



67th Annual Report

**May
2023**



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Department of Forestry and Environmental Resources

College of Natural Resources

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Raleigh, NC

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EXECUTIVE SUMMARY

After 67 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.

SELECTION, BREEDING, AND TESTING

Spring 2023 was a big effort for the 5th-Cycle breeding. The Cooperative bred 122 crosses, with 96 performed at the Arrowhead Breeding Center. The total number of 5th-Cycle crosses completed since 2019 is 262. Measurements of the 4th-Cycle progeny tests are about 60% complete. Through 2023, more than 60,000 trees from full-sibling families in 4th-Cycle tests have been measured, of which 374 have been chosen to be 5th-Cycle selections, a very high selection intensity. We are on track to finish 4th-Cycle test measurements in 2027. Annual BLUP results show that about 20% of the top 100 families are from the 4th-Cycle, delivering genetic gain to members. The Cooperative commenced 5th-Cycle selections in 2019, and the breeding strategy calls for 768 selections to be made through 2027.

RESEARCH

The Cooperative faculty secured a research grant (\$782,568) from the NSF-NIFA joint Plant-Biotic Interactions Program grant (Award #: 2022-67013-37717). The research team will investigate the virulence variation in the fusiform rust fungus and develop a reference genome for the community to use. The genomic selection project is underway as planned, with an increased amount of genotyping effort in 2023. Since 2021, about 2400 trees from the 4th-Cycle have been sampled for genotyping using the Pita50K array. In 2022, 16 selections carrying fusiform rust disease resistance QTL (GRID1 and GRID2) were successfully topgrafted at Arrowhead. Breeding of these trees began in 2023 to develop progeny that will be homozygous for the rust resistance alleles at both loci. The Pollen Cloud Study was planted in Winter 2022-2023 and will help us understand the impact of different pollen clouds on the performance of progeny from the same parents collected from different orchards. Periodic assessments of the **PRS**TM Calibration Studies continue. As more tests are measured through age nine years, all indications are that progeny test based measurements are good predictors of stand productivity at pre- and early-crown closure. Rust hazard maps are being developed using 3rd-Cycle and 4th-Cycle data based on the predicted rust incidence of non-improved checklots in the big BLUP analyses. The Butner Biomass/Cropping Study was measured in 2023, two years after thinning. Data are being analyzed to determine which Piedmont and Coastal families will be of value for biomass and for sawtimber production on this Piedmont site.

ASSOCIATED ACTIVITIES, GRADUATE STUDENTS, COOPERATIVE STAFF, MEMBERSHIP

The Cooperative organized a tree improvement short course in conjunction with the Cooperative Contact Meeting in November 2022 at Lake Blackshear, GA. This short course was well attended by members and even some outside of the Cooperative that were looking for tree improvement training. Another workshop was organized in December 2022, "Computing Skills in Plant Breeding". Dr. Isik, along with assistance from many other faculty, staff, and professionals, hosted a three-day Data Analysis Workshop at North Carolina State university. Teaching during the 2022-23 academic year was in full swing with TIP faculty teaching five courses on different topics in forest genetics. The "undergraduate army" continues to be a vital component of TIP's hands-on research and development work. Our graduate student program continues to flourish. Five students completed advanced degrees this year and have moved on to careers in forest genetics and plant science. Our six current grad students are making great strides to complete degrees in the coming months and years.

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A MESSAGE FROM THE CO-DIRECTORS



Dr. Fikret Isik

2022-2023 has been another year with significant changes in the Cooperative Tree Improvement Program! We hired Trevor Walker as a Co-Director of the Cooperative starting February 1, 2023. Trevor has been with the Cooperative for over seven years as a PhD student and on the staff as Tree Improvement Manager. He successfully organized and implemented logistically complex breeding and testing tasks while doing high-quality research. We are delighted to have Trevor on board to lead the Cooperative.

And Trevor's appointment was very timely! When Trevor was getting ready to start his new position, I knew I had been awarded a Fulbright U.S. Scholar Program Science/Technology award to South Africa for the 2022-2023 academic year. I started the program at the University of Pretoria at the beginning of March until the end of the year. Luckily, we have Steve. He did not hesitate to step in and is serving as interim Co-Director to help Trevor and the staff during the transition. Austin Heine, our Operations Manager for years, took a position with ArborGen in January 2023. We thank Austin for his service to the Cooperative and wish him the best in his career. Sarah Conner, a masters student with the Cooperative, finished her degree in December 2022 and joined us as a PhD student is helping out in the interim with the operations and ongoing research along with Austin Quate who began his Masters in the fall.

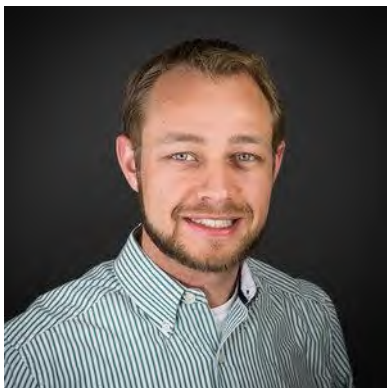
The Cooperative is as strong as ever. The Cooperative's 5th-Cycle Breeding is underway and going strong. About 60% of the 4th Cycle of testing has been completed, and we expect to wrap up the fourth cycle by 2028. Data analysis results show that new selections are ranking high for various traits, a strong indication that we are delivering genetic gain to our members and the forestry sector in the South. We also increased the genotyping efforts this year for the genomic selection project. Although we lost two members (research and contributing), we gained two members (Langdale Forest Products Co. and Reliable Source Seeds & Transplants) who joined the Cooperative in 2023.

On the research side, in collaboration with plant pathologists, we secured an NSF-NIFA joint grant (Plant-Biotic Interactions Program, Award #: 2022-67013-37717) in 2022 to study the virulence variation in the fusiform rust fungus (*Cronartium quercuum* f. sp. *fusiforme*). As part of the experimental workflow, we will generate the most contiguous reference pangenome of the pathogen ever published using long-read PacBio sequencing. Our high-quality pangenome of fusiform rust may also be beneficial in comparative genomic studies with blister rust caused by *Cronartium ribicola*, an introduced pathogen species in the same genus as fusiform rust. All North American white pine species are susceptible to this devastating disease.

Our members deserve all the credit for the success of our program, and we never take their support for granted. I look forward to returning to work with a great team in Raleigh in 2024.

Fikret Isik

May 2023



Dr. Trevor Walker

The Cooperative is truly an amazing place to work. Our members are diverse and oftentimes competitors, but we all have the same goal: increase forest productivity. This gives us a sense of community and motivates me to work hard every day. It is refreshing to work with a group that values innovation and is always striving for improvement.

I am excited to take on a new leadership role in the Cooperative as Co-Director. I think it may be valuable (and telling) to share a bit of history concerning the new Co-Director of the Cooperative! I obtained my B.S. in Forest Management at Stephen F. Austin State University in the piney-woods of East Texas. Under the guidance of Dr. Dean W. Coble, I did an M.S. in Forest Biometrics. Dr. Coble encouraged me to take every statistics class I could, a move that paid dividends in a world that has become more data-driven. My M.S. research evaluated the impact of pine tip moth and was supported by the Texas Forest Service Forest Pest Management Cooperative. Next, I worked in the environmental services sector including wetland delineation and mitigation. I really enjoyed this work, because everyday I was in the woods, oftentimes in the swamiest and loneliest places! Anthony Castilaw and Cliff Sunda taught me much about project management and work ethic.

I couldn't stay in the woods forever; I needed to be in front of a computer analyzing data. I moved to North Carolina to work as an Inventory Analyst with Hancock Forest Management under the guidance of Jim Boyd. There I learned about database management. I became more interested in research, so I responded to a PhD student recruitment advertisement for "Quantitative Forest Genetics" distributed by Fikret Isik. I was unsure at first, as my only introduction to genetics was in seventh-grade science, but Fikret assured me that I could squeeze by with the "quantitative" part!

When starting my PhD at the Cooperative, I was amazed by the data produced, not just the amount of data, but by the rigor of the experimental designs. Fikret taught me how to produce scholarly research, and Steve taught me how to breed trees. When I am Arrowhead, I feel like Gregor Mendel up in the trees!

My new job as Co-Director is simple: maintain the momentum of the Cooperative. This requires cultivating a team of staff and students, giving them what they need to succeed. It requires listening to members as they encounter emerging issues on the front lines of forest management. It requires scholarly research to discover solutions. Finally, it requires transferring those solutions into the hands of foresters.

This is a big moment for me, so I want to send a big "thank you" to the friends that helped me get here. Here are some of the folks that I would like to spend more time in the woods with: Austin Russell, Neil Boitnott, Micky Allen, Jason Grogan, Mike Fountain, Damon Houghton, and Denise Bonnette.

Trevor D. Walker

May 2023

SELECTION, BREEDING, AND TESTING

Breeding Update

5th-Cycle Breeding is in Full Swing

Spring 2023 was the second big year of 5th-Cycle breeding. Opportunistic breeding was done in 2019 through 2021, and 2022 was the first year where pollen from a large number of 5th-Cycle selections was available. This year, the Cooperative bred 122 crosses, the greatest number so far. There were 96 crosses performed at the Arrowhead Breeding Center and the remaining 26 were done at members' breeding orchards (Table 1). This brings us to 262

“true” 5th-Cycle crosses (i.e. among 5th-Cycle selections) performed since 2019. In the Fall of 2022, we harvested seed from 43 of the 46 attempted crosses (an excellent success rate) bringing us to 50 crosses available for planting the first year of 5th-Cycle progeny tests in winter 2023-2024. We do not expect the seed success rate to be as high in Fall 2022 due to a late spring freeze that caused about 60% mortality in the conelets from 2022 breeding.

Table 1. The number of 5th-Cycle crosses done since 2019 at Arrowhead Breeding Center and at Full Members' breeding orchards.

Year bred	# Crosses at Arrowhead	# Crosses at members' orchards	Total crosses attempted	Crosses yielding seed (harvested 18 months after breeding)
2019	2		2	2
2020	5		5	5
2021	46		46	43
2022	76	11	87	<i>Expected to be poor</i>
2023	96	26	122	-
Cumulative:			262	50

Flowering was prolific at Arrowhead in 2023, with 137 of the 203 selections grafted in 2020 and 2021 available for breeding (Table 2). Of the grafts made in 2020 (three years old), 80% (80 out of 101) had sufficient flowering to breed, and of the grafts made in 2021 (two years old), 64% (57 out of 89) had sufficient flowering to breed this spring. In the last three years, the percentage of two-year-old topgraft-

ed selections with enough flowers to breed has ranged from 39% to 85%. All of these selections were grafted into 25-foot or taller 181210 interstocks to expedite flower development. Much effort and planning have gone into ensuring there is an adequate supply of mature interstock at the Arrowhead Breeding Center (see front cover of this report).

Table 2. Count of selections with adequate flowering to breed for two- to four-year-old topgrafts at the Arrowhead Breeding Center (all are 5th-Cycle selections). In 2023, flowering was prevalent in 80% of the three-year-old topgrafts and 64% of the two-year-old topgrafts. The 2019 grafts were not surveyed for flowers in 2023.

Graft year (N = count grafted)	2021 Flower survey		2022 Flower survey		2023 Flower survey	
	Flowering	Not yet flowering	Flowering	Not yet flowering	Flowering	Not yet flowering
2019 (N = 13)	11	2	8	5		
2020 (N = 101)	61	40	39	62	80	21
2021 (N = 89)			12	77	57	32
Total available for breeding:	72		59		137	

5th-Cycle Tactical Breeding Plan: A well-oiled machine

The TIP staff have developed tactical breeding plans annually since 2021 to create an optimal list of crosses that best reflects the current state of the population. The need for annual breeding plans is in part due to the rolling-front nature of the testing program that started in the 4th-Cycle (crosses were tested as seed became available) and the subsequent staggered nature of the flowering of 5th-Cycle selections (Figure 1). In spring 2023, the first three “cohorts” of 5th-Cycle selections were available for breeding, including 179 selections with flowers

available at Arrowhead and members’ orchards and 93 selections with pollen in freezer storage available for breeding (Table 3). These biological limits on breeding were input into a mating selection algorithm, MateSel, to choose the best set of crosses to balance the gain and diversity in the 5th-Cycle progeny (Figure 2). The algorithm must also consider crosses that have already been made, but potential selections are too young to participate in breeding (i.e. seeds that are still developing, seeds destined for the greenhouse, and seedlings in young progeny

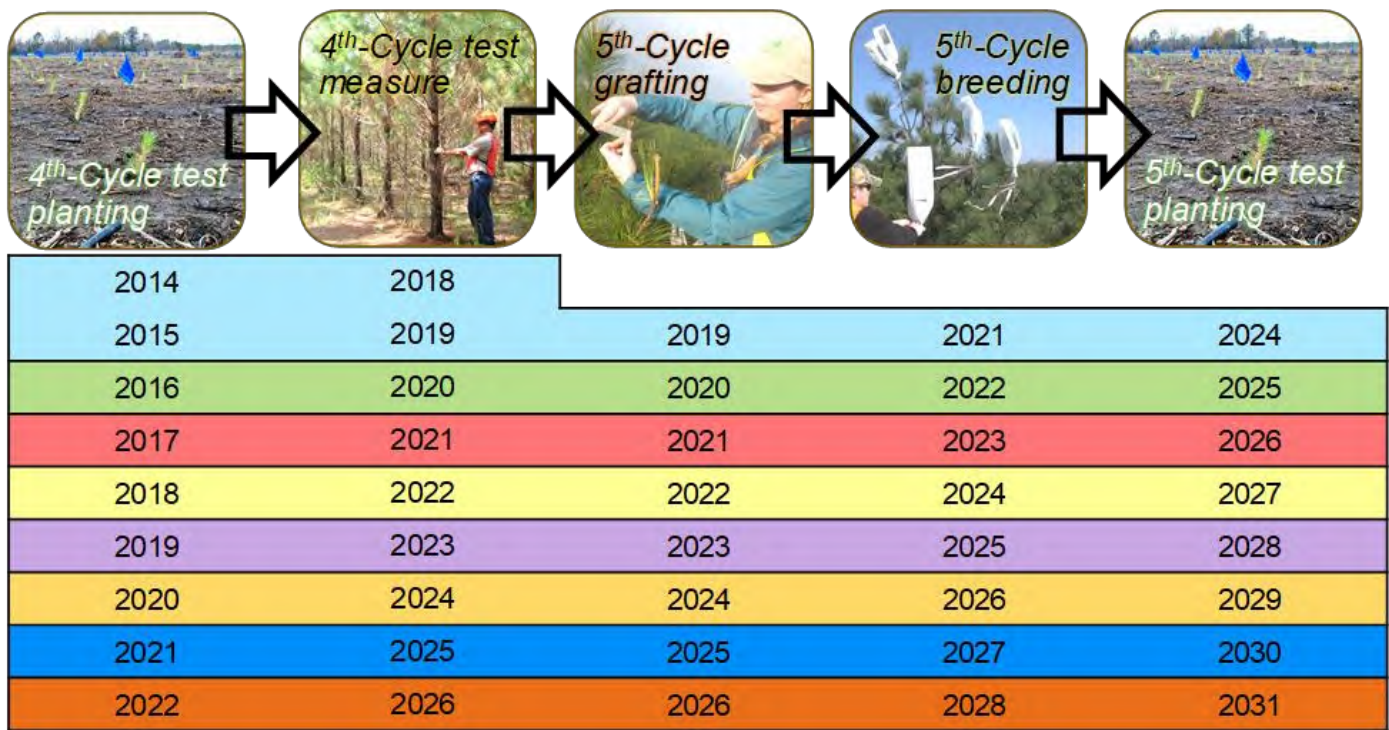


Figure 1. Idealized process flow of 4th-Cycle test planting through 5th-Cycle breeding and testing. Each color band is a cohort of families that were tested together, had selections made, flowered for breeding, and had seed grown for testing in the same year. In reality, there is considerable overlap among cohorts due to biological differences in flowering, logistical differences in test measurement, and statistical experimental designs needed to connect the population for a joint analysis.

Table 3. Pollen collection progress for 5th-Cycle selections. There will be pollen from 165 5th-Cycle selections available for breeding in 2024!

Year	# of selections with pollen at Arrowhead Breeding Center	# of selections with pollen at members' breeding orchards	# of selections with stored pollen available for breeding
2020	5		
2021	38		5
2022	50		43
2023	57	15	93
2024			165

tests). Inputting this information helps avoid making crosses that are redundant or highly related to this juvenile population (**Figure 2**). For example, if two potential crosses have the same genetic gain, the algorithm will prefer the cross that is less related to the juvenile population. The pedigree and breeding values were used to balance gain and diversity using a target degree of 35 (target degree values closer to 0 degrees result in more short-term gain, and values closer to 90 result in more diversity but less gain).

A major concern in our loblolly breeding populations is maintaining cold hardiness for northern and inland regions, because this adaptability trait tends to have an undesirable genetic correlation with our important traits for selection. For example, more cold-tolerant selections from northern and inland regions tend to have lower volume breeding values and higher fusiform rust disease susceptibility (**Figure 3**). In previous cycles, breeding zones were used to maintain adaptability (e.g. Coastal,

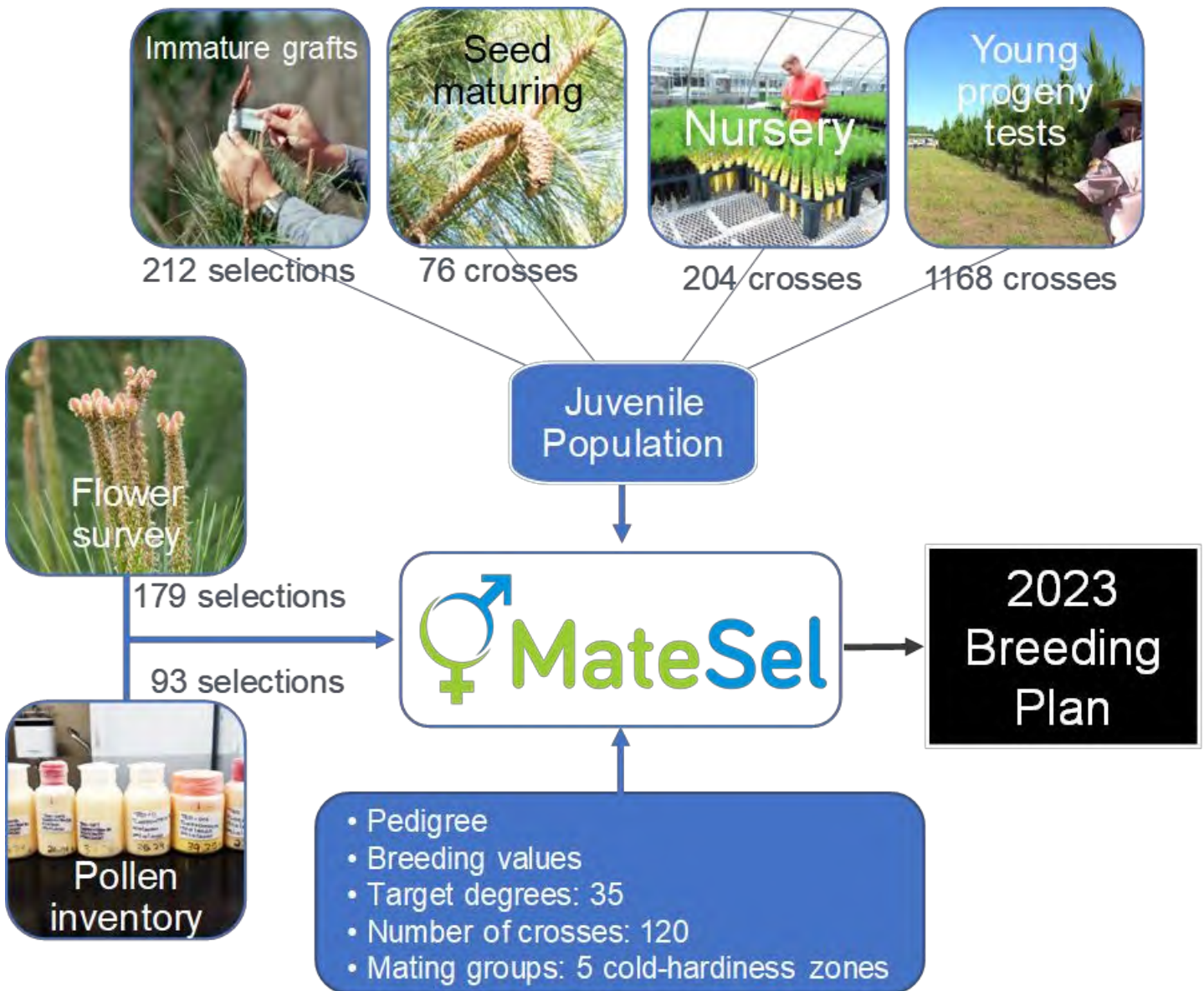


Figure 2. Process flow for developing the breeding plan using the MateSel algorithm. Inputs include flower surveys and pollen inventory to determine which selections are available for mating (and the number of times they can be mated). The juvenile population must be considered to avoid making crosses that are highly related to those already in the pipeline. The algorithm uses the pedigree and breeding values to balance gain and diversity. The mating groups option was used to manage cold hardiness using five zones and permitting mating among adjacent zones.

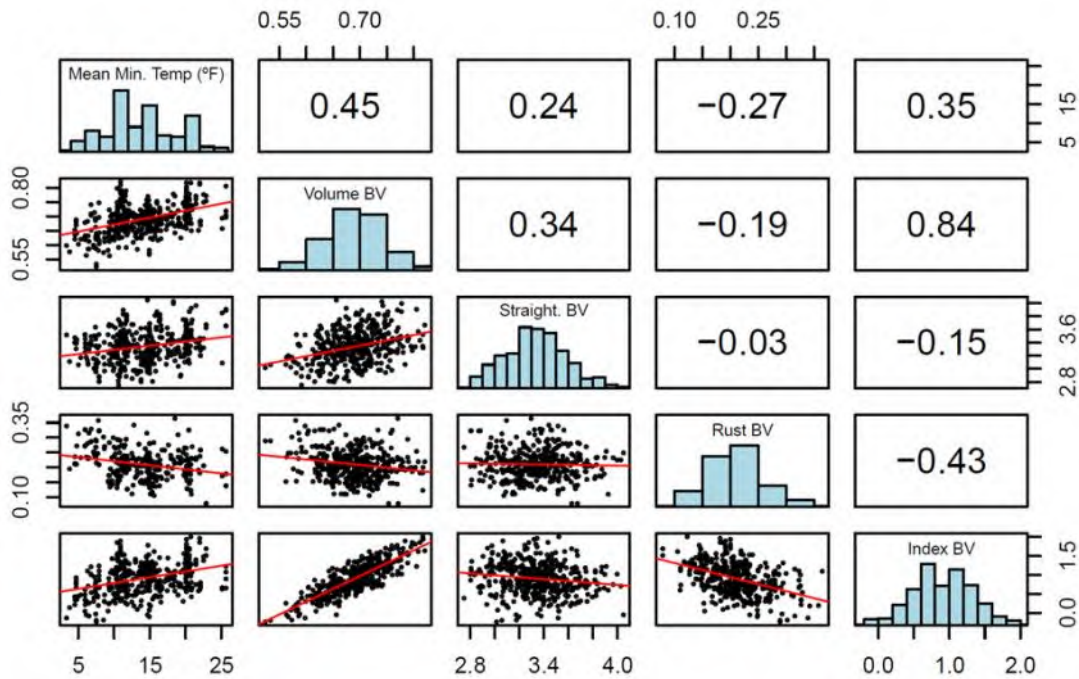


Figure 3. For 5th-Cycle selections, the relationship between mean minimum winter temperature and breeding values for volume (cubic feet), straightness (6-point scale, lower is better), rust resistance (on the probability scale, lower is better), and the index breeding value (combined volume, straightness, and rust with economic weighting). The mean minimum winter temperature of 5th-Cycle selections has undesirable correlations with volume and rust resistance breeding values, with more cold-tolerant material tending to grow slower and have more susceptibility to fusiform rust.

Piedmont, Northern) by forbidding or minimizing crosses across zone lines, but such rigid demarcations can result in a sub-optimal crossing plan. For example, a breeding zone strategy won't allow crosses among trees with similar origins but separated by the zone boundary, such as the Upper Coastal Plain and Lower Piedmont. Further, provenance origins become difficult to define after a few cycles of breeding, especially when trees from wide crosses are selected for breeding (e.g. the Cooperative's Piedmont Elite Population which included Coastal x Piedmont hybrids). A better approach is to permit crosses among *all* individuals but apply a negative weight against wide crosses in the mate selection algorithm. This was done using the Mating Groups option in MateSel and classifying each 5th-Cycle selection into a 5°F temperature bin (Figure 4). The algorithm was permitted to choose crosses among parents from adjacent mating groups, but not from more distant mating groups. A weighting of 0.85 was used (where 1 implies strict adherence to the target, and 0 implies the mating groups can be ignored). The target number of crosses per mating group was chosen to reflect the population distribution and sum to 120 crosses. The solution tended to

follow the targets closely, but a few inter-group matings were chosen (Figure 4).

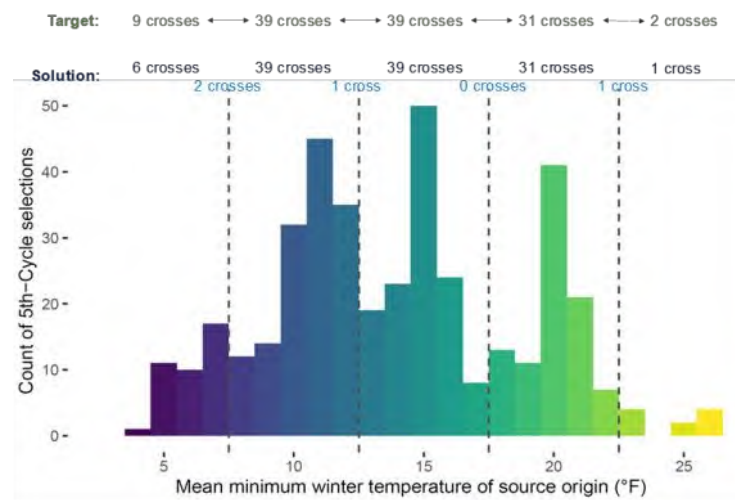


Figure 4. The distribution of mean minimum winter temperature for 5th-Cycle selections with vertical lines representing the bins used for mating groups in MateSel. The target number of crosses to do within each Mating Group bin is shown in green text above the graph. The solution (shown in blue font) did not deviate much from the target, but there were a few inter-group matings (light blue font below the arrows).

The use of tactical breeding plans to take advantage of the current state of the population (in terms of reproductive maturity and the relatedness with the juvenile population), and the use of mating groups to manage cold hardiness are some of the new innovations in the 5th-Cycle breeding strategy. The TIP breeding program has a long history of adopting innovative methods to make our breeding program as efficient as possible!

GRID Breeding Update

Three large-effect genes (QTL or Quantitative Trait Loci) for fusiform rust resistance were discovered by Lauer and Isik (2021) and aptly named GRID1, GRID2, and GRID3, acronyms for *General Rust Immunity Determinant*. These genes occur in two popular parents in the TIP breeding program, with GRID1 and GRID2 both heterozygous in one parent and GRID3 heterozygous in another parent. Because the parents are heterozygous, only half of their progeny inherit each resistance gene. In spring 2022, we initiated efforts to develop homozygous individuals (gene stacking) that could be used as parents to create 100% resistant progeny. These trees would be very valuable in seed orchards, because even open-pollinated progeny should be immune to fusiform rust.

As these genes are not known to occur in any other pedigree, crossing among related individuals (backcrosses or full-sib breeding) is required to develop homozygous progeny. In spring 2022, we were able to take advantage of GRID3 carriers from the Atlantic Coastal Elite population that had been grafted at Arrowhead in 2014 as part of 4th-Cycle breeding plan. Two GRID3-carrying progeny were backcrossed with their carrier parent, and three half-sibling crosses were done.

In spring 2023, we were able to begin breeding on GRID1 and GRID2 carriers. Last year, there were 16 progeny carriers selected from field trials (with selection that also emphasized height at age 2) and topgrafted at Arrowhead (**Image 1**). Only one of these was flowering this spring (one year after topgrafting), and a backcross was performed. Next year, we anticipate more flowering and will have pollen ready!

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

Once seeds are harvested, the inbred progeny will be genotyped using DNA markers to identify homozygous trees for the GRID genes. Seeds from the homozygous individuals will be tested using artificial inoculations at the USDA Forest Service Resistance Screening Center in Asheville, NC to confirm that they are indeed immunity donors. Prior to artificial inoculation testing, the inbred progeny can be planted in block plots to include phenotypic selection on volume and straightness. This effort represents our most promising application of marker-assisted selection in loblolly pine to date!



Image 1. Topgrafts for one of the 16 selections carrying both the GRID1 and GRID2 alleles at Arrowhead in 2022. Breeding commenced on one of these trees in 2023 to develop progeny that will be homozygous for the rust resistance alleles at both the GRID1 and GRID2 loci.

Testing Update

5th-Cycle Progeny Tests: this first season of test planting is coming up!

The first round of 5th-Cycle progeny testing is underway! Members recently sowed the seeds at their nurseries, and the seedlings are destined for the woods in the winter of 2023-2024. The objectives of the 5th-Cycle progeny testing are the same as previous cycles: 1) identify trees that have a superior set of genes to select for the 6th Cycle of breeding and 2) rank 5th-Cycle selections for deployment based on their progeny performance.

For this first round of tests, the bulk of the families were generated from the first few years of 5th-Cycle breeding completed in 2020-2021 (49 full-sibling families) and the tail-end of 4th-Cycle breeding done in 2019, dubbed the “5th-Cycle Substitutes” (85 full-sibling families) (**Table 1**). Also, a strong emphasis was placed on the inclusion of parents tested in the 3rd and 4th Cycles to provide adequate statistical connections for robust and stable breeding value estimates. This was accomplished by include 74 full-sib families from 4th-Cycle testing, with emphasis placed on families that had parents that were also tested in the 3rd-Cycle pollen mix trials. There was also some emphasis on choosing families that would

benefit from additional testing. We made certain that all of the eight years of 4th-Cycle progeny testing (2014 - 2022) were represented by the families chosen, as well as many PMX series as possible (demonstrated for the Coastal region in **Figure 1**).



***Pictured above:** Breeding for the 5th Cycle is progressing well at the Arrowhead Breeding Center, and the first 5th-Cycle tests will go to the field this coming winter.*

***Table 1.** Count of families by population that are headed to the first round of 5th-Cycle progeny tests in 2024. There is some overlap among the progeny tests in different regions, so the number of unique entries (last column) is less than the row sums.*

	2024 Cycle-5 Series			Number of unique entries
	Coastal	Piedmont	Northern	
<i>Full-sib families</i>				
5 th -Cycle Breeding	23	27	22	49
5 th -Cycle Substitutes	53	48	23	85
3 rd /4 th -Cycle Connectors	25	22	27	74
<i>Checklots</i>				
Non-improved	3	2	3	8
Improved (2 nd -Cycle)	8	7		15
Open-pollinated 5 th -Cycle	44	26	22	79
Total number of genetic entries	156	132	97	310
Rep size (anticipated)	104	99	70	

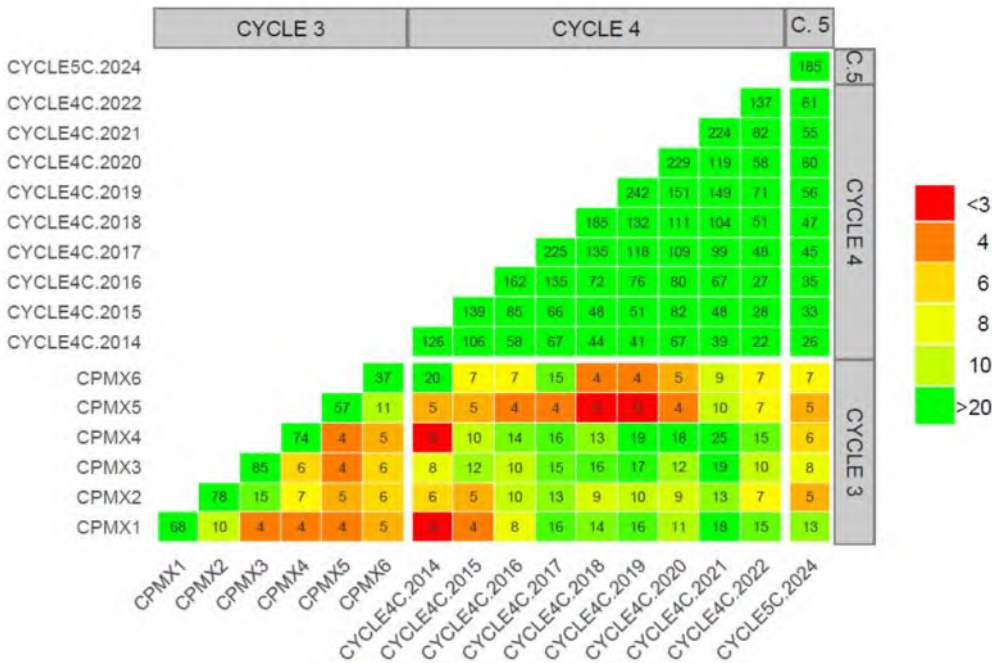


Figure 1. Counts of parents in common among the new 5th-Cycle 2024 Coastal series (CYCEL5C) and the 3rd-Cycle series (CPMX1 – CPMX6) and 4th-Cycle series (CYCLE4C, grouped by year of test planting). The 5th-Cycle 2024 series has 5 or more parents in common with all of these 3rd-Cycle series and 26 or more in common with all of the 4th-Cycle series. The pattern of strong connections in the 4th and 5th Cycles is similar for the Piedmont and Northern series. The diagonal includes the total count of parents in the series.

Overlap of families across testing regions was also considered, with 29 entries (families and checks) planned to be tested in both the Coastal and Piedmont regions, and 46 entries planned in both the Piedmont and Northern region (**Figure 2**).

An open-pollinated “sub-series” was included in this round of 5th-Cycle progeny testing. Included are seed from new selections that had too few flowers for controlled pollination, or were not chosen by MateSel when developing the breeding plan. This open-pollinated series was designed to “tag-along” to the mainline series to keep the marginal cost low. The plan is to keep the rep sizes for the full-sibling and open-pollinated series congruent and shuffle the open-pollinated reps into the series such that each members’ test assignment size increases by 3 or 4 reps. Rep sizes can be kept congruent by allowing open-pollinated families to have 2 or 3 trees in each rep (referred to as *non-contiguous single tree plots* by White et al. (2007)). This strategy simplifies the logistics by handling the open-pollinated series concurrently with the mainline series, including seed sowing, nursery growing, test organization, planting, maintenance, measurement, and analysis. Strategic use of open-pollinated series throughout the 5th Cycle can prevent delays in new selections’ breeding values when full-sibling seed production is low (e.g. due to late spring freezes like that in March 2022), or early in a breeding cycle while flower/

pollen development is still maturing. To avoid variation in pollen cloud effects, all of the open-pollinated seed for this series were collected from the Arrowhead Breeding Center, with the majority collected in the fall of 2022. The open-pollinated series includes seed from 79 5th-Cycle selections with 44 going into Coastal tests, 26 into Piedmont tests, and 22 into Northern tests (some families will be tested in multiple regions) (**Table 1**).

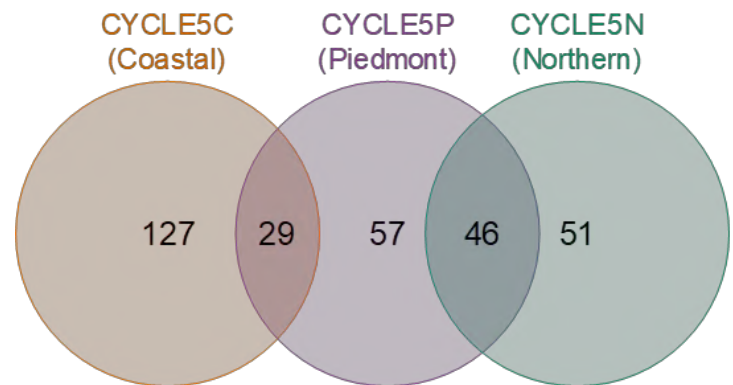


Figure 2. Number of genetic entries (families and checklots) in common among the three 2024 5th-Cycle progeny.

4th-Cycle Base Measurements: Steady does it!

Measurements of the 4th-Cycle progeny tests are about 60% complete (**Table 2**). The first 4th-Cycle tests were planted in 2014 and measured four to five years later (when the average height of trees is greater than 15 feet), starting in 2018 (**Table 2**).

Each year new measurement data come in and are included in the Annual BLUP for choosing 5th-Cycle selections and ranking the 4th-Cycle parents for seed orchard and plantation deployment. We are on track to finish 4th-Cycle test measurements in 2027.

Table 2. Count of tests measured in the 4th Cycle through 2023 by year they were planted. More than 60% of the tests have been measured.

	CYCLE4C		CYCLE4P		CYCLE4N	
	Measured	Not yet	Measured	Not yet	Measured	Not yet
2014	8		3		6	
2015	5		5		1	
2016	5		5		5	
2017	9		7		5	
2018	9		9		3	2
2019	6	3	6	4		5
2020		10		5		10
2021		7		7		1
2022		4		5		4
Total	42	24	35	21	20	22

Wood Properties Measurement Update

The Cooperative started routine assessment of progeny tests for wood density and wood stiffness (using drill resistance and acoustic velocity, respectively) in 2013 in the 3rd-Cycle pollen mix tests. In 2023, measurements of wood properties commenced in the 4th-Cycle tests that were planted in 2014-2015. The 2023 measurement season is also the first where members took the lead and measured their tests without TIP staff on site, thanks to numerous years of hands-on training by TIP staff during the measurement of 3rd-Cycle tests.

The primary objective of the Cooperative's wood properties effort is to obtain breeding values for parents to guide seed orchard and plantation deployment decisions at members' discretion. **Table 3** shows the timeline for wood property measurement in the 4th Cycle, including the number of tests to be measured each year and the number of new parents that will have data available each year. When finished, there will be wood properties on about one thousand parents used in the 4th-Cycle progeny testing.

Table 3. Timeline for wood property measurements of 4th-Cycle tests.

Measurement year	Number of tests to measure	Number of new parents with measurements	Cumulative count of parents with measurements
2023	4	241	241
2024	5	100	341
2025	8	141	482
2026	11	145	627
2027	11	91	718
2028	9	127	845
2029	10	71	916
2030	7	71	987
2031	7	30	1017

Wood properties have not been routinely included in forward selection or breeding decisions. One difficulty is that the age of selection (four to five years) is considerably less than desirable for wood property measurement (we are targeting age seven to nine years), so delays in grafting or additional grafting would be required would detract from the annual rate of gain in volume, rust resistance, and stem form. The Cooperative has taken a proactive stance on wood property measurement to monitor the population and understand the amount of gain possible given their economic importance to many of our members.

We are introducing a new trait for measurement, sawtimber potential score, to be recorded at time of wood property assessment. In previous work (Cumbie et al. 2102), we found that sawtimber potential could be readily assessed in six-year-old tests. The family differences were large and im-

portant as indicated by the half-sib family-mean heritability of 0.85. The sawtimber potential score rates the amount of defect on a tree in terms of its impact on the disqualification of sawtimber development. For example, a tree with a low fork, excessive sweep, or large gall near breast height will not produce a sawlog within the typical rotation age. A tree that is free of defect (sawtimber potential score = 1) is considered to have full sawtimber potential, even though the diameter will be much below the limit at time of assessment. A tree with reduced sawtimber potential (e.g. minor sweep or a fork about 16 feet) would receive a score of 2, because there is a defect present, but the tree will still produce a solid-wood product. A score of 3 implies “pulpwood only” due to defects that preclude solid-wood product potential. A score of 4 is a cull tree, for example a rust bush with multiple stems.

Pollen Cloud Study Planted in Winter 2022-2023

Open-pollinated seed from the same loblolly pine selection can come from different orchards that may be located in very different geographic regions (e.g. the Coastal Plain of South Carolina versus the Upper Gulf Coastal Plain of Alabama). The goal of the pollen cloud study is to better understand the impact of different pollen clouds on the performance of open-pollinated families and ensure that these differences are consistent with the assumptions made in *PRS*TM scores. This year, seven test sites were established including four Coastal series tests and 3 Piedmont/Northern series tests (**Figure 2**). We anticipate measuring these tests at age 4 to test the study hypotheses. More details about the families used, orchards represented, and experimental design can be found in the 2021 Annual Report.

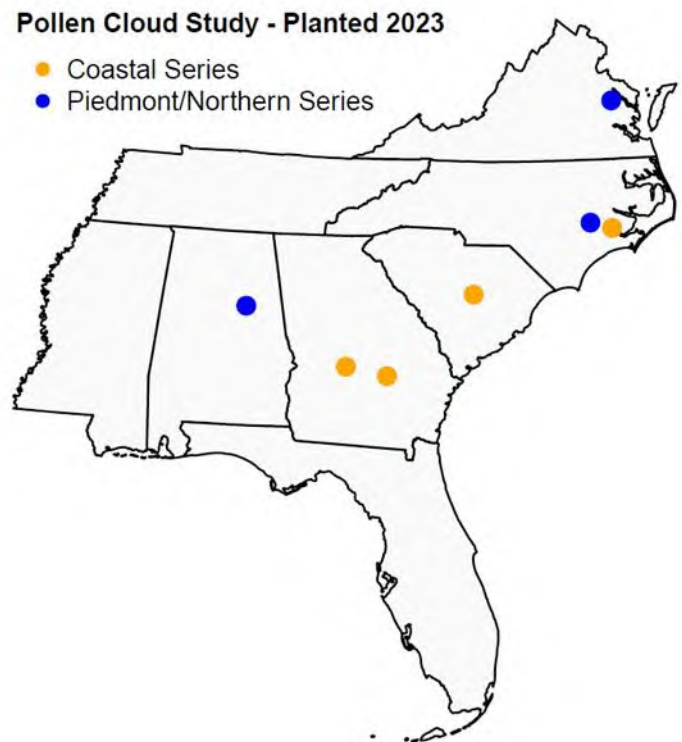


Figure 2. Test site locations for the pollen cloud study planted in 2023.

Literature Cited

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Genomic Selection Genotyping Update

The Cooperative Tree Improvement Program Genomic Selection Plan was approved in 2021 for funding by the Advisory committee. Since 2021, about 2400 trees (1200 parents and 1200 progeny) from the 4th-Cycle Coastal population have been sampled for genotyping. According to the plan, 1200 parents and 9000 offspring from the 4th-Cycle Coastal breeding population will be genotyped between 2022 and 2025. In total, about 16,500 trees, including the two-generation ACE population, will be used to train the model for genomic selection. In 2023, we plan for about 4700 4th-Cycle progeny test trees to be sampled for genotyping.

For genomic selection to work, the genetic relatedness between the training and selection populations must be strong. We used additive genetic relationships calculated from the pedigree to identify 4th-Cycle crosses related to the ~200 Coastal 5th-Cycle selections made between 2019 and 2023. The mean additive genetic relationship and the number of 5th-Cycle selections related to each 4th-Cycle cross (644 crosses in total) were calculated (**Figure 1**).

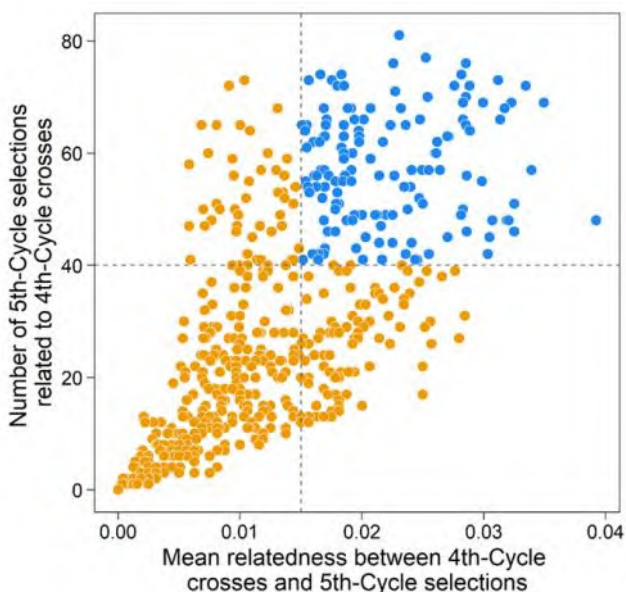


Figure 1. The mean relatedness between 4th-Cycle crosses and 5th-Cycle selections (x-axis) and the number of 5th-Cycle selections related to 4th-Cycle crosses (y-axis). Crosses (blue circles) with a mean relatedness of more than 0.015 (to the right of the dashed line) and the number of related pairs with more than 40 5th-Cycle selections (above the dashed line) were selected for genotyping.

Crosses having mean relatedness of more than 0.015 and a number of related pairs of more than 40 with the 5th-Cycle selections were selected for sampling. Based on these criteria, priority was given to the 4th-Cycle crosses having higher mean relatedness and showing greater number of related pairs with the 5th-Cycle selections (blue points on the top-right of **Figure 2**). In total, 319 crosses have been chosen for genotyping since 2021, of which 253 crosses will be sampled this year. All 319 crosses show some degree of additive genetic relationship with most 5th-Cycle selections selected (**Fig. 2**). The additive genetic relationship between 4th-Cycle crosses selected for genotyping and 5th-Cycle selections range from 0 to 0.563 (average = 0.017). The sampling was done in a way where priority was given to crosses showing the strongest genetic relatedness with the 5th-Cycle selections, followed by the crosses showing moderate to strong genetic relatedness. The sample size will be 30 seedlings per cross for crosses showing stronger genetic relatedness and 20 for crosses showing moderate to strong genetic relatedness. A number of crosses did not have enough trees in the field, and the sampling of those crosses will be completed in the coming years. The 4700 trees to be selected for genotyping in 2023 are healthy and have high accuracy of volume breeding value.

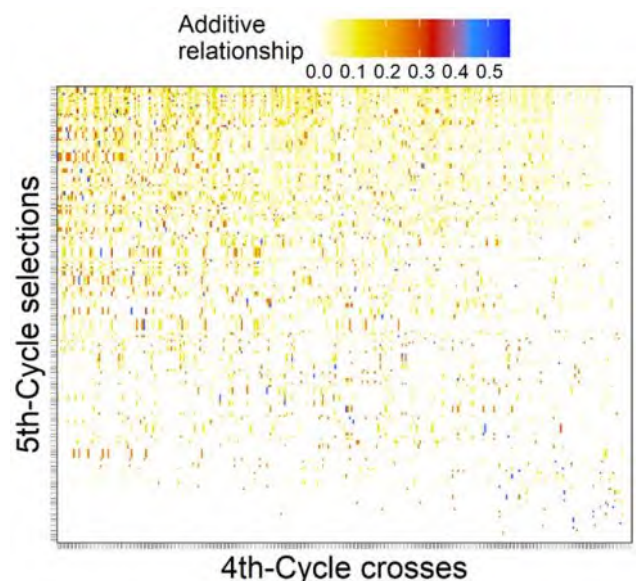


Figure 2. Heatmap of additive genetic relationships between the 319 Coastal 4th-Cycle crosses selected for genotyping and the ~200 Coastal selections made through 2023. The white color indicates no additive genetic relatedness based on the pedigree. Light yellow to dark blue indicates different levels of genetic relatedness.

5th-Cycle Selection Update

For the last four years, Full Members have expedited their measurements of 4th-Cycle progeny tests in October/November so that TIP staff can analyze the data and identify selection candidates prior to the spring grafting season. The analysis consists of two steps 1) estimating breeding values (genetic effects) for the individual trees and 2) identifying potential trees to select to balance gain and diversity in the breeding population. The former is described in detail in the 4th-Cycle data analysis section, and here we describe the latter.

Through 2023, more than 60,000 trees from full-sibling families in 4th-Cycle tests have been measured, of which 374 have been chosen to be 5th-Cycle selections, which is a very intense selection intensity (**Table 1**)! The Cooperative commenced 5th-Cycle selections in 2019, and the breeding strategy calls for 768 selections to be made through 2027. So far, there have been 374 5th-Cycle selections made through 2023, which is about 50 less than the target outlined in the breeding strategy (**Table 1**).

Of the selections made so far, 219 are Coastal-source and come from progeny tests planted in the Coastal Plain (CYCLE4C). The selections from CYCLE4P (planted in the Piedmont) and CYCLE4N (planted in Virginia and northern North Carolina) include overlapping ranges in cold hardiness, as do selections from the NE1 population. The distribution of cold-hardiness values (mean minimum winter temperature) for the 5th-Cycle selections closely resembles that of the 4th-Cycle progeny population (**Figure 1**).

The 374 5th-Cycle selections made so far come from 281 parents and 247 crosses. Their pedigree can be traced back to 327 founders (first-generation selections from natural stands or non-improved plantations) (**Table 2**). This number has increased considerably with each season of selections, while the mean additive numerator relationship has decreased. This indicates that the new selections each year contribute much diversity, which reflects the rolling-front strategy used in the 4th-Cycle progeny tests.

Table 1. Number of 5th-Cycle selections by year and series.

Year	CYCLE4C	CYCLE4P	CYCLE4N	NE1*	Total	Cumulative actual	Cumulative target
2019	29			2	31	31	43
2020	52	24	9	7	92	123	111
2021	72	42	8		122	245	225
2022**	-	-	-	-	-	245	322
2023	66	48	15		129	374	425
2024							512
2025							583
2026							703
2027							768
Total	219	114	32	9	374		

* Northern Elite (NE1) series was a clonal population derived from the 3rd-Cycle Elite Diallels for the Piedmont and Northern breeding zones. These are considered 5th-Cycle selections.

** No 5th-Cycle selections were made in 2022.

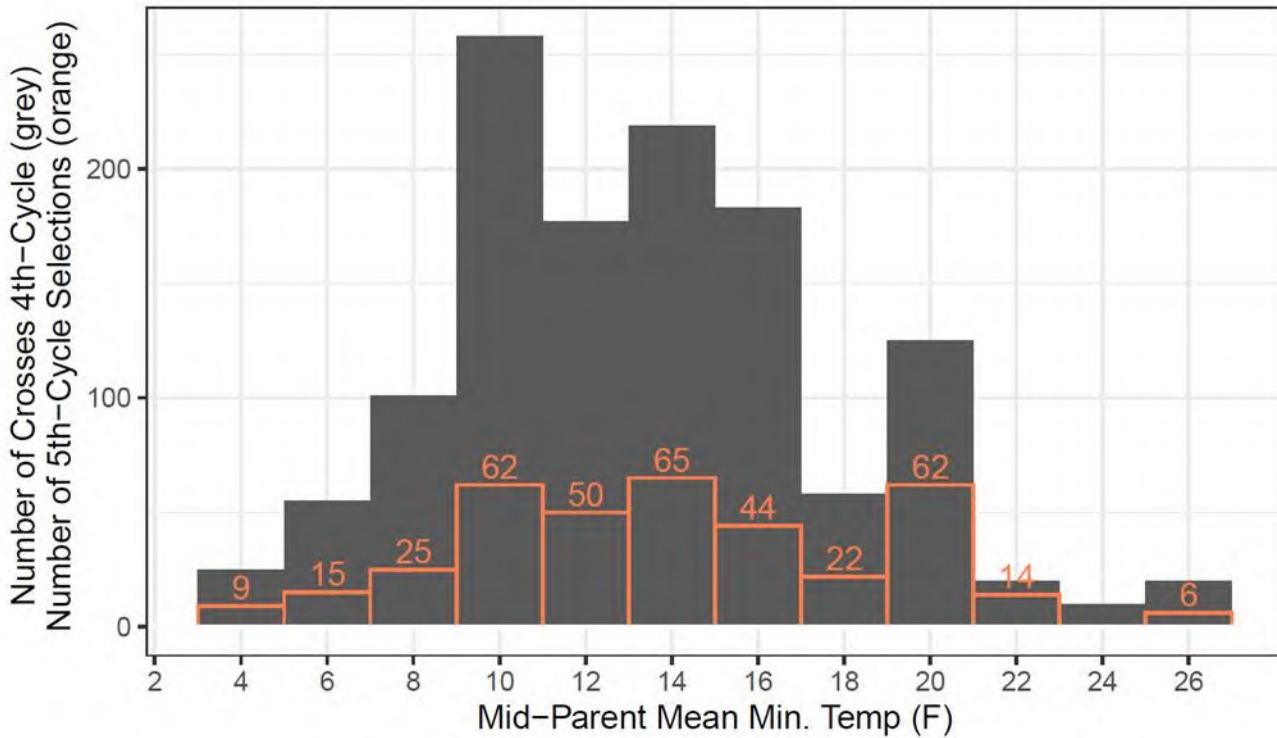


Figure 1. Distribution of number of 4th-Cycle crosses (in grey) measured through 2023 and number of 5th-Cycle selections (orange) made from those crosses.

We expect the values to stabilize towards the end of the 5th-Cycle selection program (2027). Also included in Table 2 is the status number, which is defined as half the inverse of group coancestry. The status number indicates that randomly mating the 374 5th-Cycle selections we have made through 2023 would result in a loss of diversity and progeny inbreeding similar to randomly mating 84.66 unrelated, non-inbred trees. The status number has increased by 16 to 18 trees in the last two rounds of 5th-Cycle selections (2021 and 2023), and we expect a similar annual increase throughout the 5th-Cycle selection timeline.

To estimate gain from our 5th-Cycle selections, we can compare their breeding values to those for all 4th-Cycle progeny. The breeding values come from measurements made at age 4 or 5 years and are combined to make a multi-trait index with weights for volume, straightness, and rust resistance breeding values, with less emphasis on rust for trees adapted to the colder regions. The breeding values for each trait are first standardized using Z-scores so that larger values are more desirable. For trees with a source origin minimum winter temperature warmer than 8° F, we applied weights of 60% on stem volume, 20% stem straightness, and 20% rust resistance. For those trees with colder minimum win-

Table 2. Diversity metrics for the cumulative population of 5th-Cycle selections during each selection season.

Year	Number of founders represented	Mean additive relationship	Status number
2019	50	0.0255	17.5
2020	182	0.0118	50.3
2021	252	0.0105	68.6
2022*	252	0.0105	68.6
2023	327	0.0091	84.7

*No 5th-Cycle selections were made in 2022.

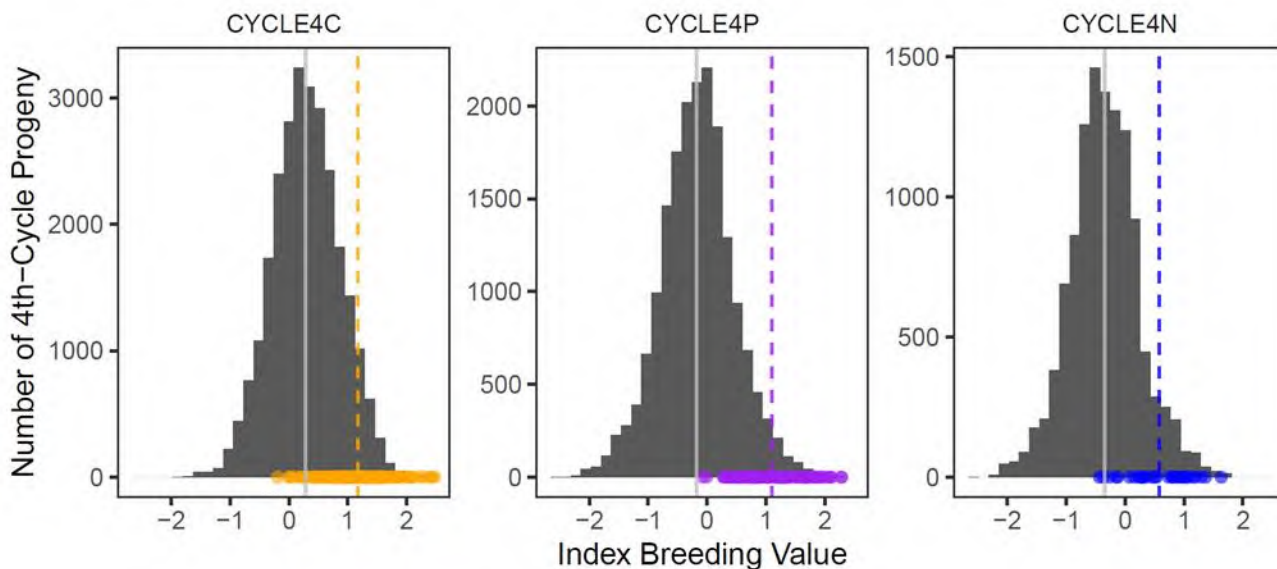


Figure 2. Multi-trait index breeding values for trees in 4th-Cycle progeny tests (gray) and those trees that were selected in each region. Vertical lines indicate the mean of the population (grey) and the mean of the selected trees (dashed, colored lines).

ter source origins, no weight was placed on rust resistance, and weights of 70% and 30% were used for volume and straightness, respectively. The distributions of candidates and selections are shown in **Figure 2**. For selections from the Coastal series (CYCLE4C), the mean of the selected population was 0.89 higher than the population. For trees selected from the Piedmont (CYCLE4P) and Northern (CYCLE4N) series, the gains were 1.28 and 0.93, respectively. These gain values correspond to the number of standard deviations above the mean in terms of index breeding value.

After TIP staff analyze the measurement data and develop a list of candidate trees for selection, members visited each tree to determine that the measurements were correct and to look for any extenuating

circumstances that might cause the tree to be unfairly ranked (e.g. large stem diameter due to an excessive number of missing competitors). In the 2023 selection season, 33 out of the 165 candidates were not selected due to inaccurate measurement data or other reasons (**Table 3**). The majority of rejections were due to the presence of a rust gull that was overlooked during measurement. There were three candidate trees rejected that had an excessive number of missing border trees; most trees with missing neighbors within the test are pre-filtered from the candidate list during data analysis.

Scions were collected at the time the selections were made and distributed to either the Arrowhead Breeding Center or to Full Members' breeding orchards for topgrafting. The 5th-Cycle Breeding Logistics

Table 3. Reasons for rejecting candidate selections in the 2023 selection season.

Reason for rejection	Number of candidate selections
<i>Rust gall present</i>	17
<i>Straightness score incorrect</i>	6
<i>Damaged/top dieback</i>	4
<i>Excessive # of missing neighbors</i>	3
<i>DBH incorrect</i>	1
<i>Huge ramicorn</i>	1
<i>Fork score incorrect</i>	1
Total	33

plan mandates that 70% of selections should be allocated for breeding at Arrowhead and 30% at members’ orchards, and the total through 2023 is very close to this target (**Table 4**).

Next year, the clone banking for 5th-Cycle selections will commence. The purpose of clone banking is to bulk up scion stocks to reduce bottlenecks for grafting selections into seed orchards once 5th-Cycle progeny test data are available. The 5th-Cycle clone banking plan calls for clone banking selections once seed have been harvested for progeny testing. The motivation for this policy is to avoid wasting effort on clone banking for selections that were not bred (e.g., due to attrition, poor flowering). Also, delaying clone banking avoids the risk of cutting too

much scion from the ortets (typically less than 6 years old) and compromising future data collection, or cutting scions from the young topgrafts and interfering with flower/pollen production. There are 90 5th-Cycle selections that are eligible for clone banking next spring (their seeds will be sown for progeny testing this spring). These selections were made in 2019 and 2020 and represent about 70% of the selections made in those years. At that rate, we anticipate 90 new selections per year will become ready for clone banking in 2025 and 2026. The clone banking process flow is shown in **Figure 3**. After clone banking, grafts will be inventoried annually for survival and signs of incompatibility, which will inform deployment decisions.

Table 4. Number of selections topgrafted by breeding orchard.

Year	Number of Selections Topgrafted at Arrowhead	Number of Selections Topgrafted at Members’ Breeding Orchards	% Workload (Arrowhead/Members)
2019	29	2	94/6
2020	91	1	99/1
2021	86	36	70/30
2022	-	-	-
2023	68	61	53/47
Total	274	100	73/27

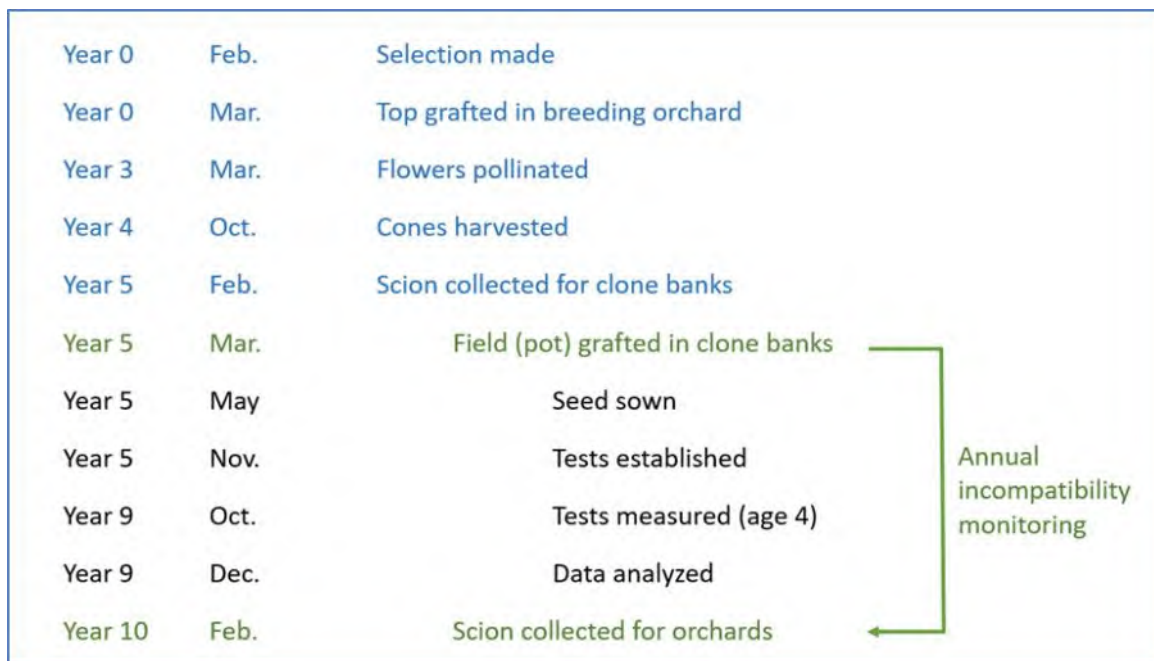


Figure 3. Clone banking process flow to ensure that ample scions are available for new orchards once breeding values for a new selection are available from progeny test data.

PRSTM Update with Fourth Cycle Data

The TIP staff initiated analysis of a new **P**erformance **R**ating **S**ystem this year and have released BETA versions for the Coastal, Piedmont, and Northern deployment areas. These new **PRSTM** versions are the first to include 4th-Cycle data and include a large number of new parents and families. Also, the Piedmont and Northern versions include families that had previously only been tested in the Coastal region. These Coastal-source families were included in the Piedmont and Northern 4th-Cycle tests where they were considered adapted (e.g., lower Piedmont and Coastal North Carolina). Users of the **PRSTM** will still need to check the families' mean minimum winter temperature against their deployment area to ensure adaptability. As expected, there are many Coastal families that do very well in the Piedmont and Northern versions, but most are not adapted to the entirety of the Piedmont or Northern deployment areas. Further, Piedmont- and Northern-source families were tested side by side in most 4th-Cycle tests, and their **PRSTM** versions now share many more families.

Previous versions of **PRSTM** contained only 2nd- and 3rd-Cycle data. The new 4th-Cycle data encompasses a small proportion (4-5% of the tree measurements) of the combined data in the 2023 analysis (**Table 1**). The smaller dataset size for 4th-Cycle data reflects improvement in testing efficiencies throughout the history of the Cooperative's breeding program, such as the adoption of single-tree plots versus 6-tree row plots and reductions of the population size through among-family selection. The 2nd- and 3rd-Cycle data were used in simulation studies to determine the appropriate number of trees and test sites per family, which led to more efficient testing in the 4th-Cycle.

Even though the 4th-Cycle dataset is only a small component of the new **PRSTM**, it has a large influence

due to its improved experimental design. A major improvement was the strengthening of the connectedness (number of families in common) among tests (**Figure 1**). Test pairs were strongly connected with each other in the 4th-Cycle data, sharing an average of 75 parents in common, which is far more rigorous than the 2nd- and 3rd-Cycle populations. Connectedness is important, as it influences the precision of comparison for families that were not tested side by side.

A significant number of new families from the 4th-Cycle data were evaluated in the BETA **PRSTM** versions, and about half of those new families rank in the top 150 for **P**roductivity (**Table 2**). There are 13 new families in the Coastal, 11 in the Piedmont, and 3 in the Northern population ranking in the top 20 for **P**roductivity. Similarly, a considerable number of new parents rank in the top 150 for volume breeding value in the Coastal (27), Piedmont (30), and Northern (21) populations, respectively.

Results from the 2023 BETA **PRSTM** were compared with the values from 2017 Coastal and Piedmont and 2019 Northern **PRSTM** to assess consistency. In general, **PRSTM** values from the 2017/2019 versions were consistent with the values from the 2023 BETA versions for all traits. The correlations ranged between 0.93 and 1. The correlation between the 2019 and 2023 productivity scores was lower in the Northern region, suggesting considerable rank changes (**Figure 2**). Most of the rank changes in the new **PRSTM** versions can be attributed to introduction of the well-connected 4th-Cycle data. The Cooperative is about halfway done measuring 4th-Cycle tests. Once all 4th-Cycle data are collected, a new **PRSTM** version will be available (2028).

Table 1. The number of tests and number of trees assessed in the 2023 BETA **PRSTM** versions.

Population	2 nd -Cycle		3 rd -Cycle		4 th -Cycle	
	# of Tests	# of Trees	# of Tests	# of Trees	# of Tests	# of Trees
Coastal	537	616K	50	67K	40	30K
Piedmont	482	512K	20	25K	33	26K
Northern	297	330K	7	8K	24	18K

Table 2. The number of new families assessed in the 2023 BETA **PRSTM** versions. About half of the new families rank in the top 150 for productivity.

Population	New families	New families in top 150
Coastal	660	78
Piedmont	1048	81
Northern	837	72

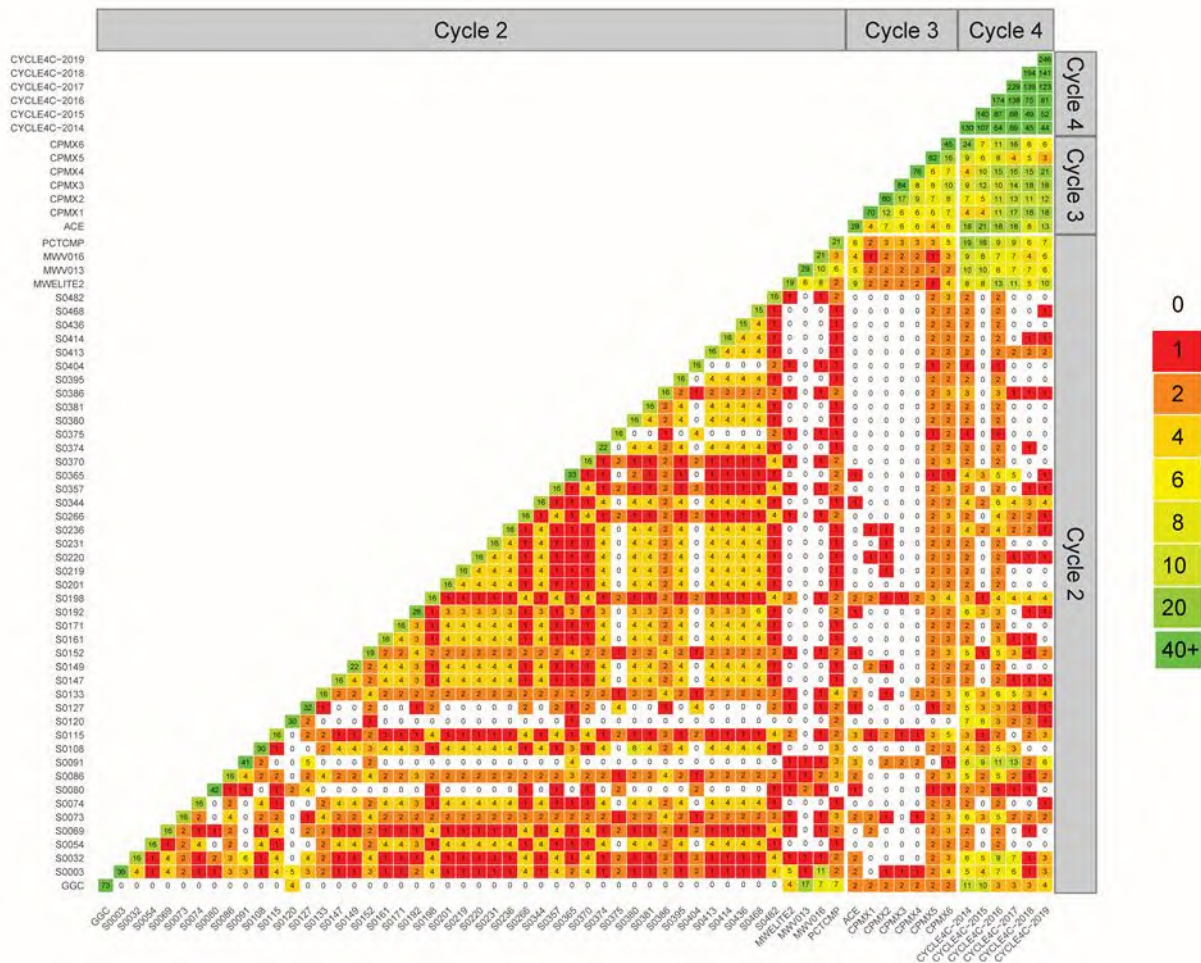


Figure 1. Number of parents shared between Coastal test series in the 2nd, 3rd, and 4th Cycle populations. Each test series is shown as a row and a column and the number of common parents between the pair of series is shown in the cell intersection. The number of parents in the series is shown along the diagonal. Series with zero to five common parents are colored red to orange to reflect that they are less connected than recommended by progeny test experimental design studies. The 4th-Cycle is characterized by very strong connectedness.

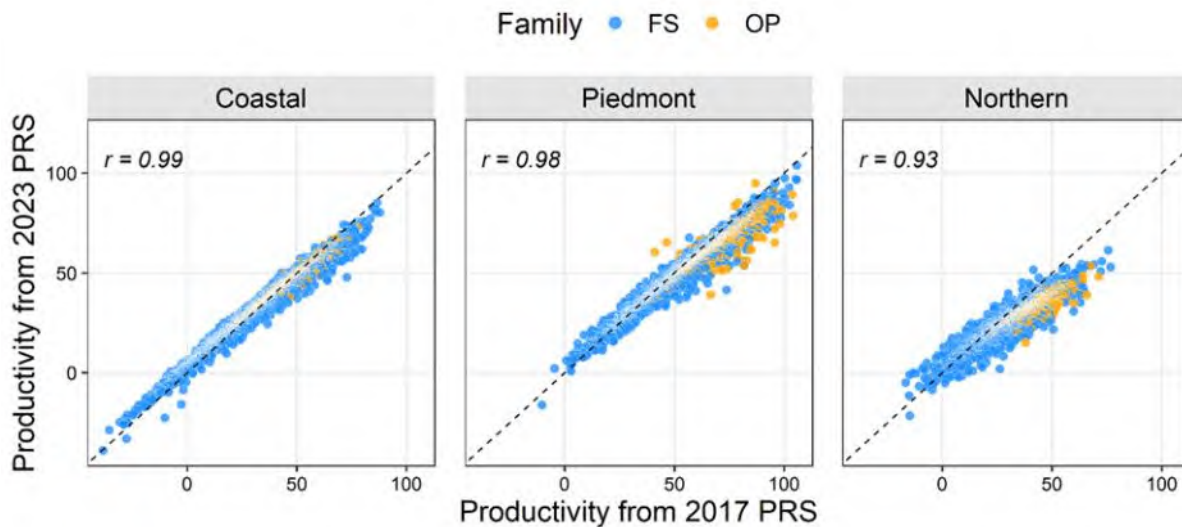


Figure 2. Relationship between productivity scores from 2017 and 2023 Coastal, Piedmont, and Northern PRS™. The productivity scores were calculated against the CCC checklot. Rank correlation coefficients are provided on the top left of the figures. Colors correspond to full-sib and open-pollinated families.

Fourth-Cycle Data Analysis

This report summarizes the analyses conducted with 4th-Cycle data collected from trials measured through 2023 and compares the results with analyses of tests measured through 2022. The purpose of the 4th-Cycle data analysis was to predict breeding values for individual trees generated by the breeding program (for 5th-Cycle selection) and their pedigree (for seed orchard and seedling deployment decisions). The Coastal (series CYCLE4C) and Cold-hardy populations (series CYCLE4P and CYCLE4N) were analyzed separately. The 4th-Cycle tests were planted 2014 – 2022 and were measured at either four or five years of age, with data for about 60% of the tests available for this analysis.

In 2023, the Coastal data had 40 tests from 436 parents (including checklots). Of these, 114 parents

were new (did not have **PRS**TM values in the COASTAL 2017 **PRS**TM) (Table 1). The cold-hardy data consisted of 53 tests, 587 parents, and 8 checklots. Of these, 301 parents did not have **PRS**TM values in the 2017 PIEDMONT & 2019 NORTHERN **PRS**TM. The large number of new parents in the Cold-hardy population is the result of new data for 4th-Cycle as well as parents that had previously only been tested in the Coastal region but were included in Piedmont and Northern tests to allow for comparisons. The pedigrees of both datasets were comprised of ancestors, parents, checklots, and individual trees. In both datasets, tests were strongly connected across test series. The median number of shared parents among test pairs was 68 in the Coastal and 59 in the Cold-hardy population.

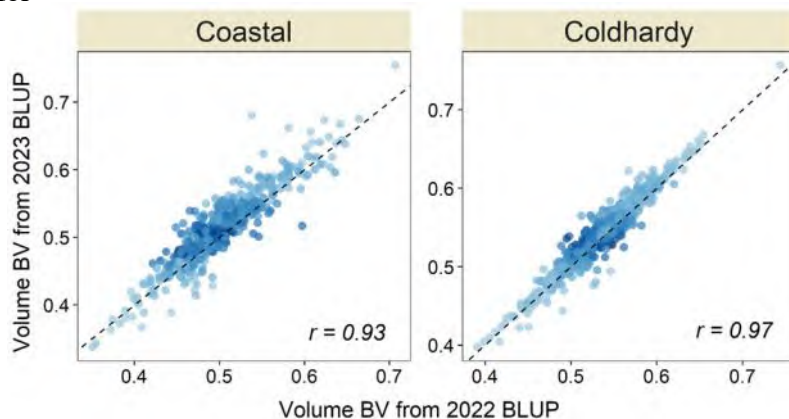
Table 1. Number of parents, crosses, tests, and trees assessed in the 2022 and 2023 4th-Cycle BLUP analysis.

	Coastal		Cold-hardy	
	2022 analysis	2023 analysis	2022 analysis	2023 analysis
Parents	359	436	549	587
Crosses	473	644	610	701
Tests	34	40	43	53
Trees	25K	30K	34K	41K

Traits presented in this summary are volume, straightness, and fusiform rust incidence (traits used in the Cooperative's index breeding value). 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 fit 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 all the trees and the pedigree.

Figure 1. The relationship between volume breeding values of the parents that were assessed in both 2022 and 2023 BLUP analyses. Pearson correlation coefficients are given in the bottom right of the figures. The dashed line represents the one-to-one relationship between the 2022 and 2023 BVs. In general, there is a strong relationship between the two years, but the 2023 BVs were slightly higher than the 2022 BVs (slope = 1.02).

Breeding values for volume, straightness, and rust were compared for parents and individual trees from 2022 and 2023 analyses. In both datasets, consistency of ranking was observed for all traits. The correlations between breeding values for volume from the 2022 and 2023 analyses were strong for both the Coastal and Cold-hardy populations (Figure 1), and the correlations for rust and straightness BVs across the two years ranged between 0.94 and 0.97.



A significant number of new parents ranked in the top 20 for stem volume assessed in the 4th-Cycle Coastal and Cold-hardy populations (**Figure 2**). In the Cold-hardy population, 13 new parents ranked in the top 20 with minimum winter temperature greater than 10 °F (not shown). Some of these new parents did not have **PRS**TM values in the 2017 **PRS**TM version.

Users must be cautious about using selections in colder regions where they may not be adapted. The mean minimum winter temperature origin of a selection is a critical criterion to consider when selecting parents for seed orchards and seedling families for deployment.

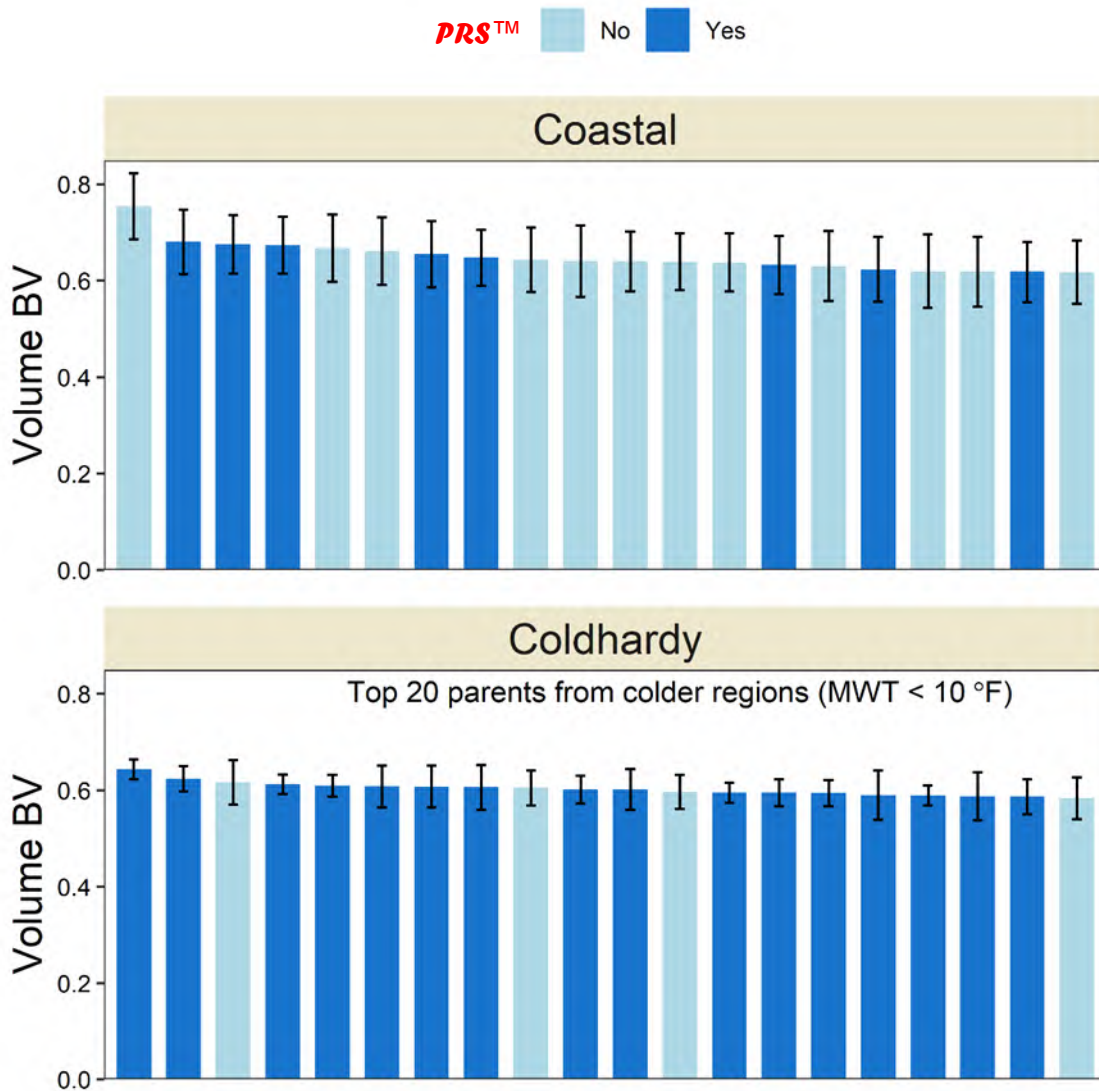


Figure 2. Volume breeding value for the top 20 parents identified in the 2023 4th-Cycle Coastal and Cold-hardy BLUP analyses. Light blue bars correspond to new parents that have been analyzed in the 4th-Cycle and have no **PRS**TM values in the current **PRS**TM system. For the Cold-hardy population, top 20 parents are shown for the list of parents with MWT < 10 °F.

ACE2 Update: Testing the genomic selection concept using the Atlantic Coastal Elite 2nd-Generation population

The ACE2 (Atlantic Coastal Elite 2nd-Generation) population was bred in 2017-2019 using selections from the ACE1 clonal trials. The motivation for this breeding was to test the genomic selection concept in a pine breeding program while also furthering an elite population that should yield excellent 5th-Cycle selections.

Genomic selection uses DNA markers with a prediction model to estimate the genetic merit of young trees much sooner than traditional phenotyping (i.e. four to six years old). The ACE2 is a great population to test the efficacy of the genomic selection concept for a few reasons. For starters, the genomic prediction model was built using the ACE1 population, which had rigorous clonal testing to provide precise phenotypes to train the model. Secondly, the ACE1 population consists of a relatively small number of parents (21), which reduces the number of haplotype combinations and should increase the predictive power of the markers. Finally, the ACE2 population is elite, and our efforts should also generate great seed orchard parents for deployment.

The predictive ability of the training model has been demonstrated in several “proof-of-concept” studies that use the ACE1 data in model building and testing (Lauer et al. 2022; Shalizi et al. 2022; Walker 2022). However, testing how well the model predicts the performance of the ACE2 population represents the “acid test” for genomic selection. This is because genomic selection models will only be valuable if they work across generations after recombination has occurred. This section provides an update on the ACE2 population.

Seedling trials

In 2021, a series of four seedling trials were planted that included 67 ACE2 crosses among about 70 ACE1 parents. The tests were planted across the southeastern US (**Figure 1**), and most are in good shape through the first two growing seasons (survival > 95%). Unfortunately, the South Carolina test experienced some Hurricane Ian damage in Fall 2022, but it should still produce valuable measurement data. The seedling tests have been assessed

annually for survival, height, and rust to give early insights on the genomic prediction accuracy.

Clonal Population

Prior to planting the seedling trials, cuttings were taken from the seedlings and rooted to produce hedges for clonal propagation. This was a novel idea that allows for seedling testing and rooted cutting clonal testing for the same set of genotypes. In the summer of 2022, a round of rooted cuttings was produced and planted in two field trials in the fall of 2022 (test locations in **Figure 1**). The count of clones with cuttings of appropriate size for testing was much lower than desired (470 clones from 32 families), so a second round was initiated in the spring of 2023.

In March 2023, we took rooted cuttings from 1325 clones from about 80 families (**Image 1**). The hedges were pruned in the summer of 2022 after the first round of cuttings. Each tree was hedged to approximately 6 inches (~15 cm). The purpose of hedging was to increase uniformity, reduce maturation, and remove apical dominance so that each tree had plenty of shoots for clonal production. Through the summer

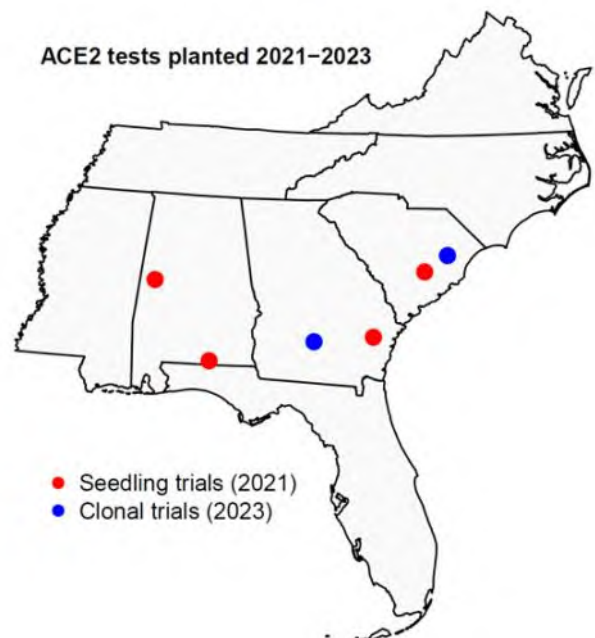


Figure 1. Test locations of the four ACE2 seedling trials planted in 2021 (red points) and the two clonal trials planted in 2023 (blue points).

and fall, we fertilized and trimmed large shoots. In February 2023, after a foliar analysis and fertilization to get the nutrients to the proper levels, we commenced the second round of cuttings. The new shoots on the hedges were approximately 2.5 – 5 inches (~6 – 13 cm) in length. Once they reached this size, they were clipped and stuck individually into Ray Leach Super Cells in the mist house at the Horticulture Field Lab in Raleigh, NC. We targeted 14 cuttings from each hedge. These ramets will be planted into ACE2 clonal trials in the fall and winter of 2023-24. Of course, this project would not have been possible without the help from our resident expert for rooted cutting production and an army of undergraduate student workers (**Images below**).



Image 1. Rooted cuttings for the ACE2 population consisting of about 1300 clones and 18,000 cuttings.



Above: Our good friend and rooted cutting expert, Dr. Barry Goldfarb, visited with us to give advice before we got started. L to R: Sarah Conner, Fikret Isik, Barry, Nasir Shalizi, Trevor Walker, and Austin Quate.



Middle: Undergraduate student workers Ashley Kerr (left) and Jenna Abernethy (right) stick cuttings for rooting.



Upper right: Undergraduate student Gray Tyrey won the award for sticking the last of the ~18k cuttings!

Lower right: Undergraduate students Meredith Flaherty (left) and Margaret Haney (right) taking cuttings from ACE2 hedges.



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Arrowhead Breeding Center Update

The continued partnership between the Georgia Forestry Commission and TIP has resulted in another very successful breeding season at the Arrowhead Breeding Center near Cochran, GA. This year, almost 100 5th-Cycle crosses were made and pollen was collected from 102 selections for next year's breeding. The weather this year was much more fa-

vorable compared to 2022 (which had a freeze event in mid-March), as demonstrated by a much higher flower survival at bag removal (91% in 2023 versus 39% in 2022) (**Table 1**). The number of bags installed by TIP staff in 2023 was 281, which is the most in the last few years as 5th-Cycle selections are becoming reproductively mature.

Table 1: Bag installation and flower counts for the 2023 and past breeding seasons at Arrowhead.

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
2023	281	2468	2249	91%	9.6	9.1

The breeding activities at Arrowhead were performed by TIP Staff with help from the Georgia Forestry Commission (**Image 1**), South Carolina Forestry Commission (**Image 2**), and the Virginia Department of Forestry. These Full Members elected to help out at Arrowhead rather than conduct breeding

in their own orchards. Their participation also presents an opportunity to learn breeding techniques from one another. We really appreciate the members' collaboration and contributions to our efforts at Arrowhead!



Image 1. Tree Improvement Program staff (Trevor Walker, Sarah Conner, and Chuck Little) and Georgia Forestry Commission staff (Anita Johnson) smile for the camera while taking a break during another busy spring at the Arrowhead Breeding Center. Anita (2nd from left) and Chuck (far right) are the local folks who make the Arrowhead Breeding Center the great success that it is. We are indebted to their hard work and dedication to the Cooperative's mission to increase value to landowners and citizens through continuous genetic improvement of forest trees.

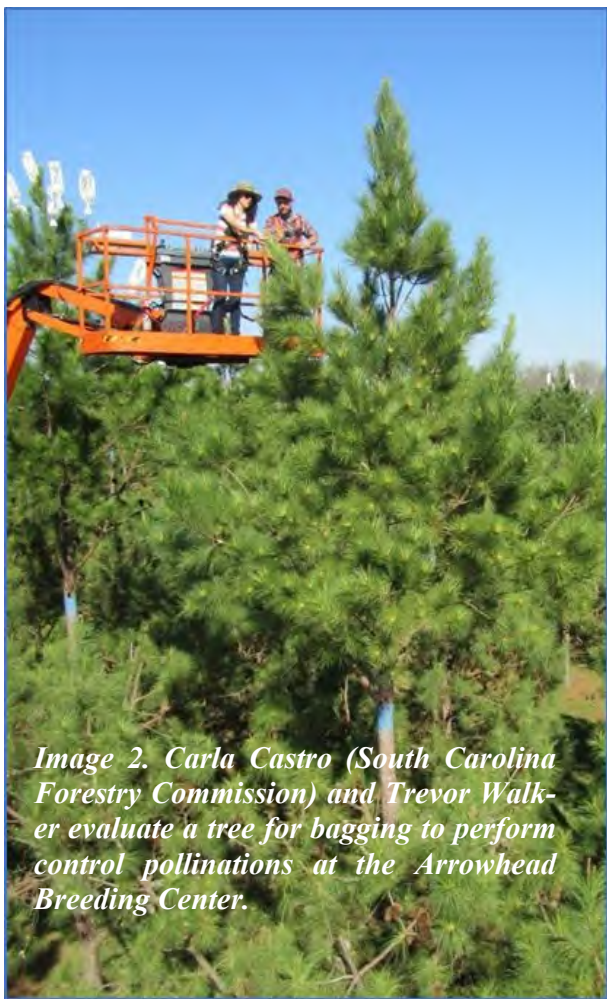


Image 2. Carla Castro (South Carolina Forestry Commission) and Trevor Walker evaluate a tree for bagging to perform control pollinations at the Arrowhead Breeding Center.

The success of a pine breeding program depends on an abundant supply of mature (25+’ tall) stock for topgrafting. At Arrowhead, TIP staff have been using 181210 as an interstock clone that promotes topgraft success and early flowering. Much planning has gone into ensuring that interstock availability will *not* be a bottleneck to turning over breeding cycles quickly (**Map 1**). In 2010, TIP staff established the “New” Breeding Orchard, which was not the best name in hindsight, and is more affectionately referred to as the “NBO”. The NBO provided around 300 compatible interstocks for the 4th Cycle and the first two years of 5th-Cycle selections.

In 2022 and 2023, the Northern Breeding Ramet Orchard (NBRO) was used for topgrafting 5th-Cycle selections, with the 2022 grafting including ACE2 genomic selections and GRID selections (see last year’s annual report for more details). The NBRO rootstock were planted in October 2014 and were additional ramets from the Northern Elite population clonal testing effort. Several selected clones were used for breeding in 2019-2022, but the vast majority of the rootstock were converted to the 181210 interstock clone with the intention of topgrafting 5th-Cycle selections. In spring 2023, 71 new 5th-Cycle selections (479 total grafts) were topgrafted into the NBRO interstock (**Image 3**). The interstock trees in the NBRO averaged 35 feet in height and were about the ideal size for

Map 1. Outlined are the Arrowhead Breeding Center orchard blocks established from 2008 to 2021. These blocks contain 18-1210 interstocks that promote of graft success and early flowering. The NBO (“New” Breeding Orchard) was established in 2008 and had 4th-Cycle selections topgrafted starting in 2014 and 5th-Cycle selections topgrafted in 2020 and 2021. The NBRO (Northern Breeding Ramet Orchard) was topgrafted with ACE2 genomic selections and GRID selections in 2022 and 5th-Cycle selections in 2023. In 2024, topgrafting will commence in the Graveyard Orchard (GYO1). An adequate supply of 25'+ interstock is required to expedite flower development, and much planning has gone into ensuring that there will be plenty of 181210 interstock at the Arrowhead Breeding Center. The map does not include the blocks RR3, CBRO, and BLOCK 4 which were used in 4th-Cycle breeding but did not include the improved interstock.

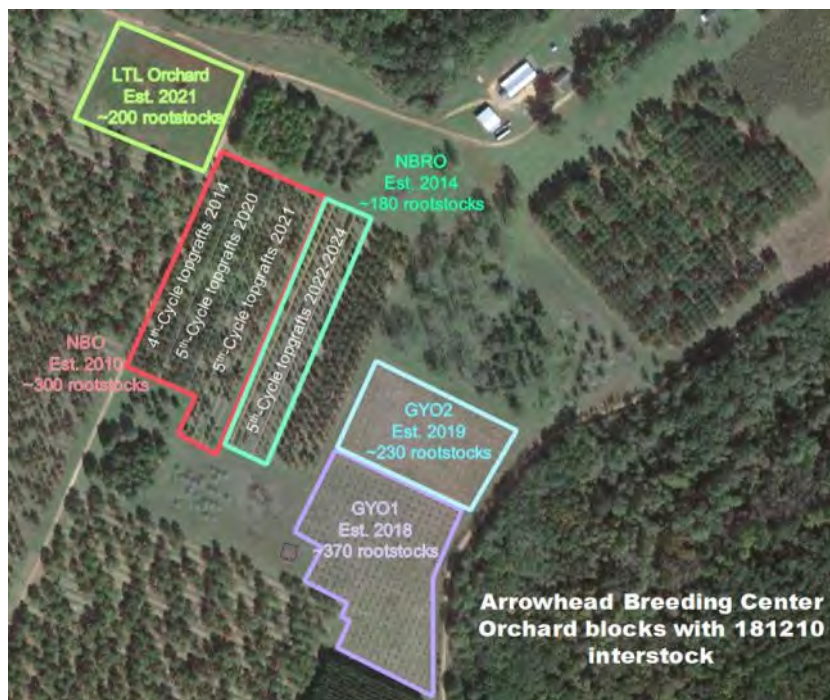




Image 3. Steve McKeand topgrafting a 5th-Cycle selection into the Northern Breeding Ramet Orchard (NBRO) at Arrowhead.

topgrafting. Our experience is that topgrafting into trees that are at least 25 feet tall will promote flowering. Trees that are taller than about 50 feet promote flowering, but access to the crowns and maneuvering to do pollinations becomes difficult. There are about 50 more interstocks in the NBRO available for topgrafting that we expect to be about 40 feet tall in 2024.



Image 4. The Graveyard Orchards were grafted with 181210 interstock scions in 2019 and 2020 and will be ideal for topgrafting 5th- and 6th-Cycle selections over the next several years.

In 2024, we will commence topgrafting in the Graveyard Orchard (Image 4), which should be at an adequate height just in time! The older block of the Graveyard Orchard (GYO1) was planted in October 2017, grafted with 181210 interstock scions in February 2019, and has about 370 trees available for topgrafting. These trees averaged 22.4 feet in height in 2023 and should be ideal for topgrafting in 2024 and beyond. The younger Graveyard Orchard (GYO2) was established a year after the GYO1, and the interstocks averaged 14.4 feet in height in early 2023. The GYO2 contains 230 interstocks that will be ready for topgrafting in 3 to 4 years.

This year, we grafted 181210 scions into a new orchard block named the “Little Orchard” in recognition of Chuck Little and his work with GFC and TIP. The Little Orchard, planted in 2021, is on track to have large interstock trees for future breeding seasons. TIP staff members worked with Ones Bitoki from the Virginia Department of Forestry to get the 200+ grafts made in 2023 (Image 5).

We had additional help from Lou McKeand this year at Arrowhead. Steve’s wife (pictured below) “volunteered” to join the crew and was a big help with pollen processing, data entry, scion preparation, cooking, and helping out wherever she was needed. We hope she comes back to visit again!





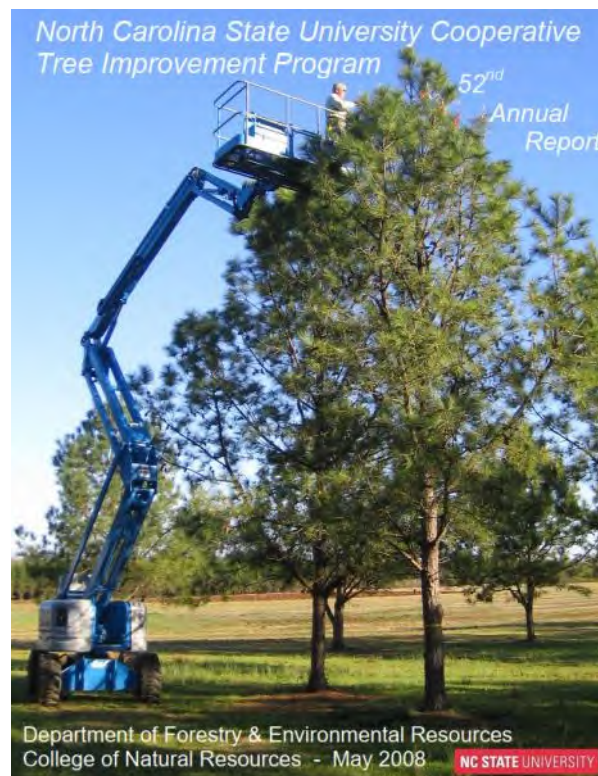
Image 5. Scions from 181210 were grafted into the Little Orchard at the Arrowhead Breeding Center. We expect/hope to have excellent graft survival with such an excellent crew of experienced and “less experienced” grafters. L to R: Ones Bitoki (VDoF) and Nasir Shalizi (TIP), Sarah Conner (TIP Grad) and Trevor Walker (TIP).

A look back: Here, here to 15 years!

While it seems like yesterday, 15 years ago was both a stressful and exciting time for the cooperative. We were in uncharted waters but opportunities were still abound, and some have been a real success story!

15 years ago the Cooperative was at a crossroads. We needed to adapt to the new forestry landscape in order to continue providing value to both forest industry and the landowners. In 2008, the partnership with Georgia Forestry Commission was sealed, and the Arrowhead Breeding Center was started. It has been a huge success, not only for the Cooperative breeding aspects but also for teaching and extension.

2008 also marked the year of the BIG BLUP and ACE. The BLUP analysis has provided more reliable breeding values and is still used today to analyze our population. ACE, otherwise know as Atlantic Coastal Elite population, was born. This original elite population was put through the paces, it was prescreened for rust, cloned, and field tested to extract within-family heritabilities and increase selection power. It worked well, and the ACE2 population followed. The experimental design of these populations have also allowed us to utilize new genomic tools in order to harness genetic selection at a potentially higher order.



To read more about what TIP was up to 15 years ago, and all other years, please visit: www.treeimprovement.org/annual-reports

SEED AND CONE YIELDS

For the last 55 years, members of the Cooperative have provided their seed and cone yields from their fall cone collection. There appears to have been an exceptional cone crop from the 2022 harvest (**Table 1**). The trend of collecting more seed from the 3rd-Cycle orchards and less seed from 2nd-Cycle orchards continues (**Figure 1**). The fall 2022 harvest marks the first year when seed from 4th-Cycle orchards were recorded. While controlled-pollinated seed have been mass produced for more than two decades, this year was the first that members provided their seed and cone yields from controlled pollinations, which constituted about 15% of the total seed harvest in 2022. As expected, yields for open-pollinated cones were higher than for controlled-pollinated cones for pounds of seed per bushel, with open-pollinated cones averaging 1.42 pounds of seed per bushel and controlled pollinated cones yielding 1.29 pounds of seed per bushel.

From 1968 through 2022, there has been a total of around 1500 tons of seed produced from members' orchards (**Figure 2**)! Note that the cycle names (2nd-, 3rd-, 4th-Cycle) refer more to the age of the orchard and not necessarily the generation of the clone that was harvested. The cycle of the orchard is the same as the set of progeny test data that was available for selecting the orchard parents. For example, the Cooperative is still in the thick of 4th-Cycle progeny testing, so very few orchards have been established or are producing seed using parents selected with this dataset. The 3rd-Cycle orchards were established using progeny test data from both the 2nd-Cycle diallel tests and the 3rd-Cycle pollen-mix tests. During the 2nd-Cycle, true 2nd-*Generation* orchards were typically established using untested 2nd-*Generation* selections and were later rogued as progeny test data became available.

Table 1: Fall 2022 cone and seed yields compared to the 2021 harvest.

Provenance / Cycle	Bushels of Cones		Pounds of Seed		Pounds of Seed per Bushel	
	2022	2021	2022	2021	2022	2021
Coastal: 2 nd -Cycle	5,899	5,111	8,814	6,369	1.49	1.25
Coastal: 3 rd -Cycle	26,533	17,145	38,175	22,986	1.44	1.34
Coastal: 4 th -Cycle	204	-	326	-	1.60	-
Piedmont: 2 nd -Cycle	2,340	2,196	3,471	3,111	1.48	1.42
Piedmont: 3 rd -Cycle	14,585	6,835	20,747	8,065	1.42	1.18
Northern: 2 nd -Cycle	-	76	-	63	-	0.83
Northern: 3 rd -Cycle	3,045	3,296	3,319	3,608	1.09	1.09
Totals	52,605	34,659	74,852	44,203	1.42 Avg.	1.18 Avg.
Coastal: Controlled pollination	7,962	-	10,530	-	1.32	-
Piedmont: Controlled pollination	1,829	-	2,308	-	1.26	-
Northern: Controlled pollination	340	-	331	-	0.97	-
Totals	10,131	-	13,169	-	1.29 Avg.	-

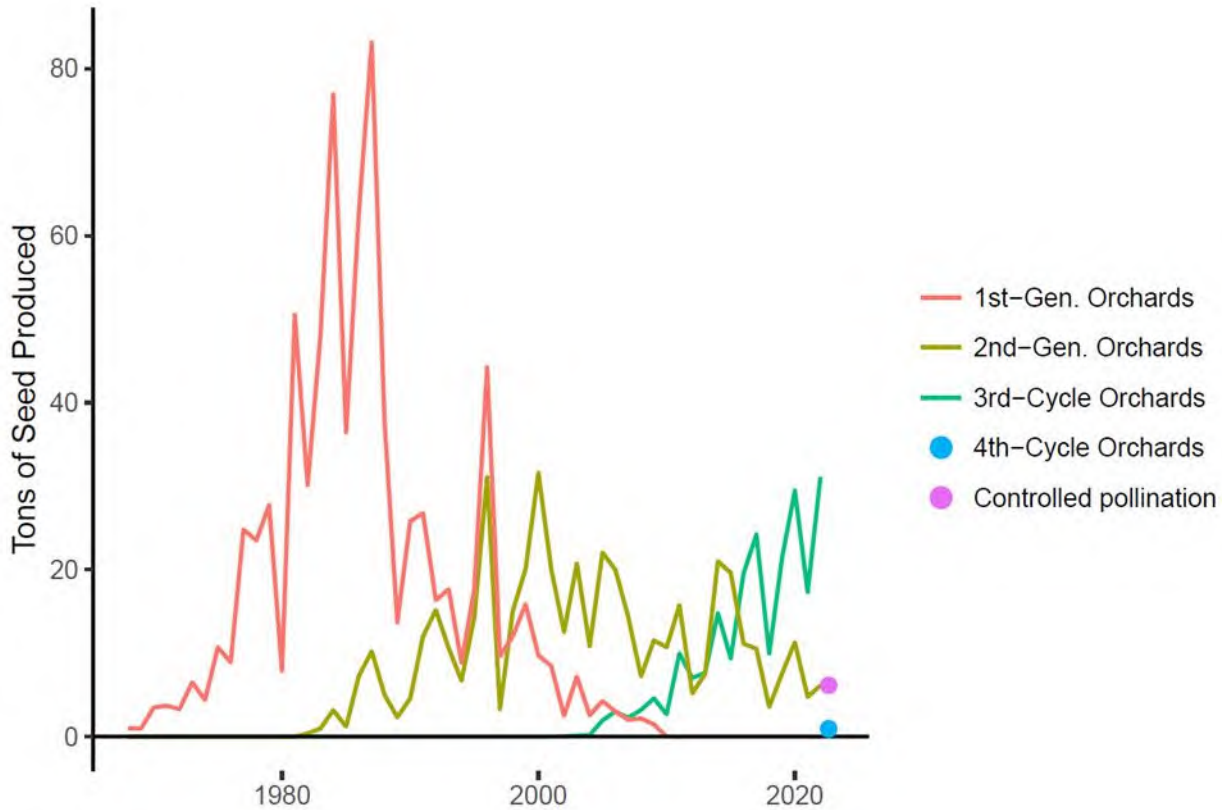
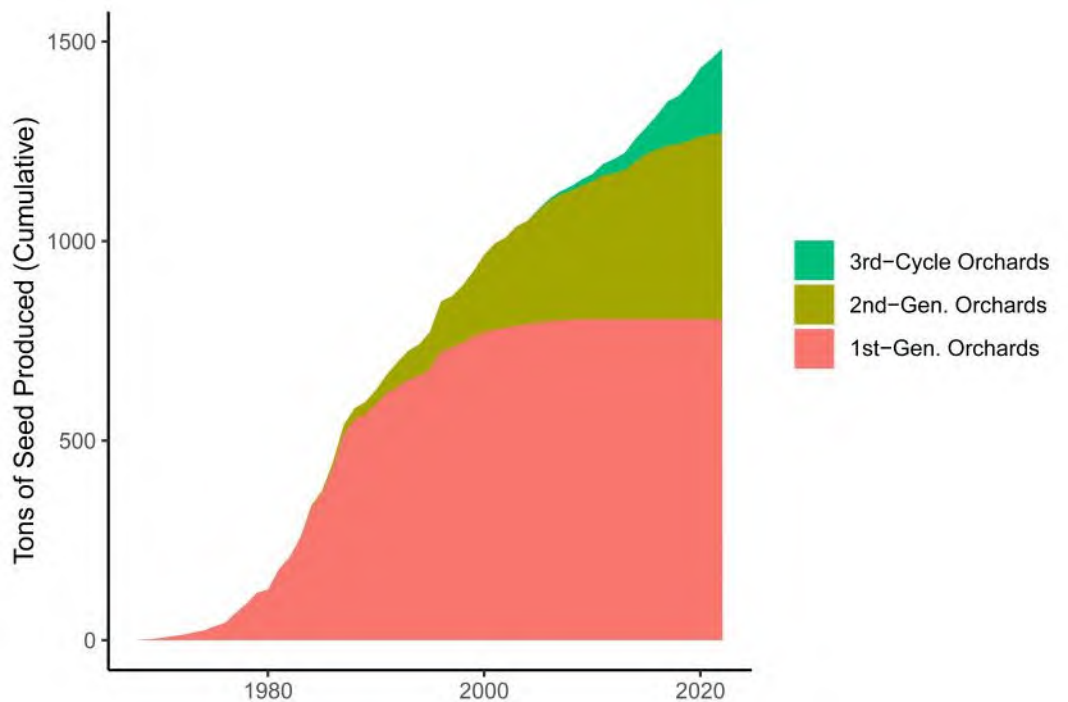


Figure 1. Since 1968, the Cooperative has tracked annual seed yields from Cooperative members. In 2022, the vast majority of seed (80%) came from 3rd-Cycle seed orchards, while the remaining 20% came from 2nd-Generation orchards and a very small amount from 4th-Cycle orchards. No 1st-Generation seed orchards have been harvested since 2009. Controlled pollinated seed made up about 15% of the seed harvested in fall 2022.

Figure 2. The cumulative seed yields over time from 1st- and 2nd-Generation orchards and the increase in production from 3rd-Cycle orchards in recent years illustrates how Cooperative members strive to collect from the most valuable parents that will yield the highest value seedlings for landowners.



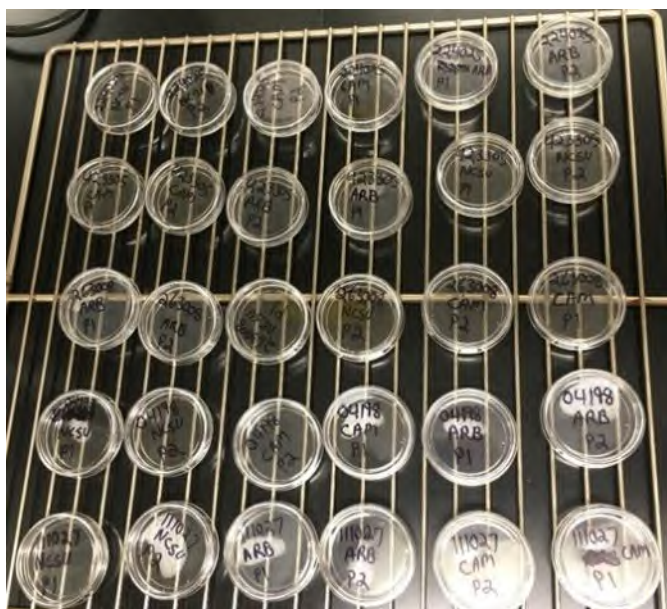
RESEARCH

Expanding our Knowledge About Loblolly Pine Pollen – Research Update¹

The Cooperative initiated two research projects (introduced in the 2021 Annual Report, pages 21-23) about the impact of pollen characteristics on filled seed production in controlled pollinations. The motivation for this research came from the Cooperative's multi-year pollination bag study, where open-pollinated cones produced a significantly higher proportion of filled seed and lower proportion of first-year aborted ovules per cone than cones from controlled pollinations (Heine et al. 2023).

The first project involved using three different tests of pollen viability. The objective was to understand

the advantages and disadvantages of each testing method for predicting the successful development of filled seed after a controlled pollination. The testing methods included respiration, impedance flow cytometry, and the standard germination test on agar (Image 1 - 3). In the spring of 2020, pollen from five male parents were applied to seven female parents at the Arrowhead Breeding Center. Cones were collected in the fall of 2021, and cone analysis was completed during the spring/summer of 2022. The number of filled seed per cone and the seed efficiency (proportion of fertile ovules producing seed) will



Images 1-3. Top left is the standard agar plate germination test, top right is the AmphaZ30 used to test impedance flow cytometry, and the bottom image is the Hansatech Oxytherm+Respiration testing machine.

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

be compared to the pollen viability test results to determine which method best predicts controlled pollination success.

The second project aims to determine if the stage of pollen development at time of collection impacts the controlled pollination success. In the spring of 2021, pollen was collected in the Rayonier Yulee Seed Orchard from five unique clones at five different stages of development, using a classification system similar to that described in Bramlett and Bridgwater (1989). Following pollen extraction, pollen was refrigerated, transported, and then applied to ten different female parents in the same spring season at the Arrowhead Breeding Center. Cones were harvested in October of 2022, and cone analysis is now underway to measure the proportion of ovules that produce filled seed. These results will be valuable for guiding pollen collection practices and yield insights on how pollen development affects controlled pollination success.



Pictured above: Austin Heine (PhD student) surrounded by cones in October of 2022 for the pollen development classification study. Cone harvest was successful leaving no shortage of future data points for cone analysis!

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Heine, A.J., T.D. Walker, J.B. Jett, F. Isik, and S.E. McKeand. 2023. Pollination bag type affects ovule development and seed yields in *Pinus taeda* L. For. Sci. 69(2):187-199. (<https://doi.org/10.1093/forsci/fxac052>)

Sometimes you have to call in the other experts...



While some folks really retire, others will occasionally step out to lend an expert eye! After several harsh freeze events in Raleigh this winter, TIP staff was worried about the ACE2 clonal hedges, and if they were up to the task for another round of taking cuttings this spring. Dr. Barry Goldfarb evaluated the hedges with TIP staff and looked for problems such as poor quality cuttings, hedge vigor, and signs of cold damage. Good research often means collaborating with other experts to make sure the projects have the best outcome. We appreciate all of the research collaborations over the years and all of those to come!

Image: Barry Goldfarb, Sarah Conner, Fikret Isik, Trevor Walker, Nasir Shalizi, Austin Quate.

PRSTM Calibration Study Update and Preliminary Analysis of Age-9 Data

The PRSTM system of the Cooperative Tree Improvement Program estimates the performance of improved loblolly pine families deviated from the performance of checklots based on progeny tests measured at four to six years of age (e.g., percent improvement for **P**roductivity score). The Cooperative Tree Improvement Program established a series of family block-plot tests in the southeastern US to better understand the impact of **P**erformance **R**ating **S**ystem (PRSTM) scores on stand growth and yield.

Progeny tests are comprised of many genotypes in either single-tree plots or six-tree row plots. An important question is how the scores estimated from these individual-tree measurements at young ages correspond with stand-level yield throughout the rotation, the actual trait of interest for landowners. The PRSTM Calibration trials were designed to evaluate this correspondence and allow biometricians to modify growth and yield models based on progeny test assessments. The study design is unique in that it focuses on testing a range of **P**roductivity scores rather than focusing on individual families, which permits the results to be applied to new families generated by the breeding program.

Table 1. The number of test sites measured by age and series for the PRSTM Calibration Study (series 555). This analysis focuses on the Coastal trials that have the most data. Analyses of the Piedmont and Northern trials are currently underway.

Age (year)	Coastal	Piedmont	Northern
1	11	6	4
2	2	2	-
3	3	2	-
4	9	4	3
5	1	1	-
6	10	5	5
7	2	-	-
9	7	2	-
10	1	1	-

In 2013 - 2014, the Cooperative planted 23 family block-plot tests across the southeastern US. As of spring 2023, nine of the trials have been measured through age nine and two through age ten (**Table 1**). A recent publication includes an analysis of the Coastal series using age six-year data (Shalizi et al. 2023). In this report, we present preliminary results from ten Coastal tests measured through nine years (**Figure 1**).

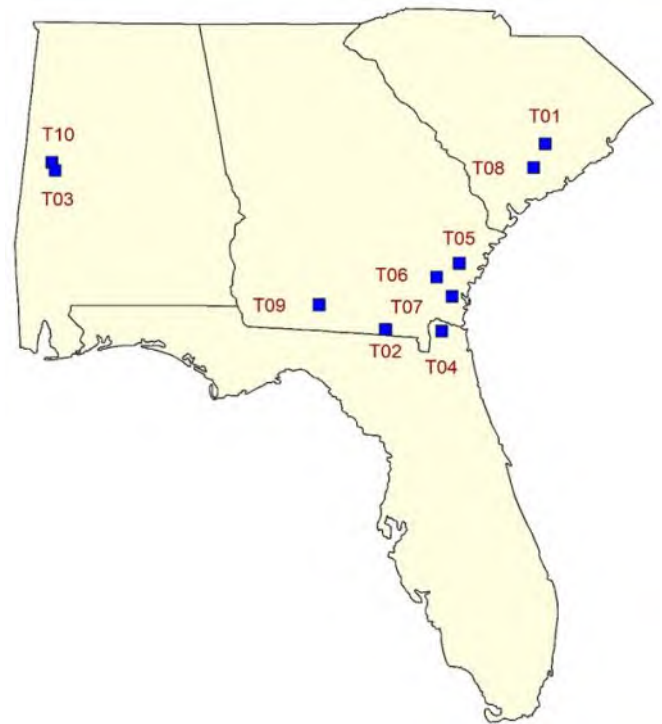


Figure 1. The geographic location of the ten Atlantic Coastal population PRSTM calibration study tests established in South Carolina, Georgia, Alabama, and Florida.

The differences in stand volume growth among families were significant and generally stable through age nine years (**Figure 2**). Overall, high **P**roductivity families maintained higher growth rates than low **P**roductivity families. The correlations between **P**roductivity scores from progeny tests and observed stand volumes from family block-plots were high at most test sites, suggesting that the predicted volume scores for families from individual-tree progeny trial measurements had similar ranking for stand-level

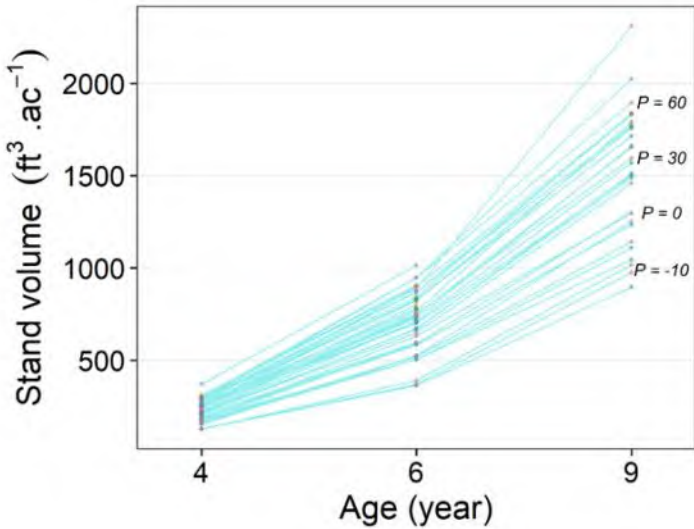


Figure 2. Predicted stand volume at ages 4, 6, and 9 for the 42 families assessed in the Coastal **PRS**TM calibration trials. **P**roductivity scores for some families are provided in the figure. The zero **P**roductivity score corresponds to the CCK checklot.

growth (**Figure 3**). However, the overall correlations were moderate at each age. This is because stand volume is not purely affected by family productivity. Other factors such as site quality, survival, and susceptibility to fusiform rust disease also affect volume per unit area. Only accounting for site effect improved the correlations considerably (**Figure 4**).

To predict volume per acre across sites at age nine, 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, survival, and site index). When assessed across sites, volume **P**roductivity scores, survival, and test site significantly affected stand volumes per acre. Survival was negatively correlated (-0.63) with the stand rust disease incidence.

These findings suggest that superior families with high **P** scores will generally perform better regardless of site quality or survival. Still, to obtain higher wood yields, landowners should plant the best families with lower rust risk and aim for high planting survival. These results are based on age four to nine year data (pre- and early-crown closure) and will be validated with data through rotation age to make the best decisions.

There have been numerous inquiries from members regarding the appropriate timing of the thinning of the **PRS**TM Calibration study. The challenge is that the thinning intensity (reduction in stand density) is confounded with the productivity score, such that faster-growing families will be subjected to a higher thinning intensity if all plots in a stand are thinned at the same time.

At a particular age, faster-growing families should have less live crown length compared to slower-growing families and could not be expected to have a fair thinning response. Thinning a subset of the plots or thinning portions within plots would be lo-

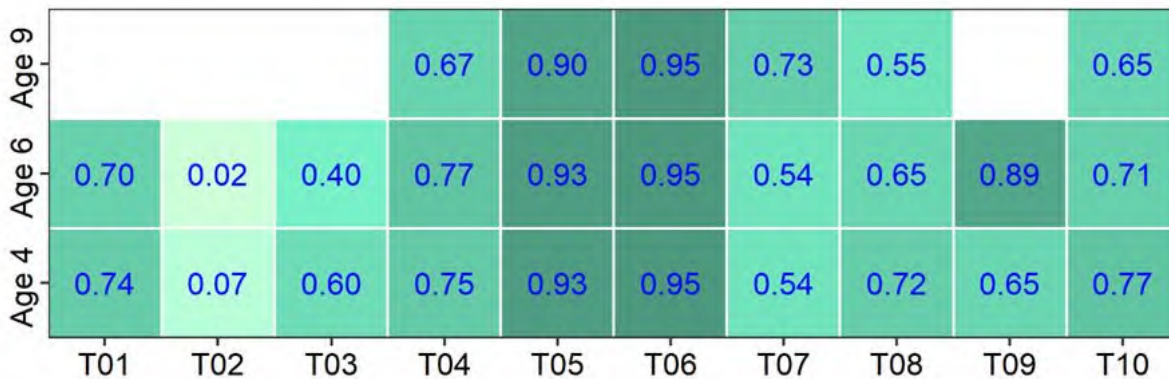


Figure 3. The rank correlation between **P**roductivity scores from progeny tests and observed stand volume from family block plots at ages 4, 6, and 9 years at each test site. Correlations were moderate to high in the majority of the test sites. Note: These correlations are based on a small sample size (9 to 12 families per site).

gistically difficult and prone to experimental error. While continuing the study as non-thinned does not represent operational deployment, it should provide the data necessary to modify base growth and yield models and test hypotheses about genetic selection effects on stand dynamics and carrying capacity (e.g., are we selecting trees that fill empty spaces

faster, or trees that are more efficient at resource utilization). Modified models could then be coupled with a thinning response model (assuming genotypes respond similarly to thinning). A more elaborate experimental design would be required to evaluate genetic variation in thinning response.

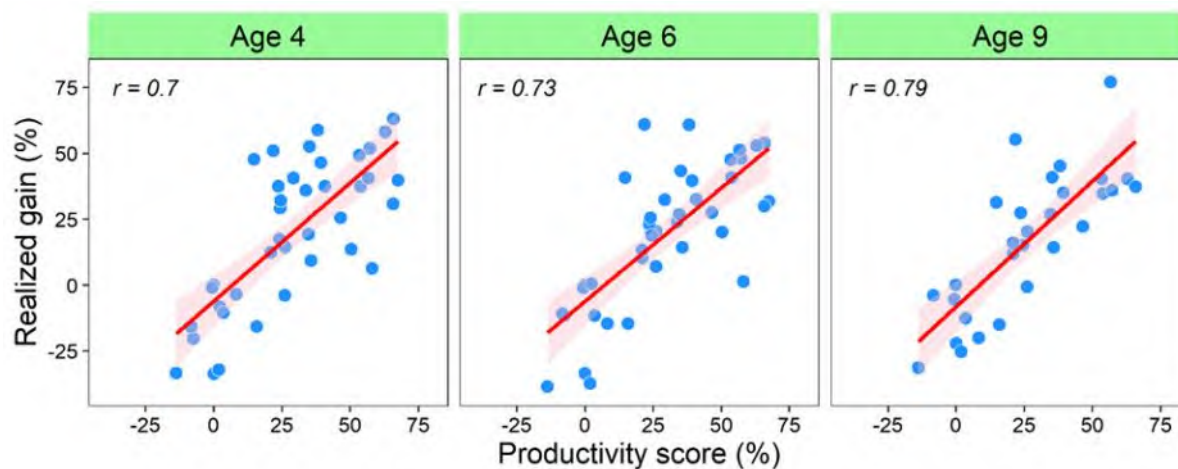


Figure 4. Across-site correlations between **P**roductivity scores from progeny tests and volume gain from family block plots at ages 4, 6, and 9 years. Rank correlations between the two variables are shown on the top left of each plot. Each dot corresponds to a predicted family mean adjusted for the site effect.

Fusiform Rust Hazard Map Update

In the last few decades, several fusiform rust hazard maps have been published (Squillace 1976; H R Powers et al. 1981; Lenhart et al. 1994; Randolph et al. 2015; Walker and McKeand 2018). These maps show a general trend of decreasing fusiform rust hazard in more northern and inland regions, and a “rust belt” of high hazard that extends from central Mississippi to central South Carolina. Periodic updates of these maps are necessary to include newer data and evaluate for changes in the hazard through time. Hazard is expected to increase with increasing silvicultural intensity (Schmidt 2003), and the effect of a changing climate adds further uncertainty. The Cooperative progeny testing data set is perhaps the best source of information for hazard mapping loblolly pine plantations in the southeastern US. This is because of the wide geographic range of test sites and the rust resistance of the genetics is well-characterized. Unfortunately, the US Forest Service Forest Inventory and Analysis data typically do not include information about the genetic resistance to

rust which can lead to an under-prediction of hazard for sites planted with resistant families.

Walker and McKeand (2018) used 2nd-Cycle progeny test data to make a hazard map for the southeastern US based on the observed performance of non-improved checklots which have no artificial selection for rust resistance. More recently, TIP staff have made maps including 3rd-Cycle and 4th-Cycle data based on the predicted non-improved checklot performance in the big BLUP analyses (**Figure 1**). Simone Lim-Hing, researcher from the University of Georgia, has also been using these data with more advanced statistical models to interpolate hazard where there are spatial gaps in progeny test data (**Figure 2a**) and using climatic variables such as consecutive frost days and water deficit to improve rust hazard estimates (**Figure 2b**). Future research plans include modelling climate variables that contribute to fusiform rust incidence and developing genotype-specific maps.

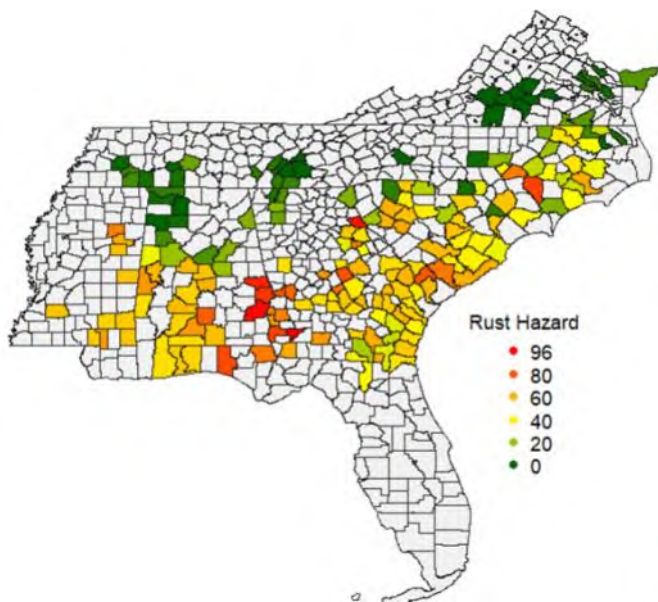


Figure 1. (left) County average rust hazard based on the local, non-improved checklots' rust incidence in 2nd, 3rd, and 4th-Cycle progeny test data using estimates from the big BLUP.

Figure 2a. (upper right) Spatially interpolated distribution of fusiform rust disease using inverse distance weighted kriging. This map was generated by Simone Lim-Hing at the University of Georgia.

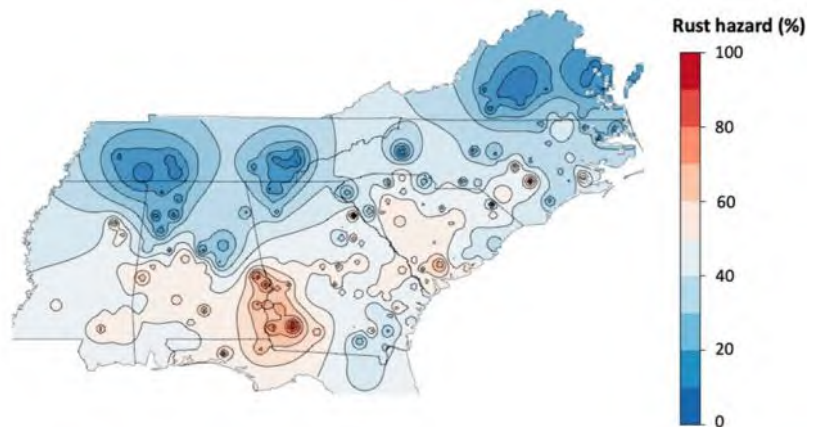
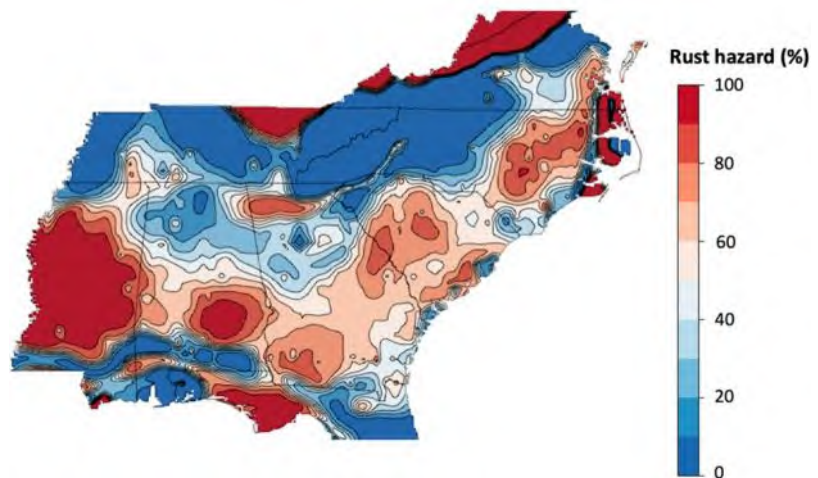


Figure 2b. (lower right) Spatial interpolation of the predicted fusiform rust disease incidence for non-improved loblolly pine using spatial and climactic predictors (consecutive frost days and water deficit). This map was generated by Simone Lim-Hing at the University of Georgia.



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AgriSeq™ Genotyping Panel for Quality Control in *Pinus taeda* Breeding¹

The Cooperative Tree Improvement Program, in partnership with Thermo Fisher, developed an AgriSeq™ targeted genotyping-by-sequencing panel for quality control in *Pinus taeda* breeding. This marker panel includes 995 single nucleotide polymorphic (SNP) markers selected from the Pita50K array. These 995 SNPs are considered high-quality because they were selected from a much larger subset based on their performance in previously genotyped populations (including the Cooperative's Coastal breeding population), as well as their spread across the genome using the linkage map developed by Lauer and Isik (2021). This genotyping panel is a cost-efficient solution for detecting pedigree errors and assigning parentage to individuals. The genotyping cost per sample using this panel is only one-third of the cost of using the Pita50K SNP array, making it a more affordable and practical option for pedigree confirmation. In this research, we present preliminary results of the AgriSeq genotyping panel for pedigree errors using the second-generation Atlantic Elite Population (ACE2).

We first calculated genetic relationships between individuals using three sources: pedigree, Pita50K genotyping array, and AgriSeq panel. The correlation between genomic relationship coefficients from the AgriSeq (995 markers) and Pita50K array (34,095 markers) was 0.90. Using the expected ge-

netic relationships based on pedigree (A_{ij}) and realized genetic relationships (G_{ij}) from AgriSeq markers, we investigated the discrepancies for individual trees. A significant discrepancy was defined when the difference between standardized values of G_{ij} and A_{ij} exceeded three standard deviations for a given pedigree category (half-sibs, full-sibs, offspring-grandparent, etc.). The frequency of significant discrepancies among individuals in the population is given in the bar plot (**Figure 1**). Trees labeled as *a*, *b*, *c*, and *d* had a noticeably high number of significant discrepancies than others in the population. These large deviations from the pedigree-based genetic relationships are likely caused by errors in the pedigree records.

Next, a principal components analysis of genomic relationships calculated from the AgriSeq markers was used to cluster full-sib families. The clustering of eight full-sib families is given in **Figure 2**. Each dot in the plot is a tree in a full-sib family, and the families are shown in different colors based on pedigree records. The four outlier trees (*a*, *c*, *b*, *d*) were also detected by the principal components analysis plot. For example, according to the pedigree records, individual *a* belongs to Family 1. However, based on the genomic relationships from markers, this individual does not cluster near Family 1, and likely belongs to Family 2 or 7.

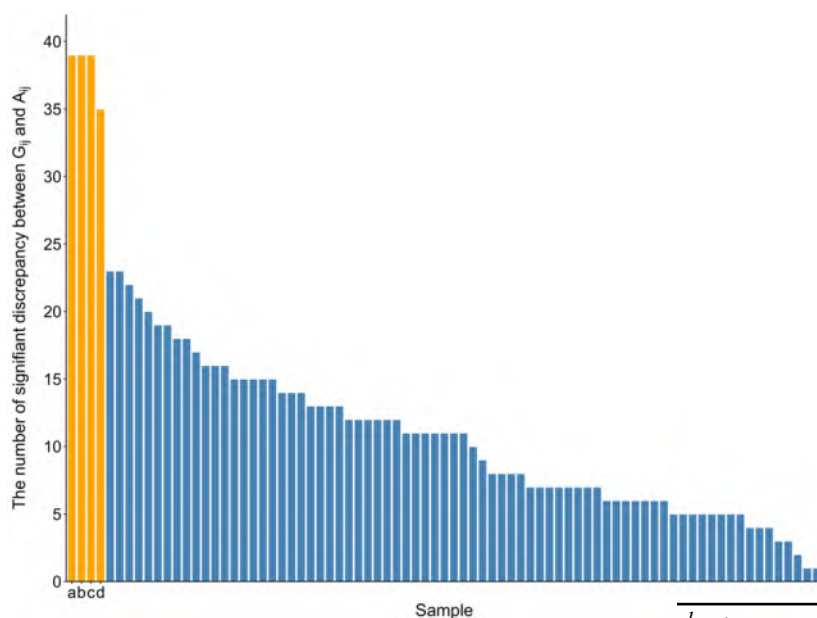


Figure 1. Distribution of the count of significant discrepancies between an individual's pedigree-based and SNP-marker based relationships using the AgriSeq panel. Four individuals labeled as *a*, *b*, *c*, and *d* had a high number of significant discrepancies, likely caused by pedigree errors.

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

² http://www.fieldgenetics.com/pages/aboutCervus_Overview.jsp

We also investigated the use of AgriSeq markers for parentage assignment with the CERVUS software. We considered using a reduced number of markers (50 to 500) to find the appropriate number of markers for parentage assignments. For each set, markers were selected based on high minor allele frequency and high genotype call rate. Parental pair assignments with unknown sex were carried out across marker sets with the software's default parameter settings. The results of parental pair assignments were compared to the pedigree, and the proportion

of consistent assignments between the CERVUS result and the pedigree were calculated (**Figure 3**). The consistent assignment accuracy was lowest at 0.69 when 50 markers were used, and the accuracy reached 0.79 when 300 markers were used.

In conclusion, the low-density cost-efficient genotyping platform produces similar genetic relationships as using Pita50K for detecting pedigree errors. The results are encouraging for operational breeding program and seed orchard/nursery quality control.

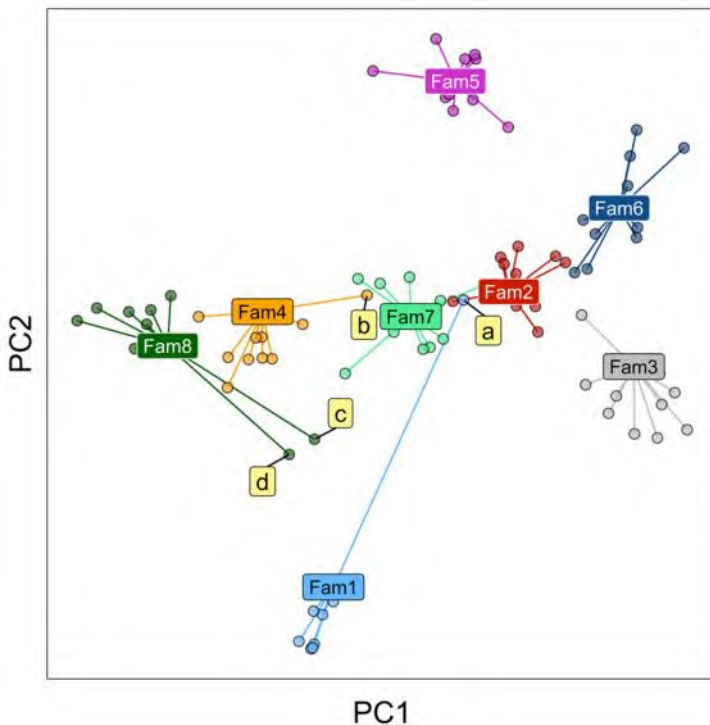
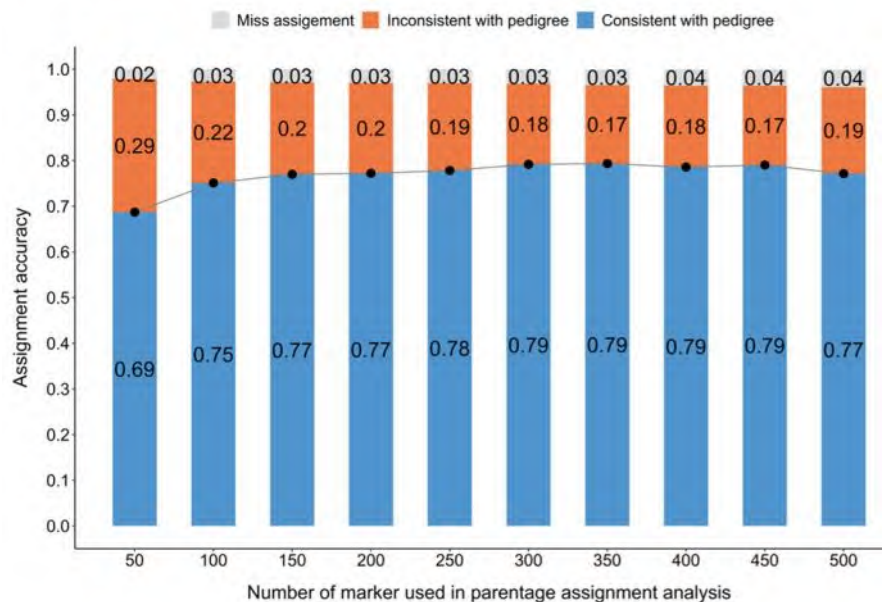


Figure 2. Clustering of eight full-sibling families using principal component analysis based on AgriSeq marker panel. Each circle represents an individual tree, and colors indicate full-sib families inferred from the pedigree. Samples a, b, c, and d deviated from their pedigree-based family clusters, indicating likely pedigree errors.

Figure 3. Parentage assignment analysis across a different number of marker sets for 663 ACE2 trees. For each marker set, the total assignments were 1326 for 2 parents of the 663 trees. For every bar, the consistent assignment between the CERVUS result and the pedigree was plotted in blue color, the inconsistent assignment was plotted in orange, and the miss assignment was plotted in grey. The number in each section represented the proportion of that assignment type. Lined dots indicated consistent assignment accuracy.

Paired parentage analysis (CERVUS) on 663 ACE2 population
N = 663 x 2 = 1326 assignments



Literature Cited

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

Butner Biomass/Cropping Study Update¹

A loblolly pine biomass/cropping study was planted by TIP staff near Butner, NC in the spring of 2012 (funded by NCDA Grant #17-072-4031). The purpose of the study is to evaluate different genetics (provenances and families) under various silviculture regimes to guide landowners looking to maximize their financial returns in both the bioenergy and sawtimber markets. The study site is in the Piedmont of NC, and a feature of the study is the inclusion of Coastal-source families to determine if the risk of cold damage is offset by their faster growth and the presence of a bioenergy market (where damaged trees can be commercially thinned). The study includes twenty families (ten from the Atlantic Coastal Plain provenance and ten from the Piedmont provenance) established at a high planting density (1,037 trees per acre). In the fall of 2020, three thinning treatments were applied: no thin, moderate thin (519 residual trees per acre), and heavy thin (288 residual trees per acre). These thinning scenarios were designed to allow comparison of scenarios focusing on biomass production versus biomass and sawtimber production.

The study has been measured every one to two years since planting (**Table 1**). This past winter, the trees were measured after their eleventh growing season, which marks two growing seasons after the thin. While the data are still being prepared for analysis, there are several observations we can share anecdotally. The no-thin treatment has fully occupied the site and has begun to experience mortality from self

thinning, as it has entered the “stem exclusion” stage of forest stand dynamics (**Image 1**). In the thinned areas, the form of the trees tends to be much better (the thinning treatment was “from below”, removing defective stems and leaving the best stems to grow into sawtimber trees). However, the thinned blocks have experienced wind damage, such as forks breaking out and wind-throw (**Image 2**), especially in the heavy-thin treatment. Stem form tends to be best in the heavy-thin treatment (**Image 3**), but trees with repeated cold damage can be found in all of the thinning treatments and provenances (**Image 4**).



Image 1. Stand density is very high in the no-thin treatment, and trees are beginning to experience competition-induced mortality.

Table 1. Measurement traits for every year this study was measured.

Year	Traits
1	Status, Total Height, Cold Damage
3	Status, Total Height, Cold Damage
5	Status, DBH, Total Height, Rust, Fork, Ramicorn
6	Status, DBH, Total Height, Sawtimber Potential
8	Status, DBH, Lean, Stem Breakage, Fork, Sawtimber Potential, Total Height, Stopper Height
9	Status, DBH, Total Height, Height to Live Crown, Stopper Height
11	Status, DBH, Sawtimber Potential, Total Height, Height to Live Crown, Stopper Height

¹ This is a summary of Austin Quate’s MS research



Image 2. The moderate-thin treatment with broken tops, broken-out forks on the ground, and some wind throw.



Image 3. (Above left) The heavy-thin treatment tends to have better residual stem form due to selective thinning and longer lengths of live crowns.

Image 4. (Above right) Repeated cold damage is a common occurrence at the Butner biomass/cropping study.

Very low seed set observed in hybrid *Pinus taeda* x *teocote*

Deployment of pine hybrids for reforestation is common in regions including Australia, Asia, South America, and Africa, accounting for up to 13% of the plantation area for some forestry companies (Dungey 2001). Successful hybrids are typically characterized by either enhanced growth from heterosis or from complementarity of traits (e.g., one species adds disease resistance or frost tolerance). Foresters in the southern US could potentially benefit from hybrids with *Pinus taeda*, as they can provide access to greater genetic variation than occurs naturally within the species and may be useful to mitigate future threats from climate change (e.g., outbreaks of invasive exotic pests/disease).

Previous research with hybrids among *P. taeda* and other *Pinus* spp. native to the US South found intermediacy in their traits, resulting in poorer productivity (Lilly et al. 2012; Dipesh et al. 2015). The lack of heterosis is likely because of their highly sympatric natural ranges (Schmidting 2007). The Mesoamerican species *Pinus teocote* may be a promising candidate for hybridizing with *P. taeda* due to its resistance to rust disease (Lambeth et al. 1997). Hybrids among Mesoamerican pines have been successful for Central/South America, but lack frost tolerance, which *P. taeda* may impart.

In spring 2021, Dr. Kitt Payn led the effort to make controlled crosses to generate hybrid *P. taeda* x *teocote* seed. Six *P. taeda* females were crossed with a mix of ten *P. teocote* pollen lots. The *P. teocote* pollen were collected by a CAMCORE member from a progeny test in South Africa in 2020. The pollen

germination rates were variable among the pollen lots, ranging between 26 and 82% (averaged 67%).

Failure of hybridization occurred within the first year after pollinating for three of the crosses, which had more than 95% of their conelets aborted by Spring of 2022 (Table 1). The remaining three crosses had nearly 100% cone survival, but seed extraction was near-zero. Two of those crosses resulted in many pops during float testing. The other cross was practically all first-year aborted seed. Example of poorly formed cones and first year aborts are shown in Figure 1. Only seven seeds were extracted from the surviving *P. taeda* x *teocote* cones in the fall of 2022 (Table 1).

A TIP undergraduate student, Jenna Abernethy, was recently awarded an undergraduate research grant to investigate the *P. taeda* x *teocote* hybrids under the guidance of Dr. Nasir Shalizi. The objective of this research is to propagate the putative hybrid seed and confirm their hybrid status using data from seedling morphology and genomic markers.



Figure 1. Example of malformed cones from *P. taeda* x *P. teocote* crosses done at the Arrowhead Breeding Center Spring 2021 that resulted in a large number of first-year aborts.

Table 1. Results of the control-pollination of *P. taeda* x *P. teocote* hybrids using a pollen mix of 10 *P. teocote* selections at Arrowhead Breeding Center.

Female	Count of flowers Spring 2021	Count of conelets Spring 2022	Cones harvested Fall 2022	Seeds extracted Fall 2022
Taeda1	66	37	36	0
Taeda2	31	0	0	0
Taeda3	48	2	2	0
Taeda4	88	23	24	7
Taeda5	39	26	26	0
Taeda6	27	0	0	0

Additionally, microscopy is planned to evaluate the pollen from both species for morphological differences (grain size and shape), which would explain poor conelet retention and low seed yield. This work is the first to explore compatibility of hybridization

between these two species. If the seed are contaminants (i.e., pure *P. taeda*), then there will be a compelling body of evidence to support that these two species are incompatible due to pre-zygotic barriers.

Literature cited :

Dipesh, K. C., R. E. Will, T. B. Lynch, R. Heinemann, and R. Holeman. 2015. Comparison of Loblolly, Shortleaf, and Pitch X Loblolly Pine Plantations Growing in Oklahoma. *Forest Science*. 61(3):540–547.

Dungey, H. S. 2001. Pine hybrids—a review of their use, performance and genetics. *Forest ecology and management*. 148(1–3):243–258.

Lambeth, C. C., W. S. Dvorak, and C. H. Young. 1997. Fusiform Rust Resistance of Central American and Mexican Pine Species Compared with Loblolly and Slash Pines — Reforestation, Nurseries and Genetics Resources. in 24th Biennial Southern Forest Tree Improvement Conference, Orlando, FL. Available online at: <https://rngr.net/publications/tree-improvement-proceedings/southern/1997/fusiform-rust-resistance-of-central-american-and-mexican-pine-species-compared-with-loblolly-and-slash-pines>; last accessed January 26, 2023.

Lilly, C. J., R. E. Will, and C. G. Tauer. 2012. Physiological and morphological attributes of shortleaf × loblolly pine F1 hybrid seedlings: is there an advantage to being a hybrid? *Can. J. For. Res.* 42(2):238–246.

Schmidting, R. 2007. Genetic variation in the southern pines: evolution, migration, and adaptation following the pleistocene. P. 28–32 in *Shortleaf Pine Restoration and Ecology in the Ozarks*, General Technical Report NRS-P-15, Springfield, MO.

Dr. Fikret Isik was Named a Fulbright U.S. Scholar

Dr. Fikret Isik, Co-Director of the Cooperative Tree Improvement Program, received a Fulbright U.S. Scholar Program Science/Technology award to South Africa for the 2022-2023 academic year.

The Fulbright Program is managed by the U.S. Department of State and the Fulbright Foreign Scholarship Board and is the U.S. government's flagship international educational exchange program. It is supported by the United States Bureau of Educational and Cultural Affairs and partner countries worldwide. The participants are chosen for their academic merit and leadership potential. They are given the opportunity to exchange ideas and contribute to finding solutions to challenges facing our communities and our world. The Fulbright program fully sponsors Prof. Isik's visit to promote cultural exchange and mutual understanding between the United States and the host country South Africa.

Dr. Isik joined the University of Pretoria in South Africa in February 2023 as a Fulbright visiting Professor. He is hosted by Professor Zander Myburg, the director of the Forest Molecular Genetics Pro-

gram in the Forestry and Agricultural Biotechnology Institute (FABI) and Future Africa Institute at the University of Pretoria. His visit is also supported by the African Centre for Crop Improvement (ACCI) and the Institute for Commercial Forestry Research (ICFR) at the University of KwaZulu-Natal.

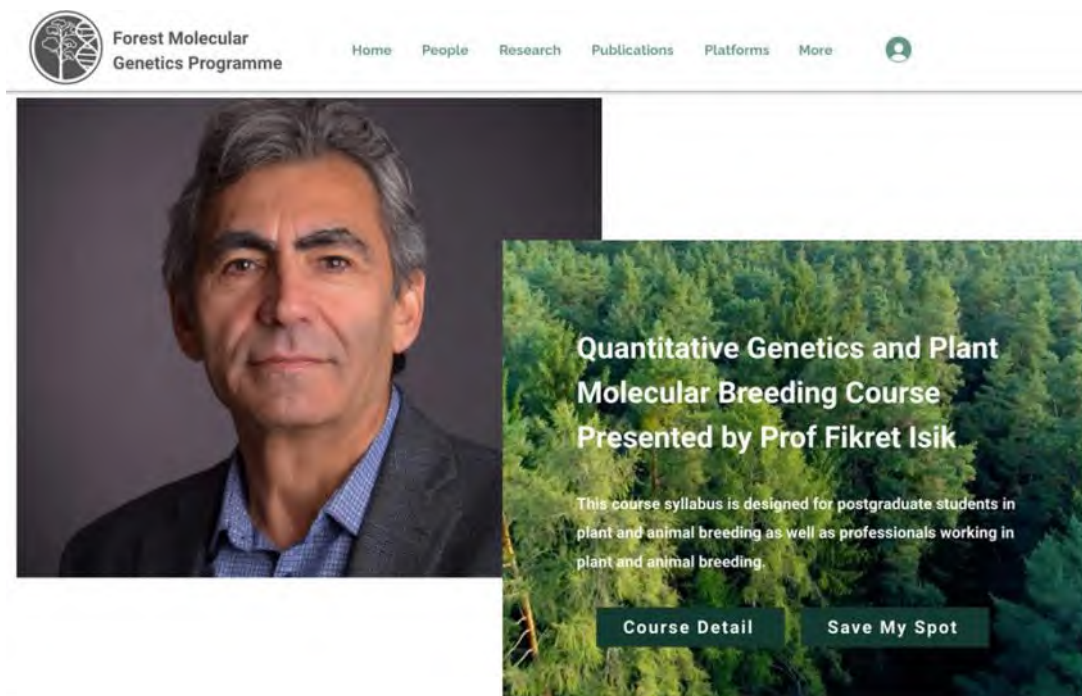
Future Africa Institute (University of Pretoria) and the African Centre for Crop Improvement at the University of KwaZulu-Natal are pan-African platforms that are set to inspire science excellence to transform Africa and the region. They aim to educate the next generation of scientists from sub-Saharan African countries. Dr. Isik is contributing to this greater cause.

As part of his sabbatical visit, Fikret is teaching an online Quantitative Genetics and Molecular Breeding course customized for graduate students and professionals in plant and animal breeding. The course focuses on quantitative genetics, the application of genomic tools, and genomic selection strategies. Two hands-on workshops will supplement the course materials to provide essential skills and

knowledge in molecular breeding. More than 40 participants from across South Africa enrolled in the course.

The course offers South African forestry professionals an excellent opportunity to gain insight into the latest research and techniques in tree breeding and genetics. It will hopefully contribute to their skill set. Ultimately, Fikret's visit will contribute to the development of the forestry industry in the country.

Fikret will also join Professor Myburg and others in a series of ongoing research projects, including developing cost-effective genotyping platforms for the South African plant and animal breeding community. While visiting the University of Pretoria and the University of KwaZulu-Natal, he hopes to establish long-term collaboration and student exchange with the North Carolina State University.



Forest Molecular Genetics Programme

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Quantitative Genetics and Plant Molecular Breeding Course Presented by Prof Fikret Isik

This course syllabus is designed for postgraduate students in plant and animal breeding as well as professionals working in plant and animal breeding.

Course Detail Save My Spot

Grants

New Grants

Nasir Shalizi, Jenna Abernethy, Sarah Conner, Trevor Walker. Genetics and Genomics Academy Summer Teamwork Mini-Grant. **Using genomic markers to confirm hybrid *Pinus taeda* x *Pinus teocote* seedlings.** \$3,000. 3/1/2023-8/15/2023.

Continuing 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. 07/15/2022-07/14/2026.

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.

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

ASSOCIATED ACTIVITIES

Meetings, Workshops, and Short Courses

In late May 2022 at the 66th Advisory Meeting in Raleigh, NC, the gang was back together again in person for the first time in two years! It felt good to be back in action with members and discussing tree improvement and forestry research in the same room. Folks were able to catch up and hear about how the group and members continued important research initiatives despite the pandemic. It was the spark we needed to get some other extension initiatives planned and executed for the coming year.

We were also able to pull together the TIP Short Course in conjunction with the TIP Contact Meeting in November 2022 at Lake Blackshear, GA. This short course was well attended by members and even some outside of the Cooperative that were looking for tree improvement training. Attendees visited the Arrowhead Breeding Center to see what new improvements had been made as well as discuss challenges and solutions that folks could take back to their own organizations. During these short

courses, there are a lot of great questions and open discussion that often prompts some really great research questions. We appreciate the folks that participate and help move forest research to new heights each time!

Another workshop was squeezed in by the end of 2022, “Computing Skills in Plant Breeding”. Dr. Isik, along with assistance from many other faculty, staff, and professionals, hosted a Data Analysis Workshop in Raleigh, NC. This was a 4-day intensive course that dove deep into data analysis challenges, software solutions, and programs that could assist folks with understanding their genomic information and better utilize the information within their breeding programs. It was attended by faculty, staff, students, and industry professionals. Those in attendance felt this was a workshop that could be given annually to help keep up with the advances in technology available to plant breeders; keep an eye out for future offerings if you missed this one!



Top left: 2023 Contact Meeting and Short Course attendees pose in front of the next round of breeding orchard at the Arrowhead Breeding Center. We want to give a big thanks to Georgia Forestry Commission and Jeff Fields for hosting the field tour. Both Chuck Little and Anita Johnson (GFC) had the Arrowhead Orchard looking majestic and it was a wonderful way to kick off our first meeting back in the woods!



Bottom left: Data analysis workshop, “Computing Skills in Plant Breeding”. These folks braved four long days of everything data analysis and even left wanting more! Stay tuned for future opportunities.



The faculty and staff members associated with the Cooperative are busy throughout the year with extension and outreach activities, both domestic and abroad. Below are records of faculty and staff invited as instructors/speakers:

- Fikret Isik. Invited speaker. Sitka Spruced Virtual Symposium - UK. “Genomic selection in *Pinus taeda* breeding in the southern USA. Lessons learned and moving forward.” (April 26-27, 2022).
- Fikret Isik (Invited talk). 15th International Christmas Tree Research and Extension Conference, Sierra Conference Center at Hidden Leaf Lake, California. (June 5-9, 2022).
- Nasir Shalizi and Fikret Isik (Invited speakers). North America Forest Genetics Society Inaugural Meeting and David Neale Symposium (June 13 – 17, 2022).
- Steve McKeand (Invited Presenter). Georgia Governor’s Office of Planning and Budget and House and Senate Budget Offices at the Arrowhead Seed Orchard and Breeding Center. Importance and value of reforestation and tree improvement to landowners and citizens of Georgia. (June 29, 2022).
- Steve McKeand and Austin Heine. The Longleaf Alliance meeting: Strategic Planning for Longleaf Pine Genetics, Andalusia, AL. (July 26-27, 2022).
- Steve McKeand and Trevor Walker. Attended the Forest Productivity Cooperative Advisory Committee Meeting in Raleigh, NC. (August 9-10, 2022)
- Fikret Isik Attended the 2022 National Association of Plant Breeding Conference. NIFA Project Directors meeting at Iowa State University, Ames, Iowa (August 7-10, 2022).
- Fikret Isik Invited speaker: Seminar on Cronartium, USDA-Forest Service Resistance Screening Center, Asheville, NC. Organized by Swedish Forest Tree Breeding and SLU (August 29, 2022).
- Trevor Walker (Webinar host). Lunch and learn with Tree Improvement Program: a webinar for American Forest Management. Where do improved genetics come from, and how should they be deployed and managed? (August 31 and October 5, 2022)
- Trevor Walker (Invited speaker). Forest Management Academy hosted by Virginia Department of Forestry for their field foresters. (September 13-15, 2022)
- Steve McKeand (Lecturer). Auburn Nursery Cooperative Short Course. (September 14, 2022)
- Steve McKeand (Invited speaker). Longleaf Biennial Conference. Wilmington, NC. A Plan for a Longleaf Pine Tree Improvement Program. (October 25-28).
- J.B. Jett, Steve McKeand, and Austin Heine (Invited speakers). IEG40: Emergent Issues in Orchard Management. (November 30-December 1, 2022)

Teaching

Faculty associated with TIP are busy each year teaching students. In the fall of 2022, Fikret Isik taught the inter-departmental graduate course, Advanced Topics in Quantitative Genetics and Breeding (FOR/CS/ANS 726). Steve McKeand took on a very heavy teaching load this spring that included FOR 411 (Forest Tree Genetics and Biology), FOR 725 (Forest Genetics), FOR 493 (Independent Study in Forest Management), and FOR 727 (Tree Improvement Research Techniques). J.B. Jett co-taught FOR 493/727 and was a huge help in making that class a success along with other guest lecturers. Eight students from the FOR 493 and FOR 727 classes traveled to the Arrowhead Breeding Center during spring break where they participated in many of the breeding, testing, and selection activities that they will need to know as tree breeders. All 4 classes as well as a plant breeding class took a field trip March 31 to the NC Forest Service nursery and seed orchard in Goldsboro, NC.

All of these classes had large numbers of students, which meant a lot more work for the faculty, but wonderful news for the Cooperative and the greater tree improvement community. Our faculty and staff continue to extend their expertise to future forestry and natural resource professionals at both the graduate and undergraduate levels.



Pictured above: Anita Johnson (GFC) is explaining the process of soil sampling and preparing soil samples for nutrient analysis for orchard fertilization to the FOR 493/727 class.

Pictured below: Dr. Barry Goldfarb showing students how to evaluate hedges for pruning and to successfully take and root cuttings for clonal replication.



Pictured left: Students in FOR 411, FOR 493, FOR 725, FOR 727, and HS 860 visited the North Carolina Forest Service. A big thanks to Bobby Smith and Preston Daly for leading a tour around the tree improvement facilities and entertaining student questions.

Staff

We had a familiar face hanging around the Advisory Meeting last May, and we are thrilled to have him back in the mix. Dr. Bailian Li stepped down as Vice Provost of International Affairs here at NCSU and returned to the department. His international experience is now serving the department with identifying opportunities for faculty and students to gain global perspectives. Bailian was also on the faculty of the Tree Improvement Cooperative for many years before taking the position with International Affairs. He is very interested in the adoption of the **PRS**TM Rating System and its impact. Dr. Li was part of the early development of this rating system for pine genetics and has been pleased with how it has impacted forest regeneration.

In September, AnneMargaret Braham retired after many years of service to tree improvement initiatives. She brought years of greenhouse and breeding program experience from her time in the Christmas Tree Genetics Program to assist us over the last year. AnneMargaret was instrumental with several of the operational activities and working with students, and her efforts were a huge help to TIP. We hope she is enjoying some travel and more time on hobbies as she joins her husband in retirement.

Dr. Nasir Shalizi officially joined the group as the Data Analyst in October 2022. He had heroically filled in the gap during the hiring freeze for this position during the pandemic, and we were thrilled to make his permanent position official in the fall. Nasir brings a whole new dynamic view to the data analyst position. We look forward to his continued contributions and expansion of this position's capabilities to make it even more impactful for forest genetics research.

Dr. Ross Whetten retired at the end of November and is now Professor Emeritus and maintains an office on Centennial campus. He's still very involved in forestry research, but he is also enjoying extra time with his family and especially his grandchildren! We look forward to working with Ross in his new capacity.

In December 2022, Austin Heine, Operations Manager, took an opportunity he couldn't refuse with ArborGen. His experience in forest management

and deep knowledge of forest genetics and nutrition made him a big catch. While he is not totally off the TIP hook as he finishes his PhD with the group, we wish him the best in his new position as Reforestation Advisor. In his absence, two folks really stepped up to help fill the gaps during these transitions. Sarah Conner finished her MS program in December and was not afraid to put her degree to use and take on the breeding and testing season among other Cooperative activities. Austin Quate, who was an undergraduate student with TIP before graduating and working with IFCO, returned this fall for his MS. Austin Q's skillset was important for keeping things moving in the greenhouse and field.

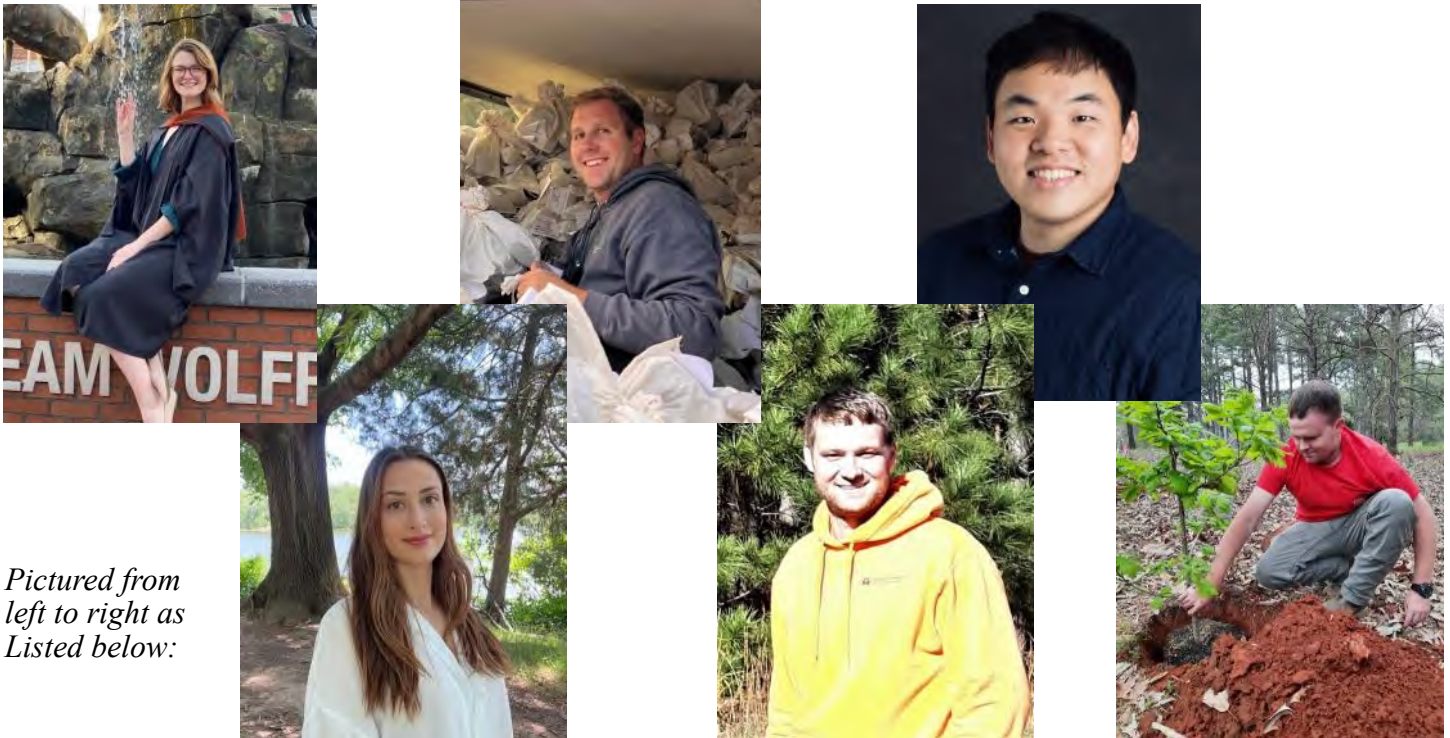
Next staff change was for Dr. Fikret Isik in February 2023. He hasn't left his position here at NCSU or with the Cooperative as Co-Director, but he did change his work location in the world! Fikret was named a Fulbright Fellow (see pages 40-41 for details). He also is writing his next book and contributing to forest genetics research around the world. We are tired just thinking about everything he is up to, but we look forward to his return in January 2024.

And perhaps the biggest change in staff also came in February 2023. Dr. Trevor Walker joined the faculty here at NCSU as an Assistant Professor and became the new Co-Director of the Tree Improvement Program. His leadership and research contributions from his years as Tree Improvement Manager will prove to be vital to the success of the program and training future forestry leaders. February is also one of the busiest times of the year for TIP, so Trevor had his hands full with TIP research along with balancing the new load of duties as a faculty member.

We hope to have the two open positions (Operations Manager and Tree Improvement Manager) posted soon with hiring planned for the summer of 2023. Dr. Steve McKeand, Dr. J.B. Jett, and Tori Brooks will remain working hard as part-timers to help with the transitions of all these new folks and newly filled positions in the coming year. We look forward to what the new folks will bring to our group and the added value to the members.

Graduate Students

We had a really busy 2022 with several students successfully completing their advanced degrees and others just getting started. Colin Jackson successfully defended his PhD in May 2022 and joined ArborGen, Inc. Adam Festa followed in June 2022 and continues his position at Bayer Crop Science. Khushi Goda followed shortly behind and successfully defended in August 2022. Piyush Pandey also defended his PhD research in November 2022 and is working with the USDA-ARS in AI Phenotyping. To end the year on a high note, Sarah Conner finished her master's program in December 2022 and is continuing as PhD student in TIP. We also welcomed two new students, Pinar Guner and Austin Quate. Below is the list of current students and their research:



Pictured from left to right as Listed below:

Sarah Conner, PhD – Sarah defended her MS research titled “Genetic Variation in Fusiform Rust Disease Resistance among Families in the Northern Breeding Population of Loblolly Pine”. She decided to continue her studies, and we look forward to her decision on a research direction as she settles into her PhD program.

Pinar Guner, PhD – Pinar began her program in fall of 2022 and has been researching the development and applications of genomic markers in tree breeding.

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."

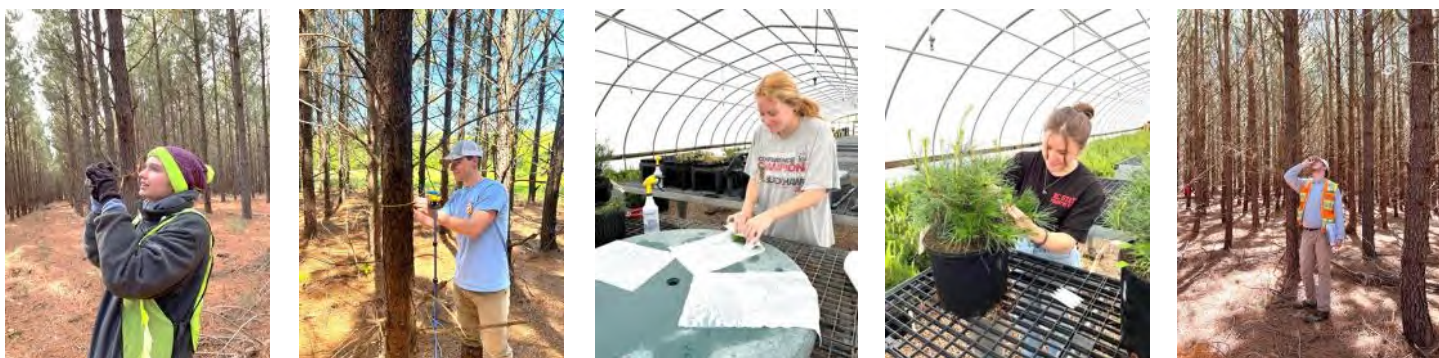
Travis Howell, PhD – Travis joined us in fall of 2021 as a shared student across the big three forestry cooperatives here at NCSU: TIP, Camcore, and Forest Productivity Cooperative. His PhD is in Remote Sensing with a focus on UAS & LiDAR data inventory and analysis on genetic trials at NC State.

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”.

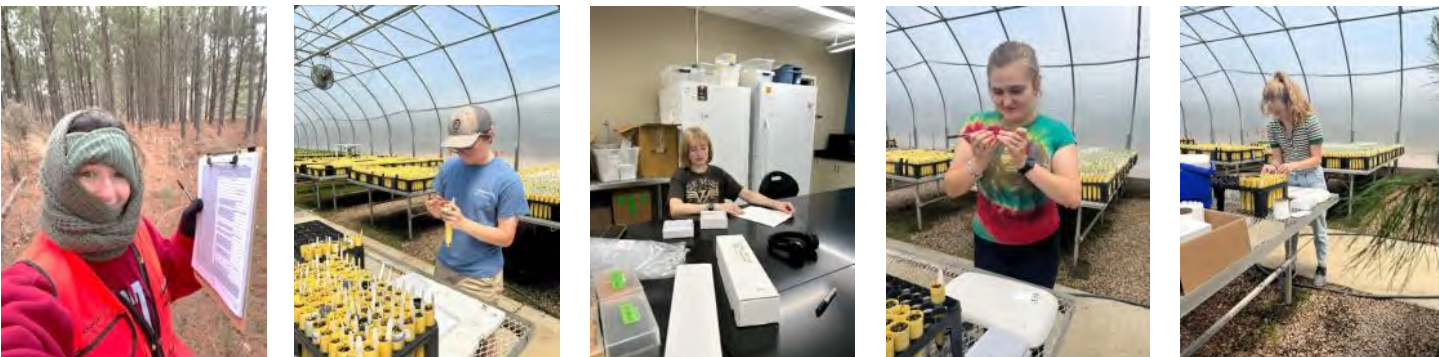
Austin Quate, MS- Austin started his master's program in the fall of 2022. His research focus is biomass cropping and effects of thinning on different families and provenances of loblolly pine in the Piedmont of NC.

Undergraduate Students

We are excited to have one of the largest undergraduate teams working for the Cooperative this year. The “undergraduate army” is a vital component of TIP’s hands-on research and development work. Over the past year, they have been hard at work with projects including ACE2 clonal rooted cuttings, measuring trees at the Butner biomass/cropping study, harvesting megagametophytes for the *Cqf* NFA project, prepping foliage samples for DNA extraction, data entry, cone analysis, and so much more! The work they do not only helps the Cooperative, but also gives them valuable experience, helping them build their resume and networking with future potential employers. For example, many of our student workers will be working internships this summer with our member companies, including J.D. Irving, Rayonier, and American Forest Management. Another three students will work with TIP over the summer to satisfy their internship requirements. We are proud of the long history of using undergraduate student workers in the Cooperative, benefitting both the students and our research!



Left: Jenna Abernethy, a sophomore majoring in Forest Management, measuring tree heights at Butner. Middle left: Gray Tyrey, a freshman majoring in Forest Management, measuring DBH at Butner. Middle: Meredith Flaherty preparing rooted cuttings, a junior dual-majoring in Environmental Sciences and Forest Management. Middle right: Ashley Kerr, a junior majoring in Forest Management, taking rooted cuttings from ACE2 hedges. Right: Conner Manning, a senior in Forest Management, taking tree height measurements at Butner.



*Left: Emily Workman, a junior majoring in Science Education, takes a selfie while starting a new page of the datasheet when measuring the Butner biomass/cropping study. Middle left: Samuel Mowrey a junior in Natural Resources, collecting megagametophyte tissue in the greenhouse for the *Cqf* project. Middle: Beka Helms, a junior dual majoring in Environmental Sciences and Biology, preparing sample collection boxes in the lab. Middle right: Makenzie Bowman, a junior majoring in Environmental Sciences, working on the *Cqf* project at the HFL. Top right: Rhianna Absher, a sophomore in Environmental Sciences, collecting megagametophytes in the greenhouse. Bottom right: Lou Beard, a junior in Natural Resources, putting stickers on collection tubes in the lab.*



Membership in the NCSU Cooperative Tree Improvement Program

This was a relatively busy year for TIP membership. In January 2022, we lost Charles Ingram Lumber Co. but gained Langdale Forest Products Co. as a Contributing Member. We also lost PRT Growing Services. Ltd. as a Contributing Member because they purchased IFCO Seedlings in April 2023. IFCO Seedlings will remain the named member and stay as a Full Member of the Cooperative. Lastly, we said goodbye to New Brunswick Tree Improvement Council but welcomed Reliable Source Seeds & Transplants as a Research Associate Member.

Full Members

ArborGen, Inc.	Rayonier, Inc.
Georgia Forestry Commission	South Carolina Forestry Commission
IFCO Seedlings	Virginia Department of Forestry
Manulife Investment Mgt.	The Westervelt Company, Inc.
North Carolina Forest Service	Weyerhaeuser Company

Contributing Members

American Forest Management	Larson and McGowin, Inc.
Blanton's Longleaf Container Nursery	Meeks Farms & Nursery, Inc.
Burgin Timberlands, LLC	Milliken Forestry Company
Campbell Global, LLC	Molpus Woodlands Group, LLC
F&W Forestry Services, Inc.	ProFOR Consulting
Four Rivers Land & Timber Company, LLC	Resource Management Service, LLC
Gelbert, Fullbright & Randolph Forestry Consultants	Roseburg Resources
Green Diamond Resource Company	Scotch Land Management, LLC
GreenWood Resources	Shoheel Land Management (Z.V. Pate, Inc.)
Jordan Lumber & Supply Company	Tennessee Division of Forestry
Langdale Forest Products Co.	Timberland Investment Resources, LLC

Research Associate Members

Arauco - Bioforest, S.A.	Nova Scotia Department of Natural Resources
GenoVerde, Inc.	Reliable Source Seeds & Transplants
J.D. Irving, Ltd	USDA Forest Service

Benefactors

Canfor Corporation

Publications of Special Interest To Members (2022 - 2023)

From our inception in 1956, there have been over 411 refereed publications written by Cooperative Scientists, Students, and Associates. Below is a list of publications from the last year. The entire list is available at our web site: <https://www.treeimprovement.org/tip-tech-transfer>

2023

- Alan, M., K. Payn, S. McKeand, and F. Isik. 2023. Genetic parameter estimates from a polymix breeding population of *Pinus taeda* L. For. Sci. (in press, available online at <https://doi.org/10.1093/forsci/fxad006>).
- Heine, A.J., T.D. Walker, J.B. Jett, F. Isik, and S.E. McKeand. 2023. Pollination bag type affects ovule development and seed yields in *Pinus taeda* L. For. Sci. 69(2):187-199, <https://doi.org/10.1093/forsci/fxac052>.
- Kloppe T., R.B. Whetten, S.B. Kim, O.R. Powell, S. Lück, D. Douchkov, R.W. Whetten, A.M. Hulse-Kemp, P. Balint-Kurti, and C. Cowger. 2023. Two pathogen loci determine *Blumeria graminis* f. sp. *tritici* virulence to wheat resistance gene *Pm1a*. New Phytol. 238(4):1546-1561, <https://doi.org/10.1111/nph.18809>.
- Shalizi, M.N., T.D. Walker, A.J. Heine, K.G. Payn, F. Isik, B.P. Bullock, and S.E. McKeand. 2023. Performance based on measurements from individual-tree progeny tests strongly predicts early stand yield in loblolly pine. For. Sci. (in press, available online at: <https://doi.org/10.1093/forsci/fxad002>).
- Whetten, R.W., K.J.S. Jayawickrama, W.P. Cumbie, and G.S. Martins. 2023. Genomic tools in applied tree breeding programs: factors to consider. Forests 14(2):169, <https://doi.org/10.3390/f14020169>.

2022

- Borthakur, D., V. Busov, X.H. Cao, Q. Du, O. Gailing, F. Isik, J-H. Ko, C. Li, Q. Li, S. Niu, G. Qu, T.H.G. Vu, X-R. Wang, Z. Wei, L. Zhang, and H. Wei. 2022. Current status and trends in forest genomics. Forestry Research. 2(1), <https://doi.org/10.48130/FR-2022-0011>.
- Culatta K., A. Krings, L.P. Matallana, and R. Whetten. 2022. Clarifying taxonomic boundaries in *Nuphar sagittifolia* (Nymphaeaceae): insights from morphology and population genetic diversity. Castanea 87(1):81-104, <https://doi.org/10.2179/0008-7475.87.1.81>
- Isik, F. 2022. Genomic prediction of complex traits in perennial plants: a case for forest trees. Chapter 18 (p. 493-520) 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>.
- 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>
- 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, M.N., 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>.
- Shalizi, M.N., K.G. Payn, T.D. Walker, F. Isik, A.J. Heine, and S.E. McKeand. 2022. Long-term evaluation of intra- and inter-provenance hybrids of loblolly pine in the Piedmont region of the southeastern United States. For. Ecol. Manag. 522 (in press, available online at: <https://doi.org/10.1016/j.foreco.2022.120469>)
- 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>

Front Cover: We are well positioned at the Arrowhead Breeding Center with hundreds of 181210 interstocks at the appropriate size for grafting 5th- and 6th-Cycle selections in the next several years.

Back Cover, clockwise:

The NCDA Bioenergy study at Butner is growing up and showing us what is possible for pines in the energy sector.

ACE2 hedges under cover for winter. The winter weather this year gave us several challenges, but cuttings were still able to be completed this past Spring.

Dr. Trevor Walker began his new role with the Cooperative in February 2023 as Co-Director of the program. One of his first duties was completing the breeding assignments he made as the Tree Improvement Manager.

