TREE
IMPROVEMENT
PROGRAM
NC STATE UNIVERSITY

66th Annual Report

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DEPARTMENT OF FORESTRY & ENVIRONMENTAL
RESOURCES - COLLEGE OF NATURAL RESOURCES

NC STATE UNIVERSITY

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Department of Forestry and Environmental Resources

College of Natural Resources

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EXECUTIVE SUMMARY

After 66 years, the North Carolina State University Cooperative Tree Improvement Program continues to provide value to the Cooperative members and to the landowners and citizens across the region. The Cooperative continues to have a strong research program in all aspects of breeding, testing, selection, and deployment. We are on track to finish the 4th-cycle breeding and testing in a record short time (14 years), with many novel methods to make the Cooperative Tree Improvement Program one of the leaders in the world.

SELECTION, BREEDING, AND TESTING

- The 2022 season was the first to have a concerted effort for 5th-Cycle breeding with 109 crosses attempted. The cold-hardiness was managed without splitting the breeding population into temperature zones as suggested in the 5th-Cycle Breeding Strategy.
- Fusiform rust QTL breeding. A rust mapping project identified two parents in our breeding program that were carriers for major-effect genes contributing to fusiform rust resistance. In spring 2022, we initiated efforts to develop homozygous individuals that can be used as parents to create immune progeny.
- We are done with the 4th-Cycle test establishment! The last tests were planted this year. In total, there were over 123,400 trees from 1,600 full-sibling crosses planted in test sites across the southeastern US. We will continue to collect data from the 4th –Cycle tests until 2027.
- The first 5th-Cycle tests will be established during the 2023-2024 planting season. About 240 crosses are expected to be available for testing. The 5th-Cycle testing plan includes open-pollinated testing of parents that are candidates for the next cycle of seed orchards.
- Atlantic Coastal Elite (ACE2) Population second-generation clonal testing is underway. About 1700 progeny were top-clipped to produced hedges for cloning.

RESEARCH

Pollen viability research and the impact of pollen development stage at time of collection on filled seed yields continued. The Cooperative staff and graduate students continued to do research and publish in using DNA markers for breeding. Trevor Walker's research suggested that genotyping is as effective as clonal testing to increase the selection efficiency within families. Sarah Conner's preliminary results revealed that families from the Northern Breeding population are susceptible to fusiform rust disease in artificial inoculation. Preliminary results are promising to implement GS in loblolly pine breeding. For example, the accuracy of genomic estimated breeding values for height at age one in the ACE2 seedling progeny was 0.40. A new genotyping platform called Pita AgriSeq™ was developed in a pilot project with ThermoFisher. The platform is promising for quality control in operational breeding and is available to members. The Pollen Cloud study is underway and is expected to be planted in winter 2023. A new NSF/NIFA research grant aims to understand virulence variation and develop a comprehensive fusiform rust reference genome.

ASSOCIATED ACTIVITIES, GRADUATE STUDENTS, COOPERATIVE STAFF, MEMBERSHIP

There are now 10 Full Members, 23 Contributing Members, and 6 Research Associate Members. Trevor Walker and Eddie Lauer successfully finished their doctorate studies. Administrators from the College of Natural Resources and the Georgia Forestry Commission visited Arrowhead Breeding Center in March 2022. TIP faculty and staff along with GFC staff presented on the research activities and the cooperative nature of our work with partners, and the impact it makes on forestry in the South.

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A MESSAGE FROM THE INTERIM CO-DIRECTORS

First… from the old gray one:

June 30, 2021 was a day I had anticipated with *very* mixed emotions for many years, my last day as a member of the greatest team on earth - the faculty, staff, students, and members of the NC State University Cooperative Tree Improvement Program. After 41 years as an employee (3 years in phased retirement) plus two years as a graduate student, my time to start a new venture with my wife Lou, and all my family had finally come. Kitt Payn had organized an informal gathering with the staff and students at the local pub to bid me adieu and watch me ride off into the sunset with my lovely bride.

My future looked to be pretty much settled. Lou and I had sold our house, and after 43 years in Raleigh, we moved to our beloved *Pungo Palace* on the Pungo River near Belhaven, NC. Our dream of time together enjoying retirement, fishing, boating, summers with grandkids, and being bums was finally coming true.

And then… on the very afternoon that we were celebrating the house closing with a champagne toast, Kitt Payn called and told me that he was leaving NC State to take a job as Senior Director of Tree R&D at TerViva, an agricultural technology company that produces pongamia trees that yield protein and oil-rich seeds similar to soybeans. What a shock this was, since I was so pleased with the job Kitt was doing as Director of the Cooperative. I don't remember all the details, but I think later that afternoon, I drank the rest of the bottle of champagne!

After many long discussions with Lou, and with her blessing, I talked with the TIP staff and Erin Sills, Department Head, and offered to help out however I could. TIP has been my professional life for so long, and I wanted to do whatever I could to contribute to the program. Long story, short, Dr. Sills asked me to come back as Interim Co-Director with Fikret for "a few months". After 37 days of blissful retirement, I was back in the saddle with my friends and colleagues in TIP.

And as usual, that saddle needed a seatbelt! As has become the norm in Tree Improvement, expect the unexpected – see details on the next page from Fikret and in the rest of this Annual Report. Fortunately, the Cooperative has the most dedicated faculty, staff, and students imaginable, so I am pleased to be back. Rest assured, our program is in very good hands during this time of transition and the search for a permanent Co-Director begins.

Steve McKeand

May 2022

Second… from the slightly less old gray one:

I have been with the Cooperative Tree Improvement Program for a long time and have seen many significant events, but this last year will be one to remember! The director of the Cooperative Kitt Payn decided to leave NC State in August 2021 to work for TerViva, and all of a sudden, I was the only full-time faculty member in the Cooperative. Without hesitation, I reached out to Erin Sills, the department head and offered help to take on more program responsibility. Erin asked me to serve as the Interim Co-Director with Steve. This was a big relief because Steve and I have worked together for many years, and I knew how much he cared about the program and how well we work as a team. Our database manager and data analyst Eddie Lauer also left, though this was expected. Eddie successfully finished his doctorate degree in August 2021 and accepted a position as Research Geneticist with PEAK Genetics in Wisconsin.

Besides the departure of Kitt and Eddie, we have seen some memorable changes and reached some milestones. To mention a few,

Erin also asked me to develop a long-term strategic management plan for the Cooperative. With input from the staff, TIP members, and colleagues, the strategic management plan was completed in October 2021, recommending co-leadership of the Cooperative for stability and continuity.

We are on track to finish the 4th-cycle breeding in 14 years, a record short period (2013-2027). The last 4th-cycle progeny tests were established in winter of 2021-22. The start of the 5th-cycle breeding began in 2021 and really took off this past spring.

The Cooperative staff developed the Genomic Selection Plan last year. It was shared with the members and revised according to their input. The plan was approved by the Advisory board, and funding was provided during the Advisory meeting in 2021. The former Director Kitt Payn was instrumental in securing a large portion of the funding necessary to implement the plan starting in the 2023 fiscal year. Logistical plans are in place to genotype over 12,000 trees for the training population and implement the plan in the 5th cycle and beyond.

Since we developed the Pita50K genotyping array for loblolly pine in 2019, we have genotyped over 8000 trees for research and genomic selection project. The genotyping efforts accelerated in the 2022 fiscal year, exceeding four thousand trees.

It is nice calling Trevor, Dr. Walker. Trevor completed his PhD and graduated in May 2022. Congratulations to Trevor and his family for this great accomplishment.

Many other projects are underway and/or have been completed and are summarized in detail in this Annual Report.

I am happy about where we are today as a tree breeding program and am optimistic about the future. As Steve said, the staff, faculty, students, and members are the most dedicated folks around. I am proud to be part of such a great team and honored to help lead it to an even brighter future.

Fikret Isik

May 2022

SELECTION, BREEDING, AND TESTING

Breeding Update

5th-Cycle Breeding Begins in Earnest

The 2022 season was the first to have a concerted effort for 5th-Cycle breeding with 100 crosses attempted. The breeding plan developed for 2022 included two new notable features: 1) slight emphasis on wood quality traits (density and stiffness), and 2) managing the cold-hardiness without splitting the breeding population into temperature zones. These features are described in detail below.

Flowering at the Arrowhead Breeding Center was not as prolific in 2022 compared to 2021, but more selections were available for breeding due to pollen collected in 2021 and modest flowering on grafts made in 2021 (Table 1). Of the five full-members that grafted 5th-Cycle selections in 2021, two members had flowers available for breeding. To expedite breeding, it is imperative that selections be topgrafted into mature rootstock. At the Arrowhead Breeding Center, we have grafted on to 30 to 40 foot (~10 to 13 m) tall interstocks of a flower-promoting clone for the last several years. We observed flower production on one-year-old grafts taken from 4-year-old selections (6 years from seed) in both 2021 and 2022 at rates of 60% and 13%, respectively.

Table 1. *Number of 5th-Cycle selections with and without flowers available for breeding at the Arrowhead Breeding Center in spring 2021 and spring 2022 by the year they were grafted. Even though the grafts made in 2019 and 2020 were older in 2022, fewer had flowers available for breeding, presumably due to weather.*

	2021 Flower Survey		2022 Flower Survey	
Selections Grafted	Total	# With Flowers	Total	# With Flowers
Grafted in 2019 (N=13)	2	11	5	8
Grafted in 2020 (N=101)	40	61	62	39
Grafted in 2021 (N=89)	-	-	77	12
Total available for breeding		**72**	203	**59**

We used the MateSel[1] algorithm to design a mating list that balanced gain and diversity. We implemented several of the features described in the 2021 Annual Report[2] (p. 8-12) when determining the list of trees to mate, including using flower surveys and pollen inventory to inform the biological limits on mating and accounting for the juvenile population (crosses already made but too young to select candidates for breeding).

Female breeding candidates included flowering 5th-Cycle selections grafted at Arrowhead and in members' orchards. Male breeding candidates included pollen lots collected in 2020 and 2021, which included 43 5th-Cycle selections grafted in 2019 and 2020 (Table 2). There was also pollen from 46 backwards selections (1st through 4th-Cycle selections) collected in 2020 and 2021 by TIP staff and members that included emphasis on wood quality traits (density and stiffness) that were measured in 3rd-Cycle tests. The purpose of including these backwards selections was to increase the size of the 5th-Cycle breeding population while adding improvements for wood quality traits.

Breeding values from the most recent BLUP analyses were compiled and an index breeding value was developed with weights of 60% stem volume, 15% stem straightness, 15% rust resistance, 5% wood density, and 5% wood stiffness. The histogram (Figure 1) shows the index breeding values of selec-

Table 2. *Pollen collection for 5th-Cycle selections in 2020 and 2021, including backward selections made in 2021 that had emphasis on wood density and wood stiffness measurements from 3rd-Cycle tests. The number of selections with pollen in storage and available for breeding in 2023 are on the bottom line.*

	Pollen Collected (# of Selections)	
Year Collected	5th-Cycle Selections	Backward Selections (Cycles 1-4)
2020 + 2021	43	46
2022	78	-
Available for 2023 breeding:	**89**	**37**

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[1] https://www.matesel.com/content/documentation/MateSelInstructions.pdf?v=20220117; last accessed May 6, 2022.

[2] https://www.treeimprovement.org/annual-reports

tions available for breeding (females as green points, male as orange points) in relation to the breeding values of the historical population (all parents in 2nd, 3rd, and 4th-Cycle progeny tests) as gray bars. The blue dots show the mid-parent values for crosses that the MateSel algorithm chose for breeding in 2022. The expected gains for each trait are shown in Figure 2. The final mating list included 109 crosses among 90 parents with a mean of 2.4 crosses per parent.

In addition to maintaining adequate genetic diversity for long-term gain, the breeding population must also contain an adequate range in cold-hardiness, as loblolly pine families from our program are deployed across a wide range of winter environments from Virginia/Tennessee to southern Mississippi/Florida (USDA plant hardiness zones 7a through 9a). In Cycles 1 through 4, this was handled by subdividing the population into breeding zones and restricting the mating to trees from the same zone (e.g. Coastal, Piedmont, and Northern). In a species like loblolly pine with clinal genetic variation from South to North and Coastal to inland, separating the population by breeding zones has disadvantages. For example, crosses are not allowed among breeding candidates that originate near the border of separate zones (e.g. the Coastal/Piedmont Fall line), even though they may result in better crosses. The continuous distribution of cold-hardiness (mean minimum winter temperature of the counties of genetic origin) for the 5th-Cycle population is shown in Figure 3.

Another disadvantage of splitting the population into breeding zones is that it ignores the co-ancestry among trees from different zones, which has increased with inclusion of selections from the Piedmont Elite and the Northern Elite populations that originated from crosses among parents from differ-

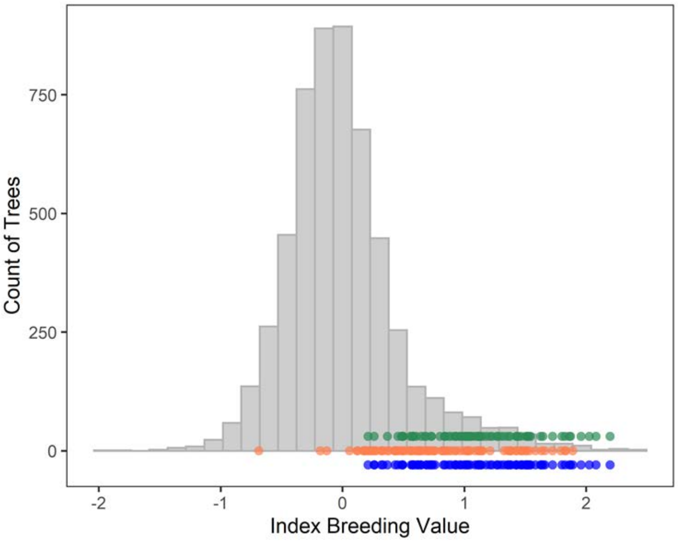

Figure 1. *(right) Distribution of index breeding values for all parents in the Big BLUP analyses (gray bars), the candidates for mating (females are green points, males are orange points), and the progeny from the 2022 breeding plan developed with MateSel (blue points). Index breeding values are in Z-score units and have weights of 60% volume, 15% stem straightness, 15% rust resistance, 5% wood density, and 5% wood stiffness.*

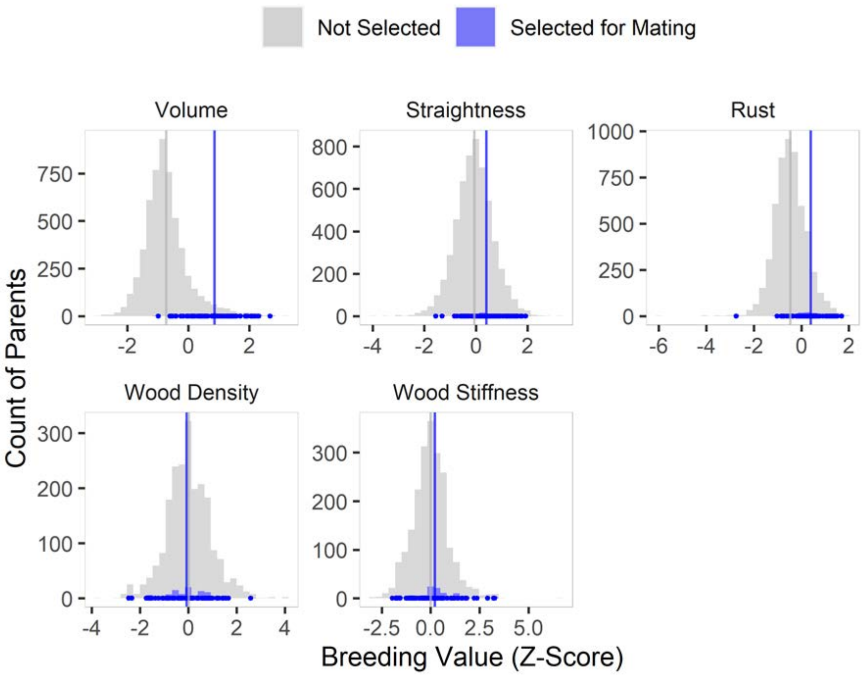

Figure 2*. Distribution of breeding values for selected parents (blue points) compared to all parents in Big BLUP analyses (gray bars) with vertical lines for mean of progeny from the 2022 cross list. Values are Z-scores where larger values are more desirable.*

Figure 3. *Distribution of mean minimum winter temperature origins for all 5th-Cycle selections (made 2019 through 2021). The values are calculated by taking the average winter temperature of the counties where the founding ancestors of a selection originated.*

ent zones. As cycles are completed and the pedigree gets deeper, assigning selections to zones will become less straightforward. Rather than splitting the population into strict breeding zones, the MateSel software has a Mating Groups option that uses a target number of crosses per group and permits intergroup crosses if their contribution to the optimal balance of gain and diversity exceeds a weighting factor. We utilized this feature to maintain a range in cold-hardiness by assigning breeding candidates to five Mating Groups that corresponded to 5°F intervals, such that Group 1 corresponded to mean minimum winter temperature values from 2.5°F to 7.5°F, Group 2 corresponded to 7.5°F to 12.5°F, …, and Group 5 corresponded to 22.5°F to 25.7°F (the maximum value in the population). The target number of crosses within each mating group was set proportionally to the number of breeding candidates in each group. Crosses were allowed among candidates from adjacent Mating Groups using a weight of 0.85 (weight equal to 0 indicates no weight against intergroup mating and 1 indicates strict intra-group mating). The resulting crosses by minimum winter temperature of the breeding candidates is shown in Figure 4, with male (pollen) candidates' values on the x-axis, female (flower) candidates' values on the y-axis, and blue 'x's indicating the crosses chosen by the algorithm. This year was the first to utilize a mating design that did not split the population into breeding zones, and we believe the Mating Group

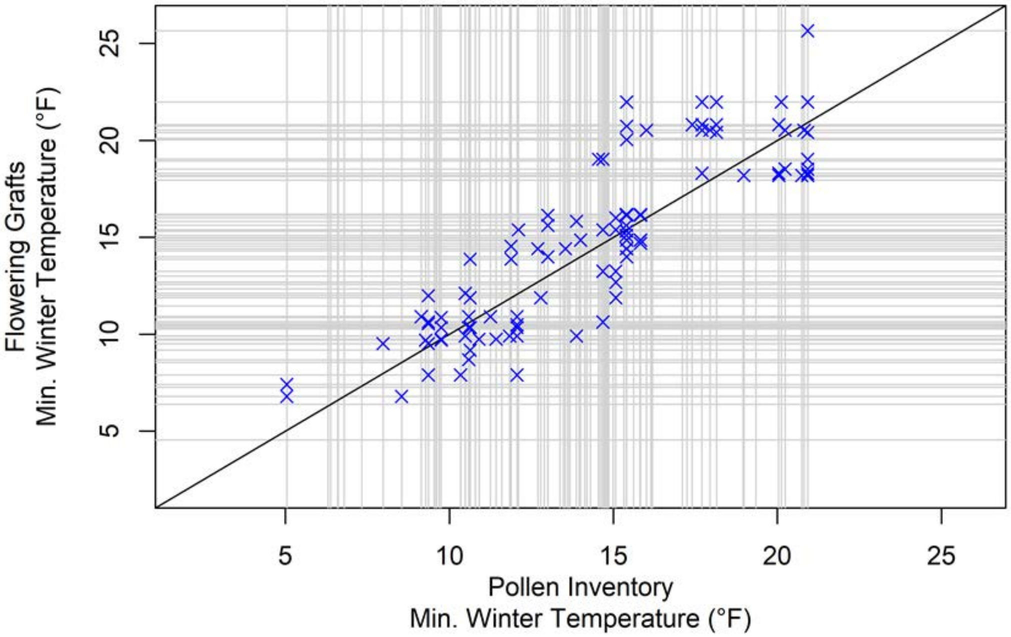

Figure 4. *Mean minimum winter temperature origins for breeding candidates (gray lines) with blue x's for crosses in the 2022 plan. The black reference line is where the temperature values for two parents are the same. The Mating Group option in the MateSel software adequately maintained cold-hardiness in the cross list and avoided 'wide' crosses without splitting the population into breeding zones.*

strategy is an improvement in managing the cold-hardiness of the population.

Unfortunately, a late spring freeze in the second week of March caused significant mortality in the crosses performed at Arrowhead this year, causing around 60% flower mortality (see Arrowhead update section). The cold damage was not as severe for 5th-Cycle crosses done in Full Members' orchards, which underscores the value in spreading the work amongst members to reduce risk. The total number of 5th-Cycle crosses expected for the first round of 5th-Cycle tests is 240 and are shown in Table 3.

Pollen collection from 5th-Cycle selections was a major effort in 2022 at the Arrowhead Breeding Center: 78 selections had pollen collected, most of them being their first year to produce pollen. None of the full-members had 5th-cycle grafts producing pollen, which was not surprising as they were grafted in 2021. The pollen inventory freezer in Raleigh presently has 89 5th-Cycle selections available for breeding in 2023 (Image 1), which will provide considerable options for making genetic gain!

Table 3. *Count of 5th-Cycle full-sibling crosses attempted by breeding region (Northern < 9°F mean winter temperature, 9°F ≤ Piedmont < 13°F, and Coastal ≥ 13°F). Crosses done in 2019 were mostly among 4th-Cycle selections and are considered "5th-Cycle Substitutes" and are broken out in the totals.*

Year Bred	Coastal Source	Piedmont Source	Northern Source	*Total*
2019	63	16	23	*102*
2020	0	1	4	*5*
2021*	25	15	6	*46*
2022*	51	28	8	*87*
Total	**139**	**60**	**41**	***240***

Image 1. Dr. *Nasir Shalizi, post-doctoral scholar, inspects a vial of 5th-Cycle pollen collected in Spring 2022. There are 89 5th-Cycle selections with pollen in the freezer available for breeding in 2023.*

GRID Breeding

The rust mapping project described by Lauer and Isik (2021) identified two parents in our breeding program that were carriers for major-effect genes contributing to fusiform rust resistance. There were three genes identified and named GRID1, GRID2, and GRID 3 for *General Rust Immunity Determinant*, as they produced broad-spectrum resistance against a diverse set of bulked rust inoculum at the USFS Resistance Screening Center in Asheville, NC (see pages 28 – 30 in the 2021 Annual Report). The parents are heterozygous (one copy of the resistant gene and one copy of the alternate), so only half of their progeny inherit the resistance gene while the other half do not. In spring 2022, we initiated efforts to develop homozygous individuals that could be used as parents to create 100% resistant progeny. These parents would provide extreme value as seed orchard parents, because even open-pollinated progeny would be immune to fusiform rust.

Because the genes are not known to occur in any other parents, crossing among related individuals (backcross or full-sib breeding) is required to develop homozygous individuals. Backcrossing progeny that are carriers with their parents and crossing among sibling progeny carriers was implemented in spring 2022 for the GRID3 line. This breeding was done using three selections from the Atlantic Coastal Elite population that were carriers of GRID3 and were grafted at Arrowhead in 2014. Two backcrosses and three half-sibling matings were made. The inbred progeny will be genotyped with the Pita50K SNP array to identify which are homozygous. Seeds from the homozygous individuals will be tested at the Resistance Screening Center in Asheville, NC to confirm that they are indeed immunity donors. Prior to Resistance Screening Center testing, the inbred progeny can be planted in block plots to include phenotypic selection on volume and straightness.

For the GRID1/GRID2 line, there were 16 progeny carriers selected from field trials (with selection emphasis on height at age 2) and topgrafted at Arrowhead (Image 2). Once flowers and pollen develop, backcross and full-sib mating will be done to create homozygous individuals.

References

Lauer, E. and F. Isik. 2021. Major QTL confer race-nonspecific resistance in the co-evolved *Cronartium quercuum* f. sp. *fusiforme–Pinus taeda* pathosystem. Heredity. 127(3):288–299.

Image 2. On left, a selection made from the Rust Mapping Population that is a carrier for major-effect rust resistant GRID1 and GRID2 genes. On right, the selection was topgrafted at Arrowhead for breeding with other sibling carriers to create homozygous progeny that could serve as parents that are expected to produce seedlings that are immune to fusiform rust!

Testing Update

4th-Cycle Testing

All 4th-Cycle tests have been planted! The 2021-2022 planting season concluded the establishment of 4th-Cycle tests. In total, there were over 123,400 trees from 1,600 full-sibling crosses planted (and surviving as of 2022) in test sites across the southeastern US. Tests were planted over a period of 8 years (2014 – 2022), and the corresponding number of reps to be measured are shown in Figure 1. Tests are measured at age 4 or 5 years (once they reach an acceptable tree size of a mean height greater than 15 feet or ~4 m), which mostly depends on the region planted as more inland/northern sites require longer to develop. The first 4th-Cycle measurements were done in 2018, and they will continue until 2027. The 2022 measurement season concluded this past spring and marks the halfway point for the test measurement schedule of 4th-Cycle crosses.

There are two objectives of the 4th-Cycle testing program: 1) identify superior individuals to use in 5th-Cycle breeding (forward selections), and 2) obtain rigorous breeding values for 4th-Cycle parents to identify winners for seed orchards/plantation deployment (backward selections). For objective 1, there have been 245 selections made so far, which are described in the 2021 Annual Report (page 8). For objective 2, there are 193 new parents (mostly 4th-Cycle selections) that will have progeny-tested breeding values for ***PRS***TM scores. The timeline for obtaining these breeding values is given in the 2020 Annual Report (page 3). While around half of the 4th-Cycle reps have been measured, the majority of 4th-Cycle selections still have not had their progeny measured because they were planted in the later tests as seed from breeding became available.

Another important feature of the 4th-Cycle testing strategy is strong overlap among genotypes from different source regions. Temperature adaptation is the major environmental feature that affects the rankings of loblolly families in the southeastern US (e.g. Florida-source families will tend to outgrow Virginia-source families when planted in Florida, but not in Virginia region due to cold damage). Previous cycles tended to test material only in the region where it originated, even if testing in a slightly colder region would be low risk based on seed source movement guidelines (Schmidtling 2001). This has left many ***PRS***TM users unable to compare Coastal families with origins near the Fall Line to Piedmont families, even though they are both viable options for their deployment planting area. Similarly, there are many Piedmont families that are adapted to the Northern testing range even though they are in separate ***PRS***TM versions. This issue was addressed in the 4th-Cycle testing strategy by including Coastal families in the Piedmont and Northern test series where they were adapted based on the mean minimum winter temperature guidelines. Similarly, a large number of Piedmont families were included in the Northern test series and vice versa to allow direct comparisons between the families from the two provenances. Figure 2 shows the distribution of mean minimum winter temperatures of families on the x-axis grouped by the mean winter temperature of the test site location. The range in family mean minimum winter temperatures at any particular test site is much greater than in pre-

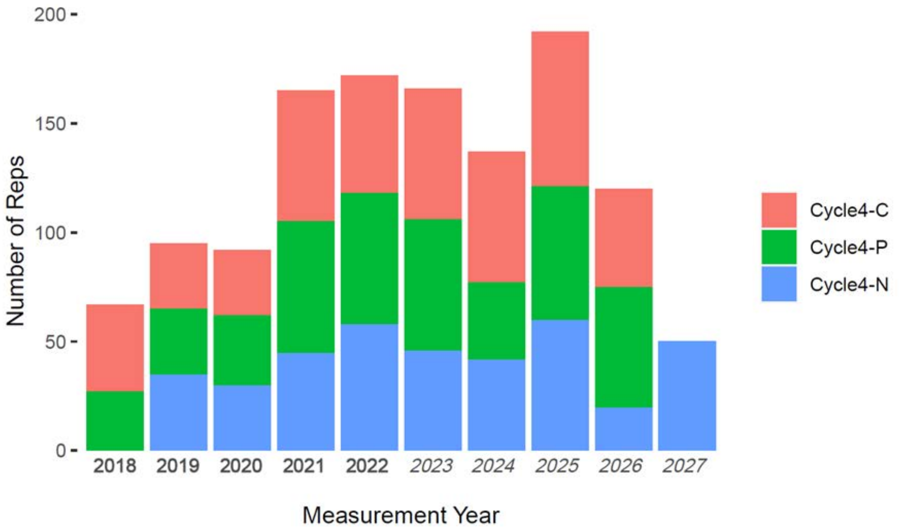

Figure 1. *Number of reps by measurement year with those already measured in bold and those scheduled in italic in x-axis. The different colors refer to the testing series, with C = Coastal, P = Piedmont, and N = Northern. Tests are measured when the average tree height ≥ 15 feet (~4 m) tall.*

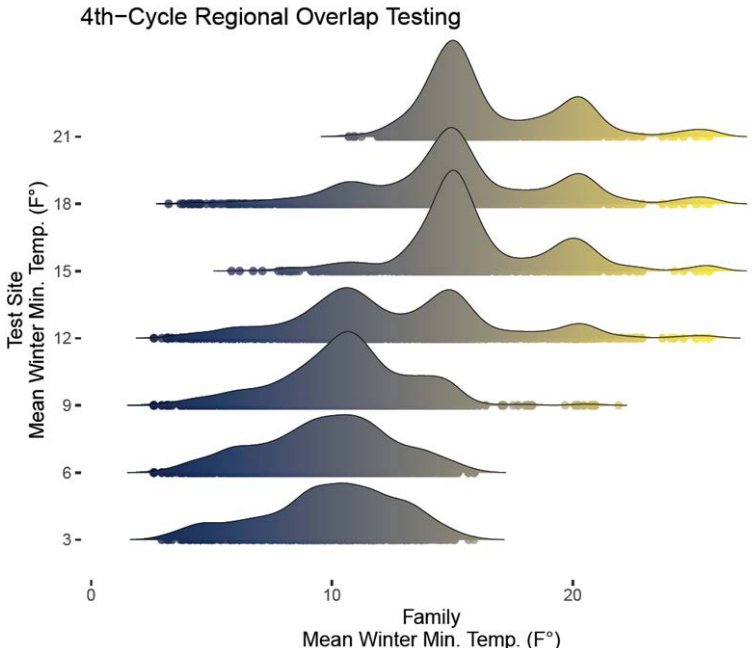

Figure 2. Distribution of family mean minimum winter temperature origin (x-axis) grouped by the mean winter temperature of the counties where they were tested. The range in family mean minimum winter temperatures is much wider than in previous cycles' test designs and permits comparing families from different breeding zones. Families with source origins that are too warm for the test site will have to be accounted for when estimating breeding values.

vious cycles' test designs. The coldest test sites contain some families that will likely be maladapted, which will need to be accounted for during the estimation of breeding values.

The first 4th-Cycle tests are old enough (8 years old) to assess juvenile wood properties using the Fakopp TreeSonic (measure of wood stiffness) and drill resistance Resistograph (measure of wood density). Based on 3rd-Cycle test pollen-mix measurements (Walker et al. 2019), these tools produce precise parental breeding values for the PRS™ and satisfactory heritability to make forward selections. The 3rd-Cycle pollen mix trials had a consistent number of progeny for each parent (the primary influencer of the accuracy of breeding values), so a sub-sampling plan was used to target 30-40 progeny per parent by measuring two tests from each series. Sub-sampling based on progeny per parent will be difficult for the 4th-Cycle tests, because they are comprised of full-sib families from an incomplete diallel that has more progeny from parents that are expected to be better. Such a design is ideal for forward selections (next-generation selections) but is logistically difficult for backward selections (e.g. PRS™ scores). This is because more reps must be measured to get an adequate breeding value accuracy for parents that were used in fewer crosses. This is illustrated in Figure 3, which shows the distribution of number of progeny per parent available for wood properties measurement by the proposed measurement year. If all of the 4th-Cycle tests are measured for wood properties, Table 1 shows the expected workload by year. We

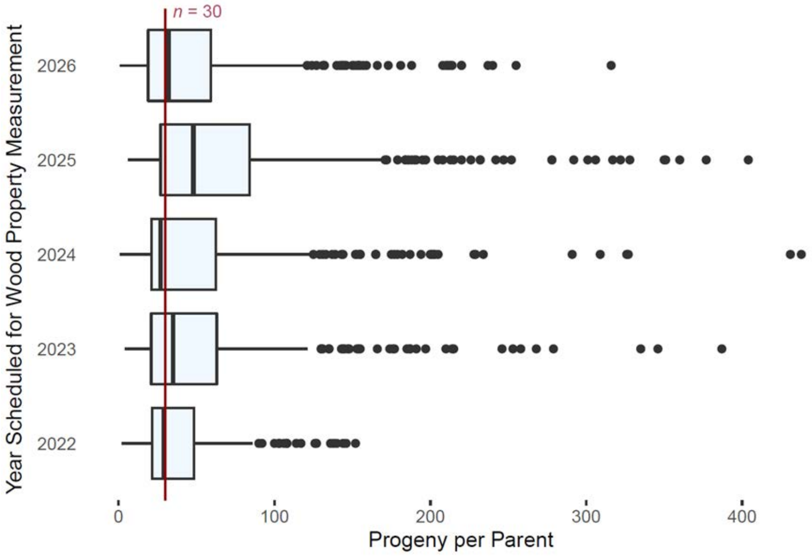

Figure 3. *Distribution of number of progeny per parent available for wood properties measurements by the scheduled measurement year. The vertical red line corresponds to 30 progeny per parent, which was the sample size for 3rd-Cycle wood property measurements. The median for each year (bold vertical line inside each boxplot) is around 30, but there are many parents that have a much larger number of progeny per parent (black dots to the right). Boxes correspond to the inner 50% of parents and whisker lines to the range of the distribution with black points for outliers.*

Table 1. *Timeline and workload (number of reps and number of trees) for wood property measurements on 4th-Cycle tests (C = Coastal, P = Piedmont, N = Northern) if all tests are measured.*

Year to Measure	Number of Reps			Number of Trees		
Wood Properties	Cycle4-C	Cycle4-P	Cycle4-N	Cycle4-C	Cycle4-P	Cycle4-N
2022	30	22		3029	1320	
2023	40	25	20	4197	3633	1619
2024	30	27	20	4551	4078	2278
2025	59	54	30	7899	7704	3518
2026	48	42	60	4640	3744	5930

are still developing a sampling strategy for assessing wood properties in these trials.

5th-Cycle Testing

Similar to the 4th Cycle, the objectives of the 5th-Cycle testing program will be to 1) identify trees to select for the 6th-Cycle of breeding and 2) rank 5th-Cycle selections for deployment based on their progeny performance. The first 5th-Cycle tests are planned to be established during the 2023-2024 planting season, where 240 crosses are expected to be available for testing (see Breeding Progress Section Table 3). The reason for no mainline progeny tests in the 2022-2023 planting season was due to a delay in 5th-Cycle breeding while new selections developed flowers and pollen. Topgrafting into mature rootstock reduces these delays, but they are still present and prolongs the availability of crosses and seed for testing.

While controlled pollination is the best method for making genetic gain in the breeding population through forward selection (Objective 1), open-pollinated families are very useful for determining the genetic merit of potential seed orchard parents for deployment of gains across the landscape (e.g. **PRS**TM score calculation) (Objective 2). A component of the 5th-Cycle testing plan is to include open-pollinated families for new selections that are candidates for the next cycle of seed orchards. The motivation for including open-pollinated families is to accelerate progeny testing of new selections that have too few flowers to make controlled-pollinations (e.g. less than four flowers). Open-pollinated seed for testing is a fast and inexpensive complement to the controlled pollination because open-pollinated cones tend to produce more seed than those from controlled pollination, so only a few cones are needed to obtain adequate seed for testing. Further, pollen takes longer to develop than female flowers, so the first few breeding seasons of new cycles will have a lag between when flowers are available and when pollen is available. These issues are illustrated in Figure 4, which includes individual-tree breeding values for volume for 5th-Cycle Coastal selections that were flowering in 2021 (on the left) and 2022 (on the right). Not all of these were bred due to sparse flowering or limited pollen, and those with higher volume breeding values tended to have more breeding. Open-pollinated testing would obtain breeding values suitable for **PRS**TM score calculation for the selections that have not yet been bred one or more years sooner by bypassing the requirement of full-sibling families for testing. Progeny tested breeding values for these selections will result in smaller standard errors and a more accurate ranking for choosing parents for seed orchards and deployment.

Open-pollinated progeny testing was used occasionally in the 2nd Cycle to obtain breeding values for parents that were not used in diallel breeding, typically with the goal of rogueing a particular seed orchard. The method was not used in the 3rd Cycle due to concerns about differences in pollen cloud composition from different orchards, so pollen-mix breeding was used. In the 5th Cycle, we propose to use cones collected from a single orchard (Arrowhead Breeding Center) to reduce variation in pollen cloud composition.

Very little open-pollinated testing was done in the 4th-Cycle, and we saw delays in the testing of 4th-Cycle selections due to limited flowering and pollen production on young grafts. Occasionally, open-pollinated families were included in the full-sibling 4th-Cycle tests, but this creates complications in the analysis, as the pollen cloud parent is unknown and must be (naively) set to the mean of the tested parents. In the 5th Cycle, we plan to use test replicates

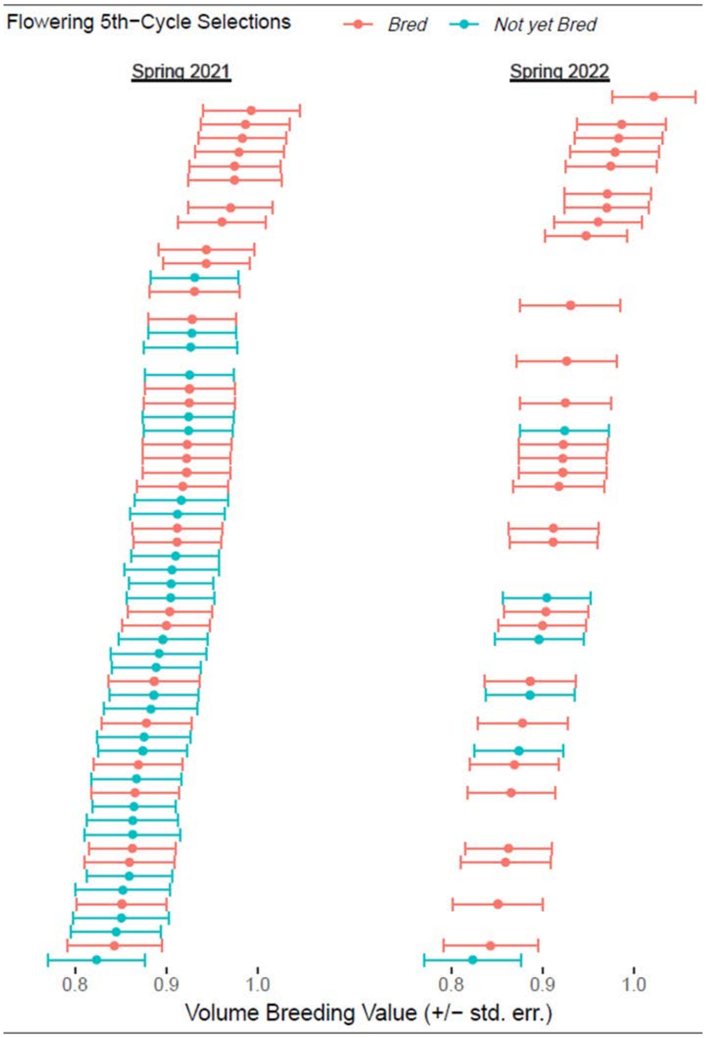

Figure 4. *The dots are volume breeding values (with whiskers for +/- standard error) of 5th-Cycle selections that had flowers available for breeding in 2021 and/or 2022. Not all flowering selections were bred due to few flowers or limited pollen. Selections with higher breeding values tended to be bred (red points) in years when flowers were available, but individual tree breeding values have wide standard errors, and we propose open-pollinated testing for those that have not yet been bred (blue points). Only selections from the 4th-Cycle Coastal tests are shown.*

that contain only open-pollinated progeny that are interspersed with full-sibling replicates on the same test site. Similar to the 3rd-Cycle pollen-mix tests, this circumvents estimating the breeding value of the pollen parents by confounding their effect with that of the test replicate. Such a strategy has a benefit over the 3rd-Cycle design in that the half-sibling and full-sibling progeny will have breeding values estimated from the same test sites and will be directly comparable. For this method to work, there must be some parents that are tested in both full-sibling and open-pollinated replicates to connect the two populations. A desirable outcome of this strategy is that no differences in seedling growing, test planting, maintenance, and measurement is required. This should result in a minimal marginal cost of testing the additional open-pollinated families and will have a great benefit of expediting ***PRS***™ scores of new orchard candidates for plantation deployment.

Pollen Cloud Study

The goal of the pollen cloud study is to better understand the differences among open-pollinated families collected from orchards in different regions and ensure that these differences are consistent with the assumptions made in ***PRS***™ scores. Foresters and nurseries can obtain the same open-pollinated family from many orchards in the Southeast, and the scores in ***PRS***™ are adjusted based on assumptions concerning the pollen cloud composition. Figure 5 gives an example of two ***PRS***™ Spec Sheets for the same open-pollinated family collected from an orchard in the Coastal Plain and an orchard in the Upper Gulf Coastal Plain. The scores for open-pollinated families are derived from the mid-parent breeding value of the female parent (known) and the breeding value of the pollen cloud, which is unknown and must be approximated. The pollen cloud breeding value is approximated using a combination of the breeding values from typical orchard parents (within-orchard pollen) and the breeding values for the wild, non-improved checklot for the region (outside-orchard/contaminating pollen). Seed source studies have shown natural genetic variation in loblolly across the Southeast (Wells 1983; Farjat et al. 2017). This variation is reflected in the performance values of the Cooperative's wild, non-improved checklots, which are bulk mixes collected in the 1970s from natural stands in eight testing-areas in the Southeast (colored regions in Figure 6). The expected differences among orchard in different regions applies to both the ***PRS***™ scores and the cold-hardiness of the family, such that the same open-pollinated family from a Coastal Georgia orchard will have a different risk of cold damage than if it were collected from central

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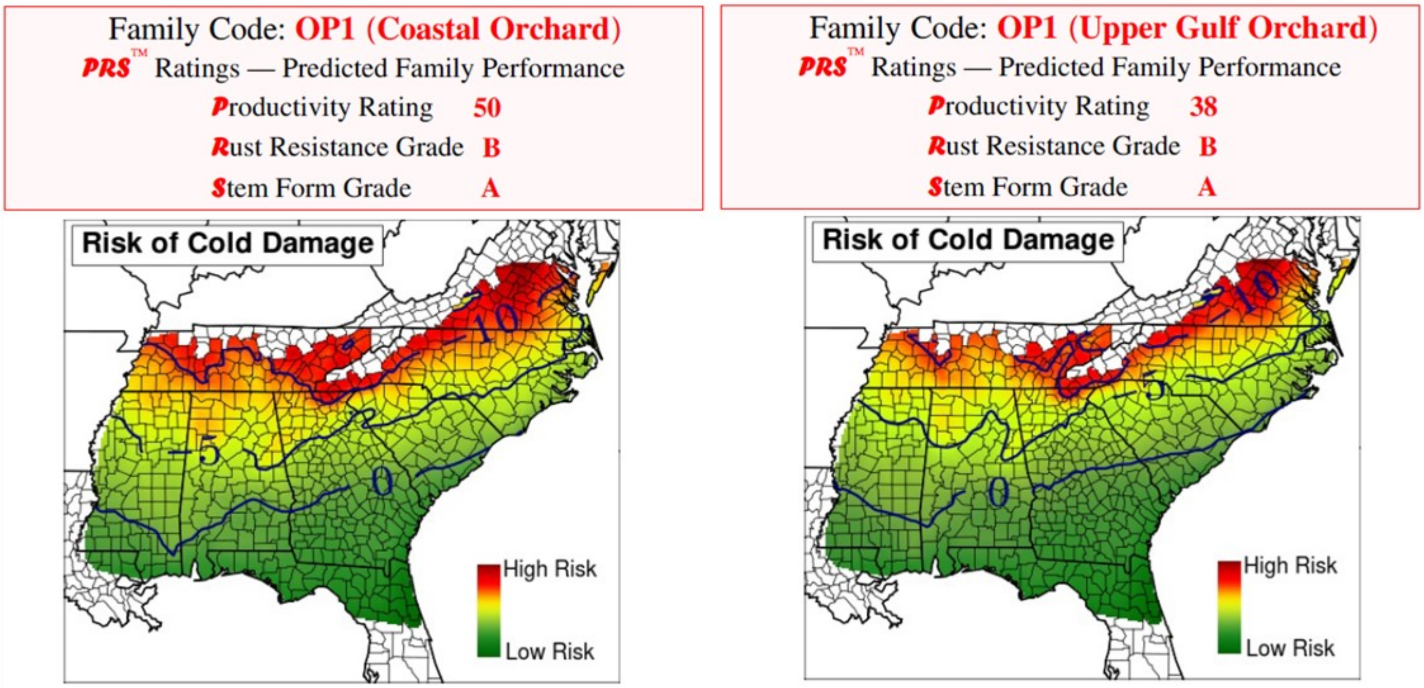
[1] Details about the checklot system can be found on pages 19-22 of the NCSU Tree Improvement Cooperative 28th Annual Report (1984) at https://www.treeimprovement.org/annual-reports


Family Code: OP1 (Coastal Orchard)
PRS™ Ratings — Predicted Family Performance
Productivity Rating 50
Rust Resistance Grade B
Stem Form Grade A

Family Code: OP1 (Upper Gulf Orchard)
PRS™ Ratings — Predicted Family Performance
Productivity Rating 38
Rust Resistance Grade B
Stem Form Grade A

Figure 5. *Comparison of* ***PRS***™ *sheets for the same open-pollinated family collected from an orchard in the Coastal Plain (left) and an orchard in the Upper Gulf Coastal Plain of Alabama (right). The Coastal Plain orchard has higher Productivity scores but is less cold hardy. The differences are based on breeding values from wild, non-improved checklots and assumptions about orchard composition and pollen contamination rates. The pollen cloud study will rigorously test these assumptions.*

Alabama (Figure 5). The influence of the pollen-cloud on cold adaptability is particularly important for Northern-source parents grafted in seed orchards in the Coastal Plain.

The first objective of the pollen cloud study is to determine if the performance of an open-pollinated family depends on the region from which it was collected, and if those differences are consistent with **PRS**™ scores. The study consists of a Coastal series and a Cold-hardy series. The Coastal series contains four open-pollinated families collected from 17 orchards across five checklot regions, and the Cold-hardy series consists of six open-pollinated families from 17 orchards across six checklot regions (Figure 6). Not every family within a series was collected from every orchard, but there are adequate connections to permit robust comparisons (Table 2).

The second objective of the pollen cloud study is to test if the performance of open-pollinated families relative to full-sibling families is consistent with the **PRS**™. This objective will usie the Coastal series by including six full-sibling families among the parents used to meet Objective 1 (H, I, J, and K in Table 2). These families provide excellent balance, represent-

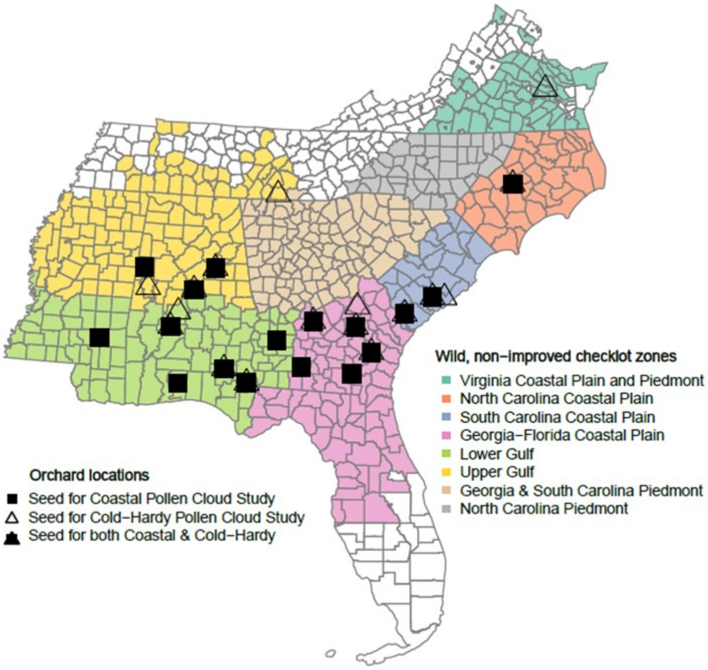


Figure 6. *Counties in the southeastern US with colors corresponding to the collection regions for bulks of wild, non-improved checklots used in* ***PRS***™*. The squares and triangles are orchard locations where seed was collected for the Coastal and Cold-hardy Pollen Cloud Study series, respectively.*

Table 2. Count of orchards where open-pollinated seeds were collected by mother parent (coded A-K) and wild, non-improved checklot region. The mother parents are sorted by their mean winter temperature origin (MWT, °F), and those in blue font will be tested in the Cold-hardy series and orange font in the Coastal series. There is adequate balance in the experimental design within each series.

Mother	MWT (°F)	Virginia	NC Coastal Plain	SC Coastal Plain	GA/FL Coastal Plain	Lower Gulf	Upper Gulf	Count
A	3.2	1		2	1	1		**4**
B	6.2	1	1	2	2	1		**5**
C	6.6	1		2	1	1		**4**
D	8.8		1	1	2	3	4	**5**
E	9.7		1	1	1	3		**4**
G	10.6		1		3	2		**3**
H	13.7		1	1	5	3	1	**5**
I	14.1		1	2	4	4	3	**5**
J	14.8			1	3	6	3	**4**
K	16.2		1	2	5	3	3	**5**
Count		**3**	**7**	**9**	**10**	**10**	**5**	

ing half of a full diallel (excluding selfs and including reciprocals) and five out of six unique combinations (ignoring which was used as female or male).

The third objective of the pollen cloud study is to determine if the cold-hardiness of an open-pollinated family depends on the orchard location, and if so, is the effect consistent with the **PRS**™. The Cold-hardy series will be used to test this objective using artificial freeze testing. The details of this study plan are to be determined.

The study seed are presently in stratification to be sown this spring and to be outplanted this upcoming winter. Each series will have 3 to 4 test sites with 30 replicates per test and around 40 trees per replicate (test site size of 1200 study trees). We are thankful to the Full Members for providing the seed and seedling growing, and are grateful for their commitments to test establishment, maintenance, and measurement for this important research project.

PRS Calibration Study

The objective of the **PRS**™ Calibration study is to translate breeding values obtained from progeny tests into yield and value per acre throughout the rotation of a stand. Most of the study sites were established in 2013, and many had their age 9 measurements completed this winter. Preliminary analysis indicates that the trials were established successfully, as the pre-canopy closure yields correlate strongly with the productivity scores from **PRS**™ (see the article in the Research section of this report). The next scheduled measurements for these tests is age 12 years. The original study plan called for a thinning to be implemented, but it is not clear how to apply a uniform thinning treatment. Faster growing families presently have much higher stand density and will have less live crown than slower growing families, which will confound the response to thinning with the family. One solution is to continue measurement of the non-thinned study trees to fully characterize their growth and yield patterns throughout maximum stand density. This will be feasible until the stands become overstocked and become a risk for fire or beetles.

The promising early results from the **PRS**™ Calibration study have some members asking when to install another round that includes 4th- and 5th-Cycle families. As we accumulate genetic gain, and productivity scores increase beyond the range in the current study, we will need to add data to the calibration equation to avoid extrapolation. Thus, the timeframe for the next round of calibration study tests depends on the success of our breeding program!

Wood Quality Measurements

Wood quality measurements for the 3rd-Cycle and ACE populations have been completed. Breeding values are presently being estimated and will be incorporated in the next **PRS**™ version (Summer 2022). The 4th-Cycle tests are next for wood quality measurements, but implementation will be difficult due to their design (see section on 4th Cycle above).

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Schmidtling, R.C. 2001. Southern pine seed sources. USDA Forest Service Gen. Tech. Rep. SRS-44, Southern Research Station. Asheville, NC. 44 p. Available at: https://www.srs.fs.usda.gov/pubs/2797

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Clone Banking Updates

The goal of archiving (also known as clone banking) is to keep a set of selections alive for future use. To achieve this, a particular selection is typically grafted in multiple locations to reduce the risk of total loss. Depending on the objective, the timeframe can be short- or long-term. The Cooperative has several clone banking efforts with differing timeframes that are implemented by the full members (Table 1). The objective of the 4th and 5th-Cycle selection archiving is to keep these clones alive until their progeny mature in the tests, and we can estimate their breeding values. Once the breeding values are calculated, members will collect scion for the winners and stock their new orchards, and the timeframe for the archive effort expires. Other archive efforts have indefinite timeframes, such as for the clones used in the 3rd-Cycle pollen mix and Resistance Screening Center checklots. The other two archive efforts are for clones used in the development of the loblolly reference genome, and clones with unique properties that may be the subject of future research.

Archiving duties are shared by the full members. Assignments are made and managed using the TIPRoot database. Each clone is assigned to at least two different full members for archiving to reduce the risk of loss. The member that owns the clone is required to archive it, and another member is assigned to graft it at a secondary location. Members are expected to update their archive progress annually. The number of assignments and the progress towards completion are shown in Table 1. Progress towards archiving the 4th-Cycle selections is not yet complete. It is imperative that these assignments are completed to reduce shortages in scion once the winners are identified. The archiving assignments for the 5th-cycle selections are planned to begin once adequate seed for testing has been harvested, so as to avoid cutting scion that encroaches on flower production and avoid clone banking selections that do not get tested (e.g. due to incompatibility, poor flowering, seed issues).

Table 1. *Clone banking/archiving efforts that are currently in effect for the Cooperative, including number of clones to be backed-up, start and end dates for the effort, the number of archive assignments, and the percent of assignments that have been completed as of May 2022.*

Archive Effort	Count	Start Year	End Year	Number of Assignments	Percent Complete
Clones that were first progeny tested in 5th-Cycle tests *	768	2023	2036	0	-
Clones that were first progeny tested in 4th-Cycle tests	193	2014	2027	379	69%
Clones used in the 3rd-Cycle pollen and checklot mixes	60	2000	Indef.	240	86%
Clones for checklots at the Resistance Screening Center	9	2018	Indef.	18	89%
Clones used to develop the loblolly reference genome	1	2014	Indef.	2	50%
Clones with extreme qualities subject of future research	3	2019	Indef.	7	43%

*Approximate numbers since not all 5th-Cycle selections have been made, and clone banking has not yet started.

Arrowhead Breeding Center Update

The Arrowhead Breeding Center continues to be a bustling area of activity throughout the year. Starting in June 2021, Anita Johnson with the Georgia Forestry Commission spent several weeks pruning back the interstock foliage to free up the recently grafted 5th-cycle selections. TIP staff returned to Arrowhead in October 2021 to plant a new future breeding orchard called the “Little Orchard” to acknowledge Chuck Little’s contributions. Seedlings from a very rust resistant family (***PRS***™ score 100, A+, A+) were grown in D-40 containers (40 cubic inches of soil) and planted with an auger. Special thanks to Graham Ford and the folks at IFCO for growing and donating these seedlings for establishing this new orchard!

Long-term planning for future breeding orchards is an activity that tree improvement managers must think about on a yearly basis. It is easy to ignore the need for future rootstock, but doing so can be costly. Since 2017, we have established over 830 rootstock trees for future breeding orchards at the Arrowhead

Image 1. *Anita Johnson with the Georgia Forestry Commission (left) and Chuck Little (TIP staff) used an auger to make quick work of planting the newly established “Little Orchard” for future breeding.*


Image 2. *Freeze damage to flowers that were inside of pollination bags at the Arrowhead Breeding Center after the nights of March 12th and 13th when the orchards got down to 25° and 29°F (left image). The right image shows newly elongated terminals that were droopy and not recovered several days post-freeze.*

Breeding Center. One year after seedling establishment, we graft 181210 scions that become the flower-promoting interstocks for grafting future selections.With the goal of topgrafting 75 to 100 selections per year and the need to have interstocks 15' to 20' tall at time of topgrafting to accelerate breeding, we are planting orchards about 7 years before they are needed for topgrafting. Topgrafting on to smaller trees will slow down the breeding cycles as we wait for adequate flowering and pollen production from topgrafts. Moving forward, we plan to begin recycling existing orchard blocks for future breeding orchard use. This emphasizes the need for tree improvement managers to be constantly thinking ahead, at least 7-8 years down the road!

Although we had a good cone crop from the Arrowhead Breeding Center in October 2021, Mother Nature was not so kind to us during the 2022 spring breeding season. Bag removal numbers indicate that 37% (88 out of 239) of the bags attempted this year failed completely. Overall flower survival at bag removal was 39% (Table 1), the lowest survival we have ever recorded. This was primarily caused by freeze damage but can also be attributed to a few bags breaking out due to extremely windy conditions. The good news is that we were able to collect all pollen from 5th-cycle selections prior to the late freeze events, putting us in good shape for spring 2023 breeding season.

Table 1: *Breeding work performed by TIP staff since 2014. Due to freeze damage this year, flower survival at bag removal was the lowest it has been (39%) since we started tracking this information.*

Year	Bags installed	Flowers bagged	Flowers at bag removal	Flower survival at bag removal	Avg. # flowers bagged	Avg. # flowers at bag removal
2014	129	1535	1237	81%	11.9	9.6
2015	161	1575	1392	95%	9.8	9.3
2016	312	3313	3195	97%	10.6	10.3
2017	496	2566	2220	87%	5.2	4.5
2018	523	4262	3889	93%	8.1	7.6
2019	419	5193	4716	92%	12.4	11.4
2020	12	98	97	99%	8.2	8.1
2021	69	661	585	89%	9.6	8.5
2022	242	1665	640	39%	6.9	2.7

Genotyping Update

The Cooperative has committed to an aggressive genotyping plan to implement genomic technologies and methods in the breeding program. The Cooperative staff developed a genomic selection plan that was shared with members in spring 2021 and approved during the 2021 Advisory meeting. The timeline for number of trees to genotype is given in Table 1. Since 2018, more than 8,000 trees have been genotyped using the Pita50K Axiom SNP Array for various projects (Table 2). The genotyping efforts can be divided into specific populations, but the data from most populations will be used for multiple objectives including genomic selection model development. All Pita50K data are stored in the TIPRoot database to be queried for ongoing projects (see page 31-32 in 2019 Annual Report). Following is a brief summary of the different projects with trees that have been genotyped the last four years:

The **4th-Cycle Coastal Breeding Population** and its pedigree were genotyped to develop genomic selection models for operational use in the mainline breeding program as described in the Cooperative Genomic Selection Plan. This includes all 5th-Cycle selections that can also serve as a baseline for pedigree quality assurance.

The **ACE1 and ACE2 Populations** were genotyped to test the predictive ability of genomic selection in a two-generation pedigree. Since the pedigree of the ACE1 and ACE2 populations overlap with the Coastal 5th-Cycle population, these data will also be useful for developing genomic selection models in the mainline population.

The **Fusiform Rust Mapping Population** consists of two large full-sib families that were used to map resistance genes (Lauer and Isik 2021). Three genes were discovered, and a strategy for marker-assisted selection is in place (see the section GRID Breeding in Breeding Update of this report).

The **Screening Array population** was genotyped to select SNP markers for inclusion on the final Pita50K array. The population consisted of 480 samples, including 36 megagametophytes from six parents, derived from the 4th-Cycle pedigree (Caballero et al. 2021).

Image 1. *Undergraduate student Sam Blumenfield training fellow students Alex Simpson and Frank Papa on needle sample preparation for DNA extraction.*

During the last year, around 1200 4th-Cycle progeny (including 5th-Cycle selections) have been genotyped. Only 96 of the 4th-Cycle pedigree have been genotyped, and the remainder are planned for fiscal year 2023. The Co-operative plans to genotype around 3000 4th-Cycle progeny each year over the next three years to develop genomic selection models. Including the ACE1 and ACE2 populations, this will provide around 16,500 genotyped and phenotyped individuals to train genomic selection models and implement the genomic selection in the 5th Cycle and beyond.

Table 1. *Proposed timeline of genotyping, phenotyping, and modeling for genomic selection in the 5th Cycle and beyond.*

Fiscal Year	Samples	Genotyping	Phenotyping	Genomic Selection
2022	1200	4th-Cycle Pedigree	4th-Cycle Progeny Tests	Model training
2023	3000	4th-Cycle Progeny Tests	4th-Cycle Progeny Tests	Model training
2024	3000	4th-Cycle Progeny Tests	4th-Cycle Progeny Tests	Model training
2025	3000	4th-Cycle Progeny Tests	4th-Cycle Progeny Tests	Prediction Model
2026	3200	5th-Cycle Progeny	4th-Cycle Progeny Tests	Genomic Selection
2027	3200	5th-Cycle Progeny	4th-Cycle Progeny Tests	GS/model update
2028	3200	5th-Cycle Progeny		GS
2029	3200	5th-Cycle Progeny		GS
2030	3200	5th-Cycle Progeny		GS
2031	3200	5th-Cycle Progeny	5th-Cycle	GS
2032	3200	6th-Cycle Progeny	5th-Cycle	GS/model update

Table 2. *Number of samples genotyped by project each year using the Pita50K Axiom SNP Array. Most samples correspond to a single tree, except in the ACE1 population where additional genotyping was required to correct the pedigree.*

Population	**2018**	**2019**	**2021**	**2022**	**Total**
4th-Cycle Pedigree				96	**96**
4th-Cycle Progeny			461	609	**1,070**
5th-Cycle Selections			19	96	**115**
ACE1		2509		100	**2,609**
ACE2				1728	**1,728**
Fusiform Rust Mapping Population		2072			**2,072**
Screening Array	480				**480**
Total	**480**	**4,581**	**480**	**2,629**	**8,170**

CITATIONS

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Fourth-Cycle Data Analysis

The purpose of this analysis was the prediction of breeding values of individual trees and their pedigree for the 4th-Cycle Coastal and Coldhardy (combined Piedmont and Northern) testing populations. The breeding values were predicted using progeny test data collected from the 4th-Cycle tests established from 2014 through 2018. The Coastal and Coldhardy populations were analyzed separately.

The Coastal data were comprised of 34 tests from 359 parents and 5 checklots of which 56 parents have no ***PRS***™ values in the COASTAL 2017 ***PRS***™. The Coldhardy data consisted of 43 tests from 549 parents and 8 checklots of which 273 parents have no ***PRS***™ values in the PIEDMONT & NORTHERN ***PRS***™. The pedigrees of both datasets were comprised of ancestors, parents, checklots, and individual trees. In both datasets, tests were strongly connected across test series.

Traits presented in this summary were volume, straightness, and fusiform rust incidence (traits used in the Cooperative's VSR selection index). Prior to multi-site analysis, simple models were fit to analyze phenotypic traits at each location. Following single site analyses, a multi-site individual-tree model was designed in which only the significant experimental design effects (rep, row within rep, column within rep) from single-site analyses were fit to the data. The multi-site models were used to estimate breeding values for individual trees in the pedigree.

Breeding values for volume, straightness, and rust were compared for parents and individual trees that had data that were included in both 2021 and 2022 analyses. In both datasets, a large degree of consistency was observed for all traits. In general, the correlation between breeding values estimated in 2021 strongly corresponded with the breeding values in 2022. The correlations ranged between 0.90 and 0.97.

A significant number of new parents ranked in the top 20 for stem volume and fusiform rust disease incidence assessed in the 4th-Cycle Coastal population (Figure 1). Similarly, in the 4th-Cycle Coldhardy population, a significant number of new parents ranked in the top 20 for stem volume, straightness, and fusiform rust disease incidence (Figure 2).

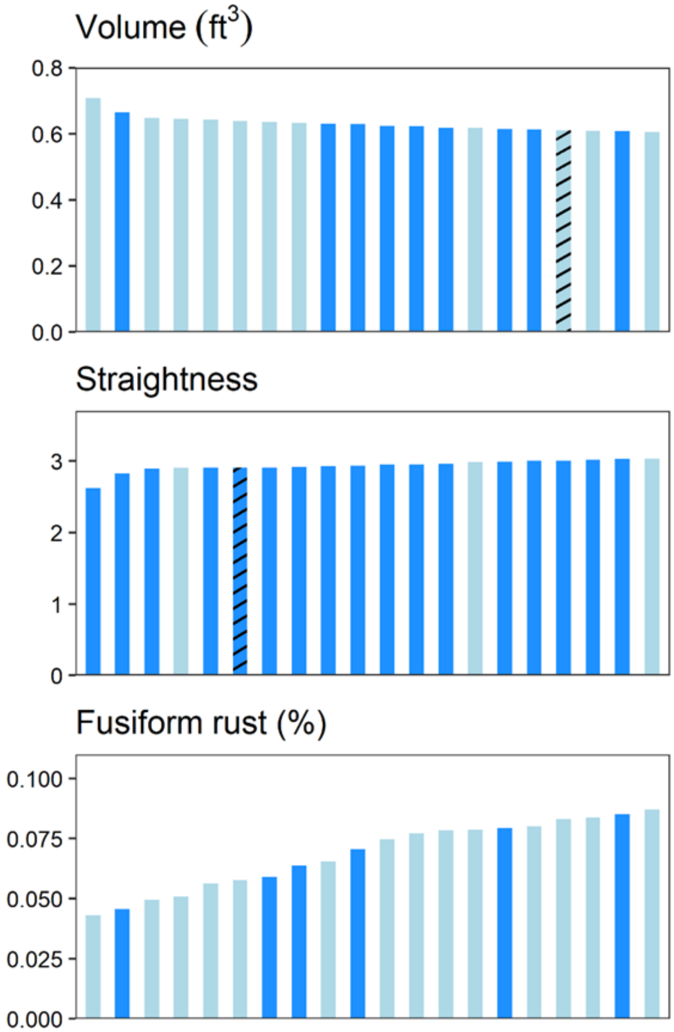

Figure 1. *Barplots showing volume, straightness, and fusiform rust disease breeding values for the top 20 parents identified in the 4th-Cycle Coastal 2022 BLUP analysis. Light blue bars correspond to the parents that have no **PRS**™ values in the current **PRS**™ system. Striped bars represent parents new to the 2022 BLUP analysis. All these data (old and new) will be included in the 2022 **PRS**™.*

Some of these new parents have no ***PRS***™ values in the current ***PRS***™ system and may be of value for inclusion into new seed orchards. As always, users in the northern and inland regions of the Southern US need to be cautious about utilizing Northern and Piedmont selections that may not be adapted. The mean minimum winter temperature origin of a selection is a critical criterion to include when deciding which of these selections to include in your seed orchard.

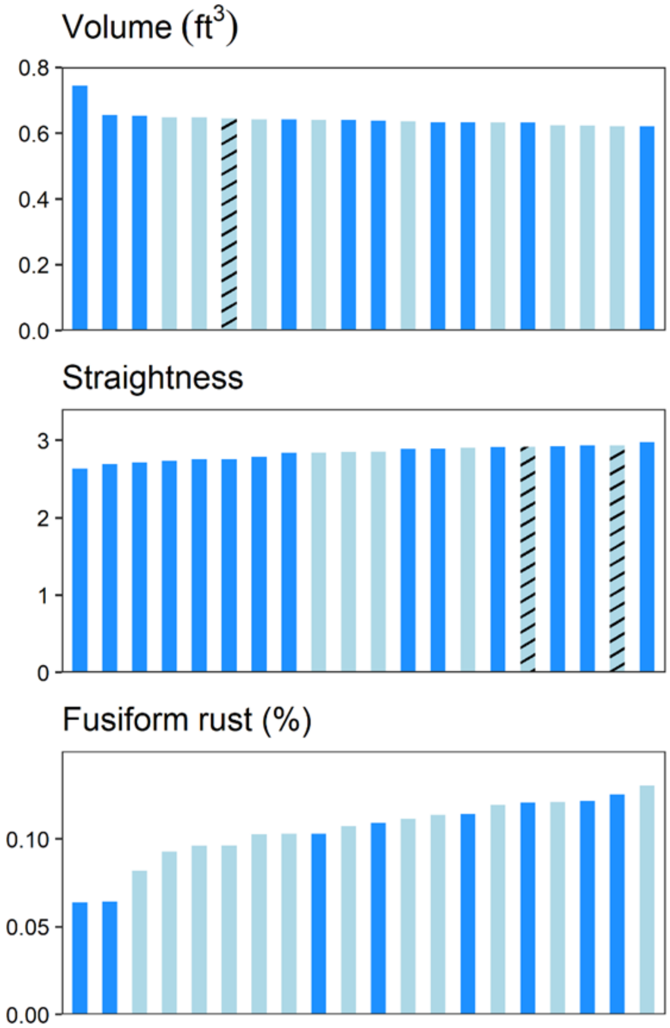

Figure 2. *Barplots showing volume, straightness, and fusiform rust disease breeding values for the top 20 parents identified in the 4th-Cycle Coldhardy 2022 BLUP analysis. Light blue bars correspond to the parents that have no **PRS**™ values in the **PRS**™ system. Striped bars represent parents new to the 2022 BLUP analysis. All these data (old and new) will be included in the 2022 **PRS**™.*

Pictured above: *A 4th-Cycle test located at Arrowhead Breeding Center at sunrise.*

Second-Generation Atlantic Coastal Elite (ACE2) Clonal Population Update

The ACE2 population was initiated and designed to test genomic selection, specifically to evaluate how well the marker-trait relationships perform after recombination when breeding next-generation progeny (more details in the Cooperative's 2020 Annual Report). In 2016 – 2019, around 183 crosses were bred among 107 ACE1 clones at the Arrowhead Breeding Center. Parents used for crosses included the 48 ACE1 clones that were selected for breeding in the 4th Cycle, as well as ACE1 clones that were selected with less emphasis on gain and more emphasis on diversity. Those crosses bred in 2016-2018 with enough seed were sown in May 2020 and planted in Fall 2020 in the *ACE2 seedling trials*. The ACE2 seedling trials include 67 crosses among 73 ACE1 clones. Survival, height, and rust incidence were measured this past winter and are scheduled for annual measurement through at least age four years.

In late summer 2020, prior to planting the ACE2 seedling trials, the seedlings were top-clipped and the clippings were rooted in the mist house at the Horticulture Field Lab in Raleigh, NC. The objective was to clone the individuals planted in the seedling trials to propagate an accompanying set of *ACE2 clonal trials*. Surviving cuttings (around 30 individuals per cross) were transferred to 3-gallon pots in the spring of 2021 so that they could be grown as hedges to produce many copies of each ACE2 clone. The hedges were grown during the 2021 growing season on the outdoor growing pads at the Horticulture Field labs. In early fall 2021, needle samples were taken from each cutting and 1728 individuals were genotyped using the Pita50K SNP Array.

Image 1*. Sarah Conner, current masters student with the Cooperative, hedging the ACE2 population in February 2022 to a height of ~ 6 inches (15 cm). Coconut fiber weed mats were used to keep out competing weeds and made clean up after hedging much easier.*

Once the cuttings had been grown to a height of approximately 18-24 inches (~45-60 cm), they were hedged for the first time in February 2022. Each tree was hedged to approximately 6 inches (~15 cm). Hedging serves two purposes: 1) all plants are cut to the same height to increase uniformity and reduce maturation effects, and 2) remove apical dominance so that each hedge produces many shoots for clonal production. In May 2022, once the hedges' new shoots reached approximately 2.5-4 inches (~6-10 cm) in length, they were clipped and stuck individually into Ray Leach Super Cells (Image 3). We targeted 14 copies from each of about 1800 hedges. The surviving ramets will be planted into ACE2 clonal trials in the fall of 2022 with a target of at least four test sites.

The motivation for developing the ACE2 clonal trials is that clonal replication in progeny testing produces more reliable individual-tree breeding values than seedling trials. The reliability of the breeding values affects the estimated predictive power of genomic selection, so it is important that we have very reliable breeding values when validating the genomic models. However, clonal trials take longer to propagate, so we established the ACE2 seedling trials quickly to avoid delays in the study. The approach we took (producing hedges from the seedling ortets) is unique in that the same genotypes in the ACE2 seedling trials will be represented in the clonal trials. The primary motivation for this approach was to reduce genotyping costs, because we only needed to genotype one set of trees that are represented in both the seedling trials and the clonal trials. Measurements from both trials will be used to validate genomic prediction models, which allows us to evaluate different strategies for tree breeding and testing in the genomics era.

Image 2. *ACE2 hedges on April 12, 2022. New shoots from each hedge varied from 2 – 8cm in length.*

Image 3. *Rooted cuttings stuck in May 2022 to be out planted in Fall 2022 in the ACE2 clonal trials. We targeted 14 cuttings for each of about 1,800 clones.*

Marine Corps Sessile Oaks (*Quercus petraea)* March to the Field

This past year, the sessile oak seedlings that have been growing for the past two years at the Horticulture Field Labs in Raleigh marched off to their new homes. These acorns were imported from Belleau Wood, France to commemorate the WWI Battle of Belleau Wood (see the 2020 Annual Report for details). To kick things off, the first sessile oaks were planted right here on campus at Reynolds Coliseum, home of the NCSU ROTC units (Images 1 and 2).

The oak trees have now been planted at various locations around the United States, and more seedlings are growing to size at the NCSU greenhouses and will be planted soon (Table 1). These trees will serve as a living reminder of the battle of Belleau Wood and the brave Marines who fought and died there.

Images 1 and 2. *Colonel John Giltz USMC (left image) planting the first sessile oak seedlings on the SW corner of Reynolds Coliseum at NCSU. The second sessile oak (right image) was planted on the NE corner of Reynolds. The tree planting took place after a ceremony acknowledging the significance of the Battle of Belleau Wood and the playing of the Marines' Hymn.*

Table 1: *Location and number of sessile oaks planted at each site. Proposed locations for planting the remaining seedlings are shown in the last row.*

Location	Number of trees Planted
Reynolds Coliseum, NCSU (ROTC) Raleigh, NC	2
The Citadel, Charleston SC	3
University of South Carolina (ROTC)	2
Marine Corps Recruit Depot, Parris Island, SC	4
2nd Bn, 6th Marine Regt, Camp Lejeune	2
3rd Bn, 6th Marine Regt, Camp Lejeune	2
Camp Johnson, NC (HQ and Montford Pt Marine Memorial)	3
School of Infantry, Camp Geiger NC	3
Camp Lejeune community center	1
Various Quantico VA locations	12
6th Marines HQ, 1stBn, 6th Marine Regt, 5th Marine Regiment, Arlington National Cemetery; National Museum of the Marine Corps, National Museum of the Medal of Honor, Florence (SC) Veterans Park, GySgt Fred Stockham memorial and gravesite (NJ), Mt Soledad	Planned for Fall 2022

SEED AND CONE YIELDS

Unfortunately, the 2021 cone crop was not one to write home about. This could have been expected, since the 2020 cone crop was phenomenal, having the highest seed yield the Cooperative has seen in 20 years. The proportion of 3rd-Cycle+ seed continues to climb each year, making up the great majority of the seed produced (Figure 1). Figure 1 shows how annual seed production has its ups and downs. Hopefully after this down year, fall of 2022 will fill up everyone's seed rooms again!

Table 1: *Fall 2021 cone and seed yields compared to the 2020 harvest. Cones collected from 4th-Cycle orchards were included in the 3rd-Cycle data, hence the* "3rd-Cycle+" *label in the text.*

Provenance / Orchard Cycle	**Bushels of Cones**		**Pounds of Seed**		**Pounds of Seed per Bushel**	
	2021	2020	2021	2020	2021	2020
Coastal 2.0/2.5	5,111	10,897	6,369	16,453	1.25	1.51
Coastal 3.0+	17,145	25,642	22,986	37,540	1.34	1.46
Piedmont 2.0	2,196	3,662	3,111	5,677	1.42	1.55
Piedmont 3.0+	6,835	11,319	8,065	16,341	1.18	1.44
Northern 2.0	76	294	63	445	0.83	0.00
Northern 3.0+	3,296	3,955	3,608	5,015	1.09	1.27
Totals	**34,659**	**55,769**	**44,203**	**81,471**	**1.18 Avg.**	**1.21 Avg.**

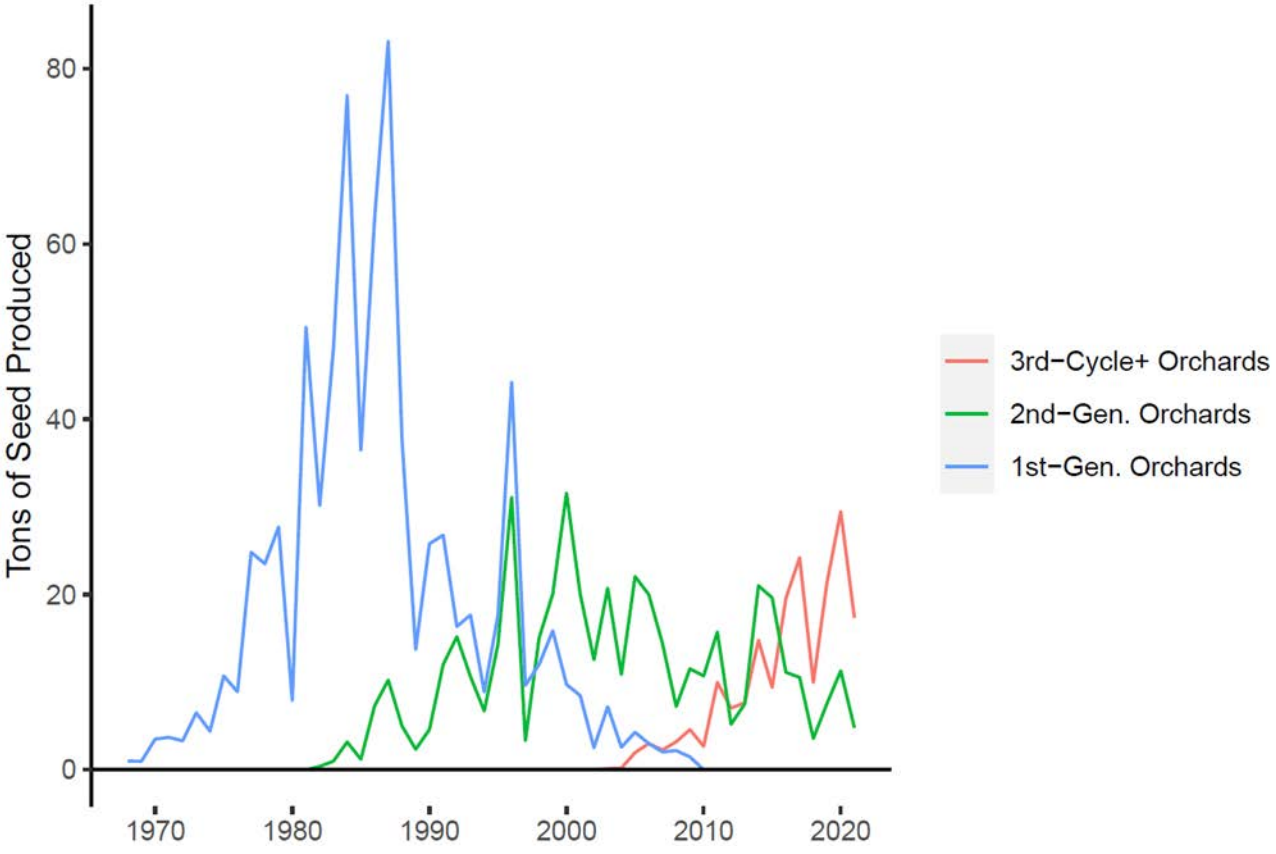

Figure 1. *Since 1968, the Cooperative has tracked annual seed yields from Cooperative members. 1st-Generation seed orchards are no longer being harvested. In 2021, 22% of the seed harvest came from 2nd-generation seed orchards, while the other 78% came from 3rd-Cycle+ seed orchards.*

RESEARCH

Within-Family Selection in Loblolly Pine is Improved with Genotyping

Progeny test data typically do a very good job at ranking families, because there are many data points (individual trees) to inform the family mean. However, ranking individuals within a family is typically based on one data point (the individual tree), so there is much less certainty. Clonal progeny testing is an option to improve within-family rankings because several copies of the same individual result in more data points and thus more reliability. Clonal testing in loblolly pine has some drawbacks, since it requires at least two extra years for propagation, increased costs in the nursery, and more land area is needed for the added replication.

Genotyping a progeny test with thousands of DNA markers may serve as a middle-ground between clonal and non-clonal progeny tests. When analyzing progeny test data, we must model relationships among trees because trees that are closely related will be more similar than trees that are unrelated. In non-genotyped progeny tests, we use the pedigree to calculate *expected* relationships. The expected relationship for individuals from the same full-sib family is 0.5, and the expected relationship for copies of the same clone is 1.0. With genotyping, we can calculate *realized* relationships based on DNA marker sharing. Realized relationships open a new door for ranking individuals within a family; because of Mendelian sampling, pairs of full-sibs may be more or less related than the expectation of 0.5. Just like cloning takes advantage of the information that trees are genetically identical, genotyping can take advantage of the information that some full-sibs are more related than others. In this way, genotyping changes the unit of replication from the line of descent to the DNA marker, which provides more information when ranking individuals within a family.

To evaluate the benefit of genotyping, we analyzed a large clonal population established and measured by ArborGen Inc. The experiment consisted of 26 test sites that included 1831 clonal progeny with around 20 to 30 copies per clone. Height, DBH, and stem straightness were measured at age 6 years. A subset of the clones (723 clones from five full-sib families) were genotyped. Expected relationships were calculated from the pedigree and realized relationships were estimated from the genotyping data. A comparison of the two relationships (Figure 1) shows that full-sibs (which have expected relationship of 0.5) vary in their realized relationships from 0.25 to 0.75. This is primarily due to the Mendelian sampling of genes during breeding, but it is also due to the statistical properties of the realized relationship estimator.

To evaluate the advantages from genotyping, models were run twice, first using the expected relationships from pedigree and again using the realized relationships from genotyping. To evaluate the impact of clonal replication, the data were subset to simulate a reduced cloning effort. The subsetting was done so that there were 1 clonal copy of each proge-

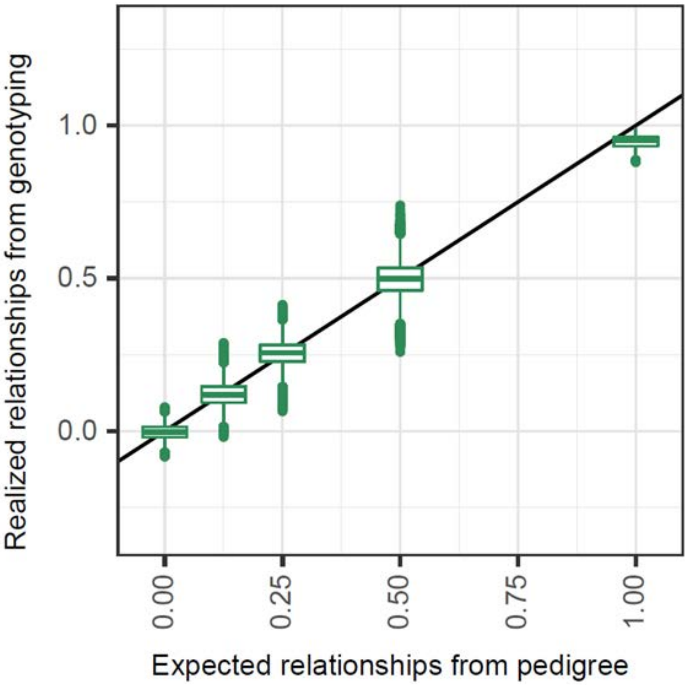

Figure 1. *Comparison of the distribution in realized relationships (from genotyping) against the expected relationships (from pedigree) for 723 genotyped progeny. For full-sibs (expected relationship of 0.5), the realized relationships range from 0.25 to 0.75 mainly due to the Mendelian sampling of genes during breeding. The black line is the 1 to 1 reference line.*

ny (analogous to no cloning), followed by 3 copies, 6 copies, 9 copies, …, 15 copies, 20 copies and 30 copies. Trees chosen for removal were done at random, and the random selection was conducted 20 times. Models were compared using the accuracy of breeding values, which is a function of the standard error of the breeding value and represents the certainty when ranking individuals.

The accuracy of the breeding values increased sharply with increased clonal replication until around six to nine copies per clone, after which it began to plateau (Figure 2). Genotyping produced a boost in accuracy when clonal replication was absent (1 copy per progeny), and the boost was similar to having 3 to 5 clonal copies per progeny. The amount of improvement in breeding value accuracy from genotyping subsided as clonal replication increased. When clonal replication was abundant (20+ copies per progeny), genotyping only modestly improved the accuracies. The improvements in accuracy from genotyping and clonal replication were due to smaller standard errors for the breeding values, resulting in a more confident ranking of individuals within a family.

While genotyping's biggest allure is genomic selection (a tool to select young individuals before they are old enough to phenotype), this study demonstrated that there is additional value when a progeny testing population is genotyped. This has implications when developing a genomic selection strategy, because the cost of genotyping training populations can be offset early on if selections are made using the rankings from realized relationships. There are also implications for clonal testing, because genotyping can replace or supplement clonal testing for species that are difficult, expensive, or time-consuming to propagate. When genotyping was used alongside abundant clonal testing, very high individual-tree breeding value accuracies were obtained that would justify grafting progeny directly into production orchards.

This is a brief summary of the Trevor Walker's PhD research that is published:

Walker, T. D., W. P. Cumbie, and F. Isik. 2021. Single-Step Genomic Analysis Increases the Accuracy of Within-Family Selection in a Clonally Replicated Population of Pinus taeda L. Forest Science. :fxab054.

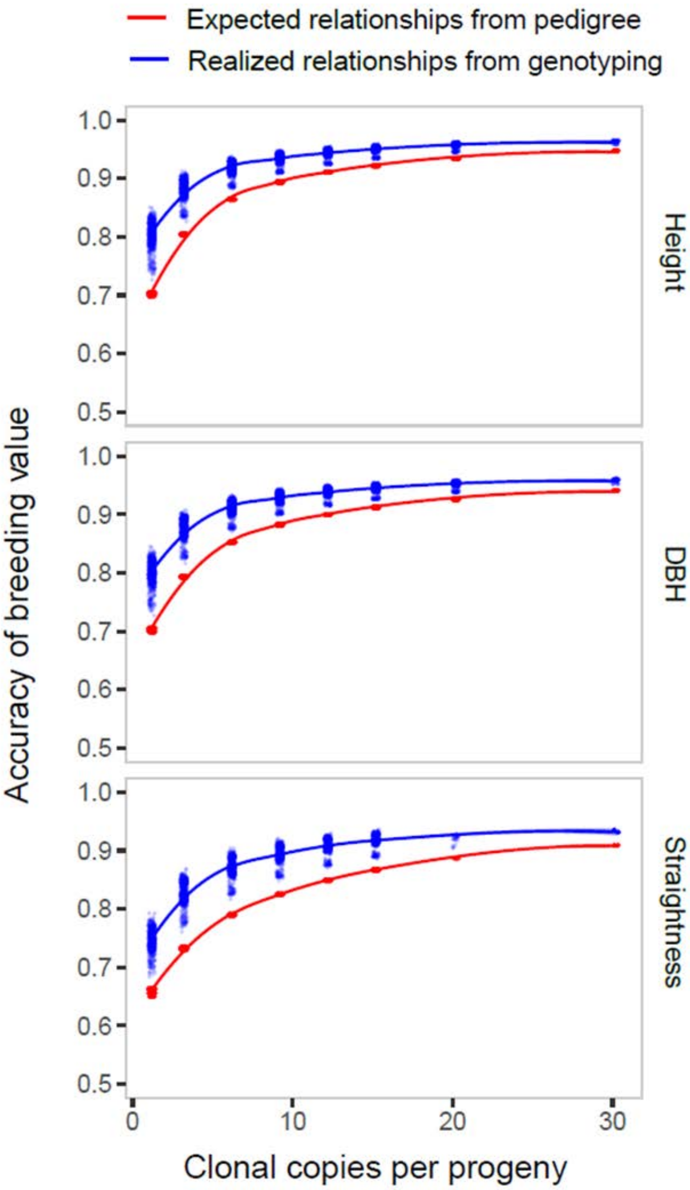

Figure 2. *Accuracy of breeding values for height, DBH, and stem straightness from a genotyped clonal population. The number of data points for each progeny was reduced to simulate clonal replication effort (x-axis). Accuracies increased sharply until around six to nine clonal copies per progeny, after which they plateaued. Relationship values were calculated with genotyping data (blue points and lines) and with pedigree (red points and lines). Genotyping produced a large boost in accuracy when there was 1 copy per progeny (i.e. no clonal replication), but that boost subsided as clonal replication increased. The lines are the moving averages.*

Fusiform Rust Disease in the *Pinus taeda* Northern Breeding Population[1]

In the Cooperative Tree Improvement Program's Northern breeding population of loblolly pine, the incidence of fusiform rust disease caused by the *Cronartium quercuum* f.sp. *fusiforme* in progeny tests is sporadic. The average disease incidence is usually below 10% in progeny tests. The underlying factor for low disease incidence in the Northern progeny tests is likely contributed by non-optimal environmental conditions for the fungus to infect trees rather than genetics. In the northern latitudes, temperature and humidity during the early spring are lower compared to more southern regions of the United States and are not conducive to infection. Low disease incidence is not ideal to separate the environmental effects from genetic effects and to predict breeding values of families for selection for diseases resistance.

To understand the level of genetic variation in the Northern breeding population of loblolly pine, we inoculated progeny of 71 half-sib families at the USDA Forest Service Resistance Screening Center in Asheville, NC. Checklots provided by the Cooperative Tree Improvement Program and two Resistance Screening Center checklots were also included. For each family, about 80 progeny were inoculated using a bulked inoculum with a spore density of 20,000 basidiospores/mL. Inoculation was conducted over three days, and each family was inoculated on all three days of inoculation.

Six months post-inoculation, the presence and absence of disease incidence (gall) were recorded on 9000 seedlings (Image 1). The overall family disease incidence was 12%, but the level of incidence varied among days (Figure 1). We observed a large variation among family means for disease incidence, ranging from 2% to 27% (Figure 2), suggesting a significant genetic effect on the disease outcome. A generalized linear mixed model was fit to data to partition the observed variation for disease incidence into genetic and environmental components and estimate the linear combinations of variance components. Family mean heritability was 0.64 suggesting that there are considerable genetic differences among Northern families for disease incidence.

Disease incidence of the Resistance Screening Center checklots were 0% for the resistant check and 7% for the susceptible check. Most families had disease incidence above the susceptible checklot, indicating a general lack of resistance to fusiform rust for these parents.

Incidence in the experiment was low, but the results suggest that artificial inoculation could be a more efficient method to separate genetic effects from the

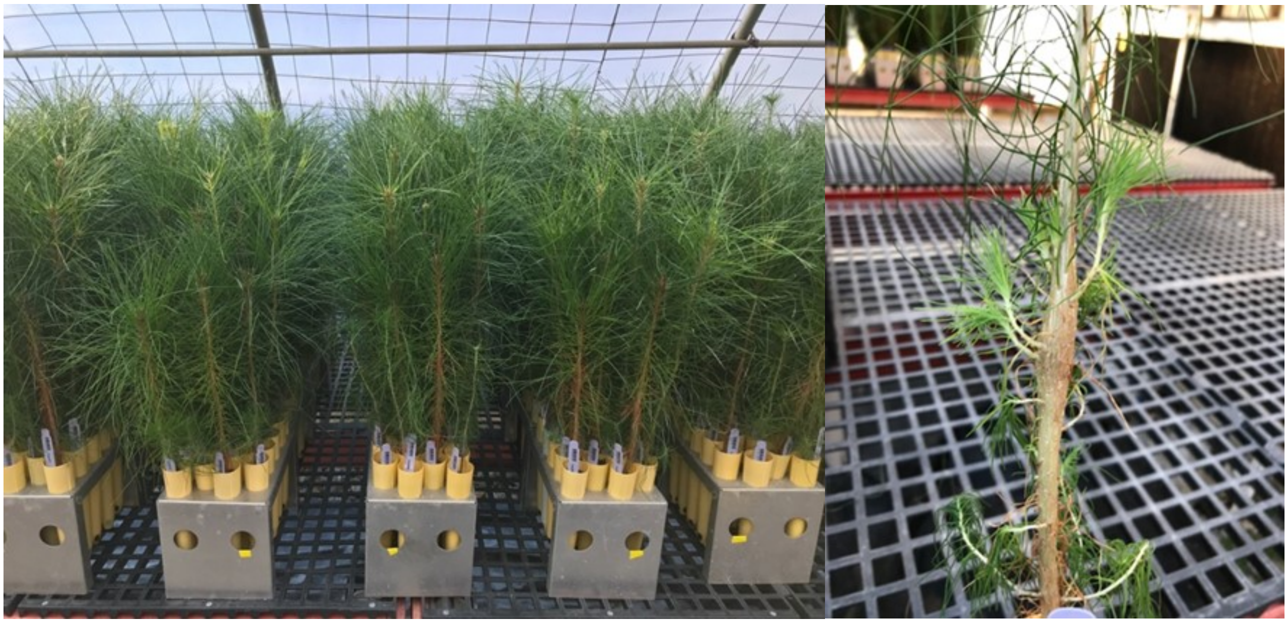

Image 1. *LEFT: Inoculated seedlings grown in the greenhouse for six months post-inoculation. RIGHT: Galled seedling six months post-inoculation.*

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[1] This is a summary of Sarah Conner's graduate research.

environmental factors for disease incidence and to estimate breeding values of these parents. With climate change, temperatures and humidity are expected to increase in the northern latitudes and may provide a more favorable environment for the pathogen to infect loblolly pine and increase the overall disease incidence in the cold-hardy loblolly pine population.

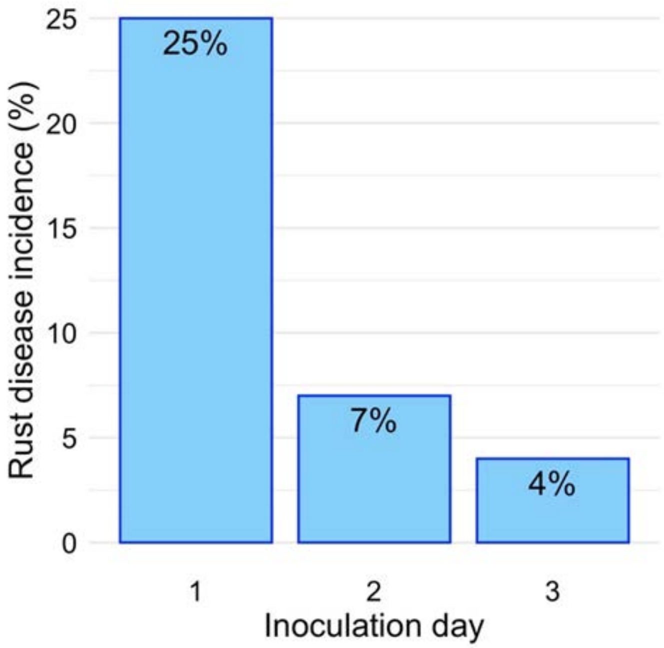

Figure 1. *(right)Mean disease incidence (percent) of rust disease at six months for three inoculation days. The disease incidence dropped from day 1 to day 2 and day 3. The overall incidence was also lower than previous artificial inoculations experiments conducted by the Cooperative.*

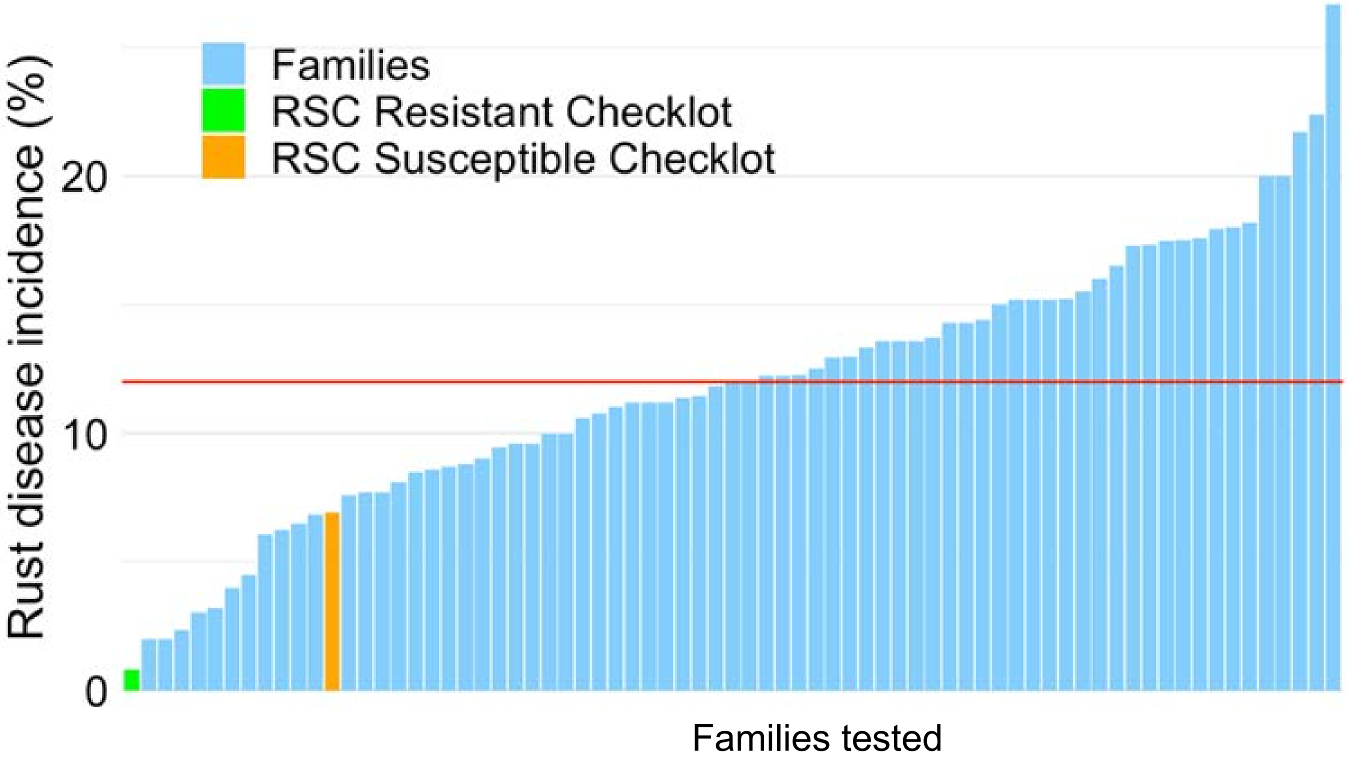

Figure 2*. Variation in rust disease incidence for families following inoculation. The resistant (green) and susceptible (orange) checklots were provided by the Resistance Screening Center. Most families had rust incidence greater than the susceptible checklot. The red horizontal line denotes the overall mean rust disease incidence.*

Genomic Selection in the Atlantic Coastal Elite Population

The aim of this genomic selection pilot study is to assess whether SNP marker-trait associations can be used to predict genomic estimated breeding values (GEBV) from one generation to the next. The linkage disequilibrium (LD) between markers and trait loci is expected to breakdown because of chromosomal recombination during the breeding, so this study goes beyond our earlier proof of concept experiments.

The Atlantic Coastal Elite (ACE) breeding population is comprised of the elite founders (21 parents), ACE1 clones (generation 1), and ACE2 seedlings (generation 2). The ACE1 population is comprised of ~2500 clones representing 51 full-sib families. One genetically identical copy (ramet) of each clone was planted at each of eight sites in the southeastern USA. This population was evaluated at age six for growth, stem quality, and fusiform rust incidence.

The ACE2 population consists of ~1900 progeny representing 67 full-sib families originating from 73 ACE1 clones. The ortets (original seedlings) of ACE2 were outplanted in winter 2021 in four field trials in the southeastern US and were assessed at age one for tree height. Before planting, cuttings from 1900 seedlings were obtained to produce hedges that are being used to produce rooted cuttings that will be identical copies (ramets) of each planted seedling. The ramets of the ACE2 clones will be out planted in progeny trials in the winter of 2022-2023.

Both ACE1 and ACE2 populations were genotyped with the Pita50K array developed for loblolly pine (Caballero et al. 2021). A total of 4044 trees have been genotyped (33 founders, 2287 ACE1, and 1724 ACE2 clones). After data filtering and quality control procedures, about 34,000 SNP markers were available. The SNP markers were used to evaluate clustering of individuals into families, construct genomic relationship matrix, and develop GS models.

Principal component analysis of SNP markers clustered ACE1 into 51 full-sib families and ACE2 into 67 full-sib families that corresponded with the pedigree (Figure 1 left). A similar trend was observed when realized additive genomic relationships were compared with the expected relationships from the pedigree (Figure 2). The three disconnected diallel mating structure of ACE1 disappeared in the ACE2 population due to breeding of trees from different diallels (Figure 1 right).

To evaluate GS in the ACE population, training models were developed using six-year phenotypic data for different traits in the ACE1 population. Whole genome regression and GBLUP models were fit to evaluate predictive ability of SNP markers by implementing different cross-validation scenarios for growth, stem quality, disease, and wood quality traits (Lauer et al., 2022; Shalizi et al., 2022; Walker, 2022). The predictive ability of SNP markers was moderate to

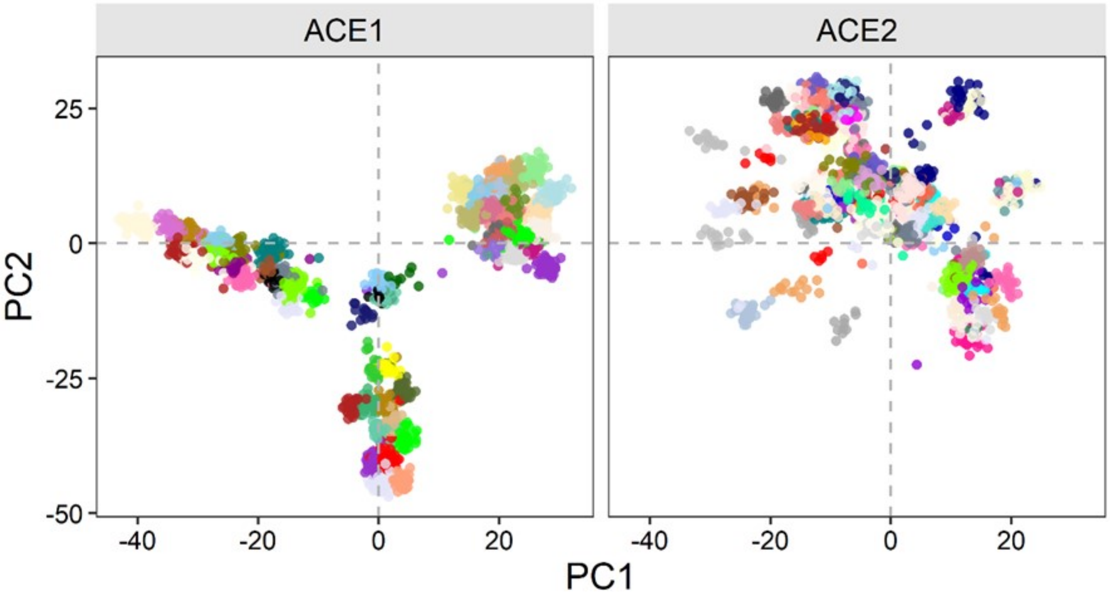

Figure 1. *Clustering of ACE1 and ACE2 clones using principal component analysis of Pita50K SNP markers. Circles represent individual clones and colors represent full-sib families.*

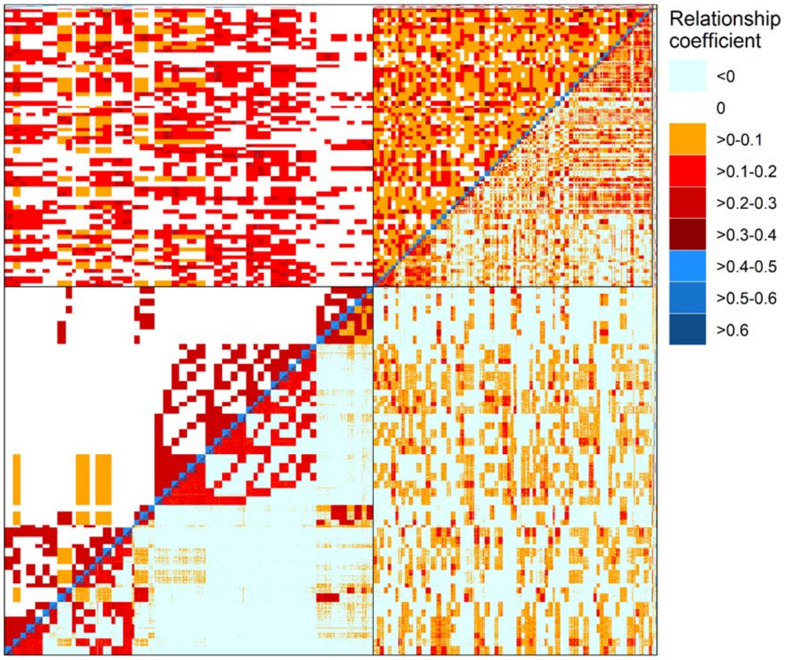

Figure 2. *Expected additive genetic relationships (**A**) derived from the pedigree (upper diagonal) and realized genomic relationships (**G**) derived from SNP markers (lower diagonal) for 4043 loblolly pine clones. The large block in the bottom left represents 2286 ACE1 clones, and the other large block on top right represent 1724 ACE2 clones. The tiny block on the top right corner are the 33 ACE founders. The blue blocks in the diagonal of the matrix represent 51 full-sib families in the ACE1 and 67 full-sib families in the ACE2 population. The red blocks in the bottom left correspond to the three disconnected diallel groups used in the mating design to produce the full-sib crosses in ACE1.*

high when the training and validation sets were genetically connected with each other. Prediction ability of SNP markers declined considerably when genetic relatedness between training and validation sets was weak (Figure 3).

In the second step, the ACE2 genotyped clones are being used as validation set in the training models developed for the ACE1 population to predict GEBVs. To validate GS, we need age four phenotypes from the ACE2 population. So far, only age one height is available from the ACE2 trees. The correlation between age one height of seedling progeny of ACE2 and their GEBV obtained from SNP markers was 0.40 (Figure 4), but this correlation is expected to improve as data accumulate, and the trees are measured at older ages. Preliminary results are promising to implement GS in loblolly pine breeding managed by the Cooperative Tree Improvement Program.

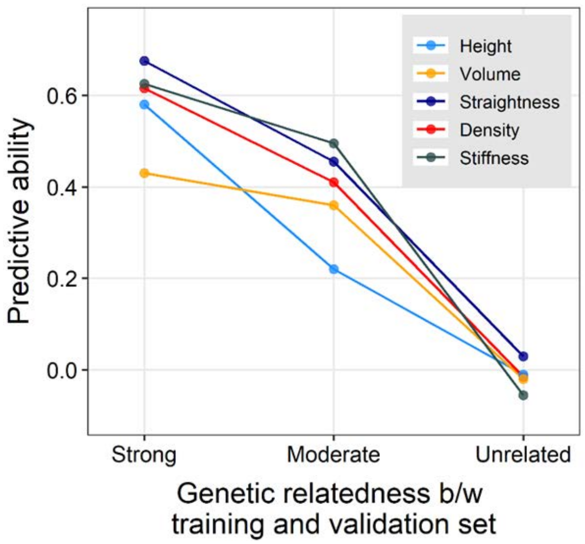

Figure 3. *Line plots showing the decrease in mean predictive ability of SNP markers as result of decay in genetic relatedness between training and validation sets for growth, stem straightness, and wood quality traits. Predictive ability of SNP markers declined considerably when the validation sets were genetically unrelated to the training sets.*

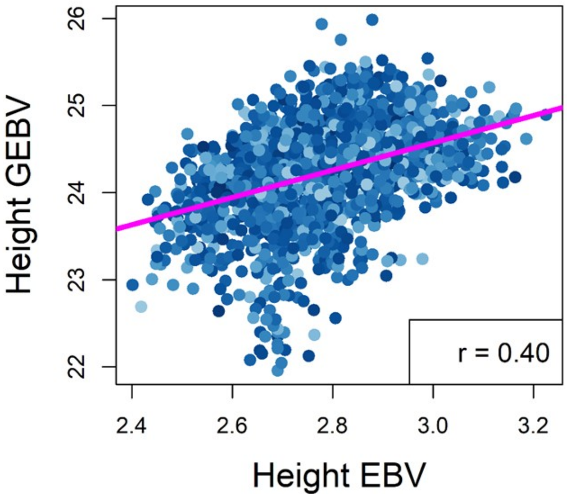

Figure 4. *Scatterplot showing correlation between estimated breeding values (EBV) and genomic estimated breeding values (GEBV) for stem height in the ACE2 population. The EBVs were obtained from age one height measurements in the ACE2 population using an ABLUP model. The GEBVs in the ACE2 population were estimated using the ACE1 adjusted phenotypes at age six years and SNP markers in a GBLUP model.*

AgriSeq$^{\text{TM}}$ Targeted GBS Genotyping Platform For Loblolly Pine[1]

Single nucleotide polymorphic markers (SNP) are routinely used in operational plant and animal breeding for quality control, marker-aided selection, and genomic selection. The loblolly pine SNP array (Pita50K) designed in 2019 is a wonderful resource, and the Cooperative has extensively used Pita50K for research and for genomic selection. The cost per sample using Pita50K is still high for some operations such as quality control (parentage analysis). The Cooperative Tree Improvement Program and Thermo Fisher Scientific have collaborated on a pilot project to develop a more cost-efficient genotyping platform using AgriSeq$^{\text{TM}}$ targeted GBS technology. We finalized the design of AgriSeq $^{\text{TM}}$targeted GBS panel in March 2022. The panel design was paid using resources from the NIFA-Genomic Selection (NIFA Award No. 2019-67013-29169) and McIntire-Stennis projects.

AgriSeq$^{\text{TM}}$ targeted GBS panel delivers genotypes based on variant allele frequencies from amplicon sequences alignment. This technology has advantages over others in its ability of discovering additional SNPs within targeted regions, the changeability of markers in panel by adding or removing primers easily, and the higher sample scalability as the number of markers in the panel tested decrease.

About 1000 high quality SNP markers from Pita50K array were selected to test with AgriSeq$^{\text{TM}}$ technology. The marker set selected covered 12 linkage groups of loblolly pine. A total of 56 million reads were amplified from which 92.8% of the reads aligned correctly to the region on reference genome. For targeted regions, the average coverage was 201x, and the uniformity was 89.1%, which is the percentage of the bases in the region that had at least 0.20 times mean coverage. A population of 192 fourth cycle trees with known pedigree was used to test the Pita AgriSeq$^{\text{TM}}$ panel. The overall sample call rate of the Pita AgriSeq$^{\text{TM}}$ marker panel was 85.5%.

To validate the utility of the Pita AgriSeq markers for loblolly pine, we compared it with the high-density Pita50K SNP array for marker quality measures, realized genomic relationships, and clustering of individuals into families. Realized genomic relationship matrices for the 192 fourth-cycle samples constructed from high-density Pita50K markers and low-density Pita AgriSeq markers strongly corresponded with each other (Figure 1).

The principal component analysis further supported strong agreement between AgriSeq$^{\text{TM}}$ and Pita50K markers. The Pita AgriSeq$^{\text{TM}}$ markers clustered in-

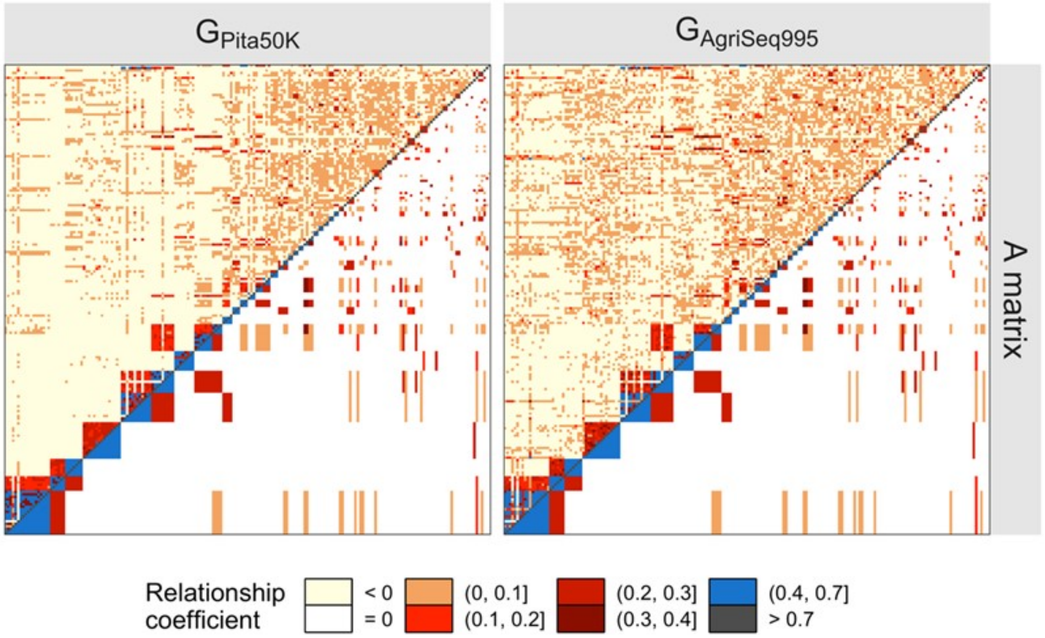

Figure 1. *Expected additive genetic relationships derived from the pedigree (lower diagonal) and realized genomic relationships derived from Pita50K (left) and Pita AgriSeq (right) SNP markers (diagonal and upper diagonal) for 192 loblolly pine trees.*

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[1] *This is a summary of Yu-Ming Lin's PhD research.*

dividuals into eight full-sib families corresponded with the clustering of individuals from Pita50K markers (Figure 2). These results suggests that Pita AgriSeq^{TM} genotype platform is a promising platform for fingerprinting and parentage analysis tasks.

The Pita AgriSeq^{TM} genotyping platform is less expensive than the Pita50K array and can be a better alternative for genotyping in the Cooperative Tree Improvement Program. A pilot project is also underway to test the utility of the Pita AgriSeq^{TM} genotyping platform for genomic selection in loblolly pine breeding. The platform is available to the Cooperative Tree Improvement Program members to genotype their trees in their operations.

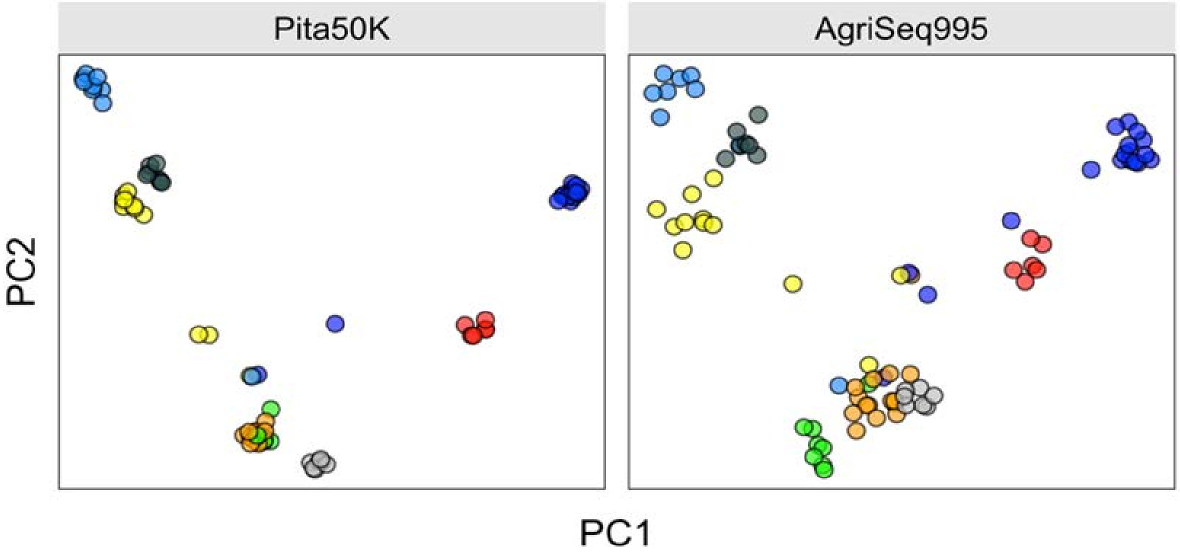
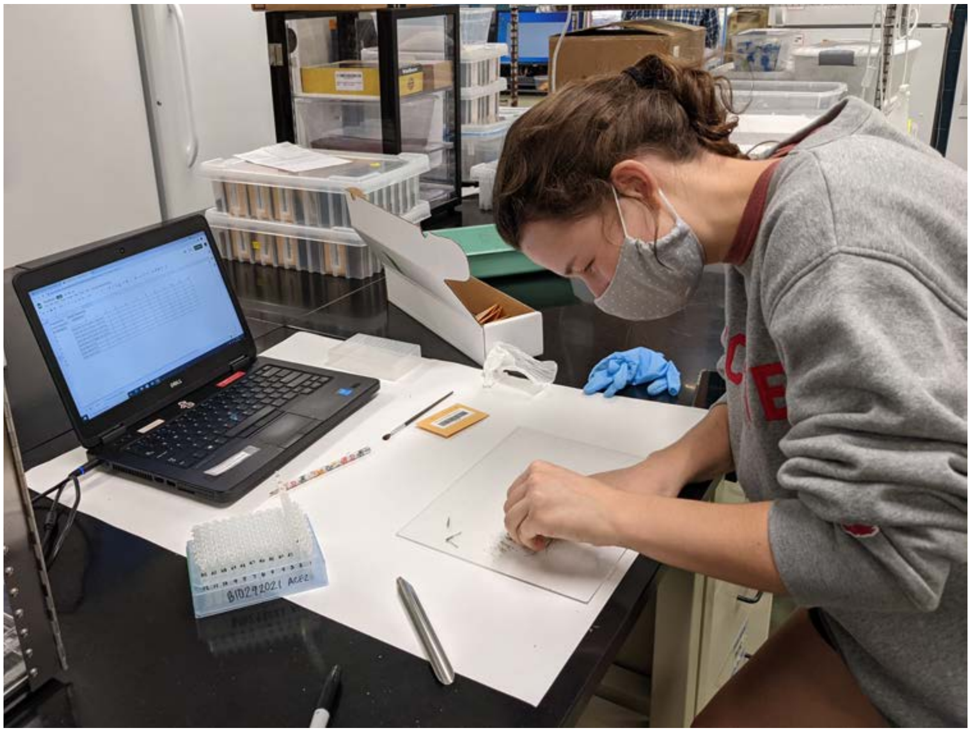


Figure 2. *Spectral decomposition of genomic relationships among full-sibs in eight families based on Pita50K (left) and Pita AgriSeq^{TM} (right) markers. The principal component analysis of marker data clustered trees into groups corresponding with the pedigree. Trees consistently away from the family clusters in both sets might be errors in the pedigree, or they might be half-sibs.*

Pictured right: *Undergraduate student Emily Workman prepares needle sample tissue for DNA extraction.*



PRS™ Scores Strongly Predict Early Stand Yield

The **P**erformance **R**ating **S**ystem (**PRS**™) estimates the predicted performance of improved loblolly pine families deviated from the performance of checklots for multiple traits. The **PRS**™ abbreviation is also used to denote **P**roductivity (individual-tree volume score), resistance to fusiform **R**ust disease score, and stem **S**traightness score. The **PRS**™ score is estimated from breeding values based on the progeny tests measured at four to six years of age. Among the traits assessed, volume is the most important performance rating score, because it can differ on a stand-level basis at rotation age. One of the important questions is whether the performance rating scores for volume estimated from single-tree measurements at an early-age correlates with stand-level performance, the actual trait of interest for landowners. More importantly, is the ranking of families based on progeny tests maintained to rotation age?

In 2013, the Cooperative established a series of family block-plot tests in the southern US to calibrate **PRS**™ scores. These trials will be used to develop a protocol to allow biometricians to regularly modify growth and yield models to better estimate yields and stand values based on progeny test assessments. The main objective is to assess the association between predicted volume scores from progeny tests and actual stand volumes from stand-level performance in large family-plot plantings.

For this study, 39 full-sib families and the CCK checklot from the Atlantic Coastal Plains population were assessed at nine family-plot tests in the southeastern United States (Figure 1). The families had a broad range of **PRS**™ scores for **P**roductivity, fusiform **R**ust disease incidence, and stem **S**traightness. At each test site, 8 to 12 full-sib families were randomly assigned to the sites using multiple-tree family plots planted in 100-tree to 144-tree plots. Not all families were planted on all sites, but all sites had full-sib families with a wide range of volume scores. In total, 94 family plots comprised of ~10,600 trees (excluding borders) were assessed at age six years.

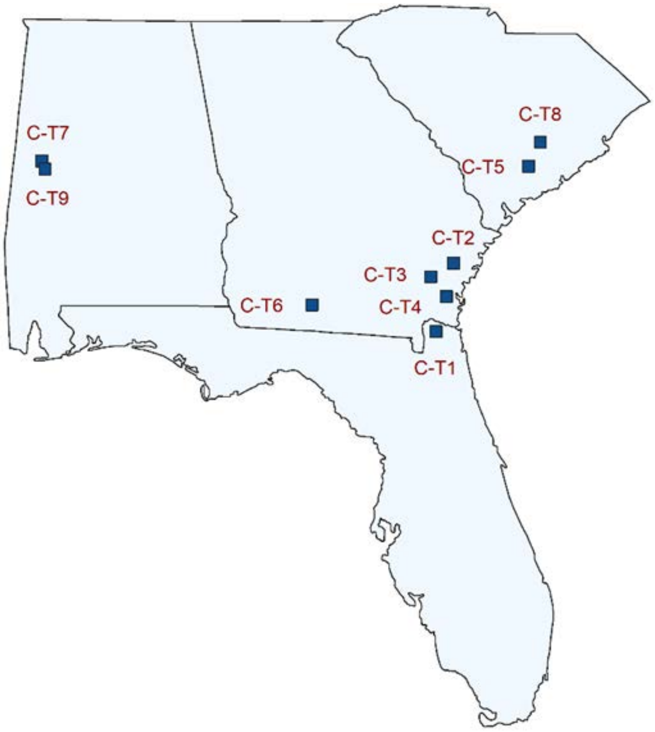


Figure 1. *Geographic location of the nine Atlantic Coastal population* **PRS**™ *calibration study trials established in South Carolina, Georgia, Alabama, and Florida.*

The correlations between **P**roductivity scores from progeny tests and observed stand volumes from family-plots was high at most test sites, suggesting that predicted volume scores from individual-tree performance maintained ranking for stand-level growth (Figure 2).

To predict volume per acre across sites, a multiple linear regression model was fit to explain the relationship between observed stand volume and a set of predictor variables (**P**roductivity score, observed stand rust incidence, **R**ust score, rust risk index, survival, and site index). Rust risk index is a statistic developed from the expected rust incidence of a particular family at a test site, the **R**ust score for the CCK checklot, and the **R**ust score for the families.

When assessed across sites, volume **P**roductivity scores, fusiform rust disease risk, and site index significantly affected stand volumes per acre. On average, a 10% increase in **P**roductivity score, a 10% decrease in rust risk, and a 10-foot improvement in site index will result in an increase of 50, 38, and 180 cubic feet of wood volume per acre, respectively (Figure 3).

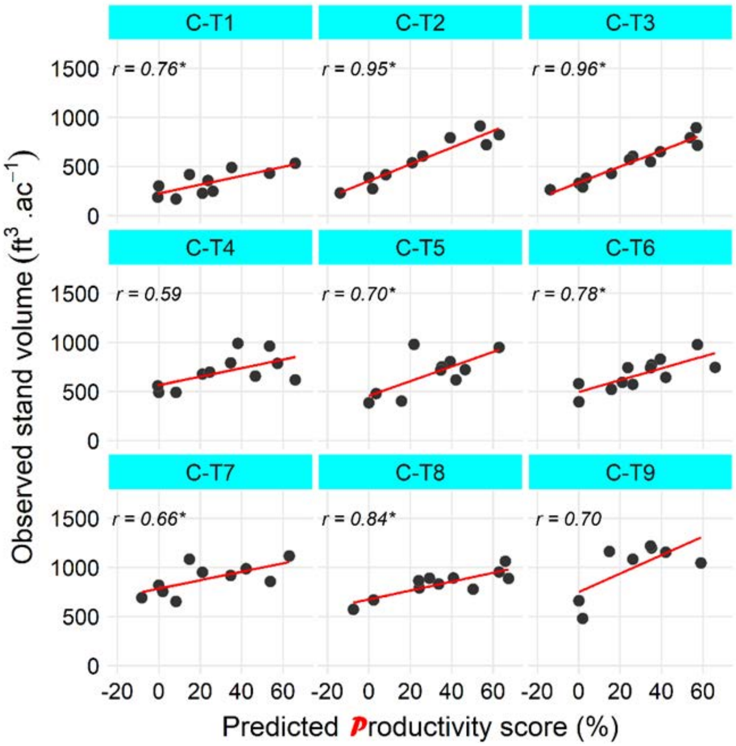


Figure 2. *Scatterplots and linear fit of predicted Productivity score from progeny tests and observed stand volume from family plots at age six years at each test site. Pearson product moment correlations between the two variables are shown on top left of each plot. Asterisks indicate significance at α = 0.05.*

Landowners should consider site quality and risk of rust disease when planting improved loblolly pine trees in plantations. Superior families with high P scores will generally perform better regardless of site quality or rust disease risk, but to obtain higher wood yields, landowners should plant the best families with lower rust risk on the most productive sites. These findings, based on age six years data (pre-crown closure), are encouraging and will be validated with the data through rotation age to make the best decisions.

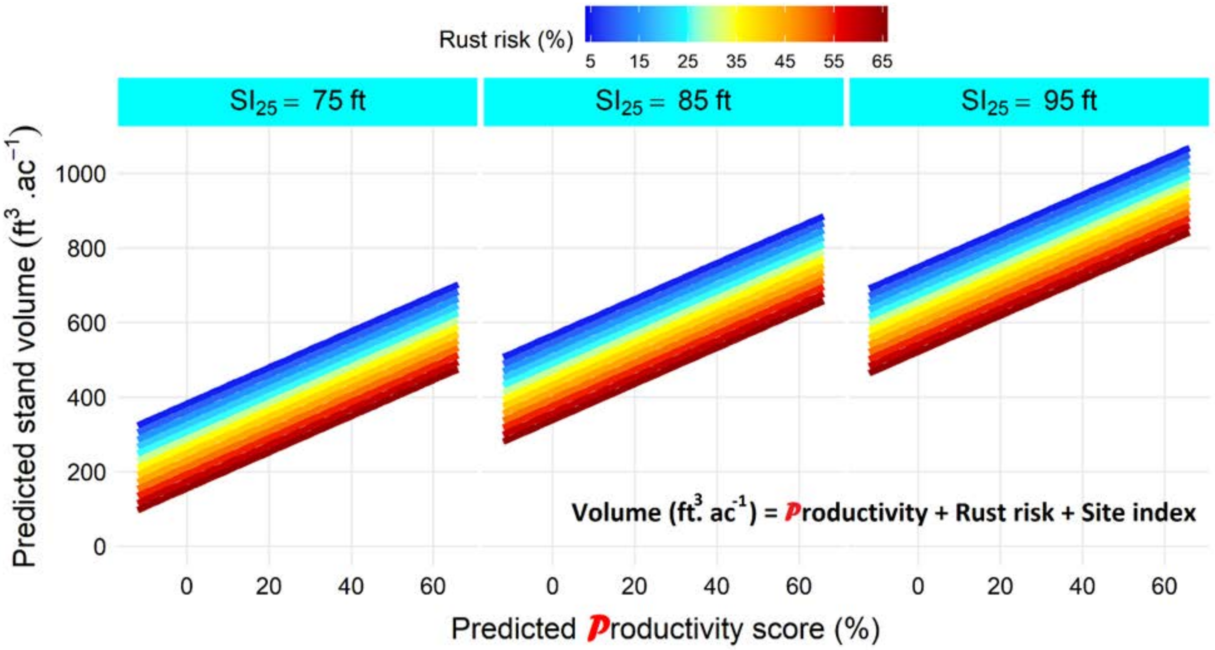


Figure 3. *Predicted stand volume as a function of Productivity score, rust risk incidence, and three site index (SI_{25}) values from the linear regression model. Families with high Productivity scores and lower rust disease risk planted on good sites yield higher wood volumes per acre.*

Estimation of Genetic Parameters for Complex Traits from Multi-Environmental Trials of Loblolly Pine in Brazil[1]

Loblolly pine is one of the most important planted tree species in Brazil. Despite the species' commercial importance, there have been very few formal assessments into genetic parameter estimates and genotype by environment interactions. In this study, 37 trials that spanned two generations and multiple series in southern Brazil were analyzed. There was a total of 270 full-sib families and 225 open-pollinated families generated from 176 founder parents. The families were a part of an internal breeding program of a company in Brazil, and the parents were mostly selected from land race populations.

The trials were largely disconnected with very few parents shared among test series, so genetic parameters were estimated by dividing the dataset into testing series (groups) of well-connected trials. The testing groups were analyzed using differing variance/covariance model structures as a part of a model building exercise to identify the best model for genetic parameter estimation. Breeding value estimation was performed in a combined analysis using all tests. The genetic variances were fixed by using the average of the estimated values from the testing group analyses.

Three truncation selection scenarios were implemented to obtain genetic gain estimates using breeding values from the final combined model. The top five individuals from each selected family were used as selection candidates for the base population to avoid high average coancestry in the selection population. Truncation points were set at 1.25, 1.5, and 2 standard deviations above the base population mean.

Genetic parameters varied greatly depending on the testing group assessed. Estimates of narrow-sense individual-tree heritability ranged between 0.15 and 0.44 for volume and between 0.24 and 0.40 for height. Type B genetic correlations were greater than 0.87 for the traits, indicating that there was essentially no discernable genotype by environment interaction across tests.

Genetic gains across three truncation selection scenarios ranged between 14% and 19% for volume (Figure 1). A substantial amount of gain can be made without compromising genetic diversity or inflating inbreeding in the selection populations (Table 1). Scenarios resulting in larger gain resulted in populations with less genetic diversity, reducing the number of parents represented in those populations.

This study represents the largest formal assessment of genetic parameters and genotype by environment interaction for loblolly pine in Brazil. In the future, genetic field tests should be well-connected to predict the breeding values that are more precise.

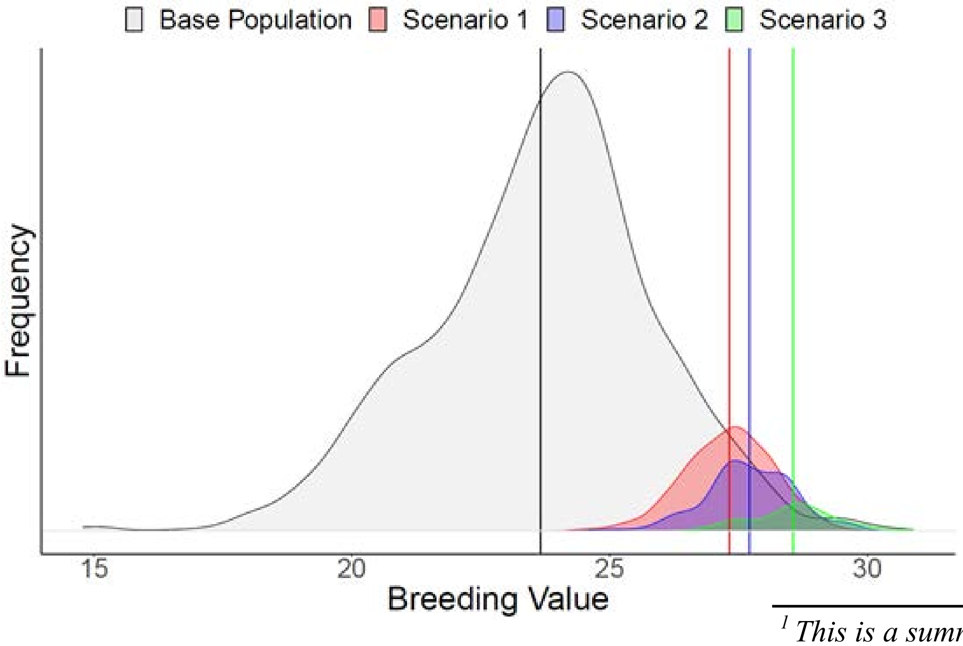


Figure 1. *Distribution of breeding value for volume in the base selection population (grey) and expected distribution of future breeding values for each selection scenario. Scenarios 1, 2, and 3 were selected at 1.25, 1.5, and 2.0 standard deviations above the mean, respectively. The vertical lines are the expected means of selected trees in each scenario. Each selection scenario provides substantial improvement over the base population.*

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[1] *This is a summary of Colin Jackson's PhD research*

Table 1. *Summary statistics for volume breeding values for each truncation selection scenario and the base selection population. The mean breeding values (standard error), percent gain over the base population, and inbreeding coefficient for each scenario are presented. Genetic gain (favorable) and average inbreeding (not favorable) increases with increased selection intensity.*

Scenario	Mean BV (SE)	Percent Gain	Inbreeding
Base	23.7 (0.044)	-	0.0023
1 (Truncation 1.25 SD)	27.3 (0.061)	14.1	0.0006
2 (Truncation 1.5 SD)	27.7 (0.072)	15.6	0.0009
3 (Truncation 2.0 SD)	28.7 (0.101)	19.0	0.0011

Analysis of Gene Expression Differences Between Eastern and Western Loblolly Pine[1]

Selecting the appropriate seed source for a given geographic region is critical to ensuring productive southern pine plantations. It has long been known that eastern and western loblolly pine seed sources show differences in economically important traits such as drought tolerance, growth rates, and disease resistance. Understanding the basis of these local adaptations is important for breeders, because current climate modeling suggests there will be an increase in the number and length of hot dry periods during the growing season by the year 2050. Typical rotation ages for loblolly pine plantation are generally around 25 to 30 years, so plantations established now will face these new challenges near the end of the typical rotation. A single breeding cycle takes 12 to 14 years, so there is not a lot of opportunity to breed loblolly pine to adapt to the projected changes in temperature and rainfall regimes.

Breeders have made crosses between eastern vs. western loblolly pine parents, with the goal of selecting progeny that combine the faster growth of eastern sources and the drought tolerance and disease resistance of western sources. This effort could be enhanced by a better fundamental understanding of specific genetic differences between eastern and western seed sources. To work toward this goal, we conducted RNA-seq on 11 OP families from the Western Gulf Cooperative (WG) and 12 OP families from the NCSU Tree Improvement Program (TIP). Seedlings were grown in flats in the TIP greenhouse, and assessments were made at age three months. RNA was purified from pooled samples of 15 to 30 seedlings, and at least three replicate pooled samples were collected per family. Gene expression was measured by sequencing libraries prepared from the RNA samples, followed by comparing the DNA sequencing data with a collection of putative "transcripts" assembled from previous similar RNA-sequencing experiments with loblolly pine. "Transcripts" are not genes but are produced from genes – each gene can produce multiple transcripts, which are generally quite similar to each other but not identical. About 78,000 transcripts were tested for differential abundance between the eastern and western families, with appropriate statistical adjustment for multiple testing, and a significance threshold of $p < 0.05$ after adjustment.

Approximately 3,623 transcripts were upregulated, and 4,619 were downregulated in the WG families relative to the TIP families. In order to understand the possible functions of transcripts detected, we aligned them to genes from the model plant *Arabidopsis*. The collection of 78,000 loblolly pine transcripts contains multiple examples of several transcripts that are all similar to the same *Arabidopsis* gene; we do not know if these similarities represent different alleles from loblolly pine or members of gene families. To simplify analysis of differences between the upregulated and downregulated pine transcripts, only the *Arabidopsis* genes unique to either the upregulated or downregulated categories

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[1] *This is a summary of Adam Festa's PhD research*

were kept. This resulted in 1520 unique *Arabidopsis* genes being identified as similar to upregulated pine transcripts, and 1653 unique *Arabidopsis* genes were identified as similar to pine transcripts downregulated in WG families relative to TIP families.

Arabidopsis is a model organism, so we can leverage public databases in order to decipher the possible functions of these genes. Gene Ontology (GO) analysis is used as a system for classifying genes into categories based on either molecular function, biological process, or cellular components. We looked at the biological process GO categories and conducted a statistical analysis to see which were preferentially enriched or depleted in one provenance relative to the other. Additionally, these *Arabidopsis* genes are also mapped to the Kyoto Encyclopedia of Genes and Genomes (KEGG), which helps to classify genes that encode enzymes which participate in particular metabolic pathways.

There were about 17 GO terms describing biological processes related to genes that were down regulated in WG relative to TIP families, including defense response, water transport, and protein phosphorylation. A total of 32 GO biological process terms related to genes that were up regulated in the Western Gulf seedling sources and included response to cold, response to water deprivation, and response to light stimulus. When exploring the KEGG pathways, we found several pathways related to Salt/Drought stress that were upregulated in the Western Gulf families. In addition, we found downregulated pathways related to brassinosteroid biosynthesis which plays a role in cell elongation and cell division, which may be related to the growth differences observed between eastern and western seed sources.

Further curation of results and linking back to the literature is currently underway; however preliminary results show several interesting pathways that are related to the known differences between eastern and western provenances. As a final point, these findings describe fundamental differences between provenances in the beginning of seedling development, without any treatments applied. These detectable differences at a young age suggests that it may be possible to develop methods for early selection and to combine traits of interest from both seed sources.

Grants

New Grants

Steve McKeand, Fikret Isik, Ross Whetten, on a North Carolina Department of Agriculture and Consumer Services grant. Loblolly Pine Biomass Cropping Study: Years 10-11. $53,611. 1/2022-12/2023.

Fikret Isik, Ralph Dean, Amanda Hulse-Kemp, Peter Balint-Kurti, on a NSF/NIFA Plant-Biotic Interaction Program grant. Population genomics of race non-specific disease resistance in the endemic *Pinus-Cronartium* pathosystem. $782,568. 06/15/2022-06/14/2026.

Continuing Grants

Justin Whitehill, Fikret Isik, Jack Wang, Kitt Payn, Ross Whetten. McIntire-Stennis Program. GRO FORTH: Genomic Resource Optimization for Forest Health. $200,000. 01/01/2021-12/31/2025.

Kitt Payn, Steve McKeand, Fikret Isik, Ross Whetten, on a North Carolina Department of Agriculture and Consumer Services grant. Loblolly Pine Biomass Cropping Study: Years 8-9. $80,291. 1/2020-12/2021.

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.

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, 2/15/2018-8/14/2021.

ASSOCIATED ACTIVITIES

Meetings, Workshops, and Short Courses

With extended pandemic restrictions, we continued to meet with folks virtually throughout the year. For the first time, and hopefully last, we held the 65th Advisory Meeting online June 2-3, 2021. We were optimistic about an in-person Contact Meeting planned for the fall, but that too was not meant to be. The second online TIP Contact Meeting was held December 2-3, 2021. The silver lining for the virtual format was that it allowed many folks from member organizations to attend meetings and workshops that would not typically be able to attend in person. The trend of seeing new faces in meetings kept us from online meeting fatigue, so thank you all for your patience and participation.

We were able to host a meeting at the Arrowhead Breeding Center for our administrators in CNR and FER in early March. Over the last two years, our college and department installed new leaders, and we wanted to relate the impact our program has on forestry in the Southeast. The new administration members included Dean Myron Floyd, Associate Dean of Research Rob Scheller, Department Head Erin Sills, and Associate Department Head Chris Moormon. We joined forces with Jeff Fields and the Georgia Forestry Commission, and they brought their new State Forester, Tim Lowrimore and Georgia State Representative Danny Mathis. TIP faculty and staff along with GFC staff presented on the research activities and the cooperative nature of our work with partners, and the impact it makes on forestry in the South. It was a great crowd, and it felt even better being back in the field talking about forest genetics. We want to thank Jeff Fields and his team for their contributions to the successful day at Arrowhead!

TREE IMPROVEMENT PROGRAM
NC STATE UNIVERSITY

Pictured above and right*: In March, we hosted administrators from the NC State University College of Natural Resources and the Georgia Forestry Commission. We had a beautiful day at the Arrowhead Breeding Center to show off how landowners and the forestry community benefit from our breeding and research activities at Arrowhead.*

The faculty and staff members associated with the Cooperative are busy throughout the year with extension activities, both domestic and abroad. The pandemic continued to restrict many of these activities, but we were able to fit in some outreach work where there were opportunities. Below are records of faculty and staff invited as instructors/speakers:

- Fikret Isik (Invited speaker): Bahcesehir University, Istanbul, Turkey, Molecular Biology and Genetics Student Club, Molecular Biology and Genetics Conference. July 3rd, 2021.
- Fikret Isik (Keynote speaker): Symposium on second cycle breeding strategy of Pinus brutia in Turkey. September 27-29, 2021, Ankara, Turkey. Organized by the General Directorate of Forestry of Turkey and Forest Research Institute.
- Fikret Isik (Invited speaker): Forest Tree Breeding in the Age of Genomics. Swedish University of Agricultural Sciences Breeding Network kick-off workshop. October 21st, 2021.
- Fikret Isik. 2021 Society of American Foresters Convention. Innovative Forest Health Tools for Ecosystem Resilience and Restoration Session. In Conjunction with the IUFRO Division 7.03.11. “Enhanced Disease Resistance of Planted Forests Achieved through Collaboration of Breeding and Forest Health Programs”. November 4, 2021.
- Fikret Isik. AtlanTIC Breeding Strategy Workshop. October 12, 2021. Gave a presentation about genomic selection.
- Trevor Walker. SFTIC 2021, “Application of MateSel software in a loblolly pine breeding program”. June 2021.
- Steve McKeand. Georgia Landowner Summit, Georgia Forestry Association, "Choosing the Appropriate Seedling Genetics to Meet Your Management Objectives". Dec. , 2021, Jekyll Island, GA
- Steve McKeand. Southeast Society of American Foresters Annual Meeting, "Seedling and Genetic Options - Pick Carefully. You’ve Got One Shot to Get It Right!". Feb. 2022, Pine Mountain, GA

Teaching

The Cooperative faculty and staff continue to extend their expertise to future forestry and natural resource professionals by teaching courses at both the graduate and undergraduate level. This year the university reopened the campus to face-to-face classes. Fikret Isik taught Introduction to Data Analysis in Natural Resources (NR 554) for the last time in spring 2021. This fall, he will be offering the inter-departmental graduate course Advanced Topics in Quantitative Genetics and Breeding (FOR/CS/ANS 726). Ross Whetten always has a full spring semester of teaching courses: FOR 350 (Ethics in Natural Resource Management) and BIT 815 (Analysis of Deep Sequencing Data Analysis). Steve McKeand came back to teach FOR 411 (Forest Tree Genetics and Biology), undergraduate tree improvement course, and the graduate level FOR 725 (Forest Genetics) this spring; both classes took a field trip to the NC Forest Service nursery and seed orchard in Goldsboro, NC. The distance education option allowed for two USDA Forest Service employees from Mississippi and Arkansas to take the FOR 725 course; one remote student even made the field trip to NCFS!

Pictured above: *Bobby Smith (NCFS) is explaining the process of extracting and preparing seed to the FOR 411/725 class.*

Staff

In July 2021, Dr. Kitt Payn let us know he would be stepping down as TIP Director and taking a position in industry the very next month and working with pongamia (*Millettia pinnata*) trees. Within that same week the TIP Data Analyst, Eddie Lauer, let us know he too would be leaving shortly after his PhD defense to take an industry position with a cattle genetics company. As those of us remaining hunkered down and tried to strategize how to navigate the next year being down two very important people/ positions, two other very important people leaned in. Upon hearing the news, as he did when Steve stepped down as Director in 2018, Dr. Isik stepped up and took on interim leadership of the Cooperative. Dr. McKeand was fully retired for a whole 37 days before he came back to help the group continue the mission of teaching students and bringing member and landowners the best trees in the South. Between the two leaders, they were given the new title of Interim Co-Directors of the Cooperative Tree Improvement Program, and lead they have.

Others in the group stepped up in a big way and stretched themselves thin to cover the voids. Dr. Trevor Walker and Dr. Nasir Shalizi took on the additional data analysis work, and Austin Heine picked up more of the breeding and testing coordination. All three also assisted our graduate students with their research while simultaneously working on their PhD research, defending their PhD, or publishing their doctoral research, and working from home and helping with very young children during a pandemic…it has been an adventure!

Another person in the mix is a familiar face to our program, AnneMargaret Braham. She decided to get back to her loblolly roots and jumped right in to assist Austin with several of the operational activities and working with students. She brought years of greenhouse and breeding program experience with her time in the Christmas Tree Genetics Program here at NCSU. Like any good rescue, her timing was perfect to help keep our stretched out faculty and staff from snapping!

As for J.B., and Ross, they continued their roles keeping the program going forward. J.B. continued his sage “advice and counsel” to members regarding orchards and testing efforts, and his input on many research projects kept staff and students progressing in their work. Ross’s contribution to research efforts and his working with students continued to be a major benefit to the program. It was gratifying seeing everyone join forces to keep things afloat.

We hope to have the open positions (Co-Director and Database Manager) posted soon to relieve our staff of their extended watch. There was a state mandated hiring freeze in place since the beginning of the pandemic that prevented any movement in hiring. The freeze was lifted in the January 2022, and we are now another number in the flood of HR requests that thawed out from the freeze. We are excited for new beginnings and what new folks will bring to our group to add to our already out of this world faculty and staff!

Visitors

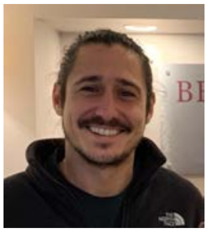
Despite the worldwide pandemic, we had a brave soul that was willing trek halfway around the world to spend some time with and learn from the group. Dr. Yağmur Biricik arrived from Turkey in February 2022. She is slated to be here for six months to work on some of her research objectives in collaboration with NCSU faculty and staff. Yağmur works for the Marmara Forestry Research Institute in Istanbul under the General Directorate of Forestry. Her research is on wood properties and wood anatomy of *Pinus nigra* in Turkey.

***Pictured right:**: Dr. Yağmur Biricik*

Graduate Students

Despite another year of pandemic hurdles, we continued to have a strong group of graduate students. Eddie Lauer successfully defended his PhD in August 2021, and both Sinem Satiroglu and Ugur Memis followed with completing their masters programs in December 2021. Tree Improvement Manager, Trevor Walker successfully defended in April 2022. Below is the list of students and their research:

Sarah Conner, MS – Sarah made big strides this year in her research project titled **“**Genetic Variation in Fusiform Rust Disease Resistance among Families in the Northern Breeding Population of Loblolly Pine”



Adam Festa, PhD candidate – Adam returned to finish after leaving for industry in 2017. His research titled, “Modeling methods for prediction of genetic value and breeding efficiency in tree improvement”. He is expected to finish in summer 2022.

Khushi Goda, PhD candidate – Khushi joined our group in 2017 and continues her research titled “Optimal Mate Selection and Managing Inbreeding in *Pinus taeda*”.

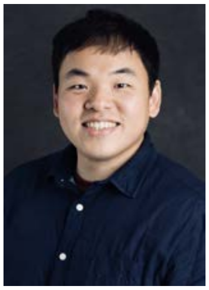
Austin Heine, PhD – Austin continues his research objectives, and the title of his research is "Expanding our Knowledge about Loblolly Pine Pollen to Increase Filled Seed per Cone from Controlled Pollinations."

Travis Howell, PhD – Travis joined us this fall as a shared student with Camcore and Forest Productivity Cooperative. He is working on remote sensing objectives for managed forests.

Colin Jackson, PhD candidate – Colin began his joint research with TIP and Camcore and plans to defend in May 2022. His research title is “Comparison of Genome Wide Association and Whole Genome Regression Methodologies in Loblolly Pine”.

Piyush Pandey, PhD candidate – Piyush has a joint research project with TIP and Biological and Agricultural Engineering. His research is titled “High throughput phenotyping of loblolly pine using hyperspectral imaging”. He is expected to finish in summer 2022.

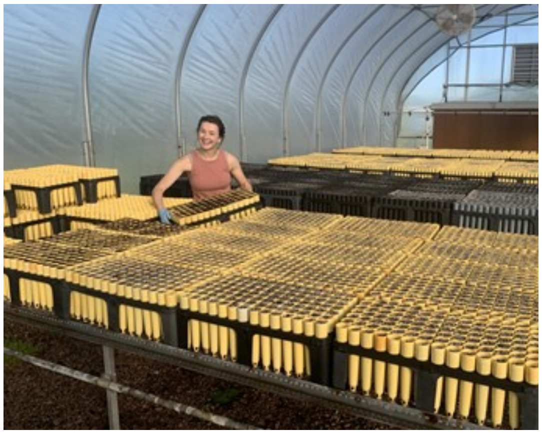
Trevor Walker, PhD candidate – Trevor successfully defended his PhD in April 2022, “Quantitative genetics and the efficiency of SNP markers for within family selection in *Pinus taeda*”.



Yu-Ming Lin, PhD – Yu-Ming continues his PhD research on “Correspondence between genomic relationships from a low-density targeted genotyping-by-sequencing (GBS) and high-density SNP marker panels in loblolly pine”.

Undergraduate Students

Undergraduate students were finally able to return to campus in full form in Autumn of 2021 and have picked back up where they left off before COVID. The *undergraduate army* has been a critical component of the TIP team over the past year. Their activities include potting, fertilizing, weeding, inventorying the ACE2 clonal population, collecting foliage samples, preparing plates for DNA extraction, entering data, processing cones from Arrowhead Breeding Center, performing cone analysis for pollen viability research, and other various lab, greenhouse, and field activities. Although some great students will be graduating this year, we recently hired on a new cohort of students who will fill their shoes!



***Images, clockwise from top left corner: Alex Simpson**, graduating in May 2022 with a B.S. in Natural Resources: Ecosystem Assessment helping to hedge the ACE2 clonal trial in February of 2022. **Sam Blumenfeld**, graduating in May 2022 with a B.S. in Natural Resources, concentration in Policy & Administration with minors in Forest Management and Political Science. Sam is shown here potting up trays for the Northern Pollen Cloud Study. Anne-Margaret Braham (TIP staff, left) and **Jenna Abernethy** (right). Jenna is a freshman majoring in Forest Management with a concentration in Ecology. Jenna is helping AnneMargaret fill trays for the upcoming ACE2 clonal stick. **Ashley Kerr** is a sophomore majoring in Natural Resources, concentration in Ecosystem Assessments with a minor in Forest Management. Ashley is filling trays for the upcoming ACE2 clonal stick. **Diego Rosado** is graduating May 2022 with a major in Biological Sciences, concentration in Human Biology. Diego is shown hedging the ACE2 population, and he has also been a big help in DNA plate prep. **Emily Workman** is a sophomore majoring in Science Education with a concentration in middle grades. Emily is organizing trays for the ACE2 rooted cutting effort.*

Not pictured are Devin Bhatt a freshman majoring in Computer Science and Adam Challgren a freshman majoring in Life Sciences (Genetics Intended).

Membership in the NCSU Cooperative Tree Improvement Program

Membership remained pretty steady with only a couple of notable events. This doesn't happen often, but we had a name change for a longtime Full Member. Hancock Forest Management changed names to Manulife Investment Management Timberland and Agriculture, Inc. In January 2022 we lost Dougherty & Dougherty Forestry Services as a Contributing Member.

Full Members

ArborGen, Inc.
Georgia Forestry Commission
Manulife Investment Mgt.
IFCO Seedlings
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
Burgin Timberlands, LLC
Campbell Global, LLC
Charles Ingram Lumber Co.
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
PRT Growing Services, LLC
Resource Management Service, LLC
Roseburg Resources
Scotch Land Management, LLC
Shoeheel Land Management (Z.V. Pate, Inc.)
Tennessee Division of Forestry
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
USDA Forest Service

Benefactors

Canfor Corporation

Publications of Special Interest To Members (2020 - 2022)

From our inception in 1956, there have been over 405 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: https://www.treeimprovement.org/tip-tech-transfer

2022

Jackson C., N. Christie, S.M. Reynolds, G.C. Marais, Y, Tii-kuzu, M. Caballero, T. Kampman, E.A. Visser, S. Naidoo, D. Kain, R.W. Whetten, F. Isik, J. Wegrzyn, G.R. Hodge, J.J. Acosta, and A.A. Myburg. 2022. A genome-wide SNP genotyping resource for tropical pine tree species. Mol. Ecol. Resour. 22(2):695-710. https://doi.org/10.1111/1755-0998.13484

Isik, F. 2022. Genomic prediction of complex traits in perennial plants: a case for forest trees. Chapter 18 in: Complex Trait Prediction, Methods and Protocols. Ahmadi, N. and J. Bartholomé (Eds.). Methods in Molecular Biology 2467. Springer Protocols. Humana Press, NY. 648p. https://doi.org/10.1007/978-1-0716-2205-6.

Lauer, E, J. Holland, and F. Isik. 2022. Prediction ability of genome-wide markers in *Pinus taeda* L. within and between population is affected by relatedness to the training population and trait genetic architecture. G3 Genes| Genomes| Genetics. 12(2):1-12. https://doi.org/10.1093/g3journal/jkab405

Shalizi, MN, K. Payn, and F. Isik. 2022. Genetic linkage between the training and selection sets impacts the predictive ability of SNP markers in a cloned population of *Pinus taeda* L. Tree Genetics & Genomes 18(1):1-17. https://doi.org/10.1007/s11295-021-01532-9.

Walker, T.D., W.P. Cumbie, and F. Isik. 2022. Single-step genomic analysis increases the accuracy of within-family selection in a clonally replicated population of *Pinus taeda* L. For. Sci. 68(1):37-52. https://doi.org/10.1093/forsci/fxab054

2021

Alan, M and F. Isik. 2021. Genetic relationships between terminal shoot length, number of flushes and height in a 4-year-old progeny test of *Pinus brutia* Ten. Annals of Forest Science, 78(2). https://doi.org/10.1007/s13595-021-01040-9

Caballero, M., E. Lauer, J. Bennett, S. Zaman, S. McEvoy, J. Acosta, C. Jackson, L. Townsend, A. Eckert, R.W. Whetten, C. Loopstra, J. Holliday, M. Mandal, J.L. Wegrzyn, and F. Isik. 2021. Toward genomic selection in *Pinus taeda*: Integrating resources to support array design in a complex conifer genome. Appl. In Plant Sci. 9(6): e11439. https://doi.org/10.1002/aps3.11439

Chan J.M. and F. Isik. 2021. Genetic variation in frost tolerance, *Uromycladium acacia* rust resistance, and growth in an *Acacia mearnsii* population. For. Sci. 67(5):574–586. https://doi.org/10.1093/forsci/fxab032

Gräns, D., F. Isik, R.C. Purnell, I.M. Peszlen, and S.E. McKeand. 2021. Genetic variation and the effect of herbicide and fertilization treatments on wood quality traits in loblolly pine. For. Sci. 67(5):564–573. https://doi.org/10.1093/forsci/fxab026

Lauer, E, and F. Isik. 2021. Major QTL confer race-nonspecific resistance in the co-evolved *Cronartium quercuum* f. sp. *fusiforme–Pinus taeda* pathosystem. Heredity: 127(3):288-299. https://doi.org/10.1038/s41437-021-00451-8

Lauer, E., A. Sims, S. McKeand, and F. Isik. 2021. Genetic parameters and genotype-by-environment interactions in regional progeny tests of *Pinus taeda* L. in the southern USA. For. Sci. 67(1):60-71. https://doi.org/10.1093/forsci/fxaa035

Lu, Y., K.G. Payn, P. Pandey, J.J. Acosta, A.J. Heine, T.D. Walker, and S. Young. 2021. Hyperspectral imaging with cost-sensitive learning for high-throughput screening of loblolly pine (*Pinus taeda* L.) seedlings for freeze tolerance. Transactions of the ASABE 64(6): 2045-2059. https://doi.org/10.13031/trans.14708

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New beginnings...a theme we are taking to heart this year! We are exploring a new way to select and breed trees based on genomic data. To accomplish breeding timelines, we have assistance from all members, some onsite at the Arrowhead Breeding Center. We even have new-ish leadership for the Cooperative.

Front cover: As the sun sets on the 4th-cycle, the very first genomic selections are in! ACE2 selections have been selected based on genomic markers and topgrafted at Arrowhead. We look forward to making crosses with these selections in the future.

Back cover: Arrowhead had some familiar faces down south this year! We appreciated the contribution to the breeding efforts this year from a few of our members. Above: Ones Bitoki from the Virginia Department of Forestry is inspecting interstocks in the Graveyard orchard. Below: Anita Johnson from the Georgia Forestry Commission is bagging flowers with Carla Castro from the South Carolina Forestry Commission. Truly a cooperative team!