NORTH CAROLINA STATE UNIVERSITY COOPERATIVE TREE IMPROVEMENT PROGRAM

63rd Annual Report | May 2019

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May 2019

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EXECUTIVE SUMMARY

The 63ʳᵈ year of the Cooperative Tree Improvement Program observed the appointment of Kitt Payn as the new Director, following the phased retirement of Steve McKeand.

SELECTION, BREEDING, AND TESTING

5ᵗʰ-Cycle selections are underway! A total of 30 selections were made this year. Scion material from these selections have been grafted at the Arrowhead Breeding Center.

The last crosses for the 4ᵗʰ-Cycle breeding populations were completed this spring with 98 crosses made in a final push to ensure sufficient seed availability for these families. This brings the projected total number of crosses included in the 4ᵗʰ-Cycle testing to **1,639**. Breeding for the Atlantic Coastal Elite (ACE) Genomic Selection population was concluded with an additional 74 crosses made, bringing the projected number of crosses to 120 (with ≥150 seed expected).

Age 4 measurements have been completed for 12% of the Coastal crosses and 5% of the Piedmont crosses in the 4ᵗʰ-Cycle tests. The first set of Northern crosses will complete age 5 measurements in 2020.

A new version of the **PRS**™ for the Northern region was released. The number of parents increased by more than 21% relative to the 2010 version, and the number of full-sib families increased by 18%.

A Mean Minimum Temperature tool was added to the **TIPRoot** database. A new pollen cloud called the "Upper Gulf Coastal Plain of Alabama (UG)" was also added to the database.

RESEARCH

The Cooperative is progressing with the development of a screening array for loblolly pine. A total of 642,275 SNP markers were submitted to ThermoFisher for quality control in March, 2019. A subset of 423,695 markers were included on the screening array, and these markers will be screened using a population of 480 *Pinus taeda* selections. The genotyping array, Pita50K, is expected to be available in mid-September.

Breeding value predictions from clonally replicated tests and half-sib progeny tests were compared. The relationship between predictions from clonal data and half-sib progeny data for volume based on independent and combined analyses were $r = 0.59$ and $r = 0.97$, respectively.

New software called PineBreed is being developed. It utilizes pedigree-based relationships to create an optimal mating list for monoecious species like loblolly pine.

ASSOCIATED ACTIVITIES, GRADUATE STUDENTS, COOPERATIVE STAFF, MEMBERSHIP

SC Forestry Commission returned as a Full Member. Green Diamond joined as a new Contributing Member, but we lost White City nursery, following their purchase by International Forest Company. There are now 10 Full Members, 21 Contributing Members, and 7 Research Associate Members. Graduate students' contributions to the program continue to be critical. We continue to teach short courses and workshops to members and colleagues at NCSU and around the world.

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A MESSAGE FROM THE DIRECTOR

THE CYCLE CONTINUES

The Tree Improvement Cooperative has a proud, long standing history of excellence. We are widely recognized for our innovative research, efficient application of new technology, and providing tangible value to our members. Core to our success is a culture of collaboration and relationship building. I feel privileged to have joined the Cooperative in November, and look forward to our continued success.

It has been an exciting year for the Cooperative. We made the first 5th-Cycle selection; a momentous step forward for our breeding program. The symbolism achieved in performing the first selection *together* during the Contact Meeting, epitomizes the cooperative strength of this Program.

We, today, are standing on the shoulders of our predecessors. The legacy of Dr. Bruce Zobel, who founded the Cooperative in 1956, will continue for generations to come. His work and teaching impacted so many of us in the national and international community. More recently, the Cooperative flourished under the leadership of Dr Steve McKeand. During a time when significant changes in forestland ownership were taking place in the South, Steve successfully formed the Contributing Membership option, catering for forestry companies, nurseries, and organizations who sought information about the genetic value of loblolly pine. Today, our Contributing Members form an integral part of the Cooperative.

Dr Fikret Isik led the team as Interim Director last year for the months prior to my arrival. He has played a key role in ensuring my smooth transition into the position, and I am grateful to Fikret and the team for the warm welcome I have received. Their enthusiasm and dedication to their work demonstrates their strong commitment to the Cooperative.

In late November, the team presented an excellent Tree Improvement Short Course. Their wide range of expertise is a tremendous asset to our Program. The Contact Meeting, sandwiched in the middle of the Short Course, was a great success, providing an effective communication platform for progress made during the year, and updates on new developments. The gathering also provided an excellent opportunity for me to meet many of the members shortly after my arrival. Although I was meeting individuals for the first time, they demonstrated the familiar core values that I have witnessed amongst forestry practitioners the world over. All can be characterized by a respect for communities and the environment, and a genuine passion to create long term value. Subsequently, I have had the opportunity of visiting member organizations, and becoming familiar with their individual operations. I look forward to more visits as the year progresses.

During the early part of 2019, our team set out on a selection-trip across the South. In partnership with our members, 30 selections were made. Scion material from these selections has since been grafted at the Arrowhead Breeding Center. Other notable achievements during the year include the release of an updated version of the **PRS**™ for the Northern region, the continued progress toward the development of the Pita50K SNP genotyping array, and Fikret being awarded a USDA-NIFA grant in support of the pioneering genomic selection work in loblolly pine.

Looking ahead, I eagerly anticipate additional value being provided to our members. The efficient manner in which the Cooperative has moved the breeding cycle forward will be further enhanced by continued research underpinning the tree improvement process. We are today's custodians of the Cooperative's genetic resource. It is our responsibility and privilege to ensure the legacy of this Program continues for the benefit of generations to come.

Kitt Payn, May 2019

SELECTION, BREEDING, AND TESTING

Fourth Cycle Breeding Progress

The 4th- Cycle breeding effort is officially complete! Breeding for the 4th-Cycle began in 2013 and was 97% complete after spring 2018 (see the 2018 Annual Report). Cooperative members were not assigned breeding in 2019, but TIP staff performed mop up/insurance breeding at the Arrowhead Breeding Center (98 crosses). The annual number of crosses attempted and their success rate throughout the 4th-Cycle breeding effort are shown in Table 1. The success rate (defined as a cross yielding at least 60 seeds) was not spectacular, and prolonged the breeding cycle. Reasons for less-than-ideal crossing success included late frosts that killed tender flowers, insect damage, hurricane damage, grafts with incompatibility issues, clones with flower/pollen morphology issues, and stored seed lost in a warehouse fire. The cross success rate also went down as the cycle progressed, as the strategy shifted from installing many pollination bags per cross to more crosses with fewer bags each. While it took longer than planned, we still "got-er-dunn"!

Now that 4th-Cycle breeding has been completed, we wanted to reflect on the origins of the population that was created. The list of parents contributing to the 4th-Cycle population is displayed by their selection origin in Table 2. The majority of these parents were selected for breeding using a differential evolution algorithm, MateSelect, 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 generations. Use of the MateSelect algorithm was a fundamental change in Cooperative breeding, because all available selections (regardless of generation) were considered for breeding based on their genetic merit and contribution to a diverse gene pool. The largest source of parents used to create the 4th-Cycle population were plantation selections (28.2% of parents). These parents were previously bred in diallels using random-mating with less than spectacular mates, and thereby did not produce elite individuals for forward selections. The MateSelect algorithm recognized this deficiency and selected the parents to breed again with better mates. The next largest class of parents came from 3rd-Cycle selections, followed by the 2nd-Generation selections, 4th-Cycle selections, and 1st-Gen selections from natural stands. There are also important contributions from the Cooperative's elite populations, especially the Atlantic Coastal Elites. A number of proprietary forward selections were offered for Cooperative breeding by MeadWestvaco, Plum Creek and CellFor. Selections from Georgia Forestry Commission/US Forest Service (USFS) rust resistant population were included in 4th-Cycle breeding. There were also parents selected from the highly productive, rust-resistant Livingston Parish, LA seed source. Finally, there was one parent that was selected from a selfed population and then outcrossed with other elite parents. This highly diverse set of parents has produced an excellent selection base for the 5th-Cycle!

Table 1. *Breeding effort throughout the 4th-Cycle. Relatively low success rates lengthened the breeding cycle and could be attributed to many factors. Cones from the 2018 breeding will be harvested fall 2019. Some crosses were successful after multiple attempts, which is not reflected here.*

Year	Crosses Attempted		Crosses Successful		Success Rate	
	Members	TIP Staff	Members	TIP Staff	Members	TIP Staff
2013	97	29	73	21	75%	72%
2014	278	62	166	43	60%	69%
2015	248	69	156	35	63%	51%
2016	246	183	167	134	68%	73%
2017	185	129	83	60	45%	47%
2018	242	216	*	*		
2019		98		*		

Table 2. Count of parents of the 4th-Cycle population by their selection origin (the test/population from which they were selected). The 4th-Cycle population includes trees that are in progeny tests, seedlings recently sown for next year's progeny test, and maturing cones in the orchards from 2018 and 2019 breeding.

Selection Origin of Parent	Population			Percent
	Coastal	Northern	Piedmont	
1st-Gen. Natural Stand	41	9	1	5.2%
1st-Gen. Plantation Selection	108	70	98	28.2%
2nd-Gen. Selection (from Tester Pop.)	100	40	25	16.8%
3rd-Cycle Selection (from 2nd-Gen. Diallel)	24	32	18	7.6%
3rd-Cycle Selection (from Plantation Diallel)	80	51	116	25.2%
4th-Cycle Selection (from Full-Sib Block Plots)	39	12	14	6.6%
Livingston Parish, LA	9			0.9%
Lower Gulf Elite Pop. Selection	2			0.2%
Piedmont Elite Pop. Selection	6	1	10	1.7%
Atlantic Coastal Elite Clone	42		2	4.5%
GFC/USFS Rust Resistant Pop.	6			0.6%
MeadWestvaco Internal Selection	9			0.9%
Plum Creek Internal Selection	10			1.0%
CellFor Internally-Tested Clone	4			0.4%
Selfed Pop. Selection	1			0.1%
	481	**215**	**284**	**100.0%**

Through detailed pedigree records and selection grade sheets, we can also evaluate the source of the earliest known ancestors of the 4th-Cycle population (founders). Figure 1 shows the proportion of genes that make up the 4th-Cycle crosses by the founder's selection origin. The first selections made by the Cooperative were from natural stands in the 1950s-1970s. Those selections contributed 43%, 40%, and 16% to the 4th-Cycle Coastal, Northern, and Piedmont populations, respectively. To increase the diversity of the original population, a large effort was made in the 1970s and 1980s to make selections from plantations that came from non-improved wild seed sources. These plantation selections had the benefit of an environment that was conducive to selecting excellent genotypes; they were more uniform, even-aged, and in some cases the infection of fusiform rust was very high, allowing identification of resistant/tolerant genotypes. These factors were expected to produce a higher heritability of the traits compared to natural stand selections. The plantation selections were the largest contributors to the three 4th-Cycle populations, which signifies the importance of this effort. The remaining founders include selections from Livingston Parish, LA seed source and selections that originated from outside the NCSU Cooperative.

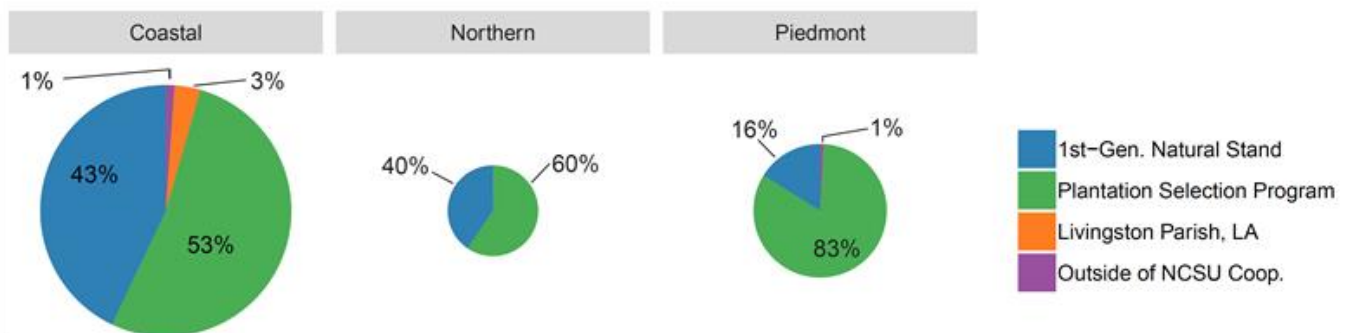## 4th- Cycle Population Founder Origins

Figure 1. The expected proportion of the genome (expected additive genetic relationships), contributed by the founders, in the 4th-Cycle population. The size of the pie chart corresponds to the population size (number of crosses). The population includes trees that are in progeny tests, seedlings recently sown for next year's progeny test, and maturing cones in the orchards from 2018 and 2019 breeding.

ACE2 Breeding

There were 74 additional crosses bred among Atlantic Coastal Elite (ACE) parents by TIP Staff in spring 2019, bringing the expected total number of crosses to 120 crosses (with 150 seed). This second generation of ACE has been dubbed ACE2, and will be the basis for the Cooperative's genomic selection research. The ACE2 crosses bred in spring 2019 were done to fill in gaps in the population to provide adequate structure to estimate associations between DNA markers and traits of interest. ACE2 breeding is expected to conclude in 2019.

Pollen Collection

The pollen-mix parents used in the 3rd-Cycle were collected during spring 2019 to allow for the re-creation of the 3rd-Cycle pollen mixes (CPMX, PPMX, and NPMX for the Coastal, Piedmont, and Northern regions). The purpose of collecting this pollen was to allow for future production of more seed for the internal checklots (CCK, PCK, NCK), which are invaluable for benchmarking genetic gain and developing new *PRS*™ versions. We would also like to have these pollen mixes on hand for breeding that requires a standardized pollen mix.

Pollen was also collected at the Arrowhead Breeding Center to initiate a "Selfing Awareness" trial for forward selections (seven crosses were made in spring 2019 using fresh pollen). A major consideration for orchard managers is the tendency for grafts to produce viable seed from self-pollination, because this seed will likely show depressed characteristics from inbreeding. Ideally, selfed individuals will not produce viable seed due to embryonic lethal genes. Classifying forward-selections as unlikely to produce selfed seed versus likely to produce selfed seed should help orchard managers more effectively design their orchards for open-pollinated seed production.

Testing Update

4th-Cycle Testing Progress

The primary objective of the 4th-Cycle testing design is to identify the best trees to use as forward selec-tions for the 5th-Cycle breeding. The 4th-Cycle testing strategy is a rolling-front, where new crosses are prepared for field tests as soon as their seed becomes available. The projected timeline for the testing of the 4th-Cycle population is shown in Table 3. The first year of 4th-Cycle test planting was 2014, and included crosses from the tail-end of the 3rd-Cycle (bred from 2005-2011) and other "substitute" cross-es. The first 4th-Cycle Breeding was done in 2013, and those crosses were first planted in 2016, and we will phenotype them at age four years (Spring 2020).

As of spring 2019, the 4th-Cycle breeding has been completed. Around 60% of the crosses have been planted. We have collected data at age four from 12% and 5% of the Coastal and Piedmont crosses, respectively. The Northern population is assessed at age five years to allow expression of the phenotype; the first set of crosses were measure done 2019 but will have complete data in 2020. The Coastal population will have 103% of the target number of cross-es planted in 2022, while the Piedmont and Northern populations will have 91% of the target number of crosses planted in 2022. The number of progeny tests established after 2022 will likely go down for a period as the 5th-Cycle population is mated. This is good news for the measurement crews; recall that the Cooperative decided to double the test establishment load in 2017, which implies that the test measurement load will double starting in 2021-2022.

Timeline to Identify New Seed Orchard Parents

Another objective of the 4th-Cycle testing design is to obtain breeding value estimates for the 4th-Cycle parents to determine which parents will be the best for seed orchards. The timeline for new parents that will have breeding value estimates is shown in Table 4. Parents are shown when they are projected to have at least 45 progeny measured. The majority of new parents are 4th-Cycle selections and 3rd-Cycle selections that had not yet been fully tested. There are also several new parents from our Cooperative's elite populations (Atlantic Coastal Elite, Piedmont Elite, Lower Gulf Elite). Table 4 also shows parents from members' internal programs that will have breeding values estimated. These will not be availa-

a) Coastal breeding population

Year			# of Crosses tested			
Mated	Planted	Measured	Begin	Continue	Complete	% Complete
2005-2011	2014	2018	88	-	-	-
2005-2012	2015	2019	43	83	99	12%
2013	2016	2020	124	66	138	17%
2014	2017	2021	106	141	290	37%
2015	2018	2022	112	114	372	47%
2016	2019	2023	169	99	483	61%
2017	2020	2024	56	211	559	70%
2018	2021	2025	158	134	659	83%
2019	2022	2026	66	234	819	103%
	2023	2027	-	67	885	112%

b) Piedmont breeding population

Year			# of Crosses tested			
Mated	Planted	Measured	Begin	Continue	Complete	% Complete
2005-2011	2014	2018	41	-	-	-
2005-2012	2015	2019	56	39	27	5%
2013	2016	2020	39	69	80	16%
2014	2017	2021	83	78	139	28%
2015	2018	2022	77	121	213	43%
2016	2019	2023	70	97	287	58%
2017	2020	2024	29	165	372	76%
2018	2021	2025	66	34	382	78%
2019	2022	2026	8	93	450	91%
	2023	2027	-	8	458	93%

c) Northern breeding population

Year			# of Crosses tested			
Mated	Planted	Measured	Begin	Continue	Complete	% Complete
2005-2011	2014	2019	45	-	-	-
2005-2012	2015	2020	43	38	17	6%
2013	2016	2021	37	64	46	15%
2014	2017	2022	41	78	108	36%
2015	2018	2023	21	83	145	48%
2016	2019	2024	15	69	168	56%
2017	2020	2025	32	62	205	68%
2018	2021	2026	61	24	211	70%
2019	2022	2027	22	85	274	91%
	2023	2028	-	23	296	98%

Table 3. *Projected timeline for 4th-Cycle testing, showing the count of crosses that begin testing each year (new crosses from the breeding program), the number of crosses that continue testing, and the cumulative number of crosses that complete testing (45 or more trees tested). The Coastal (Cycle4C) and Piedmont (Cycle4P) tests are planned to be measured at age 4 years, and the Northern (Cycle4N) at age 5 years. The first two years of testing included seed from the 3rd-Cycle breeding. The last year of 4th-Cycle breeding was spring 2019. Checklots and crosses tested outside their native breeding zone are excluded.*

ble for sharing among Full Members but their progeny will likely be available on the seedling market. The majority of parents continue to be tested in more crosses in years after their breeding values are first estimated, so we will need to be cognizant that parents may change ranks as more data become available.

The final number of crosses per parent is shown in Figure 2. About 50% of the new parents are tested in 1 or 2 crosses, while the other half are tested in 3 or more. Johnson (1998, Can. J. For. Res. 28:540-545) suggests that 2-3 crosses per parent is adequate to estimate GCA values when pedigree information

is available, as is the case in the 4th-Cycle population. Once a new parent has been satisfactorily tested, it will become a candidate for a seed orchard parent. When there becomes a cohort of superior parents, the next cycle of seed orchards can be established!

3rd-Cycle Wood Quality Measurements

The Cooperative began measuring juvenile wood quality in the 3rd-Cycle pollen-mix tests in 2013. Measurements are taken at approximately seven years of age, using rapid-assessment tools that assess acoustic velocity (an indirect measure of wood stiffness) and drill resistance (a measure of wood

Table 4. *Projected number of new parents with breeding value estimates by measurement year. A parent will have a breeding value estimate once they have more than 45 progeny measured. The Coastal, Piedmont, and Northern populations include 4th-Cycle selections and selections from Cooperative elite populations, such as the Atlantic Coastal Elite, Piedmont Elite, and Lower Gulf Elite.*

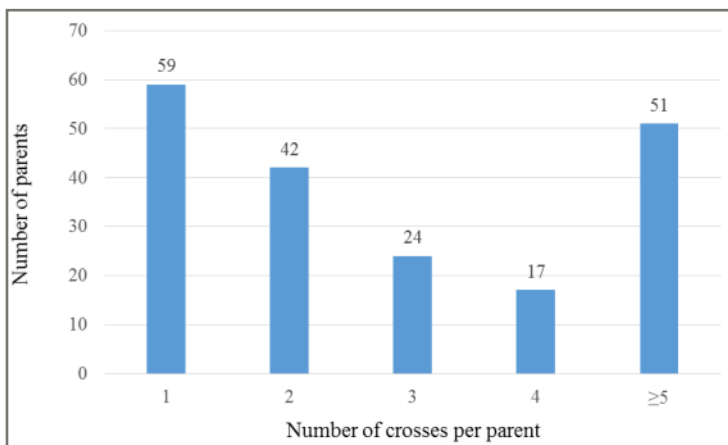| Year	Coastal	Piedmont	Northern	Internal	*Total*
2019	2	3	-	16	**21**
2020	1	15	-	2	**18**
2021	6	10	2	1	**19**
2022	7	3	7		**17**
2023	25	10	2	1	**38**
2024	16	6	-	-	**22**
2025	7	-	2		**9**
2026	12	6	-	-	**18**
2027	13	8	4	3	**28**
2028	-	-	3	-	**3**
Total	**89**	**61**	**20**	**23**	**193**

Figure 2. *Number of crosses per parent for new parents in the 4th-Cycle. About half of the parents are tested in 1-2 crosses and the other half in 3+ crosses.*

Year	Coastal	Piedmont	Northern
2013	CPMX1		
2014	CPMX2, CPMX3, CPMX4	PPMX1	
2016		PPMX2 PPMX3	NPMX1
2018			NPMX2
2019	CPMX5		
2020	CPMX6	PPMX4	

density). The 3rd-Cycle pollen mix tests are ideal for estimating parental breeding values with high accuracy. Two test sites are measured for each series (set of parents), resulting in an average of about 36 progeny per parent in at least two different environments. In 2018, the NPMX2 series was assessed, and there are three more series that will be assessed through 2019-2020 (Table 5).

ℙ𝕣𝕤TM Calibration Study

The objective of the 𝓟𝓡𝓢TM Calibration Study is to correlate breeding value estimates from progeny tests to the growth and yield of family block plantings throughout stand development. An analysis through age four years was presented in the 2018 Annual Report (pg. 19-20) and indicated that the trials were established successfully. The preliminary results showed strong correlation for growth traits and fusiform rust disease incidence. The age six year measurements were conducted in winter of 2019. At least one more measurement is planned before the trials reach stand density that requires thinning.

Northern Elite Population

The Northern Elite tests (established in 2014) were assessed for growth, stem form, and fusiform rust disease incidence this past winter. Details of the measurements and their analysis are presented in their own section of this annual report on page 11.

Longleaf

The Longleaf Pine Regional Provenance/Progeny Trial was established in 2011. Five of the tests were re-measured at age 8 years this past winter. The results, through age 6 years, were presented in the 2018 Annual Report. These 8-year data are presently being analyzed.

Table 5. *(left) Wood quality measurement schedule for 3rd-Cycle pollen-mix progeny tests. Tests are assessed around seven years of age to characterize the juvenile wood.*

The First 4th-Cycle Progeny Test Data Analysis Results

One of the major developments in the past year was the first analysis of 4th-Cycle Coastal progeny test data. At the time the analysis was conducted, measurements were available for 65 reps (12 tests) for a total of 6948 individual trees in the dataset. These were the first progeny tests established using the new incomplete row-column block design. This experimental design allowed capturing the local trends in environmental variation at a site in two directions. An individual-tree model was used to predict the breeding values of individuals and their pedigree on the same scale. Notably, these test sites were very highly connected, in that they have a large number of the same parents in common, which facilitates direct contrasts among genetic entries.

The phenotypic data included in this analysis were contributed by the Cooperative members in the winter of 2018 and uploaded directly to the TIPRoot database. Standardized queries were used to compile the dataset from our database, and ASReml software was used to predict breeding values. The linear model included site effects, rep effects, and row and column effects for each rep. We accounted for the

natural heterogeneity in genetic variances at each site, allowing each test site to have its own genetic variance. Additionally, each site had its own residual variance. Narrow-sense individual tree heritability estimates are presented in Table 1.

The model assumed a uniform genetic correlation between all pairs of sites. The individual tree model was fit to predict breeding values, which we could

Table 1. *Narrow-sense individual tree heritability estimates (standard errors in parentheses) from the 4th-Cycle Coastal analysis.*

| Site	Volume	Height	Straightness
2014 Test 1	0.38 (0.11)	0.40 (0.11)	0.21 (0.09)
2014 Test 2	0.33 (0.11)	0.39 (0.11)	0.09 (0.08)
2014 Test 3	0.21 (0.09)	0.23 (0.09)	0.19 (0.09)
2014 Test 4	0.26 (0.10)	0.44 (0.11)	0.17 (0.10)
2014 Test 5	0.20 (0.09)	0.17 (0.09)	0.21 (0.09)
2014 Test 6	0.37 (0.11)	0.33 (0.11)	0.24 (0.10)
2014 Test 7	0.22 (0.09)	0.22 (0.08)	0.10 (0.06)
2015 Test 1	0.14 (0.07)	0.14 (0.07)	0.15 (0.07)
2015 Test 2	0.13 (0.06)	0.21 (0.07)	0.11 (0.06)
2015 Test 3	0.32 (0.07)	0.28 (0.07)	0.11 (0.04)
2015 Test 4	0.17 (0.09)	0.21 (0.09)	0.10 (0.08)
2015 Test 5	0.35 (0.11)	0.39 (0.11)	0.19 (0.09)
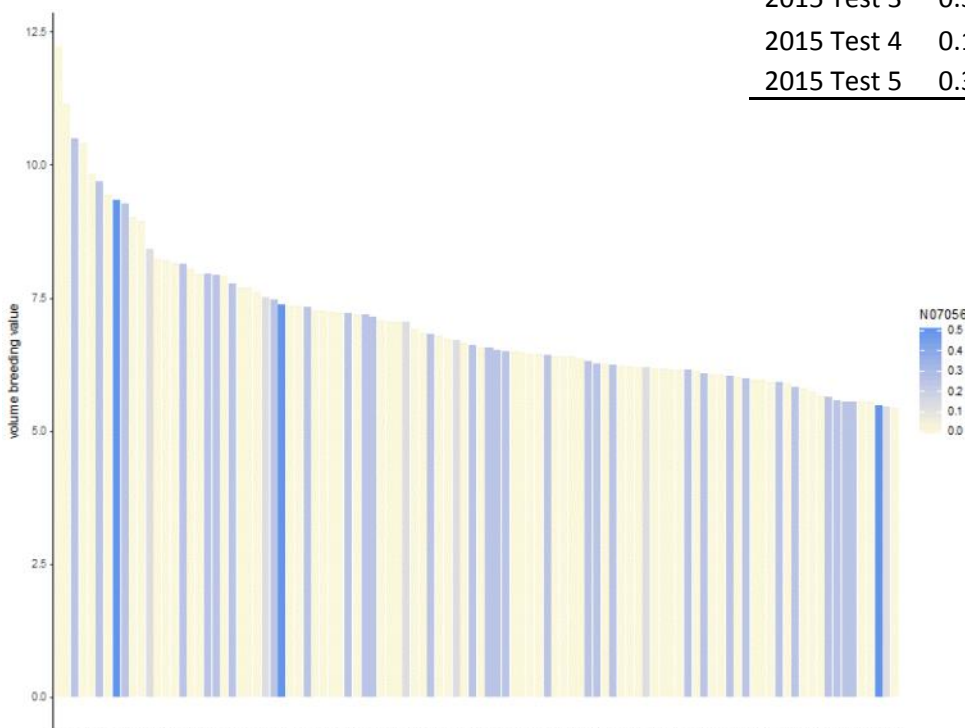
Figure 1. *Volume breeding values of the top 100 trees. Bars are shaded according to the level of relatedness with N07056. As an indication, a parent-progeny has a coefficient of relationship of 0.5, whereas a grandparent-progeny has a coefficient of relationship of 0.25.*

use in ranking selections from the 4th-Cycle progeny tests. We found that some of the model terms, such as row and column terms, were not significant for many sites. To avoid model overfitting, we are revising our models in the coming year to accommodate different effects at different sites. We are also planning ahead to the 2020 measurement season, since it may be impossible to run the individual tree model on such large datasets. One of the discussion topics at this year's 5th-Cycle planning meeting will be alternative models we can use for predicting breeding values of individual trees and their full pedigree from these large datasets (e.g. two-stage analysis or the reduced individual tree model or Reduced Animal Model in the animal breeding literature).

The 4th-Cycle Coastal data analysis yielded some surprising (or maybe not so surprising) results. Many of the top trees were related to N07056 (Figure 1). The first test series in 2014 were composed of the "Substitutes": crosses made as part of the 3rd-Cycle and crosses from members' internal breeding. Many of the substitute crosses had N07056 in their pedigree. We expect this will
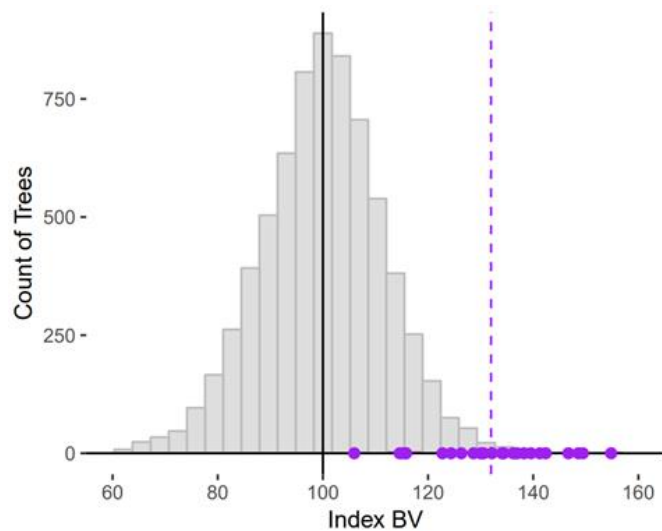
change as more data are collected from rolling front progeny tests in the coming years.

A selection index was created by giving different weights to volume (0.6), fusiform rust (0.2), and stem straightness (0.2) breeding values. Candidate selections were identified using the MateSelect algorithm (Figure 2) and were limited to families that have completed their testing through 2019. The mean of the candidate population was 32% greater than the total population mean (Figure 3). The candidate selections were then rigorously evaluated infield before being confirmed as official 5th-Cycle selections.
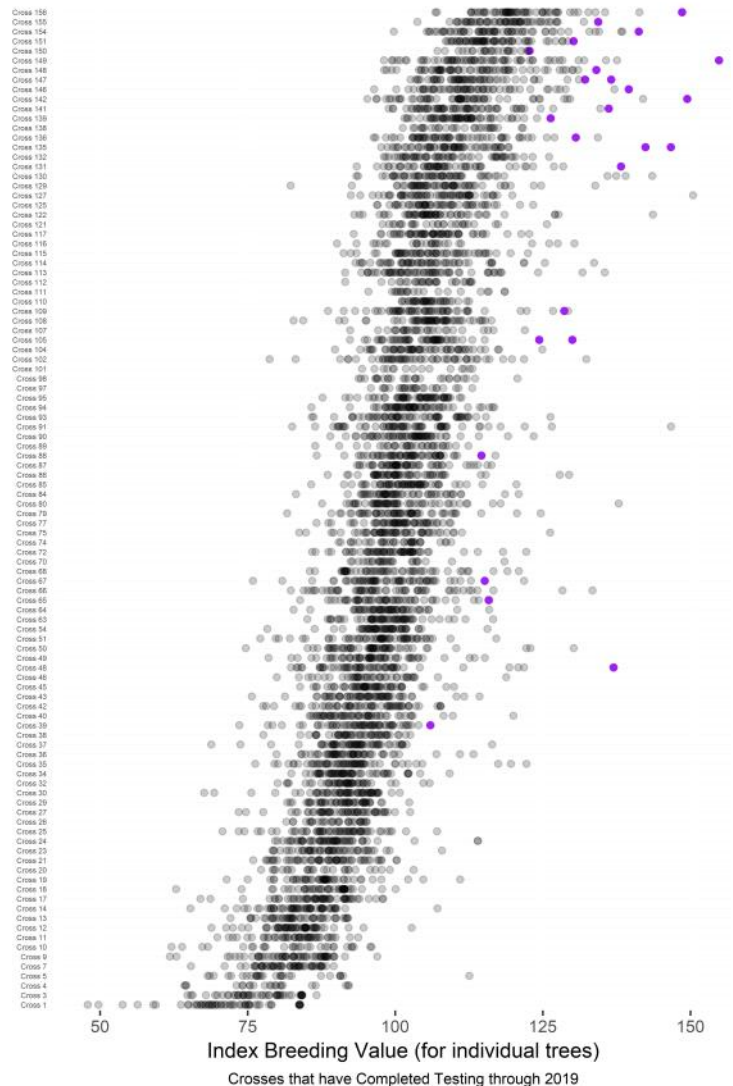

Figure 2. *Distribution of index breeding values centered on a mean of 100. The purple dots represent the candidate selections, and the vertical dashed line indicates the mean of the candidate population (132%).*

Figure 3. *Individual tree index breeding values by cross. Families are ranked by family breeding values, and candidate selections (purple points) were identified using the MateSelect algorithm.*

The 5th-Cycle Selection Process is Underway

The Cooperative has officially commenced the 5th-Cycle selection process! It was appropriate that the first selection was made during the fall 2018 Contact Meeting, hosted by IFCO in Moultrie, GA. On this exciting occasion, the members enjoyed the opportunity of participating in the in-field selection evaluation process. Steve McKeand led the charge and described the standard selection protocol used when evaluating each selection candidate (Image 1). The field visit culminated with the selection of a magnificent tree, scoring highly across all assessment criteria.

Image 1a: *(left) The first 5th-Cycle selection made during the Cooperative Contact meeting in fall 2018. The progeny test was established by IFCO. Contact meeting attendees carefully reviewing the first selection for stem straightness.* ***Image 1b:*** *(right) Fikret Isik, interim Director at the time (pictured left) and Kitt Payn, the new Director (pictured right) posing happily with the selection.*

During February and March 2019, several selection trips were made, ranging from North Carolina to Florida, and west to Alabama. At each test site, our team was met by a Cooperative member representative (Image 2). Together, we performed the in-field evaluation of candidate trees. These superior trees were identified based on the 4th-Cycle analysis. Each selection candidate was rigorously assessed for productivity, stem straightness, branch characteristics, and rust disease incidence. The candidates often looked very good, but it was important to incorporate a local neighborhood adjustment that included trees in fairly close proximity. In the occasional test, there was an adverse amount of environmental variation, attributable to poor site selection and/or sub-optimal test maintenance. Consequently, fewer selections were made from these tests. These were the exceptions, but we encourage our members to attain the high standards we have come to expect over many years of progeny testing.

In total, 30 selections were made in winter of 2019. Each selection was monumented using flagging, an aluminum tag nailed to the stem, and the GPS coordinates were recorded. An important change from previous selections is that young needles were sampled from each selection, and the extracted DNA will be used to generate a reference fingerprint for each clone (Image 3). This will enable us to verify the clone's identity as vegetative tissue is transferred from the ortet to the breeding orchards and clones banks. A small number of scions were harvested from each selection (Image 4), and these were subsequently grafted at the Arrowhead Breeding Center this spring (Image 5). Our members will be tasked with collecting additional scion next year for clone banking purposes.

Image 2: *(top left) Discussing the plans set out for the day's work.*

Image pair 3: *(top center, right) Needles sampled for DNA extraction. A reference fingerprint will be generated for each new 5ᵗʰ-Cycle selection.*

Image pair 4: *(bottom left and center) Scion collection from a 5ᵗʰ-Cycle selection (left to right Graham Ford, Austin Heine, Kitt Payn and Eddie Lauer; photographed by Steve McKeand). The harvested scion was prepared for shipment to the Arrowhead Breeding Center.*

Image 5: *(bottom right) The first 5ᵗʰ-Cycle selection grafted at the Arrowhead Breeding Center.*

Clonal Testing of the Northern Elite Population

The Northern Elite (NE) population consists of progeny from 3rd-Cycle elite breeding and sawtimber elite breeding for the Northern and Piedmont zones. The NE population was clonally tested using rooted cuttings that were initiated in February of 2013 and planted in field trials in spring of 2014. Three field trials were established in NC, TN, and VA with two ramets per clone at each test. Clonal testing increases within-family heritabilities, resulting in more power to identify forward selections. The NE population is comprised of 18 full-sib families (15 Northern and 3 cold-tolerant Piedmont) selected on the basis of volume, straightness and forking breeding values with weights of 0.4, 0.3 and 0.3, respectively. The tests also include clonally propagated checklots from five Northern crosses and seedling progeny from 41 crosses tested in the first round of 4th-Cycle Piedmont/Northern tests in order to provide adequate connectivity for a combined analysis.

Many of the Northern Elite clones had an extra ramet planted at the Arrowhead Breeding Center in 2014 at an orchard spacing to facilitate accelerated breeding. This strategy expedites breeding by allowing the clones in the orchard to mature in tandem with the ramets in the progeny tests. In February 2019, individual tree breeding values were predicted for the NE population for guiding selection, orchard rogueing, and breeding efforts. The objectives of this analysis were twofold:

1) Determine how individuals within the NE Population rank compared to the 4th-Cycle Piedmont and Northern populations with the purpose of calculating an appropriate number of selections.

2) Determine which clones should be selected from the NE Population for advanced breeding. This decision would inform rogueing and grafting at the Arrowhead orchards.

Measurement

The tests in Tennessee and Virginia were measured in the fall of 2018, five growing seasons after planting. This provided four ramets per genotype for analysis. Both sites had excellent survival, but the Tennessee test site had horrific tipmoth infestation (Image 1), and may warrant another measurement at age 6 or 7 since the effects of the damage may have affected the ranking of genotypes. The tipmoth attacks at the Tennessee site caused a high incidence of ramicorn branching (66%). The test site in Virginia was in excellent condition. It was a tour stop during the 2017 Contact Meeting, hosted by the Virginia Department of Forestry. The incidence of fusiform rust was appropriate for assessing genetic

Image 1. The Northern Elite test in Tennessee had excellent survival, but horrific tipmoth damage, with many trees looking more like bushes (shown at age 2 years pictured left). Trees were still affected at age 5, and re-measurement of this test might be warranted to improve genetic estimates.

resistance at the Virginia test site (28%), but was too low at the Tennessee site. The forking incidence was too low (less than 20%) to detect genetic variation at either site.

Clonal Breeding Values

The data were analyzed using linear mixed models with the pedigree information. In the model, an autoregressive row-column residual covariance matrix was used to account for linear environmental trends within sites. A heterogeneous genetic covariance structure was used to see how the ranking of genotypes correspond across environments.

Almost all the genetic variance was due to additive genetic effects. Non-additive genetic effects were not significant for most of the traits. Clone mean heritabilities were high for growth traits and incidence of rust disease, and ranged between 0.52 – 0.61. Clone mean heritability was low for

straightness (0.26) and was almost zero for incidence of ramicorn branches. The genotype-by-environment interaction was modest for all traits except ramicorn branching. The excessive tipmoth damage was likely responsible for the low clone mean heritability and strong GxE interaction for ramicorn branching incidence. The high clone-mean heritabilities for growth and rust indicate that forward selection from this population will likely be effective.

The full-sib families had significantly different volume breeding values, as did clones within families (Figure 1). The variation among full-sibs within a family is partly explained by Mendelian sampling effects. There was one clone with an unusually high volume breeding value compared to the rest of the individuals in the family (in cross NE2). Variation among clonal breeding values within families was not as strong for straightness score or rust incidence (results not shown).
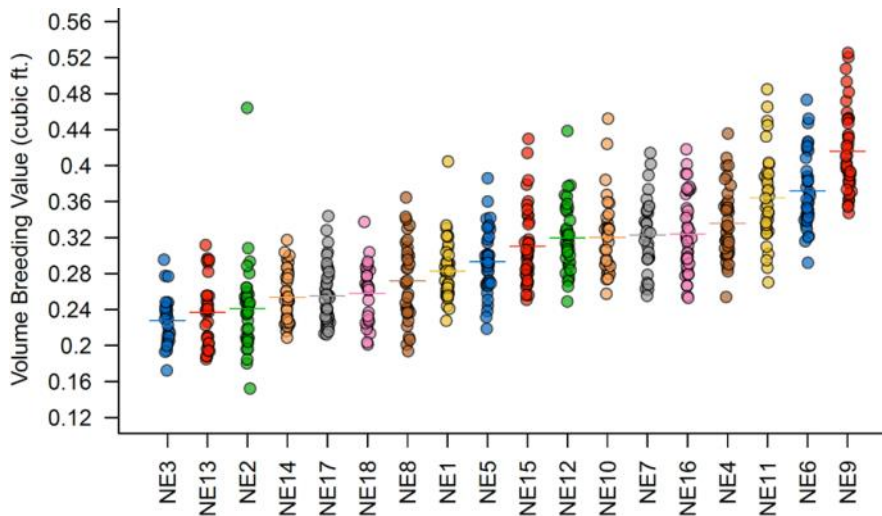

Figure 1. Clone volume breeding values within each full-sib family (NE1 – NE18), sorted by family midparent value. Circles represent individual clones and horizontal lines represent family midparent values.

Comparing the Northern Elite to the 4ᵗʰ-Cycle Northern/Piedmont Population

To evaluate how the Northern Elite population ranks compared to the 4ᵗʰ-Cycle Northern and Piedmont populations, a simpler model was used to estimate individual tree breeding values for all three populations on the same scale in a combined analysis (map of test locations in Figure 2). Many of the tests contained Coastal source families for connections with the Coastal population, therefore a cold hardy group was defined using a mean

minimum temperature threshold of 11.5°F. An index breeding value with 70% weight on volume and 30% weight on straightness score was used to rank trees. The distributions of the estimated index breeding values for the cold-hardy population are shown by cross in Figure 3.

The results of the combined analysis indicate that the cold-hardy crosses in the Northern Elite population rank similarly to the 4ᵗʰ-Cycle Piedmont and Northern populations. However, the clonal replication in the NE allows for higher accuracies

for individual-tree breeding values. This causes many of the NE clones to rank very well across the three populations, even for clones that come from crosses with less than spectacular cross midparent values. For example, if we consider a selection scenario that includes the best 20 trees, but no more than 2 trees per cross, then there are 9 Northern Elite clones included. This is likely due to the increased precision of genotypic value estimation brought about by clonal replication (less shrinkage).

Based on this analysis, some of the clones in the Northern Elite population will likely play an important role in 5ᵗʰ-Cycle breeding for the cold-tolerant population. One of the top clones was present at the Arrowhead Breeding Center and was bagged for breeding in spring 2019, and pollen was collected from another clone. The remaining clones will need to be identified in the 5ᵗʰ-Cycle strategy and topgrafted for future breeding.

Future Direction

Figure 2. (right) Map of test locations measured through 2019 used in a joint analysis of the Northern Elite and 4ᵗʰ-Cycle Piedmont/Northern (Cycle4P/ Cycle4N) populations to estimate clonal and individual tree breeding values on the same scale.
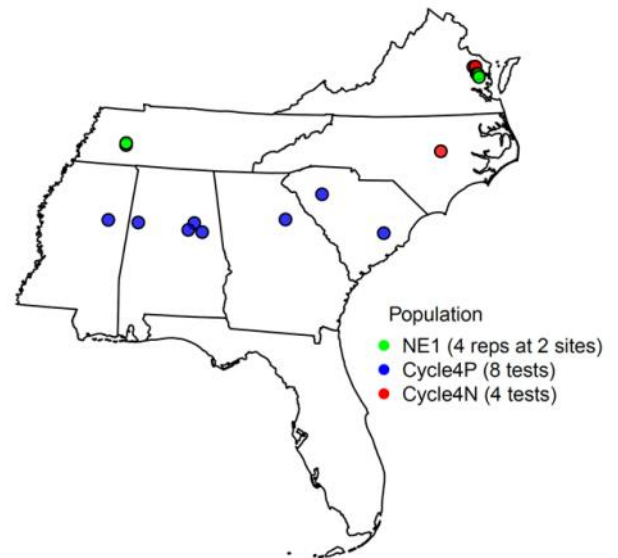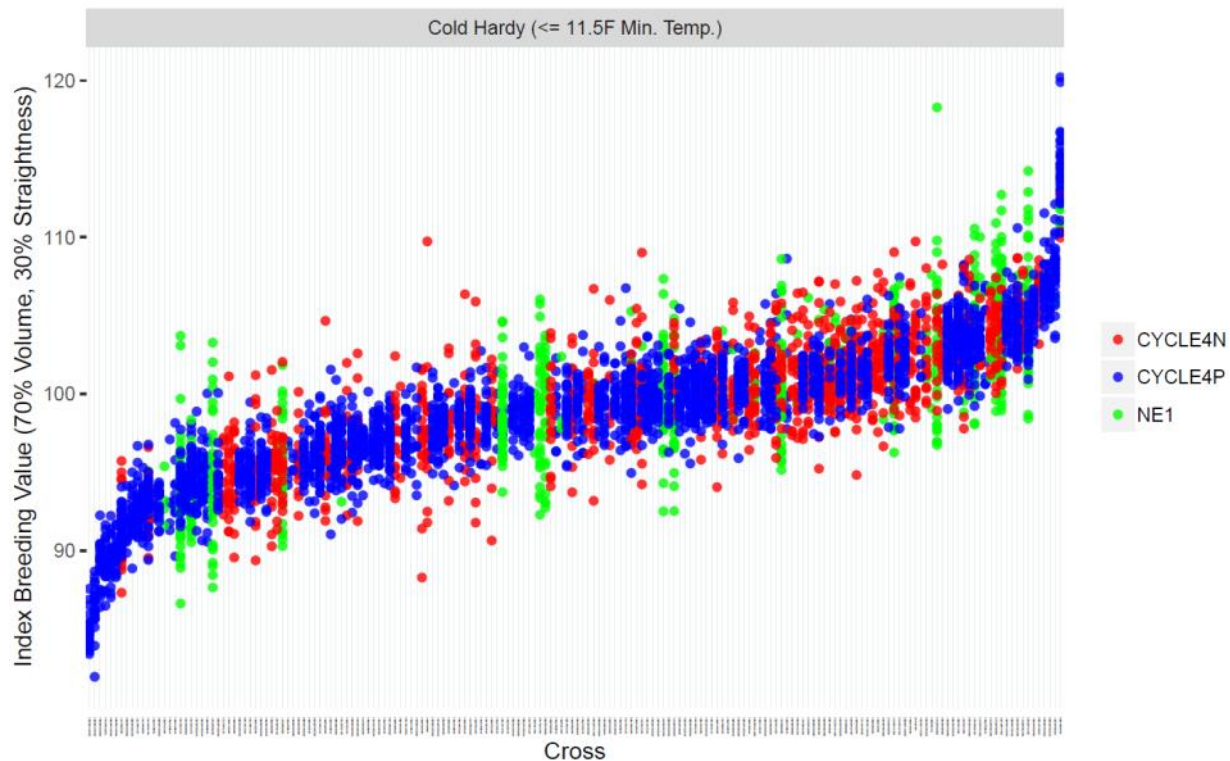

Figure 3. (below) Index breeding value estimates (70% weight on volume and 30% weight on straightness) from a joint analysis of the Northern Elite and 4ᵗʰ-Cycle Northern and Piedmont populations. Individual-tree breeding values are grouped by full-sib family and sorted by the family mean from left to right. The clonal replication in the Northern Elite population produces more confidence in partitioning within-family variation.

Loblolly Pine PRS^{TM} and TIPRoot Database Updates

Northern 2019 PRS^{TM}: A major development for our Northern population

TIP staff initiated a major effort of data gathering, formatting, and modeling, culminating in the release of an updated PRS^{TM} version for the Northern region, '2019 V1 Northern'. The 2019 V1 Northern PRS^{TM} is a vast improvement over the previous version. The number of parents increased by more than 21% relative to 2010 V1 Northern, and the number of full-sib families increased by more than 25%. Importantly, the 2019 V1 Northern PRS^{TM} comprises a number of test series not included in the 2010 V1 Northern. They are the Northern Elite clonal tests, the Northern Pollen-mix tests, as well as a number of legacy test series such as the Weyerhaeuser plantation diallel tests. In total 43 tests were added, and their locations are shown in Figure 1. The final number of tests was 302 and their genetic connectivity is displayed in Figure 2. They included 228 new parents and 296 new crosses. A total of 334,133 observations were used in the analysis.

For volume there were 1981 crosses and 1994 parents included in the analysis. Breeding values of
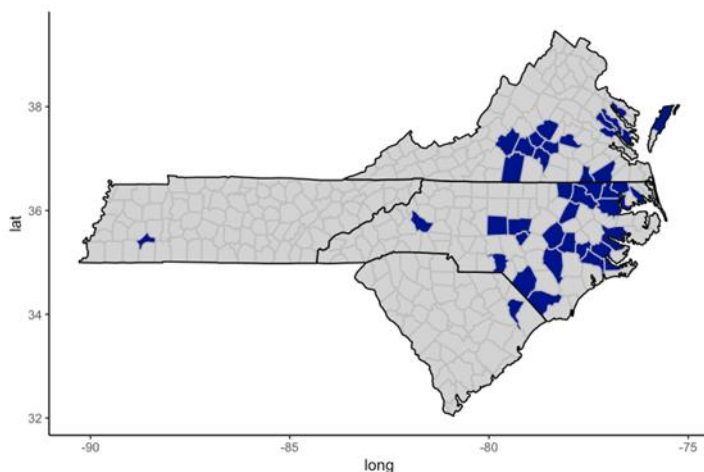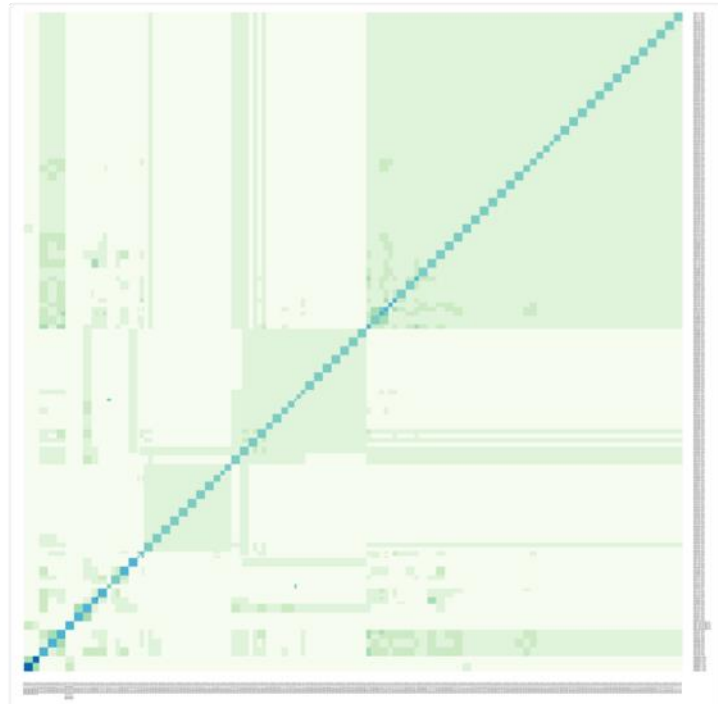

Figure 2. *Number of shared parents between all possible pairs of sites in the 2019 Northern BLUP analysis is displayed as a heatmap with darker blue colors indicating a greater number of shared parents. The diagonal represents the number of parents within each site.*

the top 100 individuals are shown in Figure 3. The y-axis is the volume breeding value centered on 100 for the population. The best parent is expected to have a progeny mean 50% better than the population mean or 115% better than the non-improved CC1 checklot.

Piedmont Upper Gulf Pollen Cloud

A new pollen cloud called the "Upper Gulf Coastal Plain of Alabama (UG)" was added to the PRS^{TM} database. Importantly, for Coastal orchards located in the Upper Gulf, this pollen cloud is more representative of the Upper Gulf region of Alabama than the "Southern Coastal Plain (CP)" pollen cloud. In the PRS^{TM} Values tool, this cloud can be selected from the Pollen Cloud drop-down menu (Screenshot 1 on the following page).

Figure 1. *Test site locations included in the 2019 V1 Northern PRS^{TM} version.*
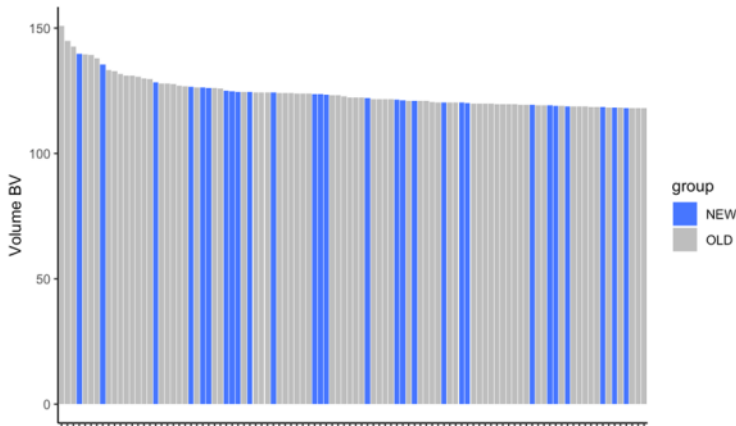
Figure 3. *The top 100 parents ranked for volume breeding value (y-axis). There are 28 new parents (blue color bars) represented in the top 100 list in the 2019 V1 Northern **PRS**™.*

NC BigBLUP: **PRS**™ values: a transition region

North Carolina is uniquely located at the confluence of the Coastal, Northern, and Piedmont Regions. Landowners in North Carolina plant seedlings based on **PRS**™ scores from all three regions. The challenge for members is that in the current **PRS**™ system, the three breeding regions are independent, and scores cannot be directly compared (e.g. a **P**=60 in the Northern Region is not the same as a **P** =60 in the Piedmont Region).

In an attempt to compare selections from these different provenances, the NC BigBLUP was created. This analysis combined test data from the states of Virginia, North Carolina, and South Carolina that included adequate connections via checklots of CC1, CC2, CC8, PCK, and/or NCK. No other area in the Cooperative has overlapping checklots similar to what exists in these trials.

Data were obtained from 464 progeny tests. A total of 530,059 observations were included in the analysis. Breeding values from the NC BigBLUP can be accessed directly on TIPRoot. **PRS**™ values were not generated for this analysis due to the complications of estimating pollen cloud effects, as it is unlikely for an orchard to contain selections from multiple regions. Seed orchards are still by-and-large specific for a given provenance. For example, northern selections from Virginia are typically only present in orchards with other cold-hardy selections. The purpose of the NC BigBLUP is to allow a comparison of the breeding values of selections from the three provenances. It is especially geared towards landowners in southern Virginia and North Carolina who own land near the boundaries of the three provenances, and who wish to transfer material from one provenance to another. These landowners will need to consider the location of the orchard from which they purchase any open pollinated seedlings.

It is recommended that the user generates a list of potential candidates by comparing breeding values from the NCBLUP, and then verify the selections are adapted to their deployment zone using the "Mean Minimum Temps" tool under "Pedigree Information" (Screenshot 2).

Screenshot 1:
The screenshot shows how to select the pollen cloud tool within the TIPRoot database.

Screenshot 2: The screenshot shows how to navigate to the NCBigBLUP data set when obtaining PRS™ information for selections.

Mean Minimum Temperature Tool

A Mean Minimum Temperature tool was added to the TIPRoot database in April, 2019. The new application is accessible via the "Pedigree Information" tab (Screenshot 3). This tool produces an estimate of the mean minimum temperature for any given cross between two parents (entered in Selection Id 1 and Selection Id 2 boxes), or of a single parent. Alternatively, the user can upload an Excel spreadsheet containing many crosses, and the mean minimum temperature is returned for each cross.

The algorithm works by finding all the most remote ancestors for the parents specified in the cross. Each of these ancestors has a geographic origin with an associated mean minimum temperature. The mean minimum temperature of the cross is a weighted average of the ancestral mean minimum temperatures, each ancestor being weighted by the relationship coefficient between that ancestor and the progeny of the cross.

Amazon Web Services Migration

During the 2018-2019 hurricane season, power outages in Biltmore Hall resulted in shutdowns of Tracker, the server which hosts the TIPRoot website. During the shutdowns, members lost the ability to log in to TIPRoot and retrieve PRS™ sheets. In order to avoid this situation in the future, we have explored the option of having TIPRoot installed on Amazon's cloud server. This is an affordable alternative to hosting a physical machine on NCSU campus, with the added benefit of guaranteed continuous service. Each "virtual machine" on Amazon's cloud servers are mirrored in multiple locations, so if any one server farm loses power, at least two other locations can serve the website. A trial "beta" version of TIPRoot is currently being tested on the cloud server and the results look positive thus far.

Screenshot 3: New Mean Minimum Temperature tool added to the TIPRoot database. This can be found in the side menu listing under the "Pedigree Information" heading.

Gene Conservation Effort

During the summer of 2018, TIP staff and students processed seedlots for the Cooperative's loblolly pine gene conservation effort (Image 1). All seedlots were derived from first-generation parents to ensure true geographic origin. The seedlots were selected from a broad geographic distribution, maximizing the number of counties represented by at least one seedlot each (Figure 1). Genetic diversity was further enhanced by selecting open pollinated families.

Seedlots were prepared for two storage categories. One group is being stored at the USDA National Seed Lab in Dry Branch, GA. These seedlots will be considered the working population and will be available for research purposes, with the necessary approval from Cooperative staff. The second group is being sent to the National Center for Genetic Resources Preservation in Fort Collins, Colorado for permanent storage in the "doomsday vault".
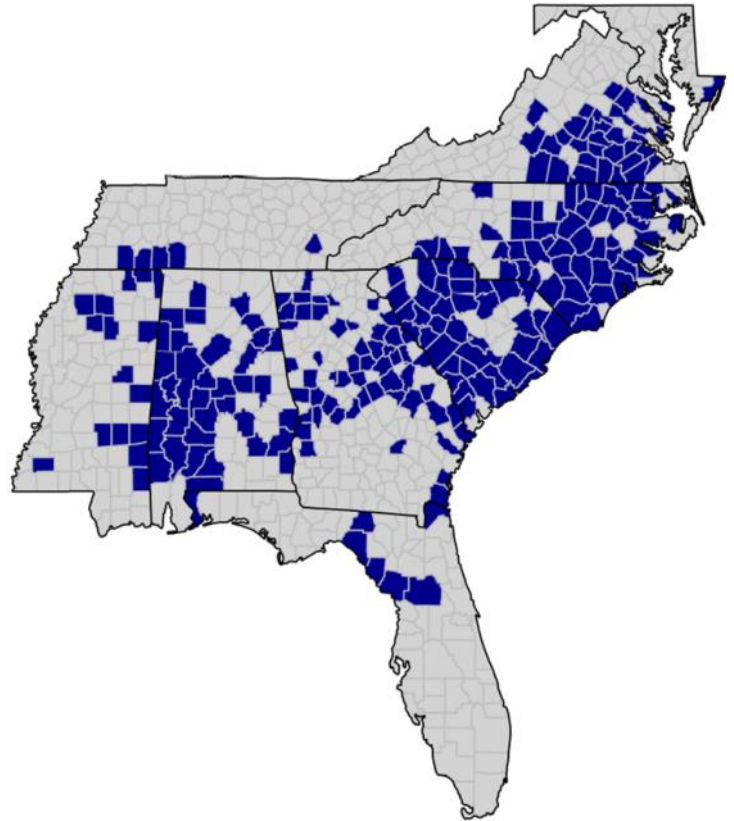

Figure 1. *Counties represented in the Cooperative's gene conservation effort. The seedlots comprised first-generation, mostly open-pollinated, families that were in the TIP lab seed inventory.*
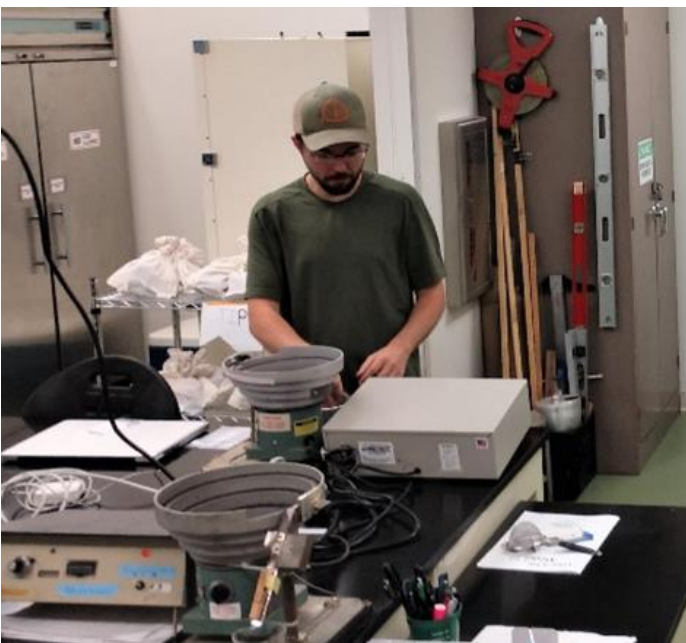

Image 1. *Graduate student, Colin Jackson, in the lab running several seed counters for the gene conservation effort. We had to source additional seed counters from our friends at Camcore to expedite the process!*

The working population sent to Dry Branch, GA consisted of 247 unique seed samples. Our aim was for 1,000 seeds for each of these samples but some had fewer than 1000. Of these 247 seedlots, an additional 2,000 seeds were pulled from 140 samples to be sent for storage at Fort Collins. Germination testing was performed on samples (approximately 200) with sufficient seeds. Based on the germination results, several seedlots will be supplemented using additional seeds available in storage, while a few others will be substituted with alternative seedlots.

Fingerprinting status report

The Cooperative faculty and staff have made progress in developing a pipeline that can confirm clonal identity among individuals, such as different ramets in a seed orchard, using DNA extracted from loblolly pine needle samples. Reagents have been designed to amplify a total of 327 fragments of DNA in a single reaction per sample. DNA sequencing of the amplified fragments from each individual allows comparison of the sequence variants in the individual against the loblolly pine reference genome assembly. The amplified fragments range between 80 and 120 base pairs in length, and there can be multiple sequence variants within each sequenced fragment. These can be analyzed together, as a multi-nucleotide haplotype, or separately as individual single-nucleotide polymorphisms (SNPs). Loblolly pine is normally diploid, with two copies of each chromosome in most cells, so the expected result is that there should be no more than two haplotypes in a single individual for a fragment that occurs in a single copy in the pine genome. The occurrence of more than two haplotypes from a single genotype is a sign that the fragment occurs in multiple copies in the genome, which presents a challenge in using the data to confirm clonal identity or evaluate genetic distance (e.g. parent-offspring relationship).

A panel of genetic variants suitable for fingerprinting has multiple uses in the context of tree breeding. The simplest use is for verifying the identity of individual ramets of grafted material in seed orchards or breeding orchards. All grafted ramets of the same clone should have exactly the same genotype at each DNA marker, but some allowance for sequencing error and other sources of experimental noise is typically made. One way of displaying the results of this use of fingerprinting data is through a cluster plot, or dendrogram, that shows the genetic distance between individuals (Figure 1). Genetic distance is a measure of the number of marker loci at which two individuals have different genotypes, so ramets of the same clone should have a very low genetic distance

(expectation of zero) from each other. A parent-offspring relationship is another example of individuals that should have relatively low genetic distance, because an offspring receives exactly half of its alleles from each parent. Figure 1 shows an example of clonal ramets that are grouped on the same "terminal branch" of the dendrogram, and a parent-offspring relationship that are on adjacent "branches". The highlighted sample is from an individual ramet that was apparently mislabeled in the field.

This process of calculating genetic distance appears to verify clonal identity among samples with confidence. The method is not as robust for identifying parent-offspring relationships, but work is in progress to develop a procedure for that objective. The time may be ripe for a coordinated effort to sample trees, develop consensus genotypes, and build a database for use in verifying clonal identity across the cooperative breeding program.
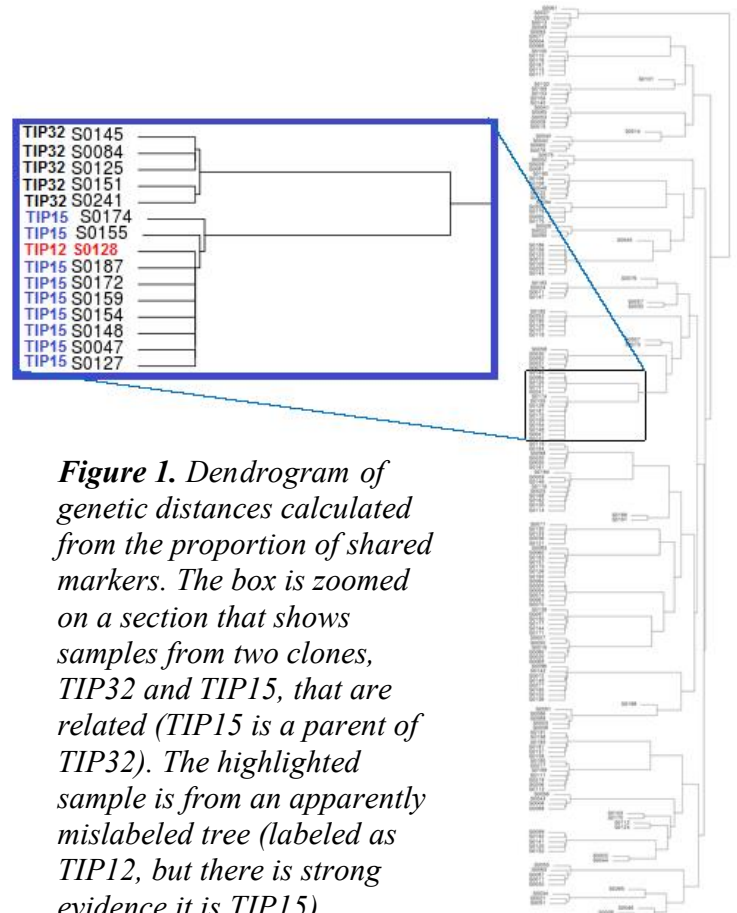

Figure 1. *Dendrogram of genetic distances calculated from the proportion of shared markers. The box is zoomed on a section that shows samples from two clones, TIP32 and TIP15, that are related (TIP15 is a parent of TIP32). The highlighted sample is from an apparently mislabeled tree (labeled as TIP12, but there is strong evidence it is TIP15).*

Arrowhead Breeding Center Update

Arrowhead, like much of the South, saw a lot of extremes over the past year. Starting with snow in January 2018 (Image 1), then Hurricane Michael, and excessive rainfall in the fall/winter of 2018-19. The warm temperatures in January 2019 led to the earliest breeding season on record. Arrowhead was thrown through the ringer this past year, but it is still standing strong.

Even with the extreme weather, Arrowhead has been a very busy place over the past year, starting in mid-summer with the completion of site preparation for the additional 4 acres of the Graveyard Orchard. A progeny test area was stumped, raked, and harrowed in time for planting an additional 235 rootstock in October 2018 (Image 2). Much of the tail end of the 5th-Cycle breeding will take place in the Graveyard Orchard.

Shortly after planting the last of the Graveyard Orchard, Chuck Little collected 321 bags of cones from 4th-Cycle breeding and ACE2 breeding performed in the spring of 2017. This cone collection effort was earlier than planned due to the threat of Hurricane Michael (Image 3). Fortunately we suffered little damage at Arrowhead except for the blown over rootstock seedlings in the Graveyard Orchard (Image 4). A special thanks to Chuck Little and Dick Young with the Georgia Forestry Commission for taking time to stake up each of these after the storm!

Image 1. (left) "The Chalet" in the snow on January 17th, 2018. A very unusual sight for this part of Georgia. The red clay really pops with an all white backdrop! Photo courtesy of Chuck Little.

This past winter-spring 2019, was once again a busy season with numerous activities taking place. Pollen was collected from 84 selections for upcoming breeding and selfing work, and to recreate the NCK, PCK, and CCK checklots. Additional clean-up breeding was completed for the 4th-Cycle and ACE2 populations, much of which had to be done due to low seed yields in the fall of 2018. Following the breeding season, TIP staff and students pruned several orchards and rogued the Northern BRO (Breeding Ramet Orchard) (Image 5) using the results from the recent analysis of the Northern Elite data. All of this pruning and rogueing work was done to prepare these orchards for use as future 5th-Cycle interstock.

Image 2. Rootstock in the Graveyard Orchard in the back, grassy area was planted in October 2017 and front was planted in October 2018). This rootstock is from a very rust resistant family and will be grafted with 18-1210 scions to be used as interstock for topgrafting many of the 5th-Cycle selections in future years.
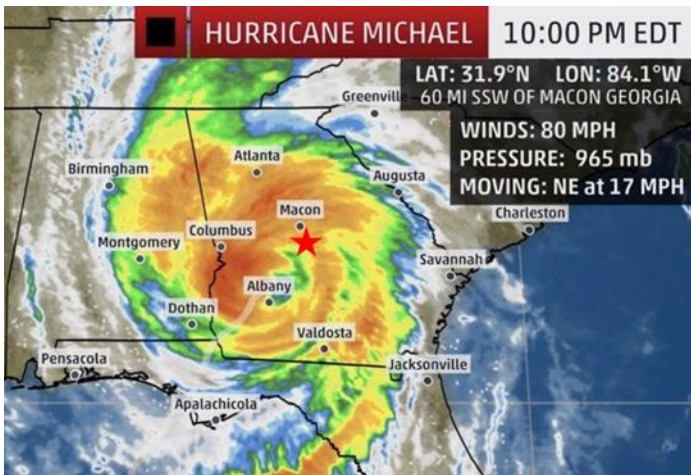
Image 3. Hurricane Michael over Georgia. Thankfully winds had weakened on approach to the Arrowhead Breeding Center(marked with a star). Image via the Weather Channel.

Steve McKeand, who thought he was retired (well, semi-retired), was put to work grafting and didn't stop for almost a month. Scions from18-1210 were grafted on all of the rootstock that were planted in the Graveyard Orchard in the fall of 2017 (~375 trees) and the remaining trees in the Northern BRO (~180 trees). Additionally, 27 5th-Cycle selections were topgrafted this spring; 5 grafts of each selection per tree will lead to a total crown conversion in a few years. The 5th-Cycle has officially commenced!

Image 5. Rogueing of the Northern BRO in March, 2019. This elimination was based on the analysis of Northern Elite clonal data. New selections were flagged and will be used for future breeding. One selection that already had flowers was bred this year at age 5. Additional trees left in this orchard that are not selections have been topgrafted with 18-1210 scion for interstock (blue flagging).

Image 4. Many rootstock seedlings were blown over in the Graveyard Orchard after Hurricane Michael. Chuck Little and Dick Young gave it their all staking up many trees to ensure the trees bounced back quickly. Photo courtesy of Chuck Little, October 11th, 2018.

Image 6. (right) Like all tree breeders in the South this time of year, we spent long hours working in the trees. Austin Heine was putting up "one more bag" in the New Breeding Orchard before the daylight was completely gone.

Genomic Selection Project Update

Cooperative staff began breeding among the Atlantic Coastal Elite (ACE) clones in 2017, creating a second-generation ACE population (known as the ACE2 population). The objective of the ACE2 breeding was to create a population that is genetically related to the ACE population for testing genomic selection methods in loblolly pine. The ACE and ACE2 populations are ideal to implement genomic selection due to the small number of founders (n=21), clonally replicated progeny established in multiple environments, and a relatively deep pedigree. The breeding of ACE2 continued in the spring of 2019 due to lower than expected seed yields for crosses made in the 2017 breeding season. Furthermore, we wanted to increase the diversity of the ACE2 population. There were 74 additional ACE2 crosses completed in 2019, bringing the total number of crosses to 120 ($\geq$ 150 seed per cross).

In the summer of 2018, TIP staff and students set out across the Southeast in search of foliage (Image 1). The objective was to collect fresh needle tissue

from each of the 2430 ACE clones and the 21 founders. After visiting three test sites, approximately 98% of the ACE trees were sampled (2382 out of the 2430), along with all 21 ACE founders. This foliage was dried over silica and then transferred into 96-well blocks (Image 2), before being shipped to the University of Florida for DNA extraction.
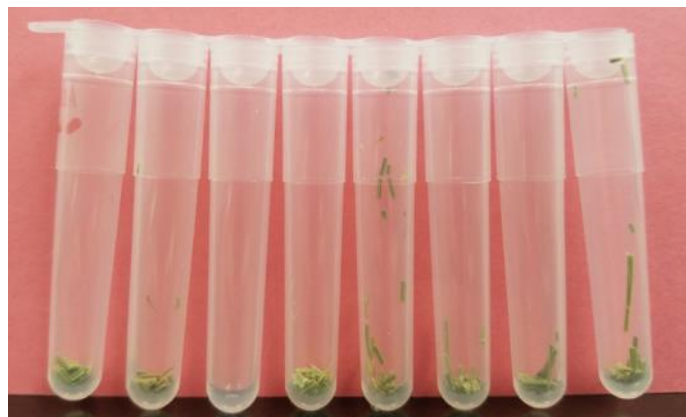

Image 2. *An 8-strip containing approximately 10 mg of dried needle tissue per cell. This represents 7 ACE clones with a control cell included, forming part of a larger 96-well block sent for DNA extraction.*

The tissue from the ACE clones and their parents will be genotyped with the Pita50K SNP array, and a genomic prediction model will be developed using the phenotypic data from eight test sites with one ramet per clone, per site. This model will be used to predict the phenotypes of 3000 ACE2 seedlings that will be genotyped with the same markers.

In February of 2019, the NIFA proposal "Genomic Selection in Forest Trees: Beyond Proof of Concept" was accepted. Funding from this grant will help cover the costs associated with the large-scale genotyping effort and allow for the testing of the ACE2 population to become a reality. TIP staff are very excited to see how this project develops!

Image 1. *Tori Brooks (left) and Maura Kaiser (right) collecting foliage samples at the Weyerhaeuser (formerly Plum Creek) ACE test in June 2018.*

SEED AND CONE YIELDS

The 2018 cone crop was much lower than previous years for most organizations. Figure 1 shows the tons of seed produced this year was lower than the past 3-4 years, regardless of orchard cycle. Seed yield has not been this low since 2012, and the lbs/bushel was the 7th lowest (1.11lbs/bushel) since 1968. We know some orchards were impacted by a late spring frost in 2017, which significantly impacted cone survival. Hurricane Michael hit during the 2018 cone harvest season and forced cone collection to occur earlier than usual, leading to issues with cone ripeness and cones not opening properly. This could explain the reduction in lbs/bushel, but may not be the whole story. Could insect damage have played a major role or did lbs/bushel decrease due to an increasing pressure to produce controlled-cross seed, which always produces lower yields than open-pollinated cones? We do not know all the answers, but one possible solution to tease out these differences is to add a column that separates out OP vs. controlled-cross seed. Controlled-cross seed production is growing each year and tracking yield differences between these two categories of cones may be useful for orchard managers in the future.

Table 1. *Fall 2018 cone and seed yields compared with the figures from 2017 harvest*

| Provenance / Orchard Cycle	Bushels of Cones		Pounds of Seed		Pounds of Seed per Bushel	
	2018	2017	2018	2017	2018	2017
Coastal 2.0/2.5	3,886	10,823	4,435	16,132	1.14	1.49
Coastal 3.0+	9,342	17,749	10,394	27,206	1.11	1.53
Piedmont 2.0	1,672	2,548	2,402	4,223	1.44	1.66
Piedmont 3.0+	5,716	12,192	6,574	18,637	1.15	1.53
Northern 2.0	383	957	296	724	0.77	0.76
Northern 3.0+	3,387	2,024	2,934	2,546	0.87	1.26
Totals	**24,385**	**46,293**	**27,035**	**69,467**	**1.08**	**1.37**
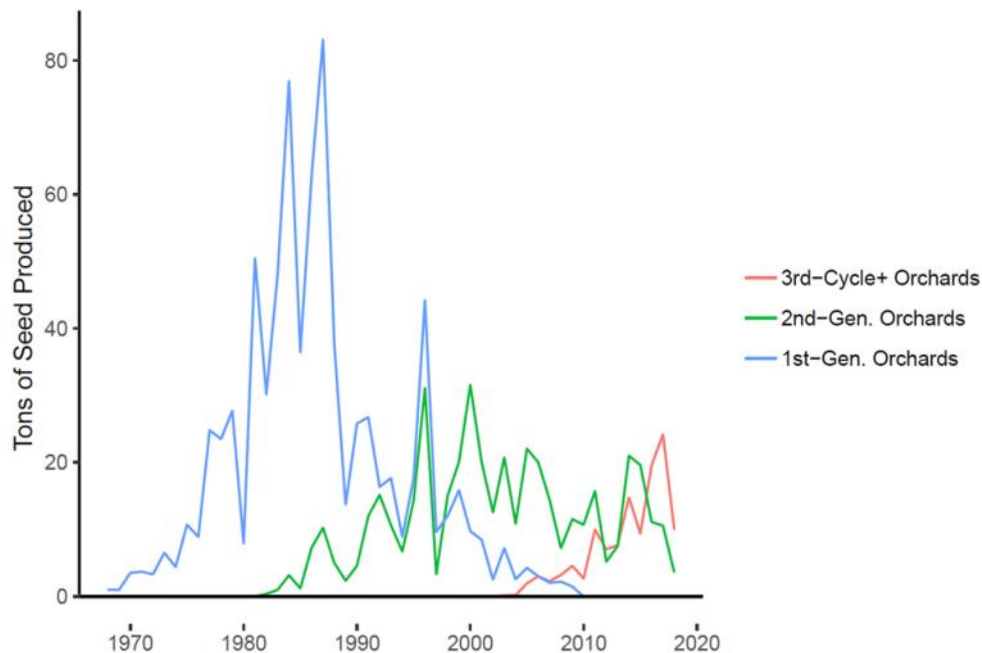
Figure 1. *Since 1968, the Cooperative has tracked annual seed yields from Cooperative members. 1ˢᵗ-generation seed orchards are no longer being harvested. In 2018 ~ 25% of the seed harvested came from 2ⁿᵈ-generation seed orchards while the other ~75% came from 3ʳᵈ-Cycle + seed orchards (includes 4ᵗʰ-Cycle orchards).*

RESEARCH

Selection Based On Clonal Versus Seedling Progeny Testing[1]

In loblolly pine, individuals can be cloned and tested in field trials for selection. Alternatively, half-sib progeny of parents can be tested in the field trials for selection. The question is how the two different propagule types affect the selection accuracy and genetic gain? To address the above question, breeding value (BV) predictions of 65 individuals based on data from clonally replicated tests and from half-sib progeny tests were compared.

The CCLONES population was established at four sites by the University of Florida Forest Biology Research Cooperative in the southern US. Approximately seven ramets of each clone were tested at each site. Clones representing a wide range of breeding values for height were selected from the CCLONES population by the FBRC. Scions were topgrafted at the Arrowhead Breeding Center in 2010, and open-pollinated seeds were collected in 2012. About 40 wind-pollinated (half-sib) progeny of the same 65 genotypes were later tested at four different locations by the NCSU Cooperative Tree Improvement Program (Figure 1).

For both the clonal trials and the half-sib progeny trials, tree height, diameter at breast height, and the incidence of fusiform rust disease (yes or no) were assessed at four years. Different linear mixed models were fit in order to partition the observed phenotypic variance into genetic and environmental effects, and predict the BVs of the same 65 genotypes from both the clonal trials and half-sib progeny tests.

Genotype means from clonal trials and their half-sib progeny trials did not differ for volume growth (Figure 2). However, fusiform rust disease incidence differed between clonal trials and half-sib progeny trials (Figure 2). We observed a larger variation for fusiform rust incidence in half-sib progeny trials than clonal trials. For growth traits, the additive genetic variance based on clonally replicated tests was about 2.5 times greater than the estimate from their half-sib progeny tests. For fusiform rust incidence, the additive genetic variance was even greater (~6 times) for clonal compared to their half-sib progeny trials.

Heritability of genotype means from clonal and half-sib progeny trials ranged between 0.88 and 0.91. Clone mean heritability estimates were slightly higher than family mean heritability estimates.
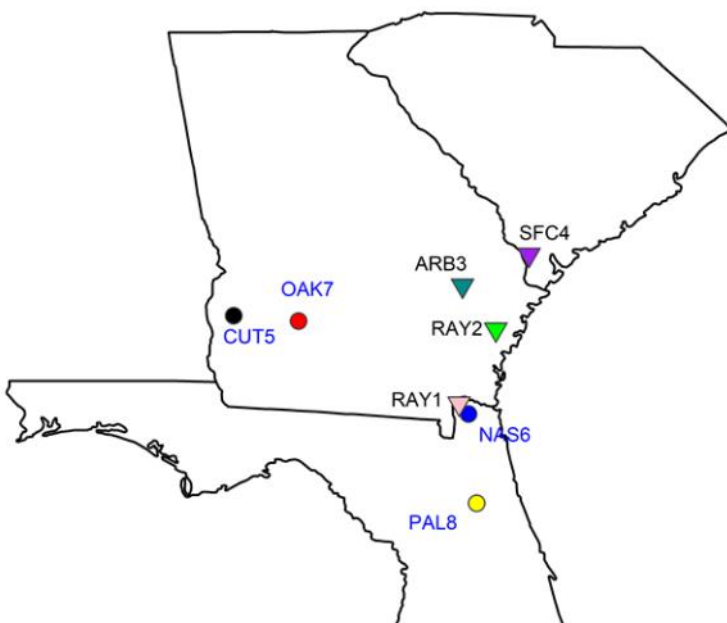

Figure 1. Geographic locations of clonal (CCLONES) and half-sib seedling (OP) trials in South Carolina, Georgia, and Florida. OAK7, CUT5, PAL8, NAS6 are clonal trials (circles) established by The University of Florida Forest Biology Research Cooperative. SFC4, RAY1, RAY2, ARB3 are half-sib progeny trials (triangles) established by members of the NC State University Cooperative Tree Improvement Program.
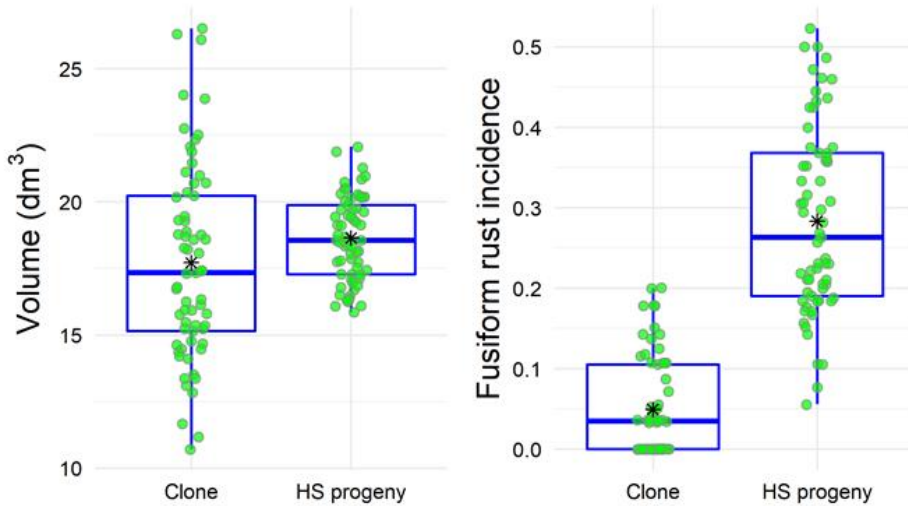
Figure 2. *Boxplots of volume (left) and incidence of fusiform rust disease (right) for clones and their half-sib seedling progeny. Horizontal line in the middle of the box is the median, and the black asterisk inside each box is the mean. Green circles represent individual clone means.*

When clone and half-sib progeny data were analyzed independently, the correlation between breeding values predictions of 65 genotypes was 0.59 (Figure 3A). This analysis ignores the GxE, assuming it is simply does not exist. It also assumes the variances of trait measured in two different trials are the same. These assumptions are likely not true. In the clonal tests, BVs are estimated from the copies of the genotypes (ramets) and from the relatives of the genotype. In the half-sib progeny tests BVs are estimated from half-sib progeny as well as from relatives. Complex additive covariance structures used for combined clone and half-sib progeny data analysis resulted in much higher correlations (Figure 3B).

The implications for seed orchard managers is important. With only a moderate to high correlation of breeding values from clonal and half-sib progeny trials in the independent analysis, the very best selections for a seed orchard could not be reliably identified based on clonal testing alone. The best selections were only identified when both the clonal and seedling progeny data were combined. Better experimental designs, such as larger number of ramets per genotype and having half-sib progeny and ramets in the same test sites might improve the correlations between BV estimated from clonal and half-sib progeny trials.
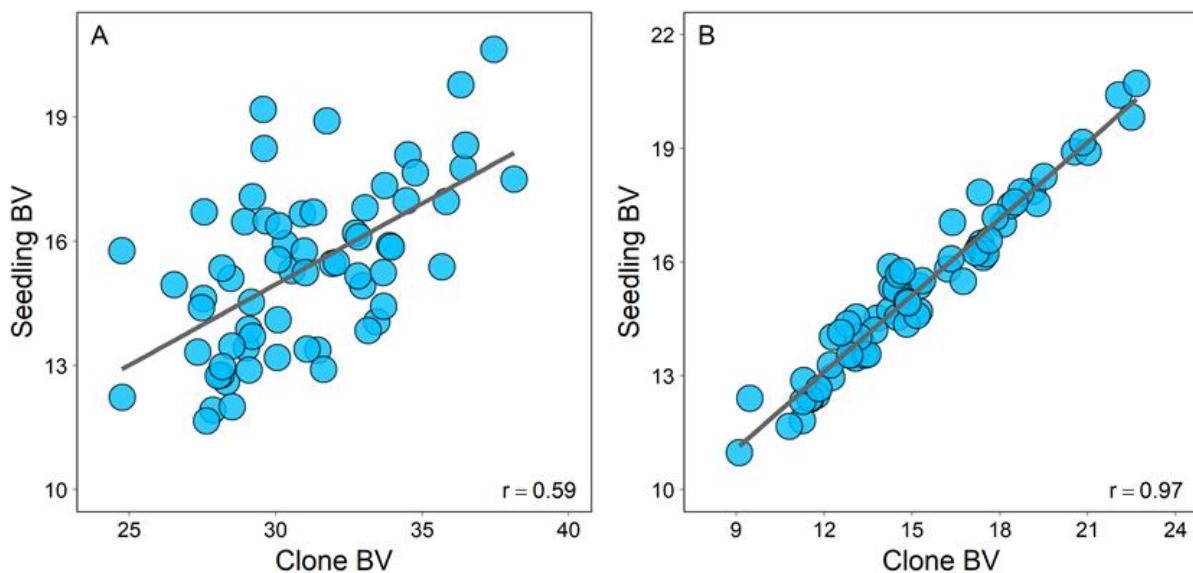

Figure 3. *Relationship between predictions from clonal data and half-sib progeny data for volume based on independent analysis (A, r=0.59) and combined analysis (B, r=0.97).*

Fusiform Rust Resistance Gene Mapping Project[1]

Fusiform rust disease is the most important disease affecting loblolly pine plantations. Understanding the genetic basis of resistance would make it much easier to select for resistant germplasm early in the breeding-testing-selection cycle. Eddie Lauer's genetic mapping project, part of the USDA-NIFA grant (PI-Isik, Award # 2019-67013-29169), aims to do just that. Two large full-sibling families of ~1500 individuals each were sent to the USDA Resistance Screening Center in Asheville, NC in March of 2018. These seedlings were inoculated with a high-density bulked inoculum (100,000 spores/ml) of *Cronartium quercuum* f. sp. *fusiforme* basidiospores. In mid-August of 2018, prior to the inoculation, TIP staff individually labeled and sampled foliage from 750 seedlings in each family, and collected random bulked samples from each family for later RNA extraction.

Images 1 and 2. The research team: Eddie Lauer, Fikret Isik (left) and April Meeks (right) collected data from the gene mapping population at the USDA Resistance Screening Center, Asheville, NC (March 14, 2019). Two full-sib families had distinct disease incidence response. One family had ~25% galled progeny, whereas the other family had ~74%.

Phenotyping the population

Just over six months after the inoculation, staff returned to Asheville to score the galls on the seedlings (Images 1 and 2). The dataset shows clear evidence of differential resistance between the families, and the ratio of diseased to non-diseased individuals suggests the segregation of large-effect resistance genes within families.

The next step is to genotype the foliage using the upcoming Pita50K array for the purpose of producing a high-density genetic map and identifying the genomic location of QTLs. Additionally, RNA extracted from bulks of resistant and susceptible individuals will be sequenced in order to identify the causal genes.

Following the measurements, the team loaded up the plants and brought them back to the greenhouse at NC State University campus (Image 3). Once in Raleigh, additional plants were labeled to bring the total number of individually sampled plants close to 1000 for each family. Since these seedlings will be genotyped, we are planning on planting the resistant trees at two locations in Georgia and South Carolina to serve as an experimental mapping population for other traits. Thanks to our members for kindly offering to help find locations and assist with the planting efforts. This population can serve as a genetic research population for years to come, and who knows what additional projects TIP graduate students will envision?

Image 3. *After artificial inoculation in August 2018 and phenotyping in March 2019 in the Resistance Screening Center, Ashville, NC plants were brought to the Horticulture Field Laboratory at NC State Campus for further research.*

Fusiform Rust Disease Incidence in the Atlantic Coastal Elite Population[1]

Use of resistant genotypes is the only feasible means of controlling fusiform rust disease in the forest environment. Full-sib progeny of the Atlantic Coastal Elite (ACE) population were initially challenged with fusiform rust inocula at the US Forest Service Resistance Screening Center (RSC) in Asheville, NC. Gall-free progeny were then hedged and established in cloned progeny field tests. Results of the artificial inoculations and field trials are summarized below.

Artificial Inoculations

In 2007, the ACE population was developed to assess short-term genetic gain for the Coastal regions of the Southeast. Twenty-four elite loblolly pine parents were mated to produce 76 full-sibling crosses. Seedlings from these crosses were then tested for fusiform rust disease susceptibility at the RSC. A broad-based inoculum source (50,000 spores per milliliter) was used for screening. A known resistant and susceptible checklot were included in the inoculation to indicate the resistance of the crosses. After nine months, the presence or absence of a gall was determined on over 9000 seedlings. Out of 76 crosses, 15 crosses (and their 3 parents) had very high disease incidence and were discarded. The remaining 51 crosses originating from 21 parents were brought to Raleigh for further fusiform rust disease assessment. The overall incidence in the data (51 crosses) was 48% at nine months. We observed a large range of parental disease means (Figure 1).

The results showed a significant effect of genetics on disease incidence. Additive effects of genes explained ~90% of the total genetic variance, whereas non-additive genetic effects explained the rest. Narrow-sense half-sib family mean heritability was 0.99 ± 0.004, whereas it was slightly lower for full-sib family mean (0.90 ± 0.036). The broad-sense full-sib family heritability was 0.95 ± 0.016. These high heritabilities suggest strong genetic differences among families.
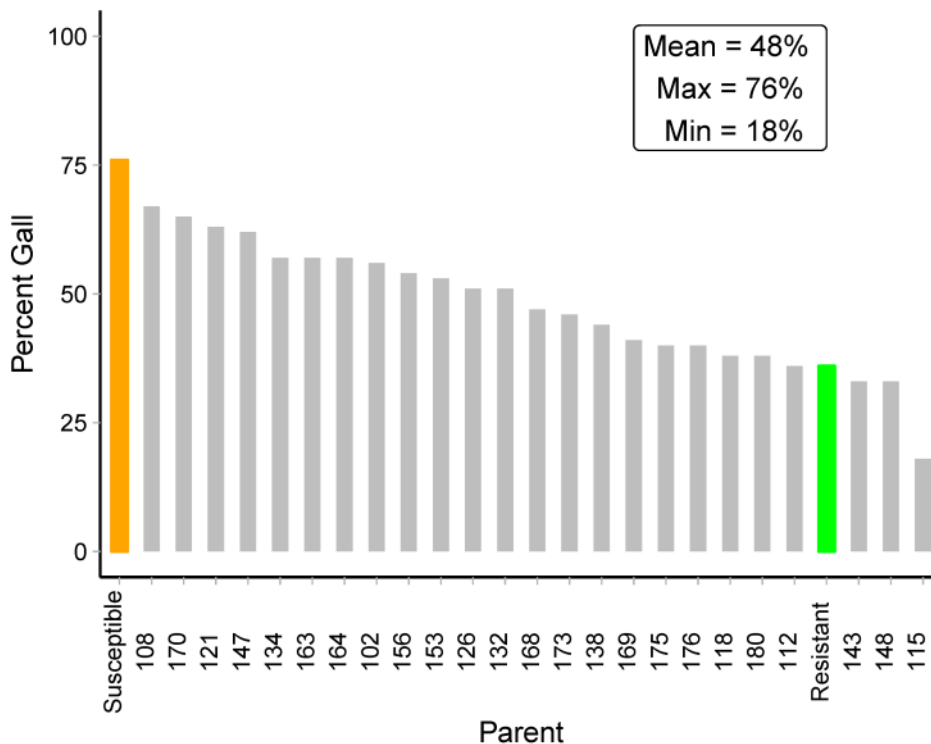

Figure 1: The mean percent gall of 24 loblolly pine parents used in screening the Atlantic Coastal Elite population at the USDA Forest Service Rust Screening Center in Asheville, NC.

Field trials

The gall-free progeny of the remaining 51 crosses (representing 21 of the 24 parents) were hedged for cloning (see the 2009 and 2010 Tree Improvement Annual Reports for details). An incomplete row-column designs was used to test ~2346 clones in the field. The experiments were established at eight locations, and one copy of each clone (ramet) was planted at each test location (Figure 2). At age four and six, the trees were assessed for height, diameter at breast height, stem straightness (1 to 6, where 1 is the straightest), the incidence of fusiform rust disease (yes=1 or no=0), stem forking (yes=1 or no=0), and ramicorm branches (yes=1 or no=0).

At age six years, the site mean fusiform rust incidence of the clones ranged from 1% (Pulaski County, GA) to 14% (Colleton County, SC), with an overall mean of 5.3% (Table 1). The checklot mean incidence ranged from 3.8% to 71%, with the overall mean of 23% across all sites. The two test sites from Colleton County, SC had the highest rust incidence (10% and 14%). Similarly, these two sites had the highest rust incidence for the checklot (48% and 71%, respectively). Artificial inoculations using bulked inocula appears to be effective to select for resistance prior to field planting since the "survivors" from the Resistance Screening Center had much lower rust incidence than the checklot. However, cloning might have contributed to the reduction in rust disease incidence since the ramets are physiologically different from seedlings (Frampton, et al 2000, SJAF 24). "
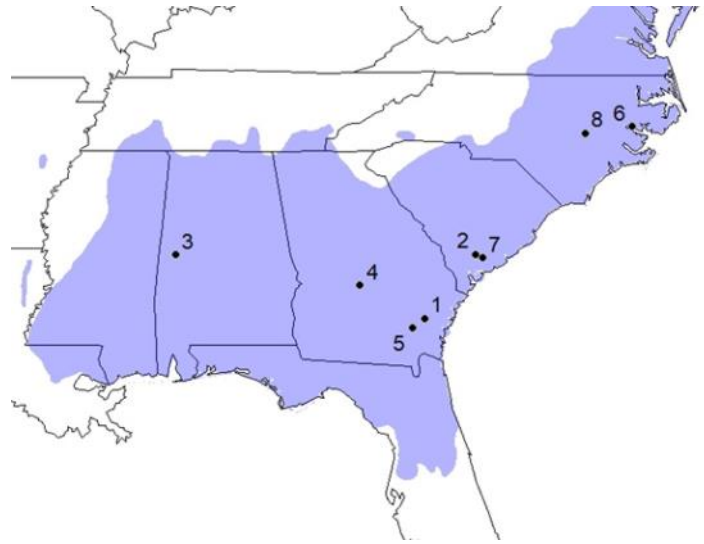
Figure 2: *Test site locations of the Atlantic Coastal Elite population. Blue shaded area is the natural range of loblolly pine east of the Mississippi river.*

Site	Location (County, State)	# of trees	Clonal Mean (%)	Checklot Mean (%)
1	Wayne County, GA	2480	1.8	11.4
2	Colleton County, SC	2480	10.1	47.5
3	Greene County, AL	2480	3.9	5.3
4	Pulaski County, GA	2480	1.6	3.8
5	Pierce County, GA	2496	3.7	23.1
6	Beaufort County, NC	2482	1.0	8.2
7	Colleton County, SC	1840	14.0	71.1
8	Wayne County, NC	1680	6.6	12.8
	Overall Mean	**2302**	**5.3**	**22.9**

Table 1: *Mean disease incidence (percent galled trees) in the field tests of the Atlantic Coastal Elite population at six years of age. Number of trees includes both clones and checklots. Clonal means percentage excludes checklots.*

Optimal Mating for Loblolly Pine: *PineBreed* [1]

Loblolly pine, like may outcrossing species, has a high genetic load and suffers greatly from inbreeding depression. When creating the mating list for future breeding cycles, it is always a challenge to balance two important but contrasting goals; increasing genetic gain and controlling the level of inbreeding. While methods and algorithms for animal breeding are well established, an efficient algorithm suited to forest trees remains elusive. Developing an algorithm to design a mating list that optimizes genetic gain while putting constraints on relatedness is imperative for loblolly pine breeding.

Towards this goal, we developed a new software package called *PineBreed* that utilizes pedigree-based relationships to create an optimal mating list. Unlike mating software developed for animal breeding, *PineBreed* allows for the same individual to be used as either a female or male candidate in the mating design. Given a curated list of selection candidates, the software is able to auto-complete the pedigree and order the completed pedigree by referencing it to the database. This step is essential to establish the relatedness between selection candidates, which would in turn define the future list of crosses. This completed pedigree is then used to calculate the additive genetic relationship matrix (A matrix) that provides a base for measures of relatedness in the mating list, such as average inbreeding coefficients and average group coancestry.

PineBreed uses a modified differential evolution algorithm that allows for the scanning of thousands of crosses to decide on the optimal mating list. By putting constraints on the relatedness of the mates (i.e. no mating between full-sibs or self-pollination allowed), a mating list is created, which allows for predicted genetic gain from crosses to be maximized and the future progeny inbreeding level to be minimized. An objective function was used as suggested by Kinghorn (Kinghorn 1998, J Dairy Sci.81).

$$Index = x'G + w_1 \frac{x'A\,x}{2} + w_2 F$$

where x is the contribution vector of the parents, G is a vector of the parent's estimated breeding values (EBVs), A is the numerator relationship matrix derived from the pedigree, and F is the mean inbreeding coefficient of the progeny. $x'G$ gives the total genetic gain expected from the progeny, $(x'Ax)/2$ is the co-ancestry of selected parents; and $w1$ and $w2$ are negative weights applied to co-ancestry and progeny inbreeding, respectively. The negative weights result in mating lists with high progeny inbreeding levels and parental coancestry but also a low index value. This is the objective function index and has no connection to breeding values. Thus, through the recursive DE algorithm, after searching the entire search space for possible mating lists and ranking them based on their index value, only the highest-ranking mating list that satisfies all constraints will be selected as the optimal mating list. The final output gives a list of mate-pairs with expected mid-parent breeding values and inbreeding level for each mate-pair. A schematic presentation of the algorithm is given in Figure 1.

To test the software, an optimal mating list for 961 selection candidates from the 3ʳᵈ-Cycle selection list was produced. The index breeding values were used for all candidates. The distribution of the candidates and mating list based on the index values depicts a 35% increase in genetic gain, resulting from the *PineBreed* optimal mating list (Figure 2). Summary statistics are provided in Table 1. Setting the minimum use for each individual to 1 (every candidate individual needs to be used as a parent at least once) to maintain genetic diversity, and including the constraint of no crosses between related individuals, an optimized mating list comprising 731 crosses was produced. A 41%

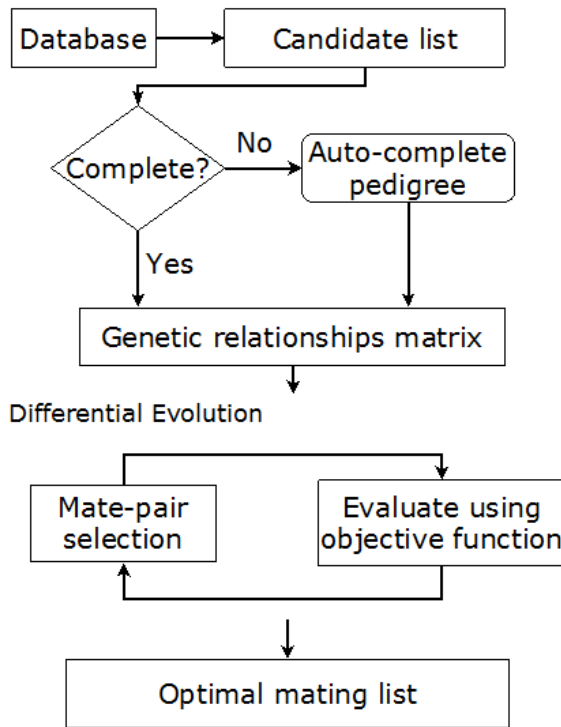[1] *This is a summary of Khushi Goda's PhD graduate research .*

Figure 1. *Schematic outline of PineBreed software. The* **TIPRoot** *Database serves as the reference. The pedigree is automatically completed and ordered simultaneously for preparing the candidate list input file. The additive genetic relationship matrix is then calculated between the candidates. Using a differential evolution algorithm, all possible mate-pairs are searched and compared to produce numerous mating lists. These mating lists are evaluated based on an objective function which considers the total expected genetic gain from progeny, the mean inbreeding levels in progeny, and the coancestry between the selected parents. The best ranking mating list that satisfies all constraints is selected as an optimal mating list.*
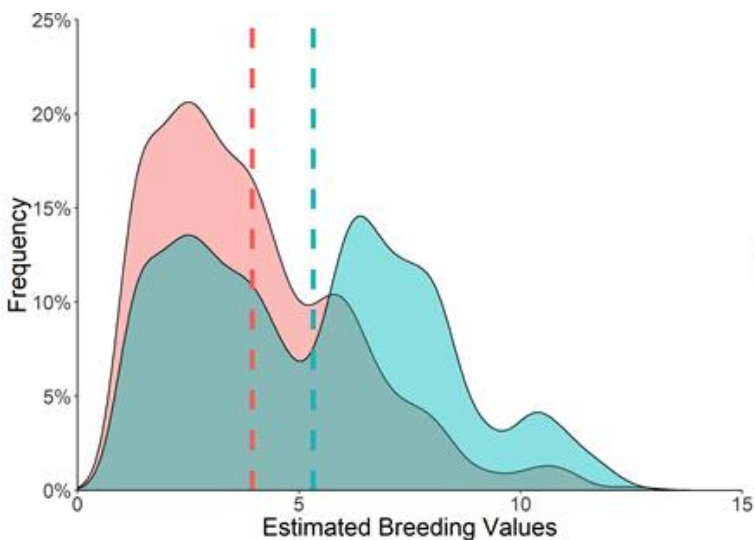
reduction in the average inbreeding coefficient ($\bar{F}$) for future progeny was observed. However, the average coefficient of coancestry between selected parents increased by 56%. This increase was a consequence of selecting candidates with higher EBVs multiple times, resulting in some proportion of the progeny population being half-sibs. Tighter constraints and better methods to control coancestry are currently being developed.

The algorithm can be utilized for breeding optimization of monoecious species to manage the inbreeding level while optimizing genetic gain. The next phase of the study will be testing the software with simulated pedigree and the development of a suite of software that is able to utilize genomic relationships derived from SNP markers.

Table 1: *Summary statistics for inbreeding (F) and coancestry (Θ) of the candidate list and resulting optimal mating list from PineBreed. The minimum and maximum coefficients did not change between the candidate and mating lists.*

Statistics	Candidate list	Mating list
N	961	731
N + pedigree	1072	1803
Min F	0.0	0.0
Average,	0.000175	0.000104
Max $\bar{F}$	0.0625	0.0625
Min Θ	0.0	0.0
Average, $\bar{\Theta}$	0.00152	0.00237
Max Θ	0.5625	0.5625

Figure 2: *(left) The distribution of the candidates (red) and progeny (blue) with respect to their EBV and mid-parent value, respectively. The dotted red line represents the average EBV of the candidates (3.94). The dotted blue line represents the average mid-parent value of the progeny (5.32). There is a 35% increase in genetic gain resulting from the optimal mating list.*

Progress on the SNP Array Design for Loblolly Pine

SNP marker discovery and design of a SNP array for loblolly pine took a great leap forward in the past year. Several major milestones in the Pita50K SNP Chip Development project were met.

The final number of markers discovered in the USDA-NIFA project (PD: Fikret Isik, Towards genomic selection in forest trees. USDA-NIFA Award #: 2016-67013-24469) was 642,275 (Table 1). About 34,290 SNPs are located within annotated coding sequences. This final list (642,275) included a set of ~150,000 SNPs discovered in 10 haploid tissue samples (megagametophytes) using whole genome sequencing by David Neale's lab at UC-Davis and Ross Whetten's USDA NIFA project "Identifying Functional Variation In Complex Plant Genomes".

Table 1. *Markers assigned to each quality category for screening*

Category	# Markers
Markers recommended on at least one strand (desirable markers)	58,155
Markers at best neutral (satisfactory markers)	99,982
Markers not recommended on either strand (insufficient information)	475,587
Markers cannot have probes made (unusable)	8,551
Total	642,275

A total of 642,275 markers were submitted to ThermoFisher for quality control in mid March, 2019. ThermoFisher used an in-house bioinformatic pipeline to assign quality metrics to each probe sequence. In addition, our in-house R pipeline was used for prioritizing the markers to be included on the screening array based on these quality metrics. This resulted in 423,695 markers being tiled on the screening array.

These SNPs will be screened using a diverse population of 480 *Pinus taeda* selections, representing three major breeding programs in the southern US: NC State University Cooperative Tree Improvement Program, Cooperative Forest Genetics Research Program at the University of Florida, and the Western Gulf Forest Tree Improvement Program. The screening project is expected to be completed by mid-June of 2019.

During the early stages of the project, a group of TIP staff, graduate and undergraduate students completed the foliage sampling of ~350 seedlings representing a wide geographic sample of the natural range of loblolly pine (Image 1). A set of 36 megagametophytes was included in the sample. They represent haploid meiotic tissue from six

Image 1. *Foliage from Specific seedlots sown in spring of 2018 was collected and transferred into tubes in 96 well format for DNA extraction.*

parents, which are also included in the array sample. These megagametophytes can be used to verify that markers in the screening array are single-copy in the pine genome, and that their alleles segregate according to Mendelian laws within some margin of error. The tissue samples were sent to University of Florida for DNA extraction. All samples met or exceeded the DNA quality criteria from ThermoFisher.

The next step will involve analyzing the genotypic data from the screening array and deciding which markers to include in the Pita50K array. We will analyze the screening array data and plan to submit the final list of 50K most informative probes to ThermoFisher by mid-July. The genotyping array, Pita50K, is expected to be available in mid-September and will be used for routine applications of fingerprinting, population genetics and/or genomic selection in loblolly pine and slash pine. This project encompassed a large amount of work from Eddie Lauer and the TIP staff, and we are very grateful the members who supported these pioneering efforts.

One of the challenges in working with genomics is how and where to store the data. To this end, an in-house database schema was developed. Information about the physical location (plate ID, well ID within each plate) and parentage of each DNA sample is stored in database tables (Image 2). Separate tables exist for storing the actual genotype data recorded for each sample. This database infrastructure will make it easy for NCSUCTIP scientists to pull out the marker data they need for any particular analysis.

```
 plate_id  | well_id | sample_id | parent1 | parent2 | db_sample_id | plate_alias
-----------+---------+-----------+---------+---------+--------------+------------
 B9SR18533 | A01     | B101      |         |         |         1921 | 9SR18533
 B9SR18533 | B01     | B133      |         |         |         1922 | 9SR18533
 B9SR18533 | C01     | BE100     |         |         |         1923 | 9SR18533
 B9SR18533 | D01     | E121      |         |         |         1924 | 9SR18533
 B9SR18533 | E01     | E132      |         |         |         1925 | 9SR18533
 B9SR18533 | F01     | F102      |         |         |         1926 | 9SR18533
 B9SR18533 | G01     | H100      |         |         |         1927 | 9SR18533
 B9SR18533 | H01     | H101      |         |         |         1928 | 9SR18533
 B9SR18533 | A02     | H107      |         |         |         1929 | 9SR18533
 B9SR18533 | B02     | H136      |         |         |         1930 | 9SR18533
```

Image 2. *Screenshot of one of tables for storing DNA sample information on the* TIPRoot *database. Parent 1 and Parent 2 ID's have been blanked out.*

Grants

New Grants

Fikret Isik, is the principal investigator on a USDA-National Institute of Food and Agriculture grant, "Genomic Selection in Forest Trees. Beyond Proof of Concept", $500,000. 02/01/2019-01/31/2024.

Continuing Grants

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

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, Ross Whetten, Kitt Payn on a North Carolina Department of Agriculture and Consumer Services grant. 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

This year we kicked things up a notch and combined the annual Contact Meeting and a Tree Improvement Short Course. We sandwiched the Contact meeting between two sections of short course to provide a more focused course for members and to add to the Contact Meeting attendance. Both events were held in Moultrie, GA and hosted by International Forest Company. Folks in attendance also got to meet the new TIP Director, Kitt Payn, who had arrived in the US just 10 days earlier. The field tour of IFCO's orchards, nurseries and field trials ended in a very sweet surprise, the very first 5th-Cycle selection! It isn't a Tree Improvement meeting without a field tour vehicle getting stuck and a great BBQ dinner…both were accomplished! We would like to thank Graham Ford, Chris Johnston, Doug Sharp, and all the IFCO staff for all they did in preparing for and assisting with the meeting. They happily took on a larger than usual event, and it was a big success!

There was a large crowd of attendees at the 62nd Contact Meeting and Tree Improvement Short Course. During the field tour attendees participated in selecting the first 5th-cycle selection at International Forest Company's progeny trial

The faculty members associated with the Cooperative are busy throughout the year teaching courses both domestically and abroad. Fikret Isik taught a 10-day quantitative genetics graduate course at Nanjing Forestry University, Nanjing, China. October 7-24, 2018. Steve McKeand followed in December 2018 and also taught a short course at Nanjing Forestry University on Genetics and Tree Improvement. Ross Whetten is steering the ship for the planning and execution of a large international meeting, the IUFRO Tree Biotechnology (https://www.treebiotech2019.org/) meeting coming to Raleigh, NC in June 2019. He is also currently serving on two Genome Canada Research Oversight Committees that are charged with reviewing research conducted by two different forestry-related Large-Scale Applied Research Projects.

The crowd of landowners and forestry professionals closely watched the demonstration of breeding skills by Kitt Payn, Steve McKeand and Austin Heine. This was a large meeting hosted by the Georgia Forestry Association and the Georgia Forestry Commission and was held at the Arrowhead Breeding Center in Cochran, GA. Since this was during breeding season, folks got a good look at the breeding activities and what goes into bringing the best genetics to the market!

TIP staff extension and outreach activities:

Faculty were invited as speakers at the following meetings:

HealGenCAR Conference, Höör, Sweden, June 2018 – Isik

IEG40 Meeting, Gainesville, FL, June 2018 – Walker and McKeand

Auburn Nursery Cooperative Annual Meeting, Auburn, AL, September 2018 – Heine

South China Agricultural University, Guangdong Provence, China, October 2018 – Isik

Chinese Academy of Forestry, Guangzhou City, China- October 2018 – Isik

Duke Silviculture Guest Lecture, Durham, NC, February 6ᵗʰ, 2019 – Heine

Forest Health Initiative, Gainesville, FL, February 2019 – Payn

In addition, Cooperative faculty and staff made over 30 visits with members, landowner groups, and forestry groups in FY 2019. Here is a list of some of the many extension and outreach activities:

Campbell Global Field Day, Hofmann Forest, April 2018 – Walker, McKeand, Lauer

Georgia Forestry Association Conference. Hilton Island, SC. July 15-16, 2018 - Isik and Heine

Milliken Forestry field day, Walterboro, SC, October 2018 - Heine and Walker

NC Forestry Association Meeting, Raleigh, NC October 10-12, 2018 - McKeand, Heine, Walker

SC Forestry Association Annual Conference, Hilton Head, SC, November 2018 - McKeand and Brooks

Georgia Forestry Association Landowner Field Day at Arrowhead, Cochran, GA, Feb. 27ᵗʰ 2019 - Payn, McKeand, Heine

NCDA BRI Research & Industry Update Meeting, Raleigh, NC April 2019 – McKeand and Payn.

Staff

It was another big year for changes in staffing here in Raleigh, but this time it came from the very top! As we reported last year, Dr. Steve McKeand stepped down as Director on July 1, 2018 and entered phased retirement (½ -time duties). Shortly before Steve's departure, the announcement was made that Dr. Kitt Payn would be the new Director and would arrive in November 2018. During this 5-month gap, all of the TIP staff stepped up to the plate and took on more responsibilities, but Dr. Fikret Isik bravely took the lead as interim director all while teaching and managing the preparation for the Contact Meeting and TIP Short Course. A big thanks to Fikret and the staff for keeping things moving along smoothly until we were back at full force. Steve returned in the summer and fall for "guest appearances" but returned in January 2019 in his new part-time (6 months on, 6 months off) appointment with the department.

Dr. Kitt Payn arrived from South Africa in November with his family, which includes his wife Carolyn and two young sons, James and Ethan. Kitt comes with extensive experience in Tree Improvement in the forest industry in South Africa, but he is no stranger to NC State Tree Improvement Program, southern pine forestry, and to many of the members. Kitt has returned to NCSU as an alumnus since getting his PhD here in Forestry in 2008 with the Camcore Program. He returned to South Africa

following his defense and was employed with Mondi Limited, SA for the past 10 years in various roles in relation to tree improvement, biotechnology, and forest genetics. Kitt has come in eager to understand our processes and how he can add value to them. We are thrilled he decided to take on the task of Director and look forward to his leadership, research initiatives, and all he brings to the Cooperative!

Pictured Above: *Dr. Kitt Payn wasted no time getting into the groove as he assisted in the breeding activities at the Arrowhead Breeding Center this year.*

Pictured Left: *TIP Staff from L to R- Kitt Payn, Fikret Isik, Austin Heine, J.B. Jett, Trevor Walker, Eddie Lauer, Steve McKeand, Ross Whetten, and April Meeks.*

Visitors

Among all of the changes in the office, we managed to bring in an international visitor to the program. Jikai Ma (pictured right) comes to us from Nanjing Forestry University in China. He has been tasked in the past with helping both Fikret and Steve during their time in China when they go to teach the short courses. Jikai arrived in late January 2019 and jumped right into helping out with projects in the greenhouse, field measurements, and breeding activities down at Arrowhead. He sat in on the Tree Improvement course, taught by Steve and Kitt, as well as Fikret's course- Introduction to Data Analysis in Natural Resources. Jikai will be with us until December 2019 when he returns to China; he has been a great addition to the TIP crew.

Teaching

The Cooperative faculty teach courses at both the graduate and undergraduate level. This spring, Fikret Isik taught his course NR554, Introduction to Data Analysis in Natural Resources, which covers general statistical procedures using SAS and R programing. Fikret taught Advanced Quantitative Genetics and Breeding (FOR/CS/ANS 726) in the fall. In the spring, Steve McKeand, assisted by Kitt Payn, 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). Each spring semester, Ross Whetten teaches FOR 350 (Ethics in Natural Resource Management), BIT 815 (Analysis of Deep Sequencing Data Analysis), and SMT483 (Capstone course in sustainable materials and technology). FOR 727 (Tree Improvement Techniques) is taught by Steve and Austin Heine and is offered every other year and will be available in Spring 2020.

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

Graduate Students

Tree Improvement Program graduates have had 100% job placement 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. Jessie Maynor took a job with Weyerhaeuser in May 2018 and returned to defend her Master's thesis in Fall 2018. We also welcomed two new Masters students in Fall 2018: Doug Dobson, who was an undergraduate worker for the last several years, and Syam Slamet who is a Fulbright Scholar. 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 2019 finish.

April Meeks, PhD – April began her program in January 2016 and her research is titled "Variation in *Cronartium quercuum* f. sp. *fusiforme* bulked inocula and *Pinus taeda* breeding populations for fusiform rust disease incidence".

Austin Heine, PhD – Austin began his PhD research immediately following his MS defense in 2018. His interest is in pollen viability and making the pollination process for full-sib seed more efficient.

Colin Jackson, MS – Colin's joint research with Camcore is titled "Comparative Genome Analysis and SNP Marker Discovery of Tropical Pines". He is expected to finish June of 2019.

Doug Dobson, MS – Doug started in Fall 2018 and knew exactly what he was getting into since working with the Cooperative staff as an undergraduate. He will be working with the Hofmann Forest genetics growth and yield data.

Eddie Lauer, PhD – Eddie began his PhD program in January 2018 and is tapping in on his genetics background. His research is titled, "Discovery of Fr Genes for Fusiform Rust Disease in Loblolly Pine".

Khushi Goda, PhD – Khushi joined our group in 2017 and continues her research 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 his research is titled "Best linear unbiased predictions of breeding values in *Pinus taeda* using expected and realized genomic relationships".

Syamsudin Slamet, MS – Syam began his program in Fall 2018 and is a Fulbright Scholar. His research is titled "Prediction of Breeding Value using Animal and Reduced Animal Model". He will be using 4th-Cycle test data for this study.

Trevor Walker, PhD – Trevor has been pushing hard and working through his prelim exams. His research focuses on efficiency of SNP markers for within family selection in *Pinus taeda*..
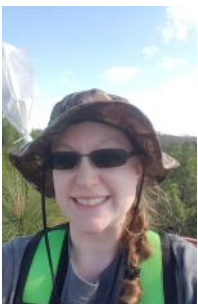
| April Meeks | Colin Jackson | Doug Dobson | Khushi Goda | Nasir Shalizi | Syam Slamet |

Undergraduate Army

Each year, the TIP staff hire on several new undergraduate students to help fill the shoes of those who have graduated. The students are critical to accomplishing the day-to-day operations of our research program, and they are the muscle that helps us get data collected for much of our research. Field measurements, pollen testing, foliage collection, seed extraction, cone analysis, inventorying and growing seedlings, and entering data are just a few job duties they have assisted with over the past 12 months. This year we had a few new additions to the group as we say goodbye to seasoned veterans. We hate to see students go but realize they are moving on to do great things!

First Row (L to R): **Haley Hollan**- Recently graduated with a B.S. in Biology. She played a pivotal role in completing the PBS study cone analysis/seed yields.

Paige Green – Graduating May 2019 in Natural Resources. She helped measure wood properties in the PMX trials.

Kenneth Dunn – Master of Forestry graduate student. Kenny is not an undergrad but has been a huge help with measuring in the field for various research projects such as at SETRES II in Scotland County, NC.

Ben Maness – Forest Management Sophomore. Ben has been helping with greenhouse work, data entry, field measurements, and even painting.

Second Row (L to R): **Austin Quate** – Graduating May 2019 in Forest Management. Austin spent many hours at the Arrowhead Breeding Center planting rootstock in the new Graveyard Orchard. He will start a job with IFCO after graduation.

Maura Kaiser – English Sophomore. Maura collected foliage for the genomic selection project from ACE tests.

Peter McNeary – Forest Management Sophomore. Pete liked using the "chainsaw on a stick" to prune orchard trees at the Arrowhead Breeding Center.

Membership in the NCSU Cooperative Tree Improvement Program

We happily welcomed Green Diamond as a new Contributing Member in November 2018. Full membership got a boost with the return of SC Forest Commission in 2018. SCFC originally left the cooperative in 2003, returned as a Contributing Member in 2014, and jumped back to a Full Member when the opportunity came. Unfortunately, we have lost White City nursery as a Contributing Member, following their purchase by International Forest Company. As always, we value the members and their support of our research and breeding activities. A list of our Cooperative partners is presented below.

Full Members

ArborGen, Inc.

Georgia Forestry Commission

Hancock Timber Resources Group

International Forest Company

North Carolina Forest Service

Rayonier, Inc.

South Carolina Forestry Commission

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

Green Diamond Resource Company

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.)

Timberland Investment Resources, 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 (2016 - 2019)

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

2019

Chan, J. M. and F. Isik. 2019. Genetic variation in resistance to *Uromycladium acaciae* fungus, growth, gummosis and stem form in *Acacia mearnsii* populations. Tree Genetics & Genomes, 15(3),35.

McKeand, S.E. 2019. The evolution of a seedling market for genetically improved loblolly pine in the Southern US   J. For. (in press)

Walker, T.D., F. Isik, and S.E. McKeand. 2019. Genetic variation in acoustic time of flight and drill resistance of juvenile wood in a large loblolly pine breeding population. For. Sci. (in press). https://doi.org/10.1093/forsci/fxz002

2018

Čepl J., J. Stejskal, Z. Lhotáková, D. Holá, J. Korecký, M. Lstibůrek, I. Tomášková, M. Kočová, O. Rothová, M. Palovska, J. Hejtmanek, A. Krejzkova, S. Gezan, R. Whetten, and J. Albrechtová. 2018. Heritable variation in needle spectral reflectance of Scots pine (*Pinus sylvestris* L.) peaks in red edge. Remote Sensing of Environment 219: 89-98 DOI:10.1016/j.rse.2018.10.001

Grattapaglia, D., O.B. Silva-Junior, R.T. Resende, E.P. Cappa, B.S. Müller, B. Tan, F. Isik, B. Ratcliffe, and Y.A. El-Kassaby. 2018. Quantitative genetics and genomics converge to accelerate forest tree breeding. Frontiers in plant science. 2018;9.

Hamlin, J., R.A. Sniezko, F. Isik, D.P. Savin, A. Kegley, S. Long, R. Danchok. 2018. Genetic variation in height and white pine blister rust resistance in sugar pine (*Pinus lambertiana*) - 15-year field trial results of three six-parent half dialers from three breeding zones. In: Schoettle, A.W., R.A. Sniezko, and J.T Kliejunas, eds. Proc. IUFRO Joint Conf.: Genetics of five-needle pines, rusts of forest trees, and Strobusphere. June 15-20, 2014 Fort Collins, CO. Proc. RMRS-P-76. Fort Collins, CO: USDA Forest Service, Rocky Mountain Research Station. p. 100.

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): 909-920. DOI: 10.1080/01621459.2017.1292179

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

Isik, F, 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. https://doi.org/10.5849/FS-2016-093R1.

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.

Building on improvement: Each year, we strive to continue building and improving what was done in the past. Some research methods date back to the beginning, and others give way to new processes and technologies. This year, new 5th-Cycle selections were made, and a new breeding cycle will begin soon, building on the continuous improvements over the last 63 years. In November 2018, we also welcomed Dr. Kitt Payn as the new Director. A new breeding cycle and new Director...the cycle of tree improvement continues!

Front cover: Tree improvement in its many stages at our Arrowhead Breeding Center in Georgia. The topgrafts of new 5th-Cycle selections (blue flags), breeding bags creating the ACE2 population to test genomic selection, background includes rootstock for new 5th-Cycle breeding orchard (Graveyard Orchard), and the final stage of tree improvement with operational control crossing activity in the distant orchards.

Back cover: (top right) The first 5thCycle selection made in Georgia at International Forest Company (Steve McKeand and Graham Ford), (bottom right) Selection and scion collection of another 5th-Cycle selection at Rayonier (David Barker and TIP staff), (bottom left) new TIP Director, Kitt Payn, jumping right into the breeding activities this spring.