests each year (Table 1). These projections are our best guesses, given that the 5th-Cycle breeding strategy has not yet been developed. We are assuming that the population size will be similar to what we have in the 4th Cycle, but this has not yet been determined.

Once the estimated number of grafts to be made each year was determined, we looked at existing rootstock at the Arrowhead Breeding Center in Cochran, Georgia. Existing orchards should provide enough rootstock for grafting selections during the first 3 years (~400), however approximately 600 additional rootstock will be needed to complete 5th-Cycle breeding. To meet this demand, a new breeding orchard was created in the Fall of 2017, the Graveyard Orchard (Image 1).

During the spring and summer of 2017, TIP staff grew rootstock of the most rust resistant full-sib

Image 1. *The Graveyard Orchard is approximately 10 acres and will be where much of the 5th-Cycle breeding will take place.*

Table 1. (Right) *TIP staff estimated the number of topgrafts that will need to be completed each year. This was done based on when adequate data will come in from 4th-Cycle tests. Different colored rows represent the estimated year that grafted selections can be bred.*

Selection Year	5th-Cycle Breeding Year	Estimated target # of selections to topgraft
2019*	2024	59
2020	2024	167
2021	2024	193
2022	2025	118
2023	2026	212
2024	2027	252
	Total # of 5th-Cycle Selections	1000

cross available in D-40 containers (Image 2). In October, graduate and undergraduate students planted the first half of this future breeding orchard (~6 acres) over their Fall Break (Image 3). The additional 4 acres are to be planted in the fall of 2018. Rootstock for this area is already up and growing here in Raleigh.

This breeding orchard was planted at a 25’ x 25’ spacing. We will graft the interstock clone (181210[1]) next spring to have the breeding orchard ready for topgrafting by 2022. The intention of this orchard is to graft a single selection into each position, fully replacing the crown of each tree with the desired selection. Approximately 3 years after topgrafting, each tree should be producing adequate flowers to complete the breeding. With this in mind, the expected life cycle of these breeding orchards is to be approximately 10 to 12 years from time of planting to time of completed breeding. Our goal is to complete breeding on each tree as quickly as possible and then recycle the orchard in a rolling front design. With proper planning, we aim to minimize space needed to complete future breeding and maximize efficiency with less traveling in bucket lifts between grafts and less time spent going up and down by breeding in smaller trees with a compact orchard design.

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[1] 181210 is an excellent interstock clone that enhances topgrafting success and flowering (See McKeand and Raley. 2000. Forest Genetics 7:179-182).

Image 2. (Left) *Rootstock shown to the left, were sown in May of 2017 at the Horticulture Field Lab in Raleigh, NC and planted at the Arrowhead Breeding Center in Cochran, GA in October of 2017. Rust resistant rootstock was grown in D-40 (40 in^3 of soil per cell) producing a very nice seedling. Assessment in spring 2018 indicated 100% survival.*

Image 3. (Right) *TIP staff, graduate students, and undergraduates planting the Graveyard Orchard rootstock.*

Big BLUP and PRS™ Values

Database Updates

There have been a few new functionalities added to TIPRoot in the past year, described below. As always, please contact TIP Staff if you ever have any questions or need assistance with the database.

Mid-Parent Values

Contributing and Full members have access to a new tool that provides the mid-parent PRS™ scores or mid-parent breeding values for a cross or list of crosses (Screenshot 1). This information was previously only available when creating a Spec Sheet. The tool was primarily designed to allow users to obtain estimates of the breeding value of untested crosses. Mid-parent values are also returned for tested crosses. A column labelled "Test Status" is present to show users which crosses from the list have been field tested (Screenshot 1). For tested crosses, users are referred to the "PRS™ Values" and "Genetic Values" tool, where they can obtain estimates that take into account the test data and specific combining ability of the cross.

PRS Version: ✔ 2017 V5 (COASTAL)

Deployment Checklot: CC2 - North Carolina Coastal Plain

Results from selection file: testMP.xlsx

Download these data: CSV Excel

Parent1	Parent2	MinTemp.	Volume	Height	Rust	Rust Grade	Strt	Strt Grade	Fork	Fork Grade	Test Status	Wide Cross
ABC	DEF	15.32	54	20	27	C	25	C	47	C	NOT TESTED	
GHI	JKL	12.33	52	14	50	E	31	B	43	A	NOT TESTED	
MN	PQR	12.92	58	21	2	A+	19	D	42	A	NOT TESTED	
STU	VWX	13.17	50	19	51	E	35	A	43	B	NOT TESTED	

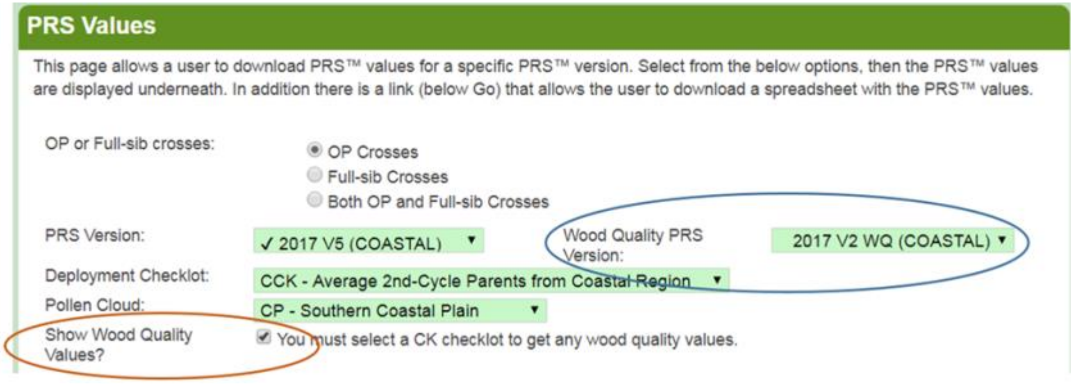
Screenshot 1. *The Mid-Parent Values tool returns the expected PRS™ scores or breeding values for a list of crosses. The expected value for a cross is the mean of the parents' breeding values. Users can get the mid-parent values for a single cross by filling in the Selection ID text boxes or by uploading an Excel workbook with two columns: "parent1" and "parent2".*

Wood Quality PRS™ Update

Contributing and Full Members will notice that the PRS™ Values and Breeding Values pages were updated to include wood quality traits. These values were estimated from 3rd-Cycle tests measured around age 6-9 years to assess juvenile wood. There is now a checkbox to include wood quality values (Screenshot 2). When checked, the user is prompted to select a wood quality PRS™ version. At this time, the Coastal and Piedmont populations each have one wood quality version, and the Northern will get a wood quality version after the final tests are measured in summer 2018. The Coastal population has estimates for around 280 families and the Piedmont has around 200 families. PRS™ values are only available for open-pollinated families and the CCK, PCK, or NCK checklot for the respective region.

There are two wood quality traits in the PRS™ system: Resistance Amplitude and Acoustic Velocity^2, which are surrogates for wood density and wood stiffness, respectively. Higher values indicate denser and stiffer wood, respectively. Check out the Wood Quality Update section in this report and the 2016 Annual Report for details on how these values are estimated.

Screenshot 2. (Right) *Checkbox option to show wood quality values (orange circle). When checked, the user will be prompted to select a wood quality PRS™ version (blue circle).*



PRS Values

This page allows a user to download PRS™ values for a specific PRS™ version. Select from the below options, then the PRS™ values are displayed underneath. In addition there is a link (below Go) that allows the user to download a spreadsheet with the PRS™ values.

OP or Full-sib crosses: ● OP Crosses ○ Full-sib Crosses ○ Both OP and Full-sib Crosses

PRS Version: ✓ 2017 V5 (COASTAL)

Wood Quality PRS Version: 2017 V2 WQ (COASTAL)

Deployment Checklot: CCK - Average 2nd-Cycle Parents from Coastal Region

Pollen Cloud: CP - Southern Coastal Plain

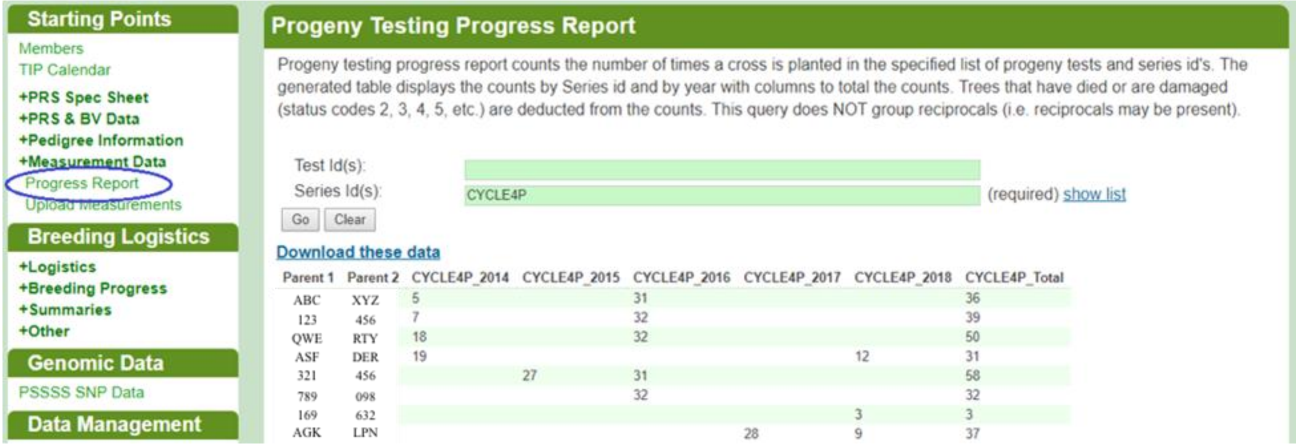
Show Wood Quality Values? ☑ You must select a CK checklot to get any wood quality values.

Testing Progress Report Query

Full members have access to a new query that displays the number of trees by genotype that have been tested for a requested series or test (Screenshot 3). The counts are subtotaled by year of test establishment, and trees that are dead or damaged (i.e. not status 1) are removed when measurements are available. While the query was built to track 4th-Cycle testing progress, the query also works for 3rd-Cycle tests. This query does not lump reciprocals, so crosses bred in both directions will show up as different rows.

Clonal Progeny Tests

TIPRoot was expanded to store data from clonal progeny tests. This functionality was tested by uploading the Atlantic Coastal Elite clonal progeny tests. This does not affect the ***PRS***™ scores, which are only available for parents that have been progeny tested.



Progeny Testing Progress Report

Progeny testing progress report counts the number of times a cross is planted in the specified list of progeny tests and series id's. The generated table displays the counts by Series id and by year with columns to total the counts. Trees that have died or are damaged (status codes 2, 3, 4, 5, etc.) are deducted from the counts. This query does NOT group reciprocals (i.e. reciprocals may be present).

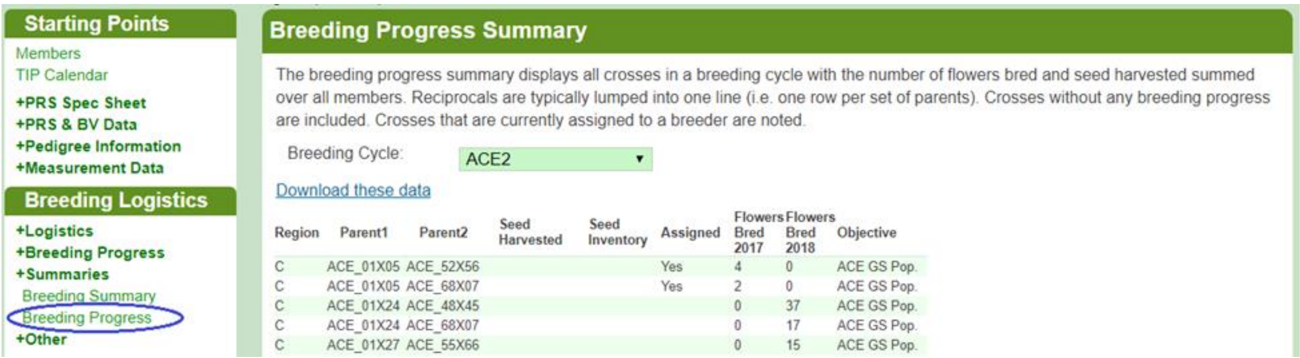
Test Id(s):
Series Id(s): CYCLE4P (required) show list

Parent 1	Parent 2	CYCLE4P_2014	CYCLE4P_2015	CYCLE4P_2016	CYCLE4P_2017	CYCLE4P_2018	CYCLE4P_Total
ABC	XYZ	5		31			36
123	456	7		32			39
QWE	RTY	18		32			50
ASF	DER	19				12	31
321	456		27	31			58
789	098			32			32
169	632					3	3
AGK	LPN				28	9	37

Screenshot 3. *Testing progress query result for the 4th-Cycle Piedmont tests (Series ID = CYCLE4P). Use the 'show list' button to see the available series choices. The number of trees by family and checklots included in the series are shown by year of planting. Trees that are dead or damaged are removed (i.e. only status 1 trees included) when status information is available.*

Breeding Progress Query

Full members have access to a new query that displays the number of flowers bred by year for all crosses in a selected breeding cycle (Screenshot 4). The breeding work is summed over cooperators so that only the Cooperative-wide work is displayed. Unless the breeding cycle plan dictates reciprocal breeding, reciprocals are summed over and lumped into one row.



Breeding Progress Summary

The breeding progress summary displays all crosses in a breeding cycle with the number of flowers bred and seed harvested summed over all members. Reciprocals are typically lumped into one line (i.e. one row per set of parents). Crosses without any breeding progress are included. Crosses that are currently assigned to a breeder are noted.

Breeding Cycle: ACE2 ▼

Region	Parent1	Parent2	Seed Harvested	Seed Inventory	Assigned	Flowers Bred 2017	Flowers Bred 2018	Objective
C	ACE_01X05	ACE_52X56			Yes	4	0	ACE GS Pop.
C	ACE_01X05	ACE_68X07			Yes	2	0	ACE GS Pop.
C	ACE_01X24	ACE_48X45				0	37	ACE GS Pop.
C	ACE_01X24	ACE_68X07				0	17	ACE GS Pop.
C	ACE_01X27	ACE_55X66				0	15	ACE GS Pop.

Screenshot 4. *Breeding progress query results for the ACE2 breeding cycle. Number of flowers bred are displayed by year bred for all crosses in the selected cycle.*

Atlantic Coastal Elite 2 (ACE2) Breeding for Genomic Selection (Update)

Cooperative staff began breeding among the Atlantic Coastal Elite (ACE) clones in 2017, creating a second-generation ACE population (ACE2 population). The objective of the ACE2 breeding was to create a suitable population for evaluating genomic selection methods in loblolly pine, with an emphasis on mating selections with the best genetic values. The ACE and ACE2 populations are ideal to test and verify genomic selection in loblolly pine due to the small number of founders (21 original parents of the ACE population), cloned progeny, and relatively deep pedigree.

The original ACE population was mated in the early 2000s from 76 crosses among 24 founders using three diallels each with eight parents (EC-1, EC-2, and EC-3 as described in the 3rd-Cycle breeding plan). The seedlings from these crosses were challenged with fusiform rust inocula at the US Forest Service Resistance Screening Center in Asheville, NC in 2007, and 13 susceptible crosses were removed from the population leaving 53 crosses for progeny testing from 21 founders. An average of 46 full-sib progeny (2362 in total) from each cross were cloned in 2007 using rooted cutting methods. A genetically identical copy of each progeny 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. Rust incidence was very low (5.4%) due to the screening of seedlings prior to planting. A total of 204 candidate clones out of ~2362 were available for breeding at the Arrowhead Breeding Center. 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 three crosses to maintain diversity.

The ACE2 breeding was carried out in spring 2017 and 2018, a summary of the crosses with satisfactory conelet survival is in Table 1. There were 68 ACE2 crosses made among 71 ACE clones. Those 71 ACE clones represented 34 first-generation ACE families originating from 18 of the 21 ACE founders. The number of crosses that each of the 18 founders of the ACE2 population are associated with is shown in Figure 2.

About 3000 seedlings from the ACE2 population will be genotyped in 2020 with the 50K SNP array being developed for loblolly pine. Tissue will be collected from ACE clones and their parents in Spring 2018 (see details in 2017 Annual Report). They will be genotyped with the same 50K SNP array. A genomic prediction model will be developed based on marker-trait association to predict the phenotypes of ACE2 seedlings.

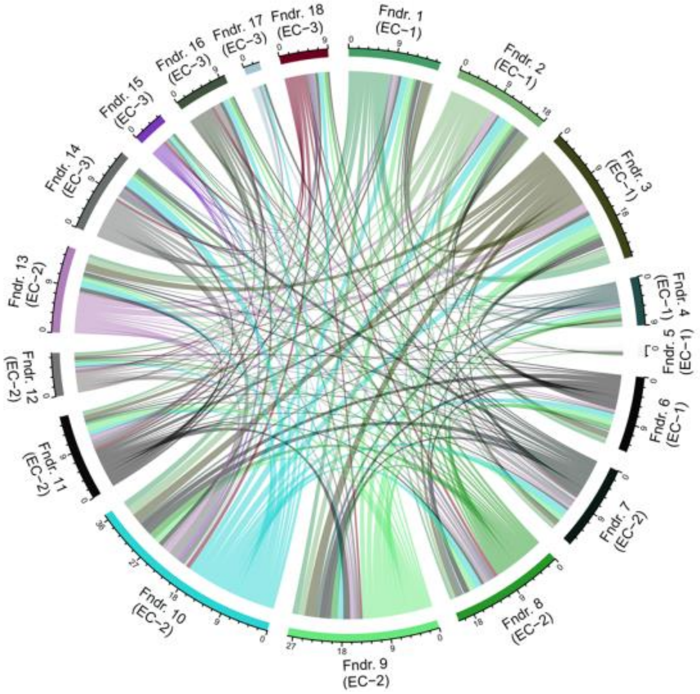


Figure 1. *Founder representation structure among ACE2 crosses with the number of ACE2 crosses where each founder appears as a grandparent on the outer arcs. The 3rd-Cycle elite diallels are given in parenthesis under the founder code. Founder 10 appeared as a grandparent in 37 ACE2 crosses, whereas Founder 5 only appeared in 2. Connecting lines indicate that both founders contribute to an ACE2 individual.*

Table 1. (Right) *Expected number of successful ACE2 crosses from breeding among ACE clones in 2017 and 2018. Additional details about the population are also provided.*

Number of ACE2 crosses made in 2017 and 2018	68
Number of ACE clones used in ACE2 breeding	71
Number of ACE crosses having a forward selection used in ACE2 breeding	34
Number of founder parents represented in ACE2 breeding	18
Expected number of seed (ACE2 population)	20,000

SEED AND CONE YIELDS

Cooperative members harvested nearly 70,000 pounds of loblolly pine seed from production orchards in 2017 (Table 1). The average yield of seed per bushel went up in all categories except in Northern 2nd-generation orchards. At approximately 12,000 seedlings produced per pound of seed, last fall's harvest has the potential to produce over 830,000,000 seedlings this year. That is enough to plant approximately 1,660,000 acres of pine plantations at 500 trees per acre! This past fall, Cycle 3+ seed comprised approximately 70% of all seed harvested while 2nd-generation seed comprised 30% of the harvest (Figure 1). This is a major shift from where the harvest was only four years ago when 2nd-generation seed still made up 50% of the total seed harvested.

Table 1. *Fall 2017 cone and seed yields compared with the figures from 2016 harvest*

Provenance / Orchard Cycle	Bushels of Cones		Pounds of Seed		Pounds of Seed per Bushel	
	2017	2016	2017	2016	2017	2016
Coastal 2.0/2.5	10,823	11,872	16,132	17,468	1.49	1.47
Coastal 3.0	17,749	14,938	27,206	22,255	1.53	1.49
Piedmont 2.0	2,548	2,913	4,223	4,664	1.66	1.60
Piedmont 3.0	12,192	7,889	18,637	10,037	1.53	1.27
Northern 2.0	957	106	724	109	0.76	1.02
Northern 3.0	2,024	6,290	2,546	6,814	1.26	1.08
Totals	**46,293**	**44,008**	**69,467**	**61,347**	**1.37**	**1.32**

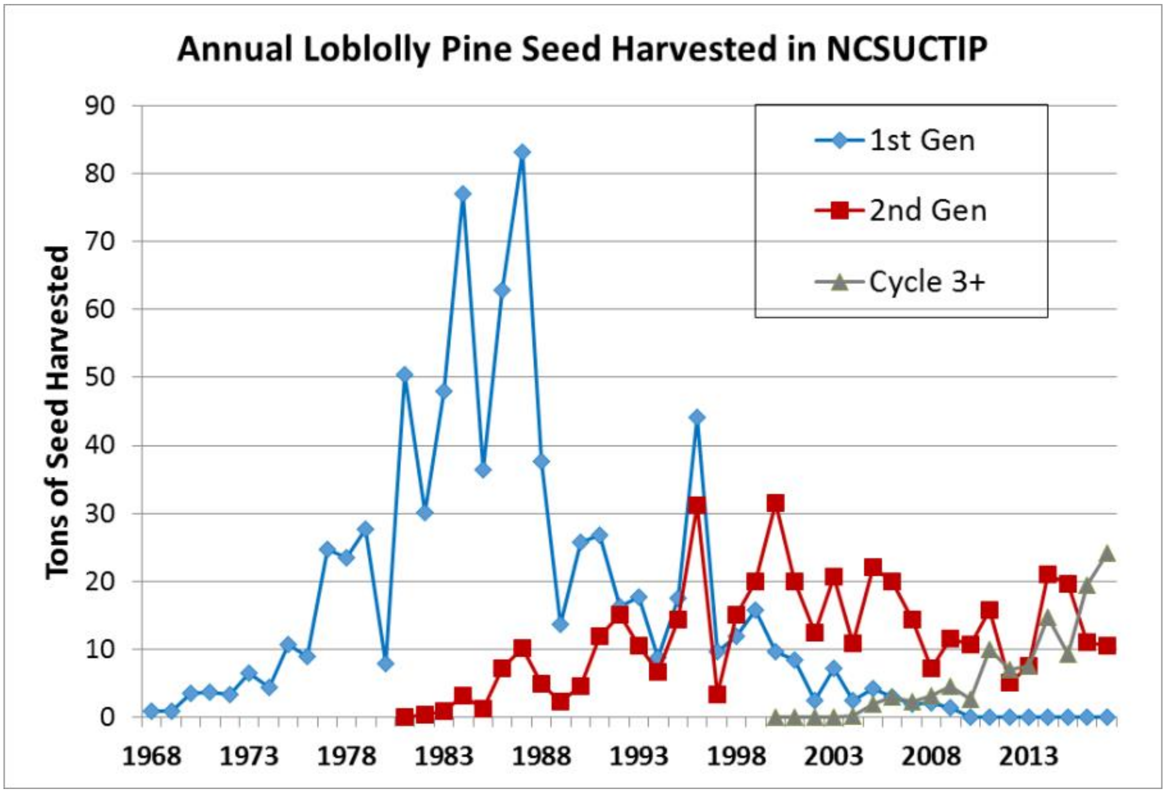


Figure 1. *Since 1968, the Cooperative has tracked annual seed yields from Cooperative members. 1st-generation seed orchards are no longer being harvested, but harvests from 2nd-generation orchards are still significant. Over the last two years, Cycle 3+ orchards have surpassed 2nd-generation orchards as the main seed producers, generating 70% of the seed in the 2017 fall cone harvest.*

RESEARCH

PBS Pollination Bag Study Update[1]

Since 2014, the Cooperative has organized and participated in the Pollination Bag Study, a cooperative effort to find a pollination bag that is superior for female strobilus survival and/or seed yield for mass production of controlled cross seeds in loblolly pine. In the spring of 2017, a third round of prototype testing began which included two new prototypes (PBS A2 and I2) along with the original PBS-A, a Lawson paper 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 with support wire.

Number of surviving conelets per bag was assessed in June of 2017, approximately four months post pollination. These data were combined with data from the previous two study years to compare strobilus survival in June for all tested bag types. Number of surviving conelets in June differed significantly (Pr<.0001) among bag types. Odds ratio estimates were calculated for each bag type compared to bag type L (the Lawson kraft paper pollination bag). The best bag for conelet survival in June was PBS-A2; female strobili bagged using PBS-A2 were 2.93 times more likely to survive to June than female strobili bagged using bag type L. Using Tukey comparisons among least squares means at an alpha of 0.05, PBS-A2 was significantly different than all other bag types for conelet survival in June, and PBS-I2 was significantly different than all bag types except for PBS-G and PBS-H.

June conelet counts indicate that the new bag design tested in the 2017 prototype study is superior to all other prototypes tested (Figure 1). The new design has a flap of material that is folded down at the top of the bag

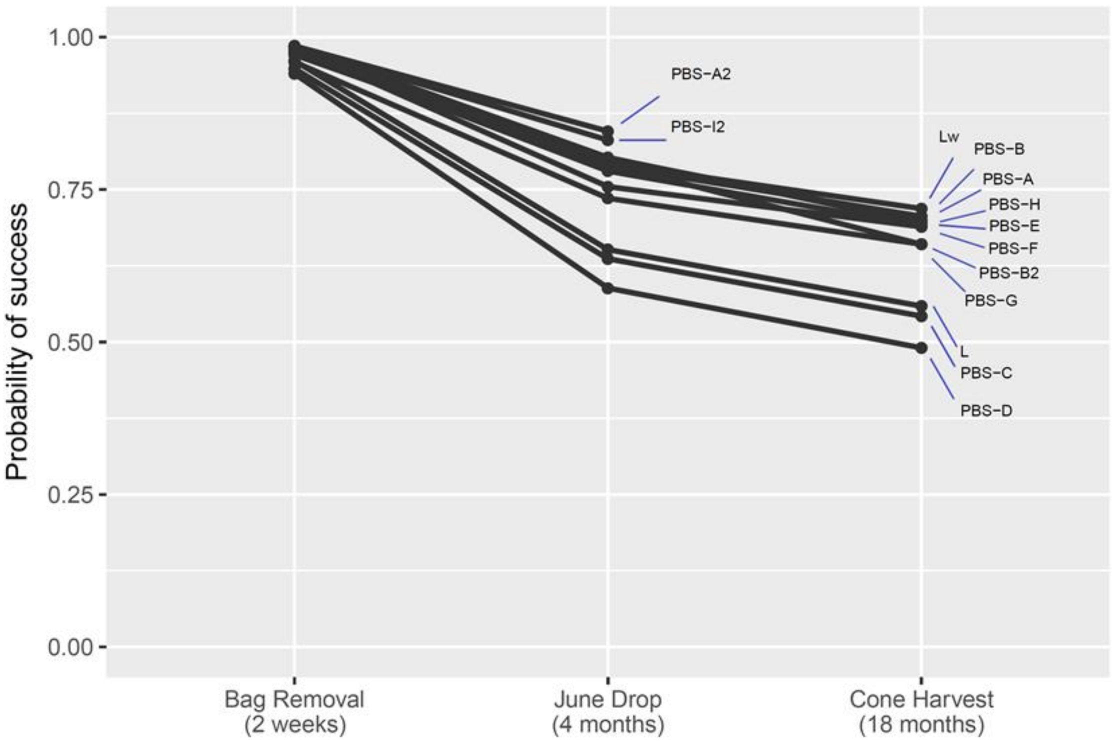


Figure 1. *Probability of female strobilus success per bag type from bag removal (two weeks post pollination) to June drop (four months post pollination) to cone harvest (18 months post pollination). The critical assessment for survival due to bag type effects appears to be at June drop, four months post pollination. The data clearly show two different groups for June drop. Bag types L, PBS-C, and PBS-D showed lower success compared to the rest of the bags. (Source: Heine, J. Austin. 2018. "Comparison of Pollination Bags for Mass Production of Controlled Cross Seeds in Loblolly Pine"http://www.lib.ncsu.edu/resolver/1840.20/35063).*

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

(Figure 2). It appears that these flaps are reducing the amount of wind that the bags are catching, making the bags more streamlined in the wind. Also, the flaps appear to reinforce the upper shape of the bag, giving it more rigidity and preventing the bag from collapsing inward. Keeping the top of the bag from collapsing inward could be important in reducing damage to female strobili as terminal shoots begin elongating, and female strobili move upwards inside of the bag.

Results from the time and motion study indicated that PBS A2 can be installed at least 30 seconds faster per bag than Lawson pollination bags with a support wire (Table 1). No significant differences were found between bag types for time pollinating. At bag removal, PBS A2 could be removed approximately 10 seconds faster per bag than Lawson pollination bags with a support wire. Saving at least 30 seconds per bag at time of bag installation means that in an 8-hour work day (28,800 seconds), a crew could install an additional 960 bags per day compared with a kraft pollination bag with a support wire. Female strobilus development can move very quickly some years (2018 was an excellent example) and being able to install additional bags could be a huge benefit!

Figure 2. *Bag type A2 (photo courtesy of Nick Muir – International Forest Company). Side flaps potentially reduce wind resistance and reinforce the upper shape preventing bag collapse.*

Image 1. (Left) *Seed orchard with PBS bags being installed operationally in large quantities.*

Table 1. *Least squares means for time to install, pollinate, and remove bag types PBS-A2 and Lawson with a support wire. Significant differences (Pr≤0 .056) for bag type was found at time of bagging and time of bag removal.*

	LS Means Time in Seconds		
Bag Type	**Bagging**	**Pollinating**	**Bag Removal**
A2	35.12	16.15	24.33
Lw	65.79	16.38	34.57
Difference	30.67	0.23	10.24

Longleaf Pine Progeny Test Update

The Cooperative initiated the Longleaf Pine Regional/ Provenance Progeny Trial in 2010, a common garden experiment with 138 open-pollinated seedlots planted in 10 test sites established in 2011. Unfortunately, the infamous drought of 2011 caused significant mortality, and only seven of the test sites were retained. Gaps were re-planted with filler trees before the second growing season to minimize variation in tree to tree competition. The map in Figure 1 displays the location of the remaining test sites as well as the county of origin of the 138 seedlots tested (where information was available).

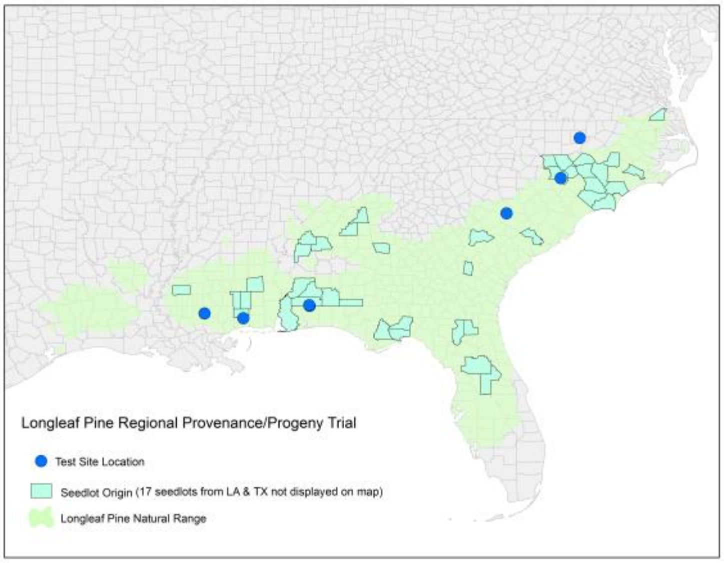

Figure 1. (Right) *Test site location for 7 test sites (blue dots) and county of selection origin (teal green color counties) for the 138 open-pollinated seedlots tested in the Longleaf Pine Regional Provenance/Progeny Trial. There are two test sites in close proximity in Escambia County, AL. There are 17 additional seedlots from LA and TX not displayed on map.*

Age 4 to 6 measurements have been made on most of the test sites through 2017. In this report, we present an analysis for tree height. Descriptive statistics for the tree height data are shown in Table 1. Test sites 8, 9, and 10 had low mean heights and high variability (coefficient of variation greater than 60%), and were dropped from this analysis.

A compound symmetry genetic variance structure in a mixed model was used to partition the variability in height measurements into site, seedlot, site x seedlot interaction, rep nested within test site, and residual error. The individual tree narrow-sense heritability was 0.09, and family mean heritability was 0.28. Low heritability estimates are likely due to poor survival. The genetic correlation across sites was 0.67. The breeding values ranged from 16% taller than the population average (a parent selection from Texas) to 18% below the population average (a selection from Mississippi) (Figure 2). There was no apparent provenance effect on the height breeding values (Figure 2), with a wide range in parental performance for height in each region evaluated. The genetic effects of height at each site are shown in Figure 3, which indicates that the best (and worst) families had relatively consistent performance across the three sites evaluated.

Continued measurements are planned for the test sites with priority given to those without an age 6 measurement. Analysis of other traits (survival, diameter, disease incidence, and stem form) are currently in the works.

Test Site	Age (years)	# Test Trees	# Reps	# of Seedlots	Avg. Height (ft.)	CV Height (%)
LL1 - 2	6	1338	10	107	7.5	23.8
LL1 - 3	6	1415	10	112	12.5	36.2
LL1 - 4	4	1669	10	120	8.8	38.3
LL1 - 8	4	571	10	74	1.4	96.0
LL1 - 9	4	662	10	64	3.4	64.2
LL1 - 10	6	657	10	57	4.4	84.4

Table 1. (Left) *Descriptive statistics for test sites used in analysis of longleaf pine tree height.*

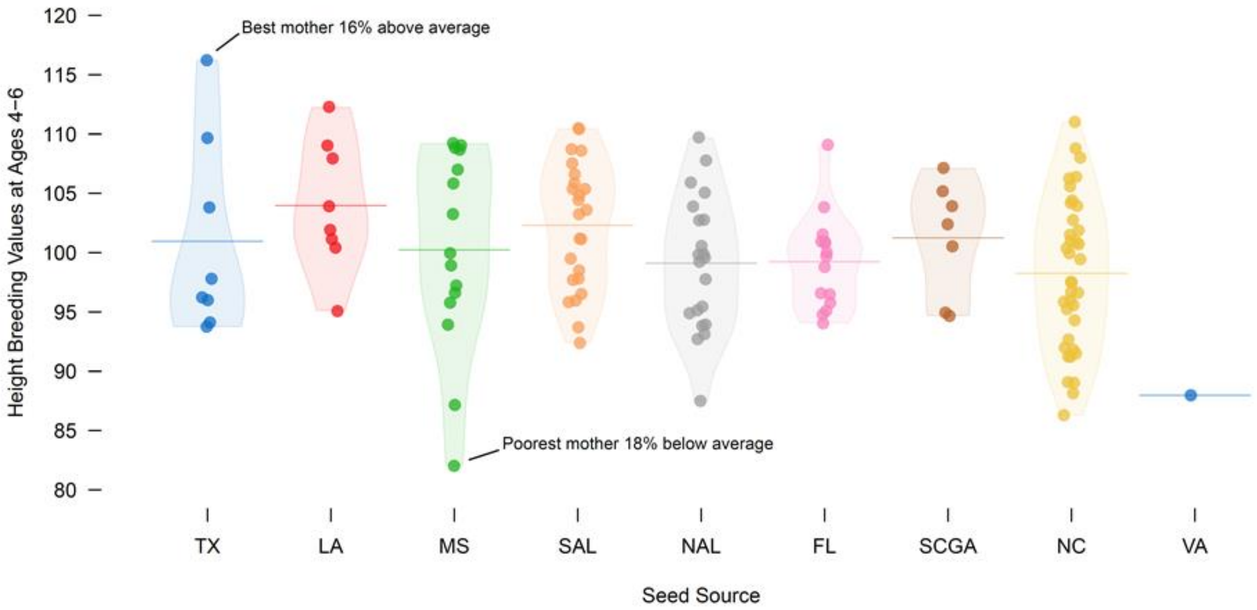

Figure 2. *Distribution of height breeding values for longleaf pine seed sources estimated from open-pollinated seedlots in a common garden experiment with 3 test sites. Provenance did not have a strong effect on breeding values, and there was a wide range in performance within most provenances.*

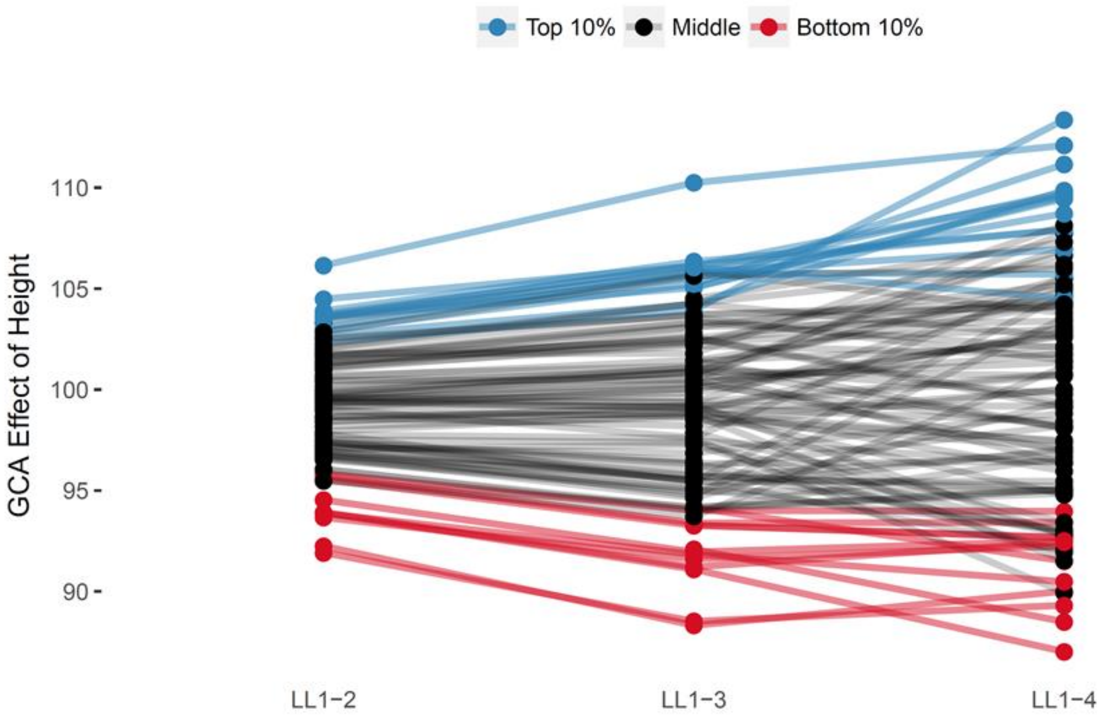

Figure 3*. Consistency of open-pollinated seedlot effects across three test sites. The top performing families are colored blue and the poorest colored red. There was some minor genotype by environment interaction (genetic correlation across sites 0.67), but the best and worst families were relatively stable across test sites.*

Loblolly Pine Biomass Genetics/Cropping Study (Butner, NC)- Year Six[1]

Loblolly pine can be an ideal feedstock species for bioenergy. It grows fast, and it does not compete with America's food production and agricultural lands. Substantial genetic differences exist among provenances and families that influence biomass/biofuel production. By planting genetically superior trees with desirable biomass/biofuel traits, it may be possible to substantially increase the amount of biomass produced at a given site.

In 2012, twenty of the Cooperative's fastest growing open-pollinated families, each with varying potential for sawtimber and biomass production, were planted at a high density spacing of 6' x 7' (1,037 trees per acre) on a northern Piedmont site at the North Carolina Department of Agriculture & Consumer Services Umstead Farm in Butner, NC. Ten families from the Atlantic Coastal Plain and ten families from the Piedmont regions were selected. For each region all families were selected for fast growth (volume), but half were also selected for good stem form to give high sawtimber quality.

A primary objective of the study is to evaluate the options of planting loblolly pine as a dedicated energy crop to provide options both for early harvest for biomass and/or longer rotations for sawtimber production. Another important objective is to evaluate the risk/reward of planting fast-growing Coastal families in an area where they are less adapted to the climate than the Piedmont families.

As expected at age three years, most Coastal families were substantially taller than Piedmont families, but were prone to forking. Soon after the third growing cycle, there were two severe ice/snow storms accompanied with extremely cold temperatures (low of 9°F), providing an opportunity to assess cold damage. Coastal families experienced more storm damage in the form of branch loss and main stem breakage (details are in the 2016 Annual Report). Since the ice storm, most Coastal families continue to outgrow Piedmont families, despite the fact they had a higher incidence of crown damage than Piedmont families.

This past winter of 2017-18, when trees were six years old, total height, diameter at breast height, sawtimber potential scores, and presence of forking and ramicorn branches were assessed. In addition, wood quality measurements were obtained. Using year five and year six data, we calculated volume per acre for each family.

For total volume yield, the difference between provenances was large. On average, Coastal families produced about 45 ft^3 per acre more at age six compared to Piedmont families (Table 1). But for sawtimber, Piedmont families had significantly more volume in trees with sawtimber potential (816 ft^3/acre) than Coastal families (763 ft^3/acre) (Figure 1). Even though Coastal families produced more total volume, they were more

Table 1. *Mean diameter, height, and volume for Coastal and Piedmont provenances at years three, five, and six.*

	Year 3		Year 5			Year 6		
Provenance	DBH (in.)	Height * (ft.)	DBH* (in.)	Height * (ft.)	Volume* (ft^3/ac)	DBH* (in.)	Height * (ft.)	Volume* (ft^3/ ac)
Coastal	-	9.20	3.71	18.8	902	4.43	24.1	1457
Piedmont	-	8.63	3.68	18.3	865	4.40	23.6	1411

* *Denotes statistical significance for provenance effect.*

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[1] *This is a summary of Jessica Maynor's Masters research funded by the NC Department of Agriculture & Consumer Services Bioenergy Research Initiative*

susceptible to storm damage and thus tended to have lower sawtimber potential than Piedmont families.

Wood stiffness (modulus of elasticity) and wood density are among the most important wood properties. TreeSonic, an acoustic wave device, was used to obtain time-of-flight as a surrogate for outer-wood stiffness. Faster time of flight values are associated with stiffer wood, so small values for time of flight are more desirable. Coastal families tended to have less variation and had significantly faster time of flight than Piedmont families (Figure 2) indicating that Coastal families had stiffer wood and potentially better quality sawtimber. Resistograph measurements were also taken to examine wood density; there was not a significant difference among the provenances, but family differences were found within each provenance (Figure 3).

For a landowner in the Piedmont, planting high volume producing families from the Coastal provenance may result in larger volume yields, but much of that yield may only be useful for biomass rather than sawtimber due to cold and ice damage. The different harvest regimes may be valuable to landowners because they can demonstrate options for planting loblolly pine as a dedicated energy crop for early harvest for biomass. If not harvested for biomass, the same stands can be thinned for sawtimber production.

The Butner trial is a long-term study that will be maintained through rotation. A clearcut and thinning treatment is planned for about age 8 to 10 years. The thinned treatment will reduce density to 259 trees per acre to allow the stand to develop into sawtimber and for future assessment. The harvested treatment will be used to assess genetic differences for biomass/biofuel production.

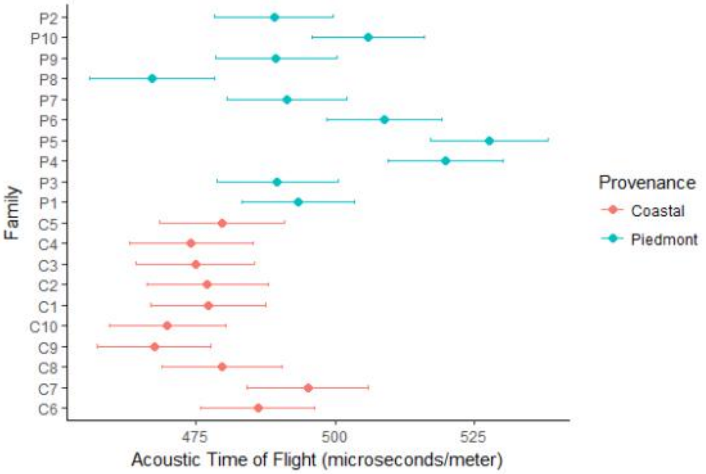

Figure 2. *Acoustic time of flight for each family by Provenance. Coastal families (pink) had less variation and faster time of flight values (i.e. stiffer wood) than did Piedmont families (blue).*

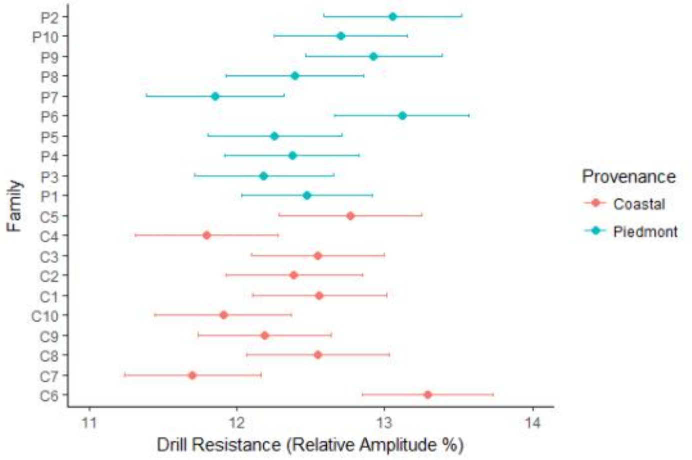

Figure 3. *Drill resistance for each family by Provenance. There was not a significant difference in drill resistance values (i.e. wood density) between the provenances, but family differences within each provenance were significant.*

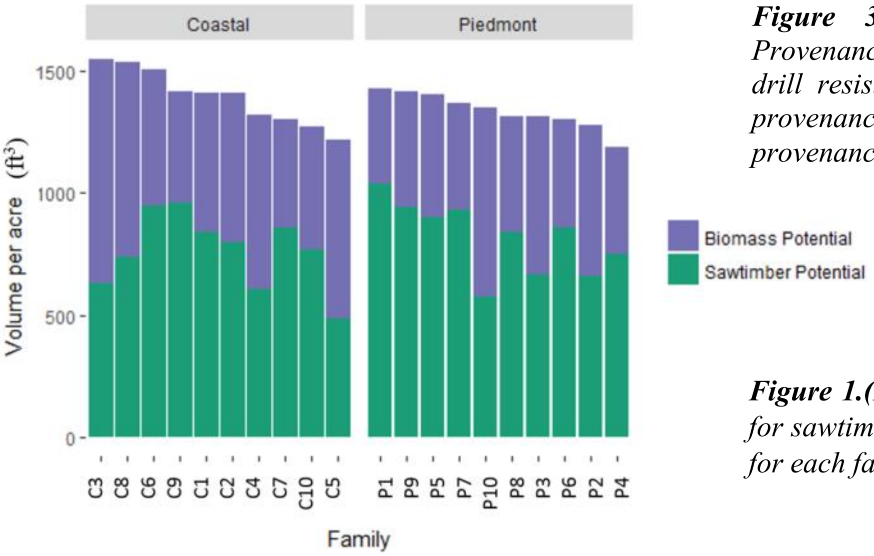

Figure 1.(Left) *Volume per acre for trees with potential for sawtimber (green bars) and for biomass (purple bars) for each family by provenance.*

PRS™ Calibration Study- Early Results Through Age 4

Translating gain estimates from progeny tests to operational plantings has been a critical need in tree improvement from its inception. As stated by Smith (2010)[1] "Evaluating genotypes based on individual tree growth traits may not account for possible differences in yield due to stand-level response to competition. The best genotype based upon single-tree-plot tests may not be the most efficient at using site resources, but may exhibit better growth by out-competing its neighbors. Planted in a pure family block, that genotype may not be the best producer, as it no longer has a competitive advantage over its neighbors…"

The Cooperative Tree Improvement Program's ***PRS***™ (***P***erformance ***R***ating ***S***ystem values) are based upon small-tree plots (single-tree, 6-tree rows, or 10-tree rows) mostly measured at 6 to 8 years of age. The genetic information from these trials is very precise for identifying good and bad genetics at young ages, but the growth traits are indirect measures of the real traits of interest: forest growth/yield and value per acre at rotation. The ***PRS***™ **Calibration Study** was initiated in 2012 with the objective to correlate predicted gains from loblolly pine progeny tests to actual volume gains through rotation age in block-plot plantings. Families from a broad range of ***PRS***™ scores for volume/productivity (***P***), fusiform rust resistance (***R***), and straightness (***S***) were planted on a range of sites across the landscape so that their stand volume and value throughout the rotation can be estimated (Figure 1).

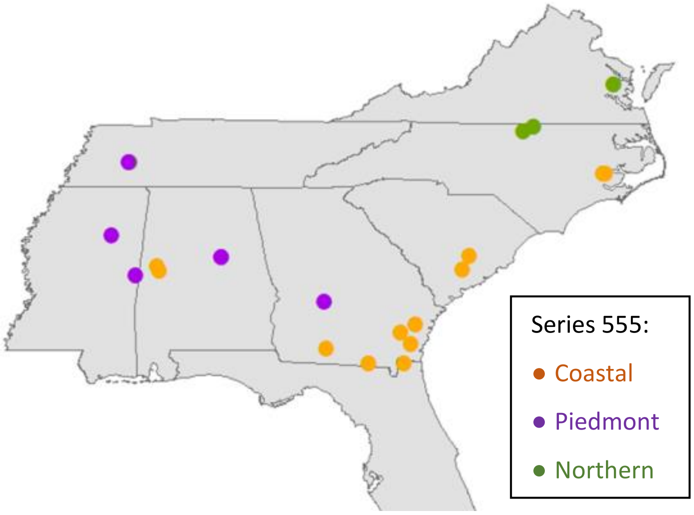

Figure 1. *The* ***PRS***™ *Calibration Study (Test Series 555) was installed at 24 locations, with 12 Coastal tests, 7 Piedmont tests, and 5 Northern tests.*

At each site, 10 full-sib families representing the range of ***PRS***™ scores were established with 144-tree block plots that will be measured through rotation (Table 1). So far, all tests have been measured at year one, and all but two tests (established in 2015) have been assessed at least once for age 3 or 4 years. The first of the year six assessments were just received in spring 2018.

Age	**status**	**height**	**dbh**	**stem rust**	**branch rust**	**straightness**	**sweep**	**forking**	**ramicorn**	sawtimber **potential**
1	X	X								
4	X	X	X	X	X	X		X	X	X
6	X	X	X	X	X	X	X	X	X	X
12	X	X	X	X	X	X	X	X	X	X
18	X	X	X	X	X	X	X	X	X	X
24+	X	X	X	X	X	X	X	X	X	X

Table 1. *Measurement schedule for* ***PRS***™ *Calibration study tests and the traits to be measured.*

Using the data available through 2017, eleven tests with age 4 data were analyzed to evaluate the quality of the tests. Family means were calculated at each site for individual tree volume, height, fusiform rust incidence, fork/ramicorn incidence, and straightness score. Correlation coefficients were calculated between the family means from the Calibration Study and the ***PRS***™ scores from the newest available version on TIPRoot database.

Strong correlations for volume productivity (Figure 2), fusiform rust incidence (Figure 3), and mean height (not shown) were observed on all Coastal sites. The Northern tests were smaller at age 4 and were not yet showing large genetic differences in volume. Rust scores were not

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[1] Smith, B.C. 2010. Genetic effects on long-term growth in loblolly pine. PhD dissertation, NC State Univ., Raleigh, 147p. (Available at http://www2.lib.ncsu.edu/catalog/record/NCSU2325119)

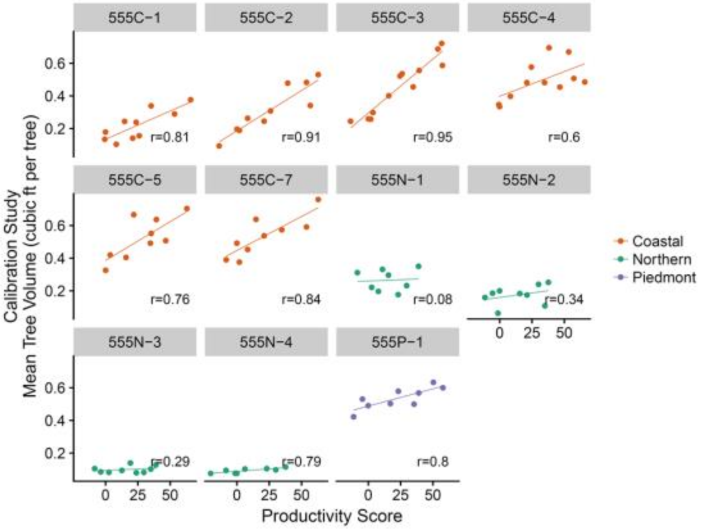


Figure 2. Scatter plots with correlation coefficients at each study site between Productivity scores and Calibration Study mean tree volume at age 4. Strong correlations were observed on all sites except for some of the Northern tests, which were small and not yet displaying large genetic differences.

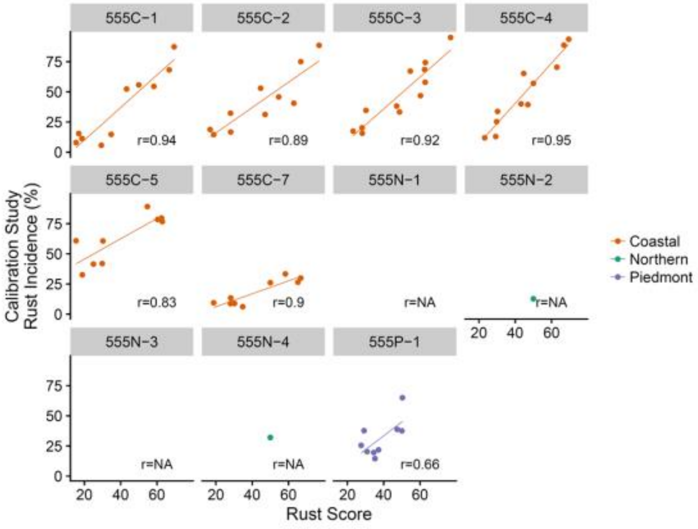


Figure 3. Scatter plots with correlation coefficients at each study site between Rust incidence scores and Calibration Study mean rust incidence at age 4. Strong correlations were observed on the Coastal sites. Rust scores were not available for Northern families due to low incidence.

available for Northern tests due to low incidence of disease in progeny tests. Straightness scores showed moderate correlations with the majority of sites having correlations between 0.53 and 0.63. Incidence of forking/ramicorn compared to the PRS™ forking score did not consistently display strong correlations at the majority of sites, but showed a strong relationship on at least one site in each region.

These results suggest that the Calibration Study has been successfully installed on uniform test sites. This finding was corroborated on the ground by a recent site visit to one of the Rayonier installations in Nassau Forest, FL (Image 1). It is also reassuring that Rust and Straightness scores calculated from single-tree plots correlate well with observations in the 144-tree block plots. This study is set to provide valuable information as older measurements are made, so stay tuned!


Full-sib family
with P = 66 over CCK

CCK (P = 0)

Image 1. (Left) *Dramatic differences in volume productivity were observed on this PRS™ Calibration Study site in Nassau Forest, FL installed by Rayonier, Inc. (Left to Right Serenia O'Berry, Austin Smith, Ross Whetten, Josh Sherrill, Fikret Isik, and David Barker – Serenia, Austin, Josh, and David are with Rayonier, Inc.)*

Wood Quality Update

The Cooperative commenced wood quality measurements on 3rd-Cycle tests in 2013. Measurements are taken at ages 6-9 years in order to characterize the wood properties of the juvenile core. The design of the 3rd-Cycle tests is very strong for estimating breeding values of parents. The assessment tools used (TreeSonic and Resistograph) allow standing trees to be measured very quickly (more than 60 per hour). The TreeSonic is a tool consisting of two probes that are inserted into the tree one meter apart with a sensor that detects the time it takes for a sound wave (initiated with a hammer tap) to pass through the tree. The speed of the sound wave is correlated with the stiffness of the wood, with faster sound waves indicating stiffer wood. It is also indicative of the amount of defect, such as knots and compression wood, which tend to slow the sound wave. The Resistograph is a precision drill that measures the amount of resistance when drilling through the wood, with more resistance indicating denser wood.

As of winter 2017, measurements have been taken for 16 tests from all three breeding zones (Figure 1). There are two more tests in the Northern region (NPMX) scheduled for measurement in summer 2018.

There was considerable site-to-site variation in TreeSonic measurements (acoustic time of flight, Figure 2), even within a testing population/region. Similarly, the variation in Resistograph measurements (drill resistance, Figure 3) among test sites was considerable.

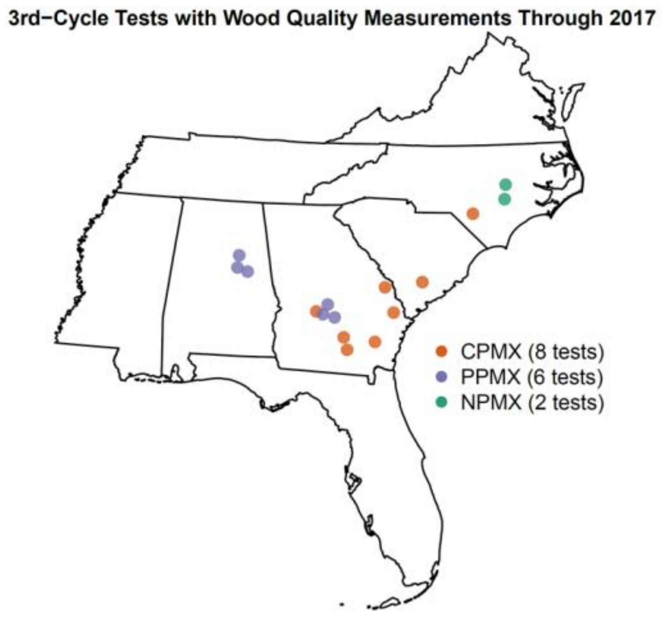


Figure 1. *Approximate test locations for 3rd-Cycle tests assessed for wood quality through 2017.*

There was also considerable genetic variation in both TreeSonic and Resistograph measurements (Figure 4). The drill resistance (surrogate for wood density) breeding values ranged +/- 20% of the population means (centered at 100 within each region) and the acoustic velocity squared (surrogate for wood stiffness) values ranged +/- 25%, with one Coastal selection having an extraordinary acoustic velocity breeding value. Larger drill resistance and larger acoustic velocity squared values are associated with denser and stiffer wood, respectively. The 3rd-Cycle checklots ranked near the middle of the population for both traits. There was no apparent genetic correlation between these two traits, which is consistent with others' findings of wood density and wood stiffness being independent among families in pine breeding programs.

Both wood quality traits were found to have stable family performance across test sites within a region (little to no genotype by environment interaction), displayed for the Coastal region in Figure 5. Families that ranked in the top 10% across all sites tended to rank very high on

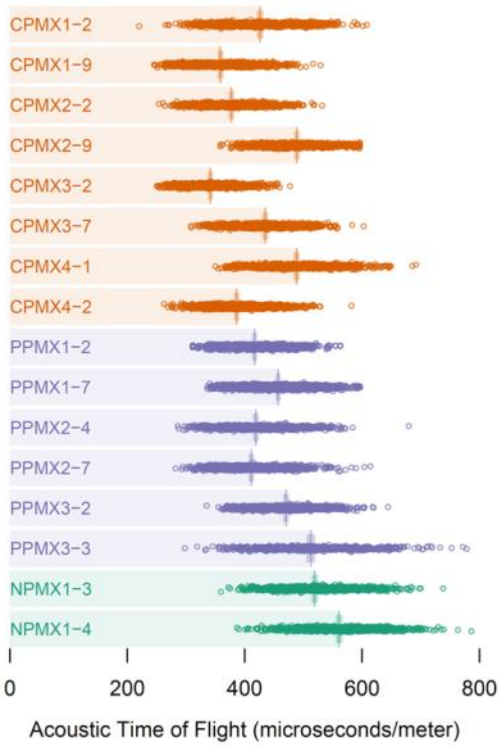
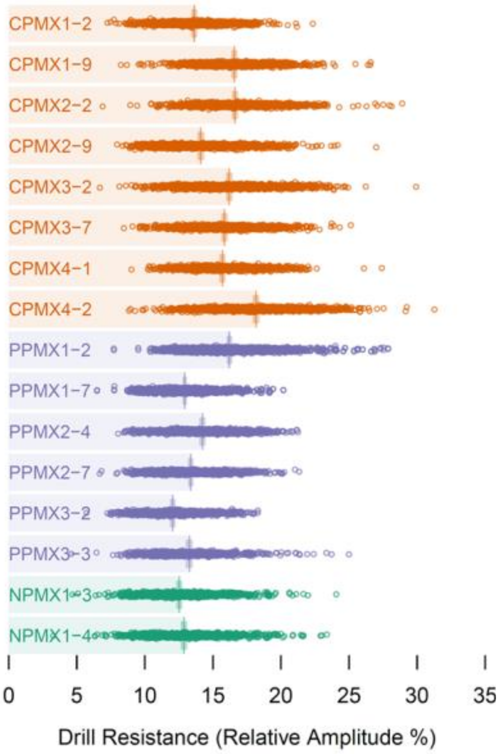


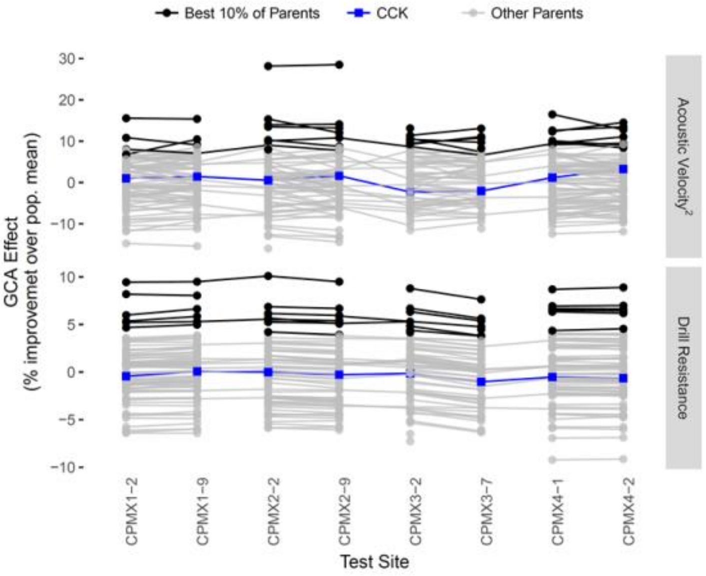
Figure 2. *TreeSonic measurements (acoustic time of flight) by test site. Vertical lines indicate the test site means, which varied considerably within a testing region/population. Acoustic time of flight is correlated with wood stiffness, with faster values indicating stiffer wood.*

individual sites, and the Coastal checklot (CCK) ranked consistently in the middle across all sites. Genetic correlation between sites was 0.85 and 0.97 for TreeSonic and Resistograph, respectively, for the Coastal tests. For the Piedmont tests, the genetic correlations were 0.94 and 0.79 for TreeSonic and Resistograph, respectively.

The wood quality breeding values are on the Cooperative's TIPRoot database for the Coastal and Piedmont populations (280 parents in the Coastal and 200 in the Piedmont). Northern population will be published for the after measurements in summer 2018.

Figure 3. (Right) *Resistograph measurements (drill resistance) by test site. Vertical lines indicate the test site means, which varied within region as much between regions. Drill resistance has a strong genetic correlation with wood density, with more resistance indicating denser wood.*





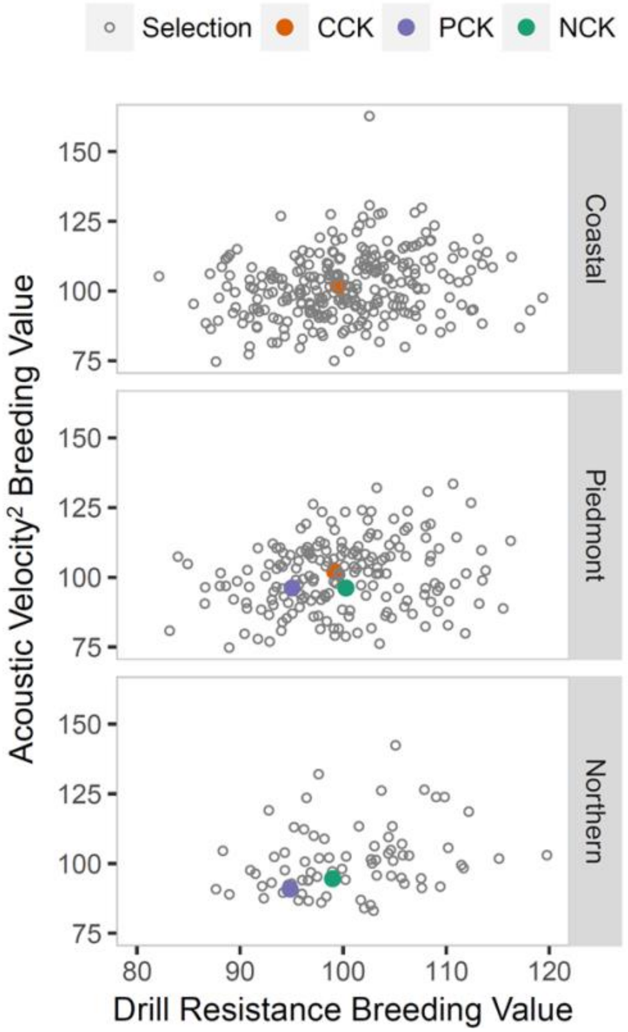


Figure 4. *Wood quality breeding values estimated from 3rd-Cycle progeny tests. There was not a strong genetic correlation between wood density (quantified with drill resistance) and wood stiffness (quantified with acoustic velocity squared). The checklots (CCK, PCK, NCK) ranked near the middle of all three populations. Breeding values are centered on 100 such that a value of 110 implies 10% above the population mean and 90 is 10% below the population mean. There was one extraordinary selection for acoustic velocity in the Coastal population.*

Figure 5. (Left) *Family effects (general combining ability) at each test site for wood quality traits in the Coastal population. Family performance was stable across test sites, and the parents that were in the top 10% overall tended to be good across the sites where they were tested (low genotype by environment interaction). The coastal checklot (CCK) consistently ranked near the middle across test sites.*

Hofmann Growth and Yield Trials- Measurements through Age 12

Clonal/Full-Sib/OP Genetics Demonstration Trial

The Cooperative established two growth and yield trials at the Hofmann Forest, NC in 2006 that were recently thinned in November 2017. The first study is known colloquially as "The Genetics Demo", because it is extremely effective at demonstrating the value of tree improvement to landowners, foresters, students, and visitors (Image 1). "The Genetics Demo" is a replicated study of 14 genotypes planted in 100-tree block plots with three replicates. Included are open-pollinated, full-sib families, and clonal genotypes that were operationally deployed at the time, as well as local non-improved loblolly (CC2) and a first-generation seed orchard mix checklot (SOM2). The planting density was 436 trees per acre on 20' beds (5' x 20'). The thinning in November 2017 targeted small and defective trees and reduced the number trees per acre to one-half of the planting density (218 trees per acre).

In the demo, significant differences in total volume were observed among genotypes, with the most productive family having a mean of 3,190 cubic feet per acre and the local, non-improved checklot (CC2) having a mean of 2,160 cubic feet per acre at age 10 prior to thinning (Figure 1). Full-sib and open-pollinated families with parents B, E, and F had the largest mean total volume before and after thinning. Some open-pollinated families, such as E and B, had significantly larger mean total volume yield than some full-sibs families. Mean total volume yield in trees with sawtimber potential (e.g. trees without major stem or branching defects) was highest for full-sib families having parent F as the pollen parent, before and after thinning.

The thinning significantly improved the proportion of trees with sawtimber potential, especially for the open-pollinated families A, E, and F, as well as the seed orchard mix checklot (SOM2) (Figure 2). The proportion of trees having sawtimber potential for the non-improved checklot (CC2) was lower than all other open pollinated and full-sib families before and after thinning (Figure 2).

There was a strong correlation between mean dominant height at age 6 and mean total volume per acre at age 10 and after thin at age 12 (Figure 3), suggesting that a site index adjustment based on young tree values would be an effective method to account for differences in genotype growth and yield through age 12. The clonal genotypes did not follow this trend.

Image 1. *The 2006 Genetics Demo at the Hofmann Forest, NC is a powerful resource for demonstrating the value of tree improvement, as visitors can see first-hand different levels of improved genotypes next to non-improved, wild-type loblolly pine.*

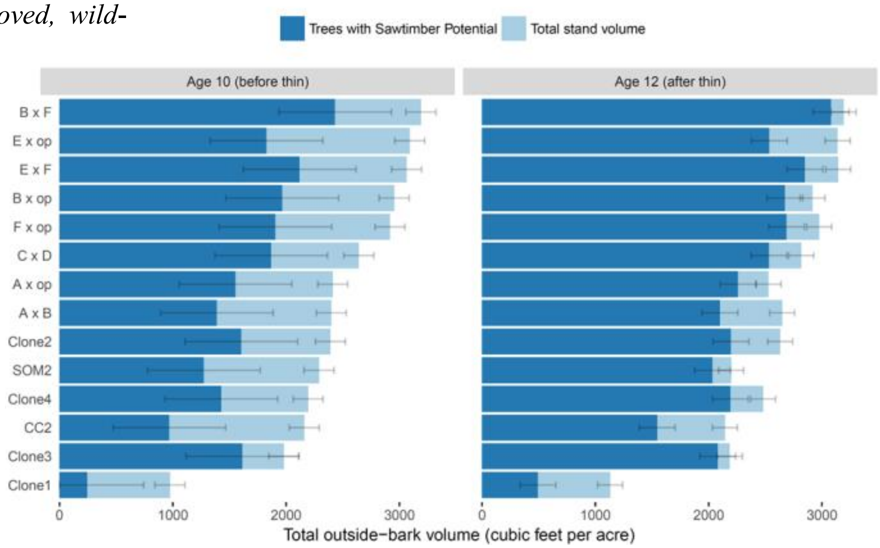


Figure 1. (Right) *Mean yield (total volume outside bark per acre) and standard errors by genotype and product potential before thin (age 10 years) and after thin (age 12 years) in the Genetics Demonstration Growth & Yield Trial at the Hofmann Forest, NC. Parent tree names are coded as A through F. OP = open-pollinated; SOM2 = seed orchard mix from* 1^{st}*-generation NC Coastal orchards; CC2 = NC Coastal non-improved checklot..*

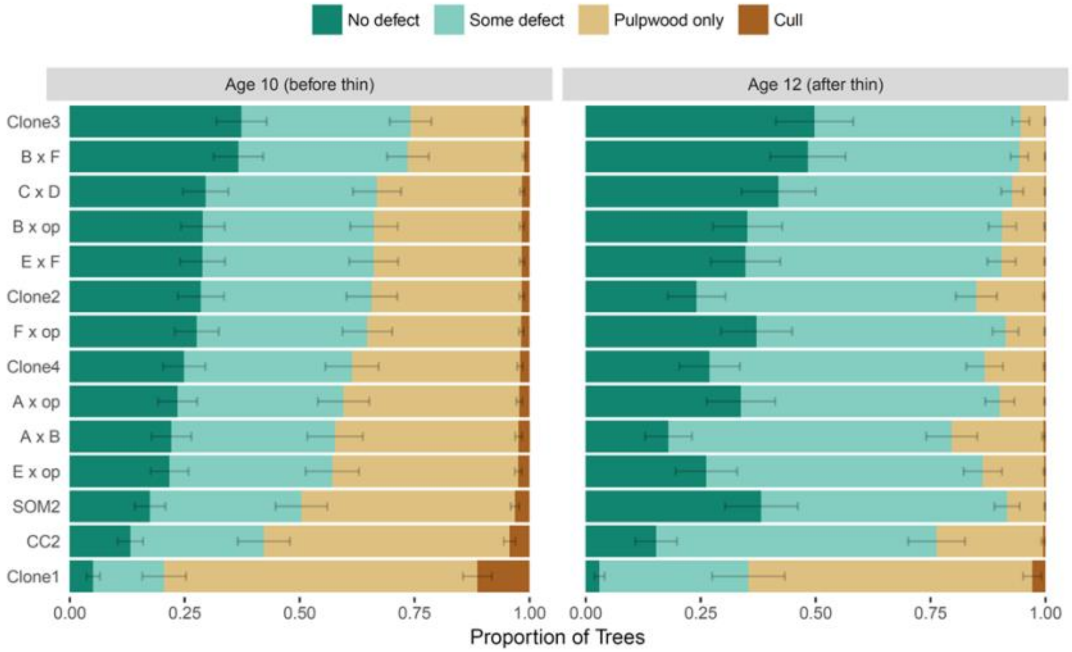


Figure 2. *(Left) Proportion of trees in each product category by genotype before thin (age 10 years) and after thin (age 12 years) at the Genetics Demonstration Growth & Yield Trial at the Hofmann Forest, NC. Parent tree names are coded as A through F. OP = open-pollinated; SOM2 = seed orchard mix from 1st-generation NC Coastal orchards; CC2 = NC Coastal non-improved checklot.*

Genetics x Spacing x Thinning Study

The second study at the Hofmann is the Genetics x Spacing x Thinning study, with the catchy abbreviation “The GST”. The objective of this study was to evaluate different levels of genetic homogeneity on forest stand dynamics and structure. Landowners can choose to plant seedling with a mixture of families, an open-pollinated family, a full-sib family, and a clonal genotype. The GST was designed to test if increasing the level of genetic homogeneity will also result in a more uniform stand. The study has 10 genotypes representing the range of genetic homogeneity, including 3 clones, 3 full-sib families (FS), 3 open-pollinated families (OP), and a second-generation seed orchard mix. There are two planting densities: 436 trees per acre (5’ x 20’ spacing) and 218 trees per acre (10’ x 20’), and there is a thin treatment and a non-thin treatment. The experimental design has three replicates on the site. Below are some preliminary results through Age 12 considering only the non-thinned plots.

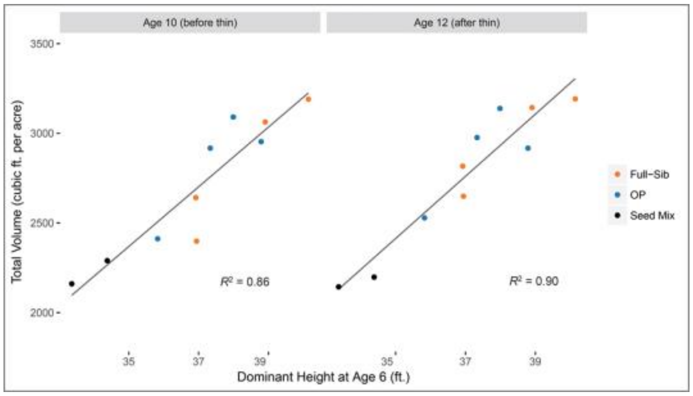


Figure 3. *The mean dominant height at age 6 explained 86% of the variation in total volume outside bark yield at age 10 (before thin) and 90% at age 12 (after thin). Seed mix includes the local, non-improved checklot CC2 and the 1st-generation seed orchard mix from Coastal NC (SOM2).*

To test the hypothesis that clones are more uniform than FS families that are more uniform than OP families that are more uniform than a mix of families, we compared the within-plot coefficient of variation (CV) for the diameter and height distributions against the mean tree size for each plot. Similar to the neighboring Genetics Demo study, significant differences in productivity were observed among the genotypes after age 6, so we compared the CV to mean tree size instead of age to evaluate how the spread of the distributions varies for a given mean tree size. The CV and mean size for the diameter and height distributions were calculated for each plot and measurement time and then modeled with a general linear model to account for the effects of replicate, genotype, and planting density. The least square means from these models were plotted to make a trajectory of the coefficient of variation as the stand develops (as the mean tree size increases), which is shown in Figures 4 and 5 for diameter at breast height and total height, respectively.

The CV of the diameter distributions dropped rapidly for all genotypes during the first five years after planting, and then began to level off and slowly rise after the mean DBH passed ~4” in the 5’ x 20’ spacing and ~ 6” mean DBH in the 10’ x 20’ spacing (Figure 4). After the mean DBH reached about 3”, the CVs for the diameter distributions tended to be higher in the 5’ x 20’ spacing plots than in the 10’ x 20’ spacing, presumably due to more competition and crown class differentiation. For a given mean DBH greater than around 3", the clonal genotypes tended to have higher CVs, corresponding to wider DBH distributions, especially at the 10' x 20' spacing. The most productive family in the study at age 12 (full-sib cross E x F) had the lowest CV of 18% in the 5' x 20' spacing and was significantly lower than the clone with the highest CV (28%) at the 5' x 20' spacing. At a mean DBH of 6" and greater for the 10' x 20' spacing, the seed orchard mix began to appear in a group by itself, and was significantly different from two open-pollinated families and one full-sib family. There was not strong evidence for consistent differences between full-sib and open-pollinated families in the spread of their DBH distributions at either spacing.

The CVs for the total tree height distribution dropped consistently through the last measurement (age 12), although there was some indication that some genotypes started to level off in the 5' x 20' spacing after a mean height around 45' (Figure 5). The height distributions did not differ drastically between the 436 and 218 trees per acre planting densities, consistent with conventional wisdom that height is less affected by stand density. Hurricane Irene hit the stand at age 6 years when trees were about 25' tall causing a jump in the CV for all genotypes. The genotype rankings for height CVs were similar to those of DBH, but the differences among genotypes were less pronounced. At the 5' x 20' spacing, only the clonal genotype with the highest mean height CV (22%) and the CV of the most productive full-sub family (E X F), which was less than 10%, were significantly different. At the 10' x 20' spacing, the clonal genotype with the highest CV had a significantly wider height distribution than all of the seedling progeny (the seed orchard mix, open-pollinated, and full-sib families). There was not strong evidence for consistent differences between full-sib and open-pollinated families in the spread of their height distributions at either spacing.

For the two clonal varieties included in this study, there was no evidence that their stands were more uniform; rather, both had substantially wider DBH and total height distributions for a given mean tree size at both planting densities. One explanation is that these clonal genotypes are displaying greater sensitivity to changes in micro-environmental conditions, while genotypes with greater genetic diversity (seed orchard mixes, open-pollinated families, full-sib families) may have some buffering to micro-site variation and thus display more stand uniformity. There was some indication that the seed orchard mix may have wider DBH distributions than the open-pollinated and full-sib families, it will be interesting to see if this because more significant as the stands develop and competition increases. The full-sib families were not consistently more uniform than the open-pollinated families through age 12 years.

The trials at the Hoffman Forest, NC are not only ideal for testing hypothesis about tree improvement and forest genetics, they are an invaluable resource for Cooperative member tours and public education. Now that these studies are in their 13th growing season, they are prime for demonstrating the value of tree improvement. It is getting time to install another demo and study at the Hofmann, so we are asking ourselves: "what hypotheses will we test next?".

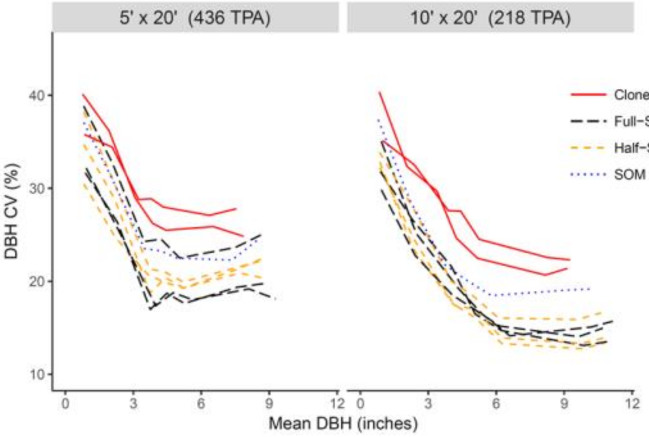


Figure 4. Mean coefficient of variation for DBH distribution against the mean DBH by planting density and genotype for non-thinned plots throughout the first 12 years of the Genetics x Spacing x Thinning study.

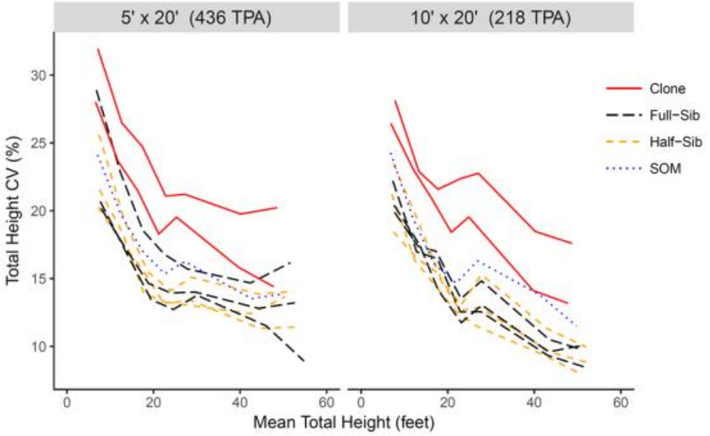


Figure 5. Mean coefficient of variation for total height distribution against the mean total height by planting density and genotype for non-thinned plots throughout the first 12 years of measurement on the Genetics x Spacing x Thinning study.

First- vs. Second- vs. Third-Generation Seedlings

One misconception some landowners have is that first-generation seedlings are all inferior to second-generation seedlings that are in turn inferior to third-generation seedlings. While it is true that the objective of a breeding program is to improve the population from one generation to the next, there is always a range in performance among families within each breeding cycle. There is also substantial overlap among families from one generation to the next (Figure 1). For example, in the Cooperative's Coastal Plain population, we have performance data for 2260 first-, second-, and third-cycle selections. For the 100 top-ranking open-pollinated families for stem volume, there were more third-cycle families (42) than for any other cycle, but there were also 19 second-cycle families, and 39 first-cycle families. Each of these groups has families that are fast growing with a range of performance for other traits.

Rather than choosing families to plant based on the generation from which they came, landowners should select families based on their genetic merit. In the NC State Cooperative, we developed the Performance Rating System (**PRS**™) about 16 years ago as a user-friendly tool to assist landowners and foresters in making the appropriate choice of genetics to plant to meet their management objectives.

Landowners have one opportunity to influence the genetic quality of the seedlings planted in a stand. If the wrong choices are made, the landowner will suffer the consequences of those decisions for 25 years or longer. For example, if a fast-growing family is selected, but it has poor stem quality, the plantation may be very acceptable for biomass or pulpwood. If markets or objectives change, then the options are limited when the stand is harvested. A stand with crooked stems or a high frequency of forking will have fewer stems that are suitable for sawtimber. As with all forest management, the first rule of forestry is to know your objectives and make decisions that optimize those management objectives.

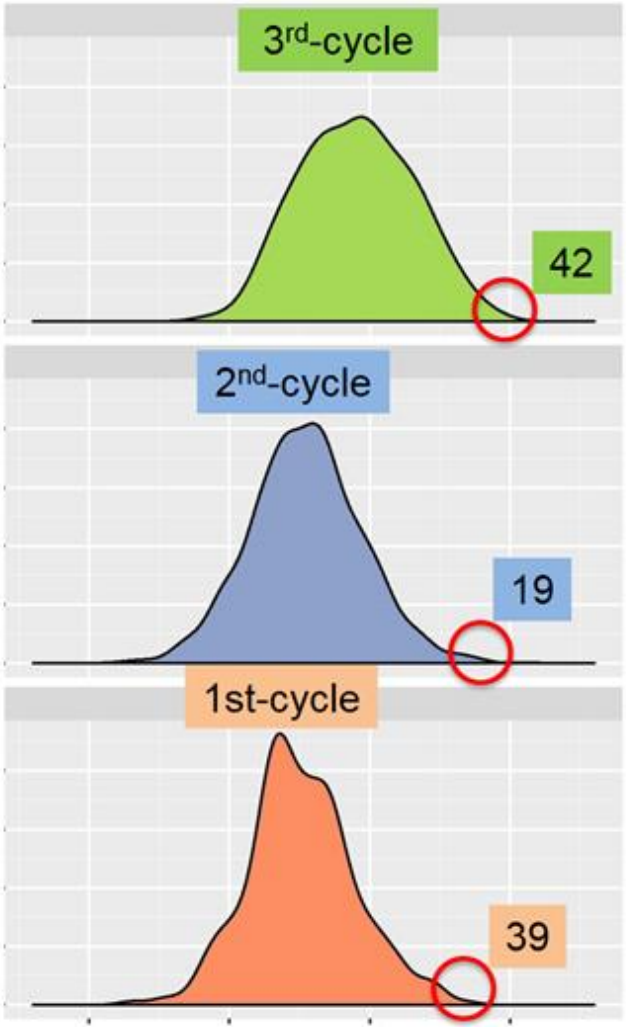
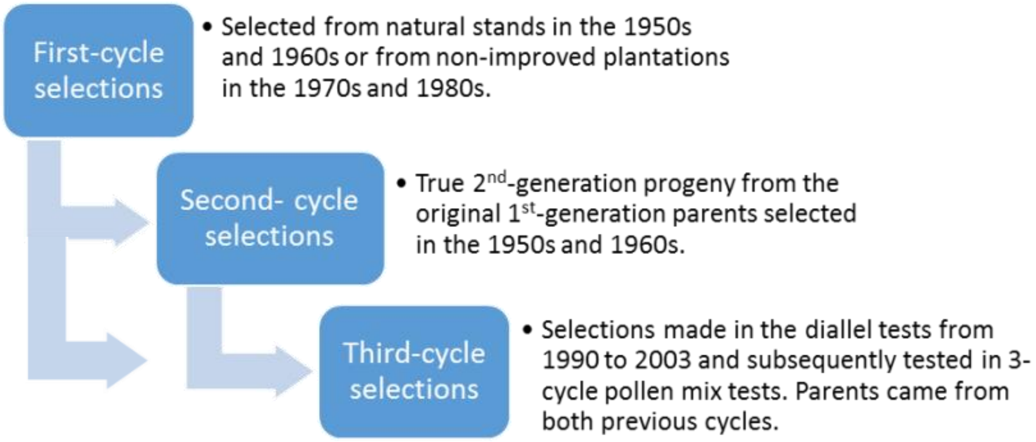


Figure 1. *Frequency distribution of* ***PRS***™ *scores for volume in the Coastal Plain breeding population. Note the large overlap from one cycle of breeding to the next. The number of selections in the top 100 for volume are shown for each breeding cycle.*

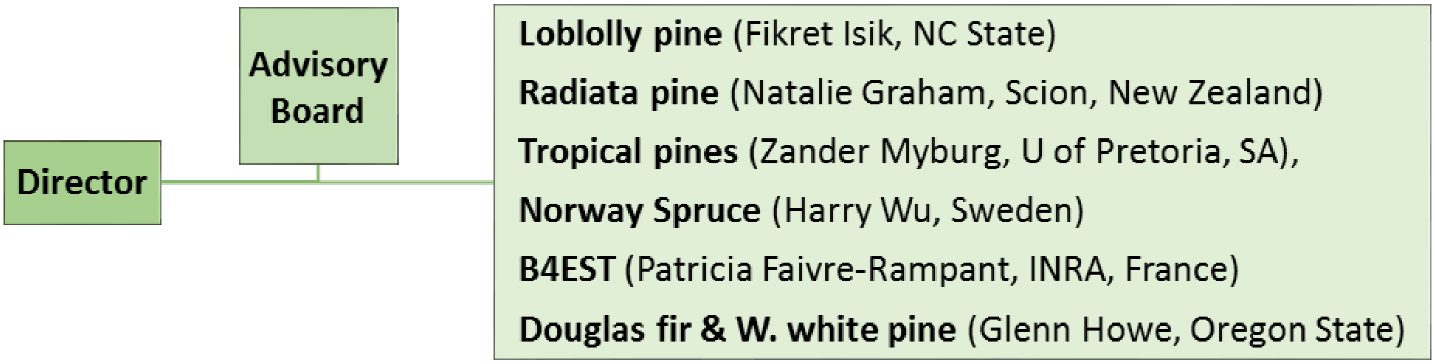


- Selected from natural stands in the 1950s and 1960s or from non-improved plantations in the 1970s and 1980s.
- True 2nd-generation progeny from the original 1st-generation parents selected in the 1950s and 1960s.
- Selections made in the diallel tests from 1990 to 2003 and subsequently tested in 3-cycle pollen mix tests. Parents came from both previous cycles.

Left: *The terms "cycle" and "generation" are sometimes used interchangeably, and it gets confusing. The diagram to the left describes what is in each of the breeding cycles.*

Progress on SNP Array Design for Loblolly Pine- Conifer SNP Consortium

Since 2016, Fikret Isik has lead the organization of the forest tree breeding community across Europe, USA, South America, and New Zealand in order to negotiate with genotyping companies to develop SNP arrays for conifers. An umbrella organization named the *Conifer SNP Consortium* was established in 2017 with the goal of creating a collaborative platform to use genomics in forest tree breeding. The Consortium includes six different groups, working on major forest trees (see diagram below). The Consortium has signed a memorandum of understanding with ThermoFisher Scientific to develop six 50K SNP arrays during the next three years, one for each species, using Axiom technology. The first two screening arrays, one for loblolly pine and one for radiata pine, as well as the 384-format genotyping arrays, will be designed during June-August 2018; work for tropical pines will commence in late 2018.



For loblolly pine, slash pine and tropical pines, a university consortium (*Pine Genomics Consortium*) is being established to jump-start genomic applications in loblolly, slash, and tropical pine breeding. The university -affiliated consortium is intended to carry out specific projects for 2-3 years on behalf of the members. The Consortium, managed by a director and board members, is a separate entity from the Cooperative Tree Improvement Program at NC State University, but Cooperative faculty will work closely with the Consortium.

There are clear benefits of membership in the Consortium. Members can access all the proprietary application SNP arrays developed by the Consortium through a genotyping service offered by ThermoFisher Scientific. Most importantly, the members can get preferential pricing of $20 per sample, compared to $44 per sample for non-members. The cost per sample does not include DNA isolation and shipping. The members can join conference calls and other Consortium meetings as part of scientific conferences or symposia. They will also have preferential access to Consortium organized workshops on data management and genetic data analyses for genomic selection.

Screening Array Project and DNA Extraction

The Cooperative Tree Improvement Program (TIP) and subsequently the Conifer SNP Consortium has been in communication with ThermoFisher-Scientific (TF) to develop a SNP array for loblolly pine. About 200,000 high-quality SNPs were identified by the NIFA funded project "Towards Genomic Selection in Forest Trees". At this time, these markers need to be screened before ThermoFisher-Scientific finalizes a 50K array.

A population of unrelated individuals was put together for the screening phase of the array development. This population represents the range of loblolly pine, and needles were collected from the following sources:

- Seedlings from all regions of the 4th-Cycle 2018 progeny tests
- Strategic set of seedlots chosen from the NCSUTIP seed inventory
- 40 Western-Gulf seedlings put together by Weyerhaeuser Co. and the Western Gulf Forest Tree Improvement Program.
- University of Florida Cooperative Forest Genetics Research Program provided additional 56 samples of slash pine and loblolly pine.

- A population of six parents, each with six offspring (36 duos) was included to test the Mendelian segregation of the SNP loci and to potentially differentiate single-locus from multi-locus markers by evaluating segregation ratios. For the offspring, haploid megagametophyte tissue is being used for genotyping to trace markers inherited from the mother parent.

The Tree Improvement Program is developing a plan to test and implement genomic selection in the Cooperative's breeding program. The Cooperative staff presented the ideas at the 2017 Advisory and Contact Meetings. We solicited the input from an ad-hoc "Genomic Selection Committee" - David Barker (Rayonier), Patrick Cumbie (ArborGen), Graham Ford (IFCO), Michael Harrington (Genoverde), and Bob Purnell (Weyerhaeuser) as well as Ross Whetten and Steve McKeand. We are moving forward with our plan to design a screening array for loblolly pine in June 2018. Once the final 384-format Affymetrix Axiom array is designed in summer 2018, the NCSUTIP, University of Florida CFGR program, and the Western Gulf Cooperative will genotype a large number of trees to test genomic selection approaches.

Image 1. (Right) *Samples taken from the newest flush of needles from the seedlings growing in the TIP greenhouse. Samples were taken back to lab for DNA extraction.*

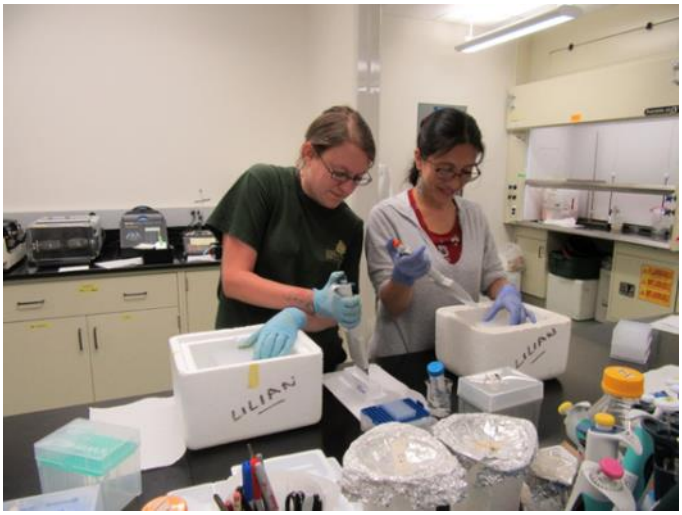


Image 2. *April Meeks (TIP Graduate student) and Dr. Lilian Mattalana (NCSU Christmas Tree Genetics) extracting DNA for the loblolly pine SNP array. Lilian has been extremely helpful teaching students and staff the appropriate protocols for DNA extraction.*

Grants

New Grants

Ross Whetten is the principal investigator on USDA-National Institute of Food and Agriculture grant, “Identifying Functional Variation in Complex Plant Genomes”, $490,000, 2/15/2018-2/14/2021.

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 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.

ASSOCIATED ACTIVITIES

Meetings, Workshops, and Short Courses

For the annual TIP Contact meeting, we headed north to historic Williamsburg, VA. The Virginia Department of Forestry hosted the field tour of the New Kent Forestry Center and their Hockley test site. We heard about VDF's ongoing contributions to restore the original longleaf genetics in Virginia that was almost totally decimated. The tour also included a stop to see an established ***PRS***TM calibration test where family differences were clearly evident! The group enjoyed a forestry related tour of historic Williamsburg and dined on early American fare in the cellar of Shields Tavern. We thank Ones Bitoki and Jerre Creighton and the VDF staff for helping make this a great meeting!

While the weather did rain on our parade through the pines it certainly didn't stop us from learning about the research trials established by Virginia Department of Forestry.

The faculty members associated with the Cooperative are in high demand and are invited to teach courses all over the world. Steve taught a short course in China at Nanjing Forestry University in December 2017 on Genetics and Tree Improvement. He also helped teach the international tree improvement short course at the University of Concepción in Chile in March 2018.

Fikret was very busy with five international workshops on genetic data analysis and utilization of SNP data in forest genetics. They included: Instituto Tecnológico de Costa Rica and Universidad de Costa Rica University (July 17-20, 2017); Institute of Subtropical Forestry, Chinese Academy of Forestry, Fuyang, China (September 28–29, 2017); Nanjing Forestry University (October 7-20, 2017), Nordic Countries Centre of Advanced Research in Forest Health and Forest Genetics to enhance Bioeconomy (HealGenCAR) workshop at Swedish University of Agriculture Sciences (November 13-17, 2017); Turkish General Directorate of Forestry, UNDP Capacity building project (December 18-22, 2017).

TIP staff extension and outreach activities:

This was a busy year for TIP staff at regional, national, and international meetings. At the 34th Southern Forest Tree Improvement Conference in Melbourne, FL, all the staff and most of the students gave presentations. In fact, our very own Austin Heine won the Bruce Zobel Award for the Most Outstanding Student Presentation.

Trevor Walker presented at the IUFRO Biotechnology Conference in Chile and at the Southern Mensurationists' meeting in Athens, GA.

Faculty were invited as keynote speakers at the following meetings:

Steve McKeand, keynote speaker, Canadian Forest Genetics Group, Edmonton, Alberta, June 2017

Steve McKeand, keynote speaker, IUFRO Seed Orchard Meeting, Bålsta, Sweden, September 2017

Fikret Isik, invited speaker, Plant Animal Genome Conference, San Diego, California, January 13-17, 2018

Fikret Isik, invited speaker, Tree Biotechnology Conference, Concepcion, Chile, June 4-9, 2017,

Fikret Isik, guest lectures at Seoul, South Kores National University, October 16-17, 2017

Austin Heine presented at a tour stop for landowners and forestry professionals as part of a forestry field tour given through NCDA Vance and Granville County Ag Extension at the Bioenergy field trial in Butner, NC. October 2017.

Austin Heine presented at the NC Forest Service Ranger Training in January 2017 in Kinston, NC to teach the value of genetics and how it impacts forest productivity.

Austin Heine and Steve McKeand taught a group of 40 high school students the value of genetics using the 2004 Genetics Demo at Sustainable Forestry Day held at the NC State University Schenck Forest, September 2017.

Austin Heine talked to approximately 40 high school sophomores and juniors at Environmental Exploration Day for the College of Natural Resources to teach them about forestry and forest genetics in March 2017.

J.B. Jett and Steve McKeand taught and organized US Forest Service Seed Orchard Workshop, Haynesville, NC, November 2017

Steve McKeand preached the tree improvement gospel at the following:

NC State University Board of Trustees meeting, Raleigh, February 2017

Breakfast with forestry colleagues and college alumni at the Hofmann Forest, September 2017

Guangxi Forestry Research Institute, Nanning, Guangxi, China, October 2017

Georgia Forestry Commission Statewide Forester Training, St. Simons Island, GA, October 2017

SC Forestry Association Annual Meeting in Greenville, SC. November 2017.

Southeast SAF meeting, Callaway Gardens, GA, January 2018

Fikret Isik represented the Cooperative at the International Conference on Selection Theory and Breeding Methodologies. March 23-24, 2017. Munich Technical University, Freising, Germany.

Fikret Isik served on a USDA-National Institute of Food and Agriculture as panel member to review research proposals, Washington, DC. November, 2017.

Fikret Isik gave a lecture on genomic relationships estimations from SNP markers during the North American Quantitative Genetics Workshop organized during the 34th Southern Tree Forest Tree Improvement Program, Melbourne, FL, June 19-22, 2018.

Trevor Walker hosted a tour of the Hofmann Forest growth and yield trials as part of the eastern Forest InSight Conference. He was also a member of a career panel for the Sustainable Forestry Day hosted by the College of Natural Resources to field questions from prospective high-school students. Trevor presented at the IUFRO Biotechnology Conference in Chile and at the Southern Mensuration' meeting in Athens, GA.

In addition, Cooperative faculty and staff made over 30 visits with members, landowner groups, and forestry groups in FY 2018.

Staff

We had another eventful year here in the office…can we say rinse and repeat? In January 2018, we added Eddie Lauer to our staff to fill the Analyst and Database Manager vacancy left by Daniel Genung in August 2017. Eddie took Dr. Isik’s Advanced Topics in Quantitative Genetics course as he was a working on his MS degree, and the tree breeding bug bit! He was set to begin a PhD program with us in January, but when the data analyst position opened, he knew it would be a great fit for him, and we are certainly glad he applied. Eddie brings an extremely strong molecular genetics and bioinformatics background, and just happens to have the database skills needed for the job. He has taken the job by the horns, including jumping in an aerial lift during breeding season!

Our Operations Manager, Austin Heine, is having a big year! Not only did he successfully defend his Masters research, he also recently won the College of Natural Resources nomination for the NC State University Award of Excellence for Public Service. His efforts in mentoring, teaching, and training both graduate and undergraduate students in forestry and tree improvement techniques, as well as his extension activities had him at the top of the heap for his value in the public service arena. His nomination now goes on to the university level for more cash and prizes. He could go all the way to the state level for a Governor’s Award for Excellence, which is the highest honor that can be awarded to a State of North Carolina employee.

As they say, all good things must come to an end. In May 2017, Dr. Steve McKeand announced he would be going into phased retirement and stepping down as director as of June 30, 2018. Steve has been at the helm since 2005 and he has been a great director, leading the Cooperative through the best of times and some tough times. A search for his replacement is underway, and we look forward to welcoming the new director soon. With phased retirement, we haven’t gotten rid of Steve quite yet, so you will still see him participating in Cooperative research and teaching for the next few years. But during his time off he will be happily spending more time with his wife Lou and his family, especially all the grandbabies he has acquired over the years!

Visitors

The Cooperative Tree Improvement Program offered a three-week training program to Turkish tree breeders Mr. Kubilay Özyalçin, Mr. Abdullah Kaplan, and Mr. Mehmet Acet on various practical applications of pine breeding in Raleigh and at the Arrowhead Breeding Center from February 11 to March 6, 2018. The visit included two weeks of training at NC State University on experimental designs, mating designs, pollen handling, container seedling production, grafting, and data analysis using R and ASReml. They also visited the Genetics x Silviculture x Thinning study at Hofmann Forest. During the third week, the group visited ArborGen and then spent six days at the Arrowhead Breeding Center. At Arrowhead, they learned about controlled pollinations, seed orchard design, orchard management, and enjoyed an orchard management bonfire (removing beetle bait from the orchards). The training was part of the United Nations Development Fund project for capacity building for the Turkish General Directorate of Forestry. Kubilay, Abdullah and Mehmet work for the Tree Seeds and Tree Breeding Research Institute, located in Ankara, Turkey. The group is in charge of starting controlled pollination breeding of Turkish red pine (*Pinus brutia*) in Turkey. We enjoyed having Kubilay, Abdullah, and Mehmet and wish them best. In their tree breeding endeavors back in Turkey.

Turkish tree breeders Abdullah Kaplan, Kubilay Ozyalcin, and Mehmet Acet at the Arrowhead Seed Orchard in Cochran, GA.

Teaching

The Cooperative faculty teach courses at both the graduate and undergraduate level. In the spring, Steve McKeand and Ross Whetten with the teaching assistance of April Meeks, taught FOR 411 (Forest Tree Genetics and Biology), our undergraduate tree improvement course, and the graduate level FOR 725 (Forest Genetics). This spring, Steve and Austin Heine taught FOR 727 (Tree Improvement Research Techniques) that included the excursion to Arrowhead during spring break (picture bottom of page 31). 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. Ross teaches 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. His inter-departmental course "Advanced Quantitative Genetics and Breeding (FOR/CS/ANS 726)' will be offered in fall 2018.

Once again, James West and Bobby Smith (far right) hosted the FOR 411, 725, and 727 classes at the NC Forest Service nursery and seed orchard in Goldsboro, NC. This annual field trip provides students with a memorable learning experience!

Graduate Students

We keep packing them in and turning them out! Tree Improvement Program graduates have had 100% job placement in the last many years, or have forged ahead into PhD programs, an excellent sign for the future of tree improvement! We had another great year for students who successfully defended their research and graduated. Serenia O'Berry finished her Master's program in Fall 2017, and Austin Heine successfully defended his MS research in March 2018. We also welcomed one new Masters and two PhD students in Fall 2017; Khushi Goda and Eddie Lauer began their PhD programs, and Colin Jackson started as a Masters student shared with Camcore. 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 in April 2017 and expects a summer 2018 finish.

April Meeks, PhD – April is working towards a better understanding of the fusiform rust and loblolly pine pathosystem and genetic basis of disease resistance in loblolly pine. Her research will focus on the pathogen variation and potential loblolly family by inoculum interactions.

Austin Heine, PhD – Austin decided to continue his studies after completing his MS project in March 2018 and is pursuing a PhD. He is still recovering and will be exploring ideas for his research, so stay tuned.

Colin Jackson, MS – Colin started in Fall 2017 and is a student we share with Camcore. His research is titled "Comparative Genome Analysis and SNP Marker Discovery of Tropical Pines".

Eddie Lauer, PhD – Eddie began his PhD program in January 2018. He is pretty focused and knows he wants to explore more in the area of fusiform rust resistance. His research is titled, "Discovery of Fr Genes for Fusiform Rust Disease in Loblolly Pine".

Jessie Maynor, MS – Jessie took to the Butner bioenergy research project as an undergraduate and made that the focus of her research. Her research is titled "Assessing Improved Loblolly Pine Families for Biomass Potential". She expects to finish in summer of 2018.

Khushi Goda, PhD – Khushi joined us from the Genetics program in the summer of 2017. Her research is titled "Optimal Mate Selection and Managing Inbreeding in *Pinus Taeda*".

Nasir Shalizi, PhD – Nasir began in Fall of 2016 and is continuing to work on clonal data analysis and one of his manuscripts (in preparation) is titled "Genetic parameter estimates from a large cloned loblolly pine population".

Trevor Walker, PhD – Trevor continues to study applications of quantitative genetics in several loblolly pine traits.

Most of the TIP graduate students were at Arrowhead except for April Meeks (in Raleigh keeping the office and lab going) and Adam Festa (Monsanto). Pictured left to right are Trevor Walker (TIP), Austin Thomas (Camcore), Eddie Lauer (TIP), Colin Jackson (TIP and Camcore), Romeo Jump (Camcore), Luis Ibarra (Camcore), Khushi Goda (TIP-Genetics), Jessie Maynor (TIP), Nasir Shalizi (TIP), Steve McKeand (old guy), and Austin Heine (TIP). Our graduate students are a key source of innovative ideas and energy., the future of forest genetics and tree improvement is in great hands with such outstanding scholars.

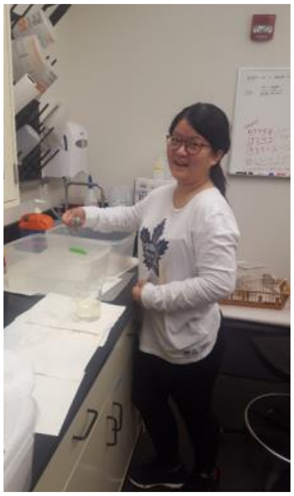
Undergraduate Army

Our Cooperative simply would not be capable of doing as much as we can on a day to day basis without the help from our wonderful graduate and undergraduate students. There has been a tradition here in the Tree Improvement Program to keep a steady flow of bright, talented, and hardworking students around to help with all aspects of our work, and we are certainly full of them right now. Some of these duties over the past year include cleaning and entering data, testing pollen in the lab, pulling seed for testing, planting future breeding orchards, greenhouse work, DNA sampling and extraction, and measuring research trials around the state of NC during fall, winter and spring breaks. We attract students from all over campus as well as right here in CNR. The current group of students 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, all 10 of them!










First row, L to R: *Austin Quate, Dwight Hilton, Maura Kaiser*

Middle Row, L to R: *Rachel Powell, Paige Green, Jonathon Taylor, Rita Li-Yen Chen*

Last Row, L to R: *Rachel Schwitzgebel, Haley Hollan, and Doug Dobson*

Membership in the NCSU Cooperative Tree Improvement Program

On a positive note, we added a new Contributing Member, Roseburg Resources Co., and a new Research Associate Member, the Nova Scotia Department of Natural Resources. Unfortunately, the Tennessee Division of Forestry abandoned all their tree improvement programs and dropped Cooperative Membership. As always, we value the members and their support of our research and breeding activities. Here is a list of our Cooperative partners:

Full Members

ArborGen, Inc.
Georgia Forestry Commission
Hancock Timber Resources Group
International Forest Company
North Carolina Forest Service
Rayonier, Inc.
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.
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
Roseburg Resources
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
Nova Scotia Department of Natural Resources
PBS International
USDA Forest Service

Publications of Special Interest To Members (2015 - 2018)

From our inception in 1956, there have been over 360 refereed 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

2018

Walker, T.D. and S.E. McKeand. 2018. Fusiform rust hazard mapping for loblolly pine in the southeastern United States using progeny test data. J. For. 116(2): 117-122.

Wang, J.P., M.L. Matthews, C.M. Williams, R. Shi, C. Yang, S. Tunlaya-Anukit, H-C. Chen, Q. Li, J. Liu, C-Y. Lin, P. Naik, Y-H. Sun, P.L. Loziuk, T-F. Yeh, H. Kim, E. Gjersing, T. Shollenberger, C.M. Shuford, J. Song, Z. Miller, Y-Y. Huang, C.W. Edmunds, B. Liu, Y. Sun, Y-C. J. Lin, W. Li, H. Chen, I. Peszlen, J.J. Ducoste, J. Ralph, H-M. Chang, D.C. Muddiman, M.F. Davis, C. Smith, F. Isik, R. Sederoff, and V.L. Chiang. 2018. Improving wood properties for wood utilization through multi-omics integration in lignin biosynthesis. Nature Communications. 9:1579. doi:10.1038/s41467-018-03863-z

2017

Duran, R., F. Isik, J. Zapata-Valenzuela, C. Balocchi, and S. Valenzuela. 2017. Genomic predictions of breeding values in a cloned *Eucalyptus globulus* population in Chile. Tree Genetics & Genomes, 13(4).

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. 112 (519):

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, J. Holland, and C. Maltecca. 2017. Genetic Data Analysis for Plant and Animal Breeding. Springer International Publishing. 400p.

Kohlway, W.H., R.W. Whetten, D.M. Benson and 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

McKeand, S. 2017. Making sense of the genetics market. Forest Landowner 76 (5): 14-19.

McKeand, S. 2017. Shopping for genetically improved loblolly pine seedlings? Look at the data first! South Carolina Forestry. The 2017 Annual Magazine of the Forestry Association of SC. p. 86-89.

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., 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. 63(5):496–503.

2016

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.

Čepl J., D. Holá, J. Stejskal, J. Korecký, M. Kočová, Z. Lhotáková, I. Tomášková, M. Palovská, O. Rothová, R.W. Whetten, J. Kaňák, J. Albrechtová, and M. Lstibůrek. 2016. Genetic variability and heritability of chlorophyll *a* fluorescence parameters in Scots pine (*Pinus sylvestris* L.). Tree Physiology 36(7): 883-895.

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.

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

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.

Xiong, J., S.E. McKeand, F.T. Isik, Jill Wegrzyn, D.B. Neale, Z-B Zeng, L. da Costa e Silva, and R.W. Whetten. 2016. Quantitative trait loci influencing stem defects in an outbred pedigree of loblolly pine. BMC Genetics 17:138. DOI 10.1186/s12863-016-0446-6

Wang, J.P., S. Tunlay-Anukit, R. Shi, T.F. Yeh, L. Chuang, F. Isik, and P.P. Naik. 2016. A proteomic-based quantitative analysis of the relationship between monolignol biosynthetic protein abundance and lignin content using transgenic *Populus trichocarpa*. Recent Advances in Polyphenol Research, 5, 89.

2015

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

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

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

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

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

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

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

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

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

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

Productivity. Our mission is to enhance productivity through breeding, testing, and selecting superior loblolly pine genotypes. We accomplish this through our breeding program, but research studies like the ones pictured are critical for real-world evaluation of the work we do. Data from these growth and yield trials also allow us to demonstrate to foresters, landowners, and students the value of different families for a multitude of traits.

Front Cover: The Hofmann Forest **Genetics-Spacing-Thinning (GST)** study (age 12-years, post thinning) is putting genetics to the test with thinning and spacing regimes, processes that land managers consider when planting families to meet landowner objectives.

Above: **Butner Biomass Genetics/Cropping Study** (age 6 years): Families from the Piedmont and the Coastal Plain are being evaluated for short-rotation bioenergy production as well as for sawtimber. *Below:* Staff and students measuring **SETRES2** at age 24 years. We are evaluating the long-term growth and yield and carrying capacity of families from the Atlantic Coastal Plain and drought-hardy Texas provenances with and without fertilization.