TREE IMPROVEMENT PROGRAM
NC STATE UNIVERSITY

NORTH CAROLINA STATE UNIVERSITY
COOPERATIVE TREE IMPROVEMENT PROGRAM

65th Annual Report | June 2021

DEPARTMENT OF FORESTRY & ENVIRONMENTAL
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65th Annual Report

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EXECUTIVE SUMMARY

At age 65 years, the Cooperative Tree Improvement Program continues to provide value to the Cooperative members, to NC State University, to the forestry and scientific communities, and to the landowners and citizens across the region.

BREEDING, TESTING, AND SELECTION

As of 2021, approximately one third of the 4th-Cycle tests have been measured, with the remainder of the tests still growing to measurement age. In winter of 2021, 122 trees were selected from 34 4th-Cycle tests, bringing the total number of 5th-Cycle selections to 245.

Many of the 5th-Cycle selections that were topgrafted at the Arrowhead Breeding Center in 2020 had an abundance of female strobili that provided an opportunity to jump-start 5th-Cycle breeding. TIP staff completed 46 crosses using 31 5th-Cycle selections as females. Fortunately, many of the one-year old topgrafts were also producing pollen. Pollen was collected from 32 5th-Cycle selections, which will permit a significant amount of 5th-Cycle breeding in spring 2022.

Cooperative members and TIP staff completed wood quality measurements on an additional five 3rd-Cycle polymix progeny tests at ages 7 to 9 years old. Data are presently being prepared for analysis, and new breeding values for wood density and wood stiffness are expected for 68 Coastal and 82 Piedmont parents.

RESEARCH

Two new pollen research projects have been initiated. The first project is investigating impedance flow cytometry as a tool to evaluate loblolly pine pollen viability. The second project aims to quantify the impact that pollen development stage at time of collection has on filled seed yields.

Results from a genomic selection (GS) study involving the Atlantic Coastal Elite (ACE) population showed the expected genetic gain from GS was approximately twice the expected genetic gain achieved by a traditional breeding strategy. The difference in gain was primarily due to a 50% reduction in the duration of the breeding cycle when incorporating a GS strategy. Importantly, prediction based on SNP markers requires strong genetic relatedness between the model training and validation populations.

The major Quantitative Trait Loci (QTL) underlying non-race-specific fusiform rust disease resistance described in the 2020 Annual Report were validated in field trials. The same regions associated with QTL in the mapping populations were associated with rust disease resistance in the ACE tests.

Hyperspectral imaging was investigated as a tool to predict the cold adaptability of loblolly pine seedlings. Hyperspectral image data were obtained from scanning an experimental population comprising 98 seedlots from Virginia to Florida. High prediction accuracy of minimum winter temperature at seed source origin was achieved ($R^2 = 0.78$) when scanning seedlings in mid-winter following a period of cold acclimation.

ASSOCIATED ACTIVITIES, GRADUATE STUDENTS, COOPERATIVE STAFF, MEMBERSHIP

We welcome Burgin Timberland Management, LLC as a new Contributing Member. There are now 10 Full Members, 24 Contributing Members, and 6 Research Associate Members. Graduate students' contributions to the program continue to be critical. We thank Steve McKeand for his tremendous contribution to our Program as he completes his phased retirement this June. We are delighted that Steve wishes to remain involved with the Cooperative on a part-time basis. Congratulations to Austin Heine, Operations Manager, and his wife Logan on the birth of their son, Luke in May.

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A MESSAGE FROM THE DIRECTOR

The Cooperative celebrates its 65th year providing tangible value to our members and the region as a whole. At the center of its success is the collaborative manner in which breeding, testing and selection activities are performed across the Southeast. The effectiveness of our tree improvement processes are underpinned by innovative research and the sharing of knowledge. The robustness of the Cooperative during the last year is a testament to the hard working collective effort of our members and staff.

It's exciting to report that approximately one third of the 4th-Cycle tests have been measured, with the remainder of the tests systematically approaching measurement age. Through the accumulation of 4th-Cycle test measurements, we have identified superior parents that can be grafted into new 4th-Cycle orchards, providing enhanced genetic quality of loblolly pine seedlings for deployment across the Southeast. Our members continue to do an excellent job at efficiently converting gains achieved within our breeding program to operational gains realized across the region.

Over the past decade, we estimate that the Cooperative has delivered a rate of operational gain of approximately 1% per year. Our mandate is to increase the rate further through the application of new technologies that promote greater efficiencies in our tree improvement processes. Recent results from a genomic selection (GS) study involving the Atlantic Coastal Elite (ACE) population indicate that substantial gain can be made by incorporating GS in a shortened breeding cycle strategy. A 2nd-Generation ACE (ACE2) population was established in the fall 2020. It serves as a validation population to verify the predictive ability of markers from one generation to the next. The Cooperative has also initiated several precision phenotyping studies investigating technologies that offer potential for the assessment of adaptability and productivity traits of loblolly pine. A key focus is the use of hyperspectral imaging for the assessment of biotic and abiotic stress tolerance in pine seedlings. I believe we will find greater synergy between these genotyping and precision phenotyping technologies moving forward. We are grateful to our Cooperative for providing the mechanism to leverage research dollars to fund this potentially high impact research.

Steve McKeand will complete his phased retirement at the end of June 2021. Steve has been with the Program since the beginning of the second-generation breeding. His contribution to the success of the Cooperative has been immense. A highlight during the past year was Steve participating in the first season of breeding among 5th-Cycle selections. We are delighted that Steve wishes to remain involved with the Cooperative on a part-time basis.

I end by acknowledging the people who make up the Cooperative. All Cooperative members, staff and students should be incredibly proud of the progress achieved. I thank you for your enthusiasm and steadfastness during a challenging but successful year. Your commitment towards our common goals ensures that our Program continues to grow from strength to strength.

Kitt Payn, June 2021

BREEDING, TESTING, AND SELECTION

Breeding Update

5th-Cycle Breeding Underway

The Arrowhead Breeding Center experienced excellent flower and pollen production this spring. Many of the 5th-Cycle selections that were topgrafted in 2020 had an abundance of female strobili that provided an opportunity to jump-start 5th-Cycle breeding. TIP staff completed 46 crosses using 31 5th-Cycle selections as females (Images 1 and 2). The pollen parents included 11 5th-Cycle selections, of which six were grafted only last year yet produced sufficient pollen for breeding. The balance of the pollen parents used in mating comprised 12 4th-Cycle selections. These selections had low representation in the 4th-Cycle testing population and would thus benefit from additional breeding. Furthermore, their pollen was readily available to apply to 5th-Cycle selections, thereby expediting progeny-tested breeding value estimation for new 5th-Cycle parents and contributing full-sibling progeny that will complement the 5th-Cycle population. In spring 2022, the plan is to utilize the MateSel algorithm to develop a mating design among the 5th-Cycle selections based on their individual-tree breeding values, pedigree, and their relatedness to the juvenile population (progeny tests yet to be measured).

In spring 2021, pollen was also collected from 3rd-Cycle backwards selections based on progeny-tested breeding values for wood quality traits (acoustic velocity and drill resistance), with lower selection weights placed on volume, straightness, and rust resistance. The motivation for collecting pollen from these backward selections was to increase the size of the 5th-Cycle breeding population while adding diversity for traits that may potentially become more economically important. Cooperative members and TIP staff collected pollen from 40 backward selections in total.

Optimizing the Mating Design to Alleviate Female Strobili and Pollen Constraints

Breeding for the 4th-Cycle required approximately five years to complete (started in 2013 and was 95% complete in 2018). While faster than previous cycles, there were several bottlenecks that were observed that could be alleviated in the 5th-Cycle breeding strategy. For example, flower production of new selections was often limiting and prohibited the multiple crosses required by the mating plan. To counter this limitation, constraints on the number of crosses can be included in the MateSel software, and the algorithm will find other females to contribute towards the optimal solution while balancing genetic gain and diversity. By running the MateSel software annually, the breeding plan can take advantage of the rolling-front design, as new selections will be available each year, and the number of crosses possible per selection can be precisely estimated with flower counts and pollen inventory. TIP Staff also noted that progeny testing for new selections was often delayed due to limited flowering that was not enough to justify controlled pollination (e.g. less than 4 flowers). Rather than ignore these cones, open pollinated seed from new selections can be included in progeny tests (when collected from a single location like Arrowhead Breeding Center) to expedite the estimation of progeny-tested breeding values and *PRS*™ scores of putative elite parents.

Image 1. *Trevor Walker installing pollination bags in anticipation for 5th-Cycle breeding to commence. In the background are pollination bags for Austin Heine's PhD research study (Photo by Steve McKeand).*

Image 2. *Pollination bags were installed on 31 5ᵗʰ-Cycle selections that were topgrafted in 2020 (Photo by Kitt Payn).*

Full Member Assistance with Cone Collection

In fall 2020, Full Members that chose to assist TIP Staff at the Arrowhead Breeding Center (rather than conducting breeding operations in their organization's facilities) began their contributions by extracting seed from cones harvested from Arrowhead (Image 3). Together, seed from 215 crosses were extracted for various Cooperative projects, including 5ᵗʰ-Cycle Substitutes, ACE2 (2ⁿᵈ-Generation Atlantic Coastal Elite), and a planned Pollen Cloud Study.

Testing Update

4ᵗʰ-Cycle Progeny Testing

The final cohort of 4ᵗʰ-Cycle progeny tests will be established this winter (2021-2022). The first 4ᵗʰ-Cycle tests were planted in 2014 using a "rolling-front" strategy, where seed are sown as soon as they are harvested (see Figure 1 for the number of full-sibling crosses planted each year). The number of new crosses planted peaked in 2019 with 287 crosses, which corresponded to a productive breeding season in spring 2016. Each cross is typically tested over two years to increase the number of representative testing environments and to provide statistical connections with the rest of the population. The majority of crosses to be planted this winter are continued-testing crosses, and the new crosses will continue their testing in the first 5ᵗʰ-Cycle tests.

As of 2021, around one third of the 4ᵗʰ-Cycle tests have been measured, with the remainder of the tests still growing to measurement age. The 4ᵗʰ-Cycle testing program demonstrated the astounding capability

Image 3: *Truck load of bags of cones harvested from the Arrowhead Breeding Center (photo by Austin Heine). We are grateful to the Cooperative members for their assistance with seed extraction.*

of our Cooperative members to robustly test the genetic value of our population, which will include a total of 1,241 replications planted across the southeastern US (Table 1, Figure 2). From 2017 - 2021, members have consistently planted 150+ replications annually. The number of 4ᵗʰ-Cycle reps planted peaked at 178 in 2017 when the Cooperative members decided to double-up on the testing effort to avoid a backlog of non-tested families. The Cooperative maintains high standards for data quality, and only around 8% of the planted reps have been abandoned due to less than satisfactory survival, a reminder of the challenges of progeny testing.

The testing strategy in the 4ᵗʰ Cycle included several innovative and cutting-edge experimental designs that we plan to carry over into the 5ᵗʰ Cycle, including rolling-front testing of new families, alpha-lattice
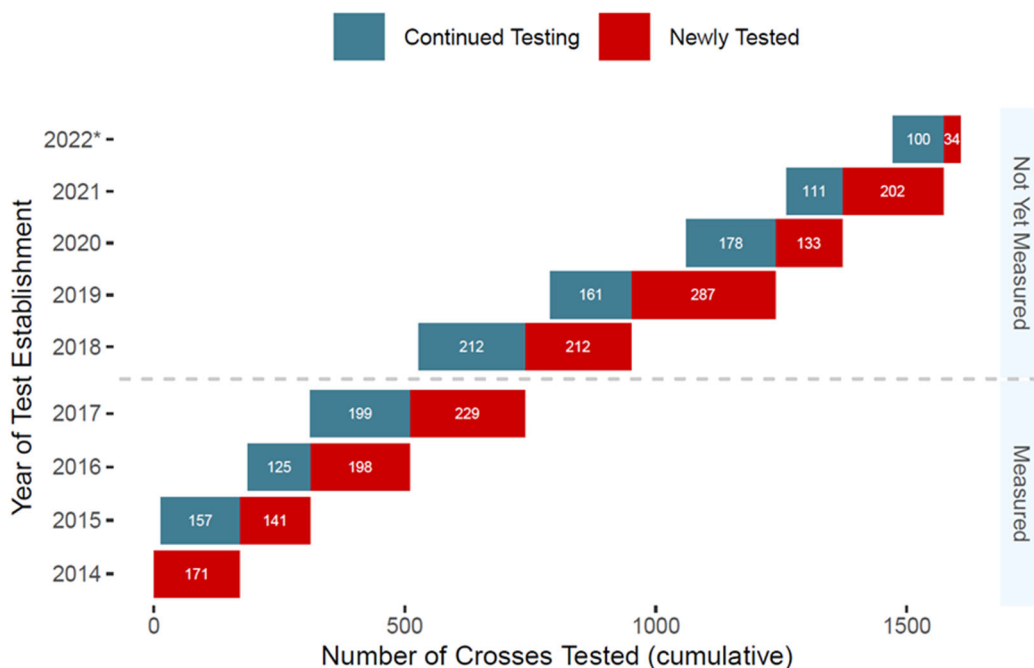
Figure 1. Number of crosses tested annually throughout the 4th Cycle (white numbers in bars). The number of newly tested crosses are in the red bars, and the number of crosses with follow-up testing are in the blue bars. Most tests planted in 2017 and earlier have already been measured. The 2022 progeny test seedlings are presently growing in greenhouses and will be outplanted this upcoming winter.

Table 1. Number of replications planted annually as part of the 4th-Cycle testing program by Cooperative members. The 2022 cohort are presently growing in greenhouses and will be planted this winter; these are the final cohort of 4th-Cycle progeny.

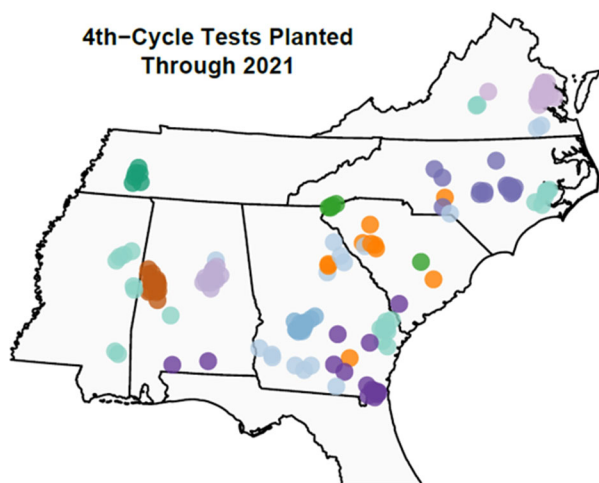|	Year Planted									
	2014	**2015**	**2016**	**2017**	**2018**	**2019**	**2020**	**2021**	**2022***	**Total**
Measured	92	65	87	108	0	0	0	0	0	**352**
Not Yet Measured	0	0	10	70	142	150	155	152	135	**814**
Abandoned	10	25	10	0	18	12	0	0	0	**75**
Total Planted	**102**	**90**	**107**	**178**	**160**	**162**	**155**	**152**	**135**	**1241**

Figure 2. Distribution of the 4th-Cycle progeny test site locations planted through 2021. Different color points indicate the ten different full members responsible for test planting, maintenance, and measurement. Cooperative members have done an excellent job testing throughout the range of loblolly pine east of the Mississippi River.

row-column incomplete blocks, and provenance overlap (see page 6 of 2017 Annual Report for details). TIP staff and members are excited to start designing the first 5th-Cycle tests next year!

3rd-Cycle Wood Quality Measurements

Since last year, Cooperative members and TIP staff completed wood quality measurements on an additional five progeny tests ages 7 to 9 years old from the 3rd-Cycle, including three Coastal tests (from series CPMX5 and CPMX6) and two Piedmont tests (both from PPMX4). Measurements included 1) acoustic velocity using TreeSonic as a measure of wood stiffness, and 2) drill resistance using Resistograph as a measure of wood density (Image 1). This will conclude the wood quality sampling effort for 3rd-Cycle tests, where the target number of two tests per series was accomplished for all series except one (CPMX6, the youngest Coastal pollen mix series).

Data are presently being prepared for analysis, and new breeding values for wood density and wood stiffness can be expected for 68 Coastal parents and 82 Piedmont parents with direct progeny measurements. At this time, there are 269 Coastal parents, 183 Piedmont parents, and 127 Northern parents with wood quality breeding values available on TI-PRoot and $\mathcal{PRS}^{TM}$. More information on the wood quality sampling effort can be found on page 15 of the 2020 Annual Report. Figure 3 displays the geographic distribution of test measurements where wood quality has been assessed.

Image 1. *Georgia Forestry Commission Staff completing wood quality measurements on 3rd-Cycle pollen mix tests using TreeSonic (left) and Resistograph (right) to assess the wood stiffness and density, respectively, of parents in the CPMX5 series.*

Figure 3. *Distribution of 3rd-Cycle tests where wood quality (wood density and wood stiffness) has been assessed at ages 7 to 9 years.*

Atlantic Coastal Elite and ACE2 Population

A series of four progeny test sites containing seedlings from the second generation Atlantic Coastal Elite (ACE2) population was established this past winter (Figure 4). Each progeny test site contained around 14 replications with 80 families per, including 67 crosses (among 73 of the original ACE clones), seven common family checklots used in the 3rd-Cycle pollen-mix tests, and six 4th-Cycle families that include six of the 48 ACE selections used in 4th-Cycle breeding. Prior to planting, cuttings from the test seedlings were rooted for vegetative propagation (more details in the ACE2 clonal propagation section, p. 37).
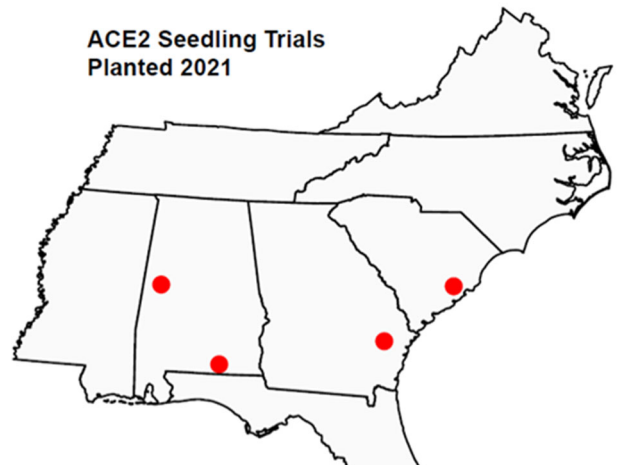

Figure 4. *Distribution of ACE2 seedling tests planted this past winter (2020-2021).*

4th - Cycle Data Analysis

Objectives

The purpose of the analysis was for prediction of breeding values of individual trees and parents for the 4th-Cycle Coastal and Coldhardy breeding populations. These breeding values were predicted using progeny test data collected from 4th-Cycle tests established from 2014 through 2017.

Datasets

Data analyzed for the 2021 selections included all tests established in or before 2017. Coastal and Coldhardy populations were analyzed separately. Most tests were measured at 4 years, while some were measured at 5 years post-establishment.

The dataset for the Coastal analysis consisted of 25 progeny tests, while the combined Piedmont/Northern (Coldhardy) dataset comprised a total of 27 tests. All progeny tests included in the analyses were generated with the MateSel® mating design, and represented seedling progeny from a mixture of open- and control-pollinated matings.

Analysis Highlights

Many of the top parents for volume in the Coastal breeding population were derived from Florida seed sources. Six of the top ten parents could trace their ancestry to northern Florida (Figure 1). This is in
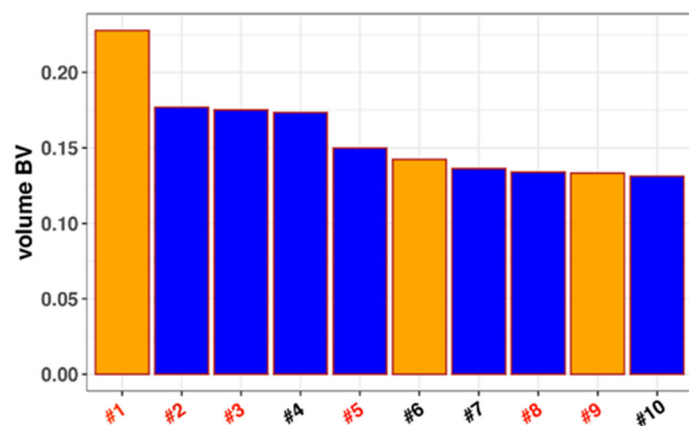
contrast to stem straightness where none of the top ten performers for stem straightness had ancestry from Florida (Figure 2). The fast growth of Florida seed sources is well known from studies of provenance-level variation in loblolly pine. The lack of representation of Florida seed sources in the top ten for straightness supports the findings of Farjat et al. (2017); namely, seed sources from northern Florida had inferior stem straightness compared to slower-growing regions such as Virginia, North Carolina, and the Piedmont.
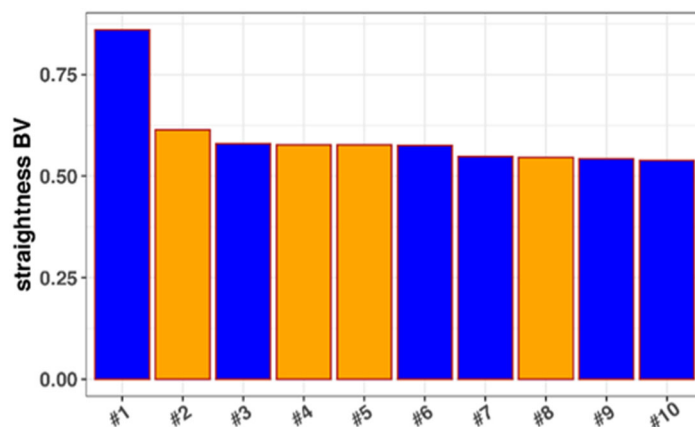

Figure 2*. Top ten Coastal parents for straightness breeding value in the 2021 Annual BLUP. For the sake of presentation, the inverse of the straightness BV is shown on the Y-axis (this makes higher scores better). Parents represented in this year's annual BLUP but not in the 2020 annual BLUP are shown with orange bars.*

Consistency with 2020 Annual BLUP

As we embark on individual tree selections from the 4th-Cycle progeny tests, one of the most important issues is consistency of modeling results among years. In 2018, the first year that individual tree selections were made from these tests, there were 12 test sites in the Coastal BLUP analysis. This number increased to 19 in 2019 and to 25 in 2020. As the number of test sites increases, how do estimates of breeding value change? The good news is that the breeding value estimates are remarkably consistent, both for parents (Figure 3) and for individual trees (Figure 4).

Figure 1*.Top ten Coastal parents for volume breeding value in the 2021 Annual BLUP. Parents represented in this year's annual BLUP but not in the 2020 annual BLUP are shown with orange bars. Parents with Florida ancestry are labelled with red numbers.*

In general, the correlations for the Coldhardy analyses were similarly high, but slightly lower than the Coastal analyses. In the Coldhardy analyses the correlations between years for volume, straightness and rust parental breeding values were 0.9, 0.89, and 0.9, respectively (data not shown).

Conclusions

The phenotypic data indicate that members of the Cooperative continue to show a high degree of attention to detail and data quality. High correlations between successive measurement years indicate that values for parents are not changing drastically, which results in more consistent individual tree values. This is very important as individual tree selections are made, since large changes in BVs for grafted selections are undesirable from a logistical standpoint. The high quality of progeny test data is a testament to the dedication and hard work of the members and staff of the NCSU Cooperative Tree Improvement Program.
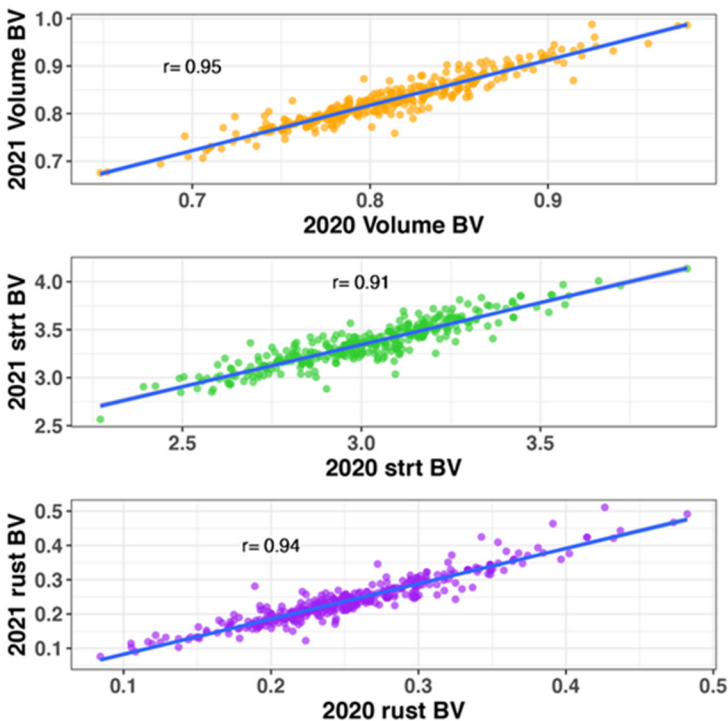

Figure 3. Consistency of breeding value estimates for Coastal parents between successive analyses for volume, straightness, and rust resistance. In each plot, the estimates from 2020 are shown on the X axis, while the estimates from 2021 are shown on the Y axis. The correlation is shown in the off-diagonal of each plot.
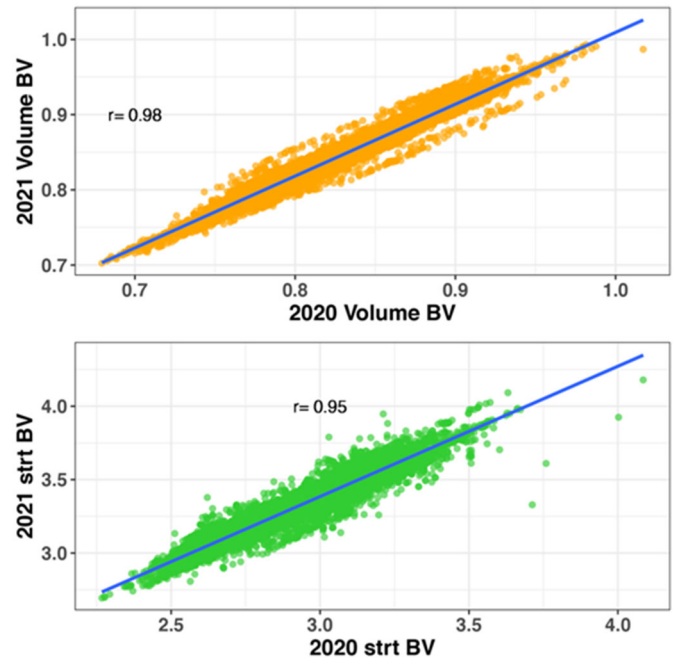
Figure 4. Consistency of breeding value estimates for individual trees between successive analyses for volume and straightness. In both plots, the estimates from 2020 are shown on the X axis, and the estimates from 2021 are shown on the Y axis. The correlation is shown in the off-diagonal of each plot.

References

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

5th-Cycle Selections Update

Overview

In 2021, TIP Staff and Cooperative members made 5th-Cycle selections (Image 1) using measurements from 4th-Cycle progeny tests established during the period 2014-2017, which included 16 tests that were measured for the first time in 2021. Data were analyzed using two individual-tree mixed-models: one model for the Coastal tests and another for the Piedmont/Northern tests. A multi-trait index with weights of 0.6, 0.2, and 0.2 for volume, straightness, and rust resistance breeding values, respectively, was calculated using standardized breeding values (Figure 1). The MateSel algorithm (Kinghorn 2011), which creates a mating plan that balances gain and diversity, was then used to generate a candidate list of selections for field evaluation by TIP staff and Cooperative Members.
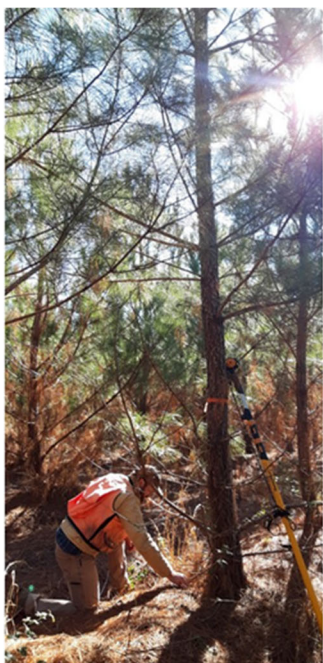

Image 1: In-field selection evaluations during February 2021. Image 1a, Graham Ford from IFCO Seedlings confirming the tree identity printed on the planting tag; Image 1b, Brandon Loomis, James Owens and George Surritte from The Westervelt Company evaluating a selection candidate; Image 1c, Austin Smith and David Barker from Rayonier collecting scion from a new selection (Photos by Kitt Payn).

While the MateSel algorithm outputs a list of crosses to make, TIP Staff utilized the output to generate a list of trees to evaluate for selection purposes. The 2021 selection season was the first occasion where TIP Staff utilized the commercial version of MateSel. The new software optimizes the selection candidate list for a rolling-front breeding program by incorporating two important features. The first feature accounts for the relatedness among the new selection candidates and the 5th-Cycle selections that have already been topgrafted by avoiding making new selections that do not add sufficient diversity to the breeding orchard unless their breeding value is outstanding. The second feature accounts for the relatedness among the new selection candidates and the young trees in progeny tests that have not yet been measured (juvenile population) by applying negative pressure on selection candidates that have close relatives in the juvenile population, such that if
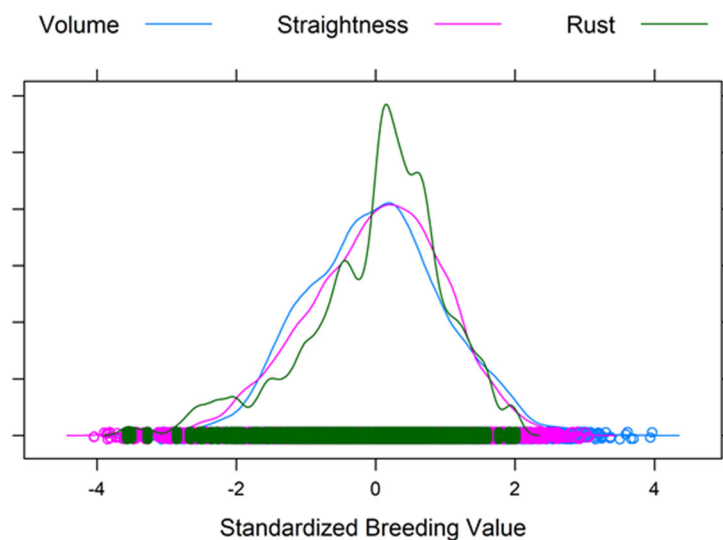

Figure 1. Distribution of standardized breeding values (standard normal/Z-scores) for traits used for the selection index in the Coastal 4th-Cycle population measured through 2021.

two individuals have the same index breeding value, the tree that is less related to the juvenile population is preferred as a selection candidate.

To evaluate the impact these two features have on the genetic gain and diversity of 5th-Cycle selections, three scenarios were assessed: 1) apply the relatedness constraint of selection candidates with respect to existing 5th-Cycle topgrafts and the juvenile 4th-Cycle population, 2) apply the relatedness constraint of selection candidates with respect to the 5th-Cycle topgrafts only, and 3) apply neither constraint. Scenario 1 resulted in the most diverse set of candidate selections, whereas scenario 3 generated a list of candidate selections that was less diverse (higher coancestry) and more related to the 5th-Cycle selections that were already topgrafted (Table 1).

Table 1. *Simulation of different scenarios of MateSel algorithm constraints using the Coastal 4th-Cycle population and candidate 5th-Cycle Selections for 2021. Accounting for relatedness with 5th-Cycle topgrafts already made in 2019 and 2020 and 4th-Cycle progeny tests not yet measured (planted in 2018 – 2021) resulted in a more diverse set of 5th-Cycle selection candidates.*

Scenario	Feature Included	Mean Coefficient of Coancestry		
		For 2021 5th-Cycle Selection Candidates	With Existing 5th-Cycle Topgrafts	With 4th-Cycle Progeny Planted 2018-2021 (Juvenile Pop.)
1	Account for relatedness with existing 5th-Cycle topgrafts + Juvenile 4th-Cycle population	0.0080	0.0065	0.0035
2	Account for relatedness with existing 5th-Cycle topgrafts only	0.0084	0.0075	0.0055
3	No constraints	0.0095	0.0090	0.0060

Incorporating the diversity constraints (scenarios 1 & 2) reduced the expected genetic gain of the 5th-Cycle selections over the 4th-Cycle population (Figure 2). There was a considerable decrease in the expected genetic gain when accounting for existing 5th-Cycle topgrafts, which indicates that there are highly-related selection candidates in recently measured 4th-Cycle tests with superior breeding values. The impact on gain when accounting for the juvenile 4th-Cycle tests in addition to the 5th-Cycle topgrafts was less pronounced, although it remains important to account for the juvenile population to deter from making selections that have close relatives to be measured in the near future, unless the mature candidate has an exceptional breeding value that warrants immediate selection. Ultimately, TIP Staff decided to utilize a modified version of Scenario 1 to develop the selection candidate list for 2021.

The target number of selections for 2021 was 114. Similar to 2020, the Cooperative implemented a two-step selection process. First, the measurement data pertaining to selection candidates (generated from the MateSel algorithm) were verified infield by the
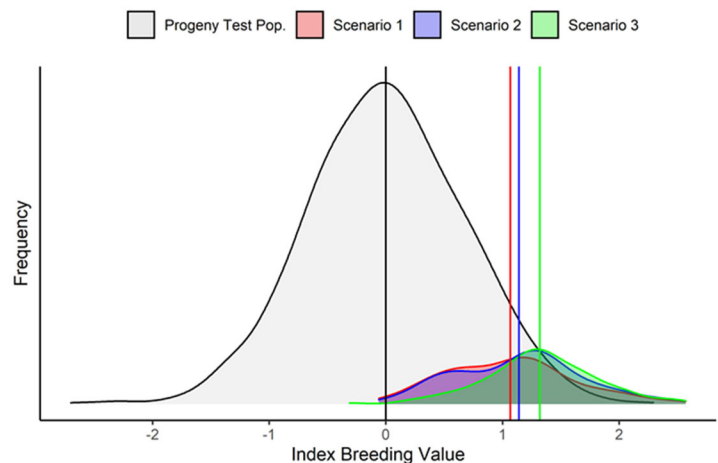
Figure 2. *Distribution of index breeding values for the entire 4th-Cycle population (gray) and trees selected by different MateSel scenarios, with vertical lines indicating the mean for each population. Scenario 1 (red) accounts for relatedness with existing 5th-Cycle top grafts and young 4th-Cycle tests not yet measured, Scenario 2 (blue) only accounts for existing 5th-Cycle top grafts, and Scenario 3 (green) excludes both constraints. Accounting for existing 5th-Cycle topgrafts had a large impact on the expected genetic gain, indicating there are highly related genotypes in the newly measured 4th-Cycle tests with better breeding values than those selected in 2019-2020.*

members. Adjustments to the candidate list were then made to ensure that only the very best trees were included in the final in-field selection evaluation step. This year, 122 trees were selected from 34 tests (Figure 3), bringing the total number of 5th-Cycle selections made through 2021 to 245 (Table 2).

Table 2: *Number of 5th-Cycle selections by year and series.*

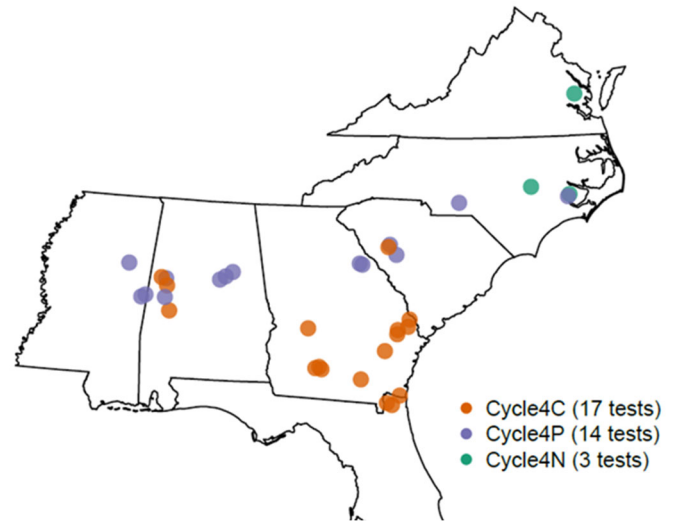| Series	2019	2020	2021	Total
CYCLE4C	29	52	72	153
CYCLE4P		24	42	66
CYCLE4N		9	8	17
NE1*	2	7		9
Total	31	92	122	245

Figure 3: *Locations of test sites with selections made in February 2021.*

Selection Summary

Pedigree Diversity

Of the 245 5th-Cycle selections made through 2021, 129 selections each represented a unique cross (single selection per cross). The balance of the selections represented 177 crosses, where two or more selections were made per cross. There were 32 4th-Cycle crosses having two selections made, 12 having three selections made, and four crosses where four selections were made.

There were 213 4th-Cycle parents that had a progeny selected for 5th-Cycle breeding. The plurality (110) of the 5th-Cycle selections were the only progeny selected from their parent (Figure 4). There were 43 4th-Cycle parents that had two progeny selected, and 60 parents with three or more progeny selected. One parent had fifteen progeny selected due to its superior index breeding value and relatively low relatedness to the rest of the population. The pedigree of the 245 5th-Cycle selections made through 2021 can be traced back to 252 founders (plus trees from wild natural stands or non-improved plantations). This is a significant increase compared to the selections made through 2020, indicating an adequate increase in diversity with the selections made in 2021.
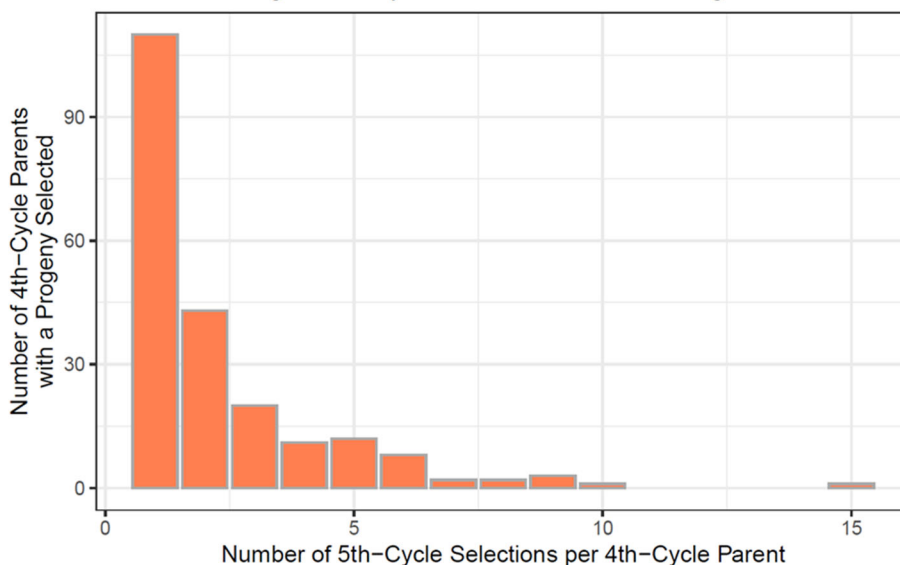

Figure 4: *Count of 4th-Cycle parents having one or more 5th-Cycle selections made from their progeny.*

Mean Minimum Temperature Origins

Selections were made across the range of mean minimum temperatures of 4ᵗʰ-Cycle crosses represented in tests measured through 2021 (Figure 5). To assure an adequate number of selections adapted to the most northern regions of the Cooperative, within the Piedmont and Northern tests, the MateSel solution had to be supplemented to bring the number of Northern selections to the target of 7 trees. This issue did not arise in 2020, as there were many top-ranking individuals across the temperature spectrum of Northern and Piedmont source origins. Notably, the distribution of selections in Figure 5 broadly represents the distribution of family mean minimum temperature in the 4ᵗʰ-Cycle tests.

Inbreeding Coefficients

Of the 245 5ᵗʰ-Cycle selections made through 2021, four have non-zero inbreeding coefficients (their parents are related). Two of these selections came from crosses where the parents were half-siblings ($F = 0.125$) and two selections came from parents that were more distantly related ($F = 0.0625$).

Expected Gain

To calculate index breeding values, the breeding value were standardized to Z-scores (standard normal) and ratios of 0.6, 0.2, and 0.2 for stem volume, straightness, and rust incidence probability were applied. The mean multi-trait index breeding value among the 5ᵗʰ-Cycle selections was 1.13 for the selections from Coastal tests, and 1.16 from
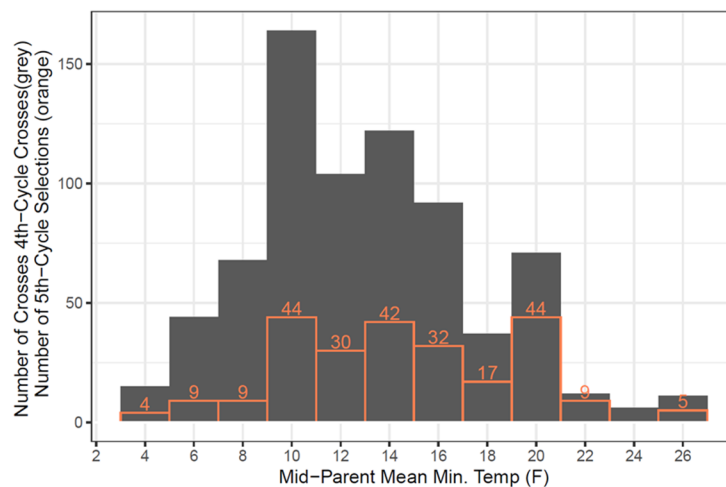

Figure 5: Number of 4ᵗʰ-Cycle crosses (in grey) measured through 2021 and number of 5ᵗʰ-Cycle selections (orange) made from those crosses by mid-parent mean minimum temperature origin.

Piedmont/Northern tests, compared to a mean of 0 for candidate trees in both populations (Figure 6). These values correspond to the number of standard deviations above the mean in terms of index breeding value.

In most crosses having a selection made, the tree with the highest index breeding value from a family was selected. There were some occasions where the presence of a fork, rust gall, other defect, or too many missing neighbors caused trees with lower index breeding values to be selected. The index breeding value of trees in the 4ᵗʰ-Cycle progeny tests, grouped by cross, are shown in Figure 7 (Coastal population).

Figure 6: Multi-trait index breeding values (60% volume, 20% straightness, 20% rust resistance) for trees in 4ᵗʰ-Cycle progeny tests (gray) and those trees that were selected (orange represents Coastal and blue represents Piedmont/Northern selections). Vertical lines indicate the mean of the population (grey) and the mean of the selected (color) trees. Trees selected in 2021 are shown above those selected 2019-2020.
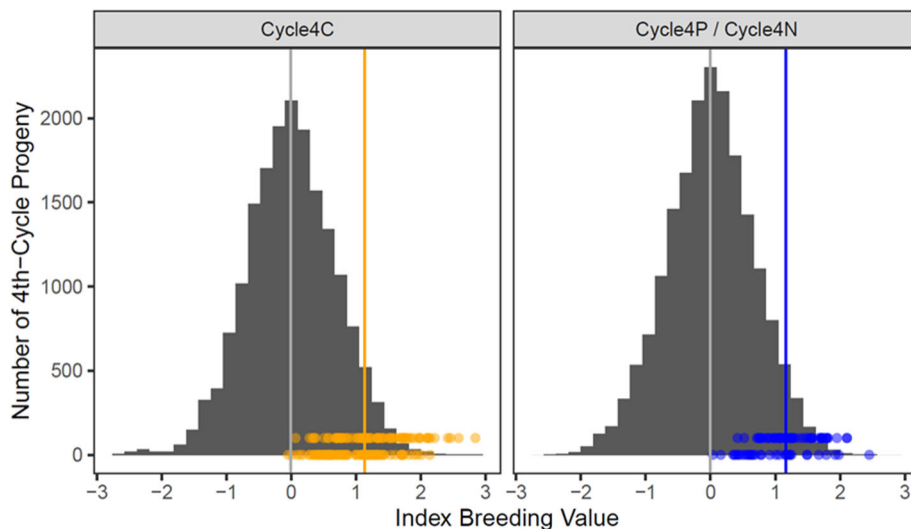
Figure 7: *Index breeding values for trees in 4th-Cycle Coastal progeny tests, grouped by their cross (very small print on y axis). Trees selected in 2021 are colored blue and those selected in 2019-2020 are colored red.*

Topgrafting Selections into Breeding Orchards

All selections made through 2020 have been topgrafted at Arrowhead Breeding Center. Starting in 2021, ~70% of the selections were topgrafted at Arrowhead, with the balance being grafted at the Cooperative Members' breeding orchards. Breeding among the flowering 5th-Cycle selections made in 2019 & 2020 commenced this past spring. We anticipate the breeding among the selections made in 2021 to commence in the next year or two. Open-pollinated seed will be collected from a subset of elite selections as soon as possible to expedite breeding value estimation for deployment purposes. TIP Staff are proud of the rapid implementation of the 5th-Cycle selection process and the thoughtful planning and action taken by Cooperative Members.
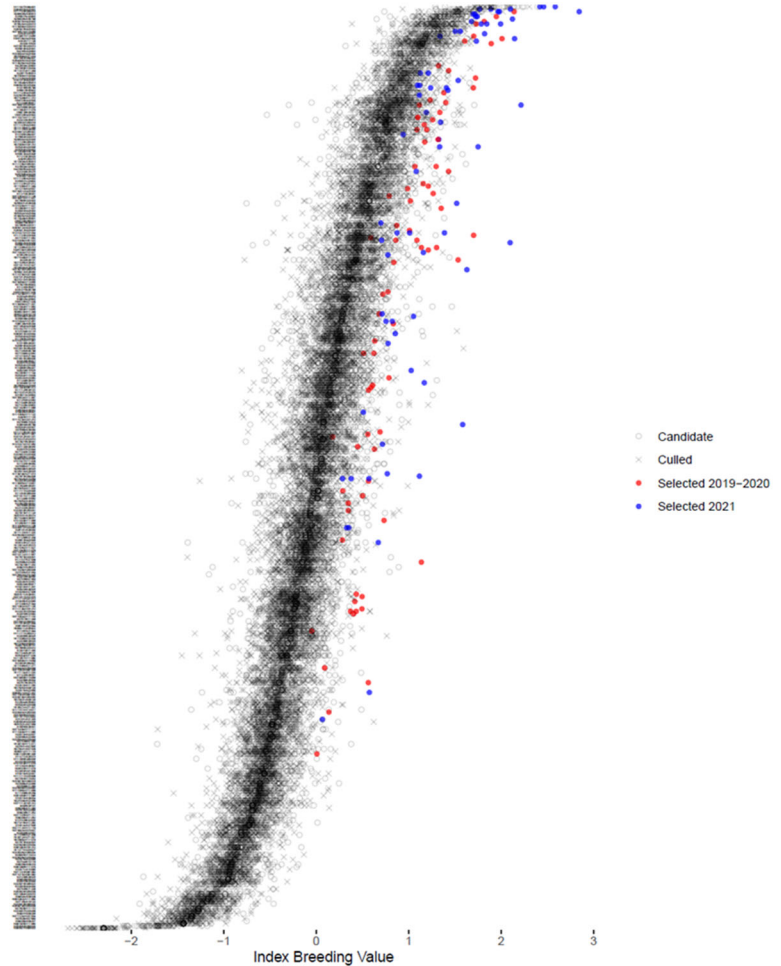
Grafting and Topgrafting at the Cooperative's Arrowhead Breeding Center

Just as last year, 2021 was a busy season for top-grafting of new 5th-Cycle selections at the Arrowhead Breeding Center in Georgia. Cooperative members and staff made 122 selections in February 2021; 86 of these were assigned to Arrowhead for breeding, and the balance were shared among Cooperative members participating in breeding. All of the Arrowhead selections were topgrafted into the New Breeding Orchard (NBO), which was established in 2009 and is now the oldest block with active breeding. The NBO was field grafted at a young age with the clone TIP2201951[1] to be used as an interstock. The target was to topgraft seven scions of each selection in hopes that the large number of shoots will result in greater strobilus production in a reduced amount of time.

Topgrafting commenced later this year compared to 2020 due to the cold, wet weather in January and February. Scion quality was noticeably better this year compared to 2020, when many scions were riddled with tip moth (*Rhyacionia* spp.) damage. The first graft was made February 27, and all grafting was done at the best time possible (e.g. dormant scion grafted onto shoots that were just starting to flush, Image 1). The last topgraft of the year was made March 11.

The location of each graft was recorded and flagged (Image 2) so that the impact of scion position can be assessed for graft success and flowering. Big dominant scions grafted into the terminal shoot appeared to be best for flowering this year, but we want to track this more closely in the future to see how grafts in lateral branches compare.

[1]Selection TIP2201951 is known to increase graft success and is very effective at promoting both female and male strobili when used as an interstock (see McKeand and Raley, 2000. Forest Genetics 7:179-182).

Image 1. *Timing for topgrafting was just about ideal this year. Dormant scions (far left) were collected in early/mid February, dipped in wax, and stored in the refrigerator. Most grafts were made when buds on the interstock shoot were starting to elongate (left picture right side) in early March. Finished grafts (right picture) were wrapped in Parafilm and will be assessed in June for grafting success (photos by Steve McKeand).*

tip moth damage. Flowering in 2021 was excellent on many of these one-year-old topgrafts (Image 3) and permitted accelerated breeding. Flower counts were made on all 107 surviving one-year-old topgrafts of 5ᵗʰ-Cycle selections in the NBO, and female strobili were found on 79% of them (85 in total). For those 85 selections with flowers, they averaged 20 female strobili per ramet.

Image 2. *The seven topgrafts of N365075 were labeled to keep track of the location of each graft: terminal graft (blue flag), three top lateral grafts (blue dot flags), three lower lateral grafts (white flags) (photo by Steve McKeand).*

The success from 2020 topgrafting is a good indication of the value of large (~40 feet or 12 m tall) TIP2201951 interstocks in the NBO. Of the 109 selections that were topgrafted in 2020, only two selections failed and required re-grafting in 2021. The overall graft success was 80%, not bad considering the large number of poor quality scions with

Image 3. *Excellent flowering was seen on the one-year-old topgrafts this year in the NBO with 79% of the 107 selections having female strobili, allowing breeding to commence with many 5ᵗʰ-Cycle selections. This topgraft had 30+ female strobili and was loaded with pollen (photo by Steve McKeand).*

Pollen production usually takes two to three years on newly topgrafted loblolly pine and is usually a major limitation to breeding progress. Fortunately, pollen was collected from 32 of the 107 one-year-old 5th-Cycle topgrafts this year (Image 4), which will permit a significant amount of 5th-Cycle breed-ing in spring 2022. There were six 5th-Cycle selections grafted last year where pollen was collected and used for breeding this spring, a testament to the success of accelerated breeding techniques implemented at the Arrowhead Breeding Center.

Image 4. Pollen collection got off to a great start this year with 32 of 107 one-year-old selections producing pollen (note all the bags in the lift, photo by Kitt Payn). For N085019 (right, photo by Steve McKeand), eight vials each with 0.5 cc of pollen were extracted from only 5 "catkin" clusters for breeding this year. More pollen clusters were collected from this selection for use next year.

The Graveyard Orchard (GYO) was planted in 2018 with seedling rootstock from a rust-resistant family; an annex was planted in 2019, and seedlings were field grafted with TIP2201951 similar to the NBO (Image 5). Of the 609 planting positions, there were only a few remaining "holes" that required re-grafting this year (58 re-grafts total). We anticipate that topgrafting new selections for breeding will take place on these interstocks in three to five years, as they reach a critical height for stimulating strobilus production.

The Northern BRO (Breeding Ramet Orchard) was planted in 2014 with rooted cuttings and was converted to TIP2201951 interstock starting in 2018. Trees in this orchard are 15 to 20 feet tall and will be topgrafted next year with selections made in February 2022.

Image 5. The Graveyard Orchard contains just over 600 ramets of an interstock clone that promotes graft success and strobilus production. Interstocks will reach the critical height for topgrafting new selections in three to five years (photo by Kitt Payn).

ACE2 Clonal Population

One of the central questions in genomic prediction is whether the marker-trait loci relationships hold when the chromosome crossovers (recombination) occur during breeding? In other words, can we use a model based on SNP markers-trait association in the Atlantic Coastal Elite (ACE) population to predict the breeding values of the next generation? To answer this question, the second generation of the Atlantic Coastal Elite population (ACE2) was produced from crosses among ACE1 clones (details are in the Cooperative's 2020 Annual Report). The ACE2 population will comprise a seedling trial series (recently established on four sites fall 2020) and a subsequent clonal trial series that will both be used for the validation of genomic prediction models.

Seed from 67 crosses among 73 ACE1 clones were harvested in fall of 2018 & 2019. The ACE2 seedlings were sown in May 2020 and were grown over the summer in Raleigh, NC at the Horticulture Field Lab. After all families reached an adequate height, the seedlings were top clipped in late August to increase seedling uniformity (Image 1). Top clipping was not performed in a traditional manner where seedling tops are all pruned at once and then clippings thrown away. Rather, at time of top clipping, 49 seedlings per tray from 67 different families were chosen to produce rooted cuttings. For each seedling, the terminal and up to three lateral branches were clipped from each seedling, dipped in a rooting hormone, and then placed into soil media and tagged to produce rooted cuttings (Image 2). Each RL Super

Image 2. Postdoctoral researcher, Dr. Nasir Shalizi, sticking rooted cuttings (photo by Austin Heine). At time of harvest, each seedling (ortet) was given a blue tag that matched the appropriate clone that was tagged with a yellow tag. This will allow us to track which seedling (now in field trials) was the origin of which clone.

Cell contained one or two cuttings with two cells stuck per seedling for a total of 12,250 cuttings (up to four cuttings per seedling).

In late November, indicated that 8,147 out of the 12,250 cuttings had rooted (~67% rooting success, which is not bad for a mid-summer harvest). In the most recent inventory in March 2021, the family with the lowest rooting percentage still had cuttings that rooted from 18 of the 49 seedlings, and 62 families had cuttings that rooted from 30 or more seedlings. The cuttings are now being planted into 3 gallon pots (Image 5) to produce hedges in order to bulk up the number of cuttings per individual for the ACE2 clonal testing effort. The cutting will be planted in replicated field trials in 2023 winter.

A big benefit of having the ACE2 clonal population derived from the ACE2 seedlings is that we only have to genotype one population for testing genomic selection using both the seedling and clonal ACE2 progeny tests.
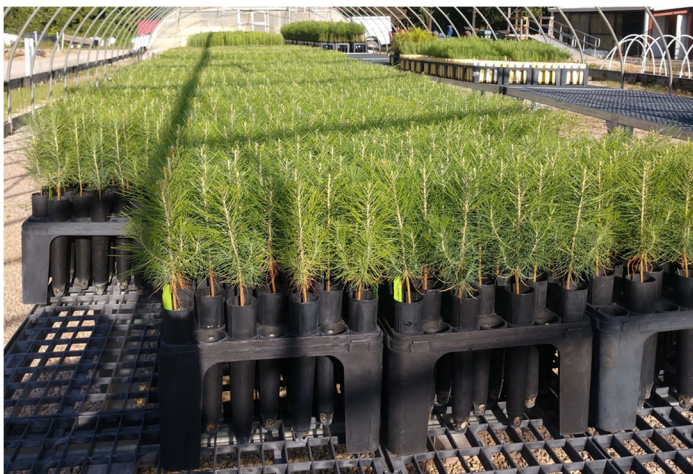

Image 1. ACE2 seedlings in August 2020 after top-clipping (to a height of 10 inches) to produce cuttings for rooting. These uniform, top-clipped seedlings were planted across four test sites the fall of 2020 (photo by Austin Heine).
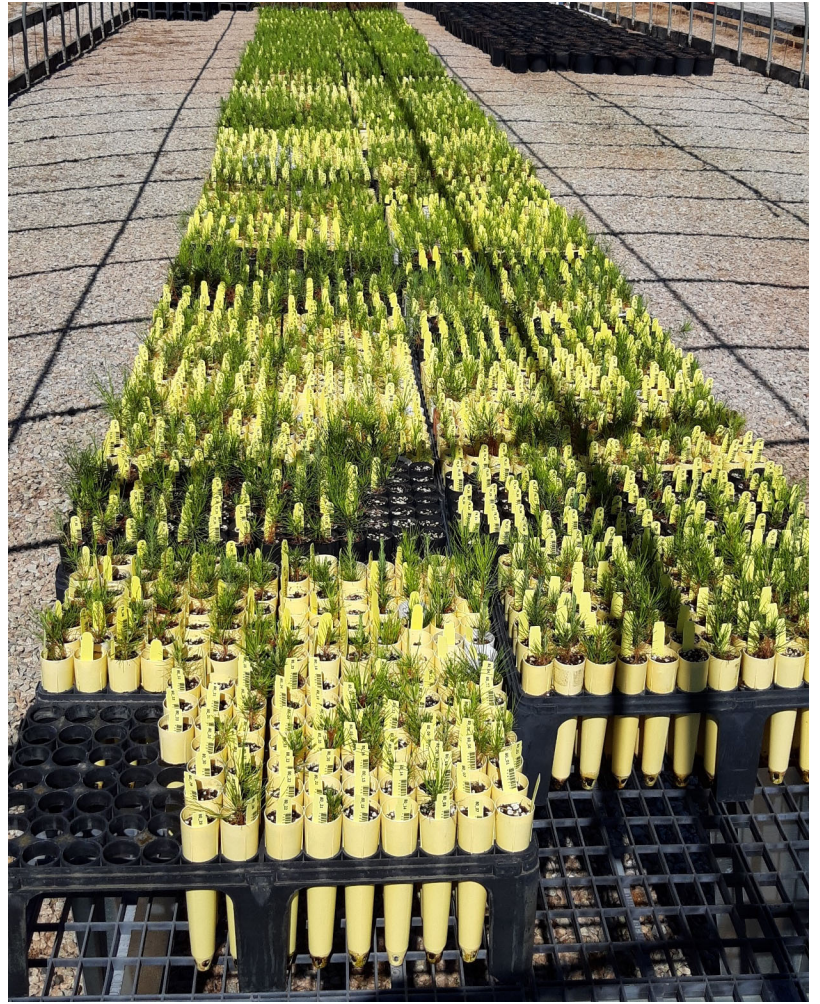
Image 3. (upper left) *Roots developed at the base of cuttings (bottom right of the photo) and were first seen on October 10th, approximately 6 weeks after sticking the cuttings (photo by A. Heine).*

Image 4. (upper right) *Successful cuttings moved to the outdoor greenhouse pad to put on growth. (K. Payn)*

Image 5. (lower left) *Undergraduates Sam Blumenfeld (right) and Alex Simpson (left) attack the mountain of soil medium required for potting up the future ACE2 hedge plants (photo: A. Heine).*

Database Updates

Progeny Test Data Reports

This year, the Cooperative made an important update to the "Progeny Test Data" section of TIPRoot. It was apparent that the hard work of progeny test maintenance and measurement for 4th-Cycle tests was disappearing into a "black box", only to resurface in analysis summary results and the Annual BLUP spreadsheets. In order to provide Cooperative members with a more immediate rundown of the data they collect, a new Data Report template was created. This template is a vast improvement over the previous versions of the report, since it includes spatial information about each rep of the progeny test, as well as more informative tables featuring rep-by-rep breakdowns of the measurement data (Figure 1, Figure 2). These tables and figures should help facilitate data diagnostics and troubleshooting, as well as provide important metrics about data quality.

Figure 2. Example of phenotype distributions for a 4th-Cycle test. A height/DBH plot is shown in the bottom right. These plots enable quick visual data diagnostics to assess test quality.

Figure 1. An example of the data summary table for a 4th-Cycle progeny test is shown here. The top line shows test-level information, such as the spatial dimensions of the reps and site means for all traits. The second table gives a rep-level breakdown, including the within-rep coefficient of variation (CV) for tree height.
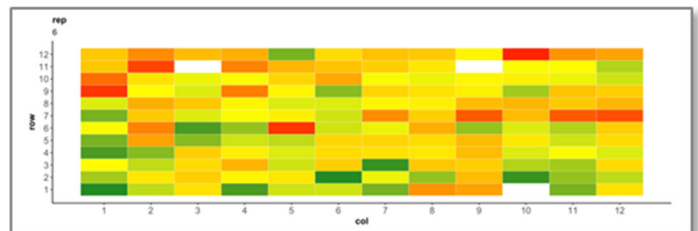
Figure 3. Raster plot showing spatial trends in a 4th-Cycle progeny test. Row numbers are labelled on the Y axis, and column numbers appear on the X axis. In this rep, the 12th row appears shorter than the other rows, and a number of very short trees appear in the corner of the first column.

In some instances, tree growth is not uniform throughout a rep of a test due to environmental factors such as moisture gradients or uneven grade. This may appear as a pocket of dead trees, or in some cases a patch of shorter or taller trees. The raster plots included in each data report help to quickly diagnose these spatial trends. Each raster plot is a heatmap of tree height deviations from the rep mean. Missing data appear as white squares. The squares are colored from red to green, with taller trees showing a greener color (Figure 3).

The new data reports can be accessed in the 'Progeny Test Data' section, which is the first link under 'Measurement Data' in the 'PRS and Pedigree' tab (Figure 4). After opening the Progeny Test Data tool, entering a test ID in the "Test ID(s)" box, or highlighting the "4TH CYCLE PROGENY" under 'Series type(s)' drop down menu, will bring you to the progeny test data table where the 4th-Cycle data reports can be easily accessed (Figure 5).

Soon to be Measured

A new feature was added for Full Members to forecast their measurement workload over time. The Soon to be Measured query provides a convenient interface to view progeny tests that have been assigned to your organization. The tool can be accessed via the fourth link under "Measurement Data" in the 'PRS and Pedigree' tab. (Figure 4).

The query for Soon to be Measured can be activated by clicking on the circular buttons next to 'Select a measurement year:', and clicking 'Go'. This brings up a list of tests that are scheduled for measurement on the selected measurement year (Figure 6).

Figure 4. The new progeny test data reports can be accessed in the Progeny Test Data link, next to the orange arrow. The Soon to be Measured tool is the fourth link down, next to the blue arrow. This tool can be used to get a quick view of a member's upcoming progeny test measurement assignments.

Figure 5. A view of a section of the Progeny Test Data table is shown above. Data reports for measured tests can be accessed by clicking on the blue 'file' link (red arrow). An .html file will automatically open on your internet browser. Likewise, the establishment reports for each test can be accessed by clicking the blue 'file' link under the 'Est. Rep.' column (yellow arrow).
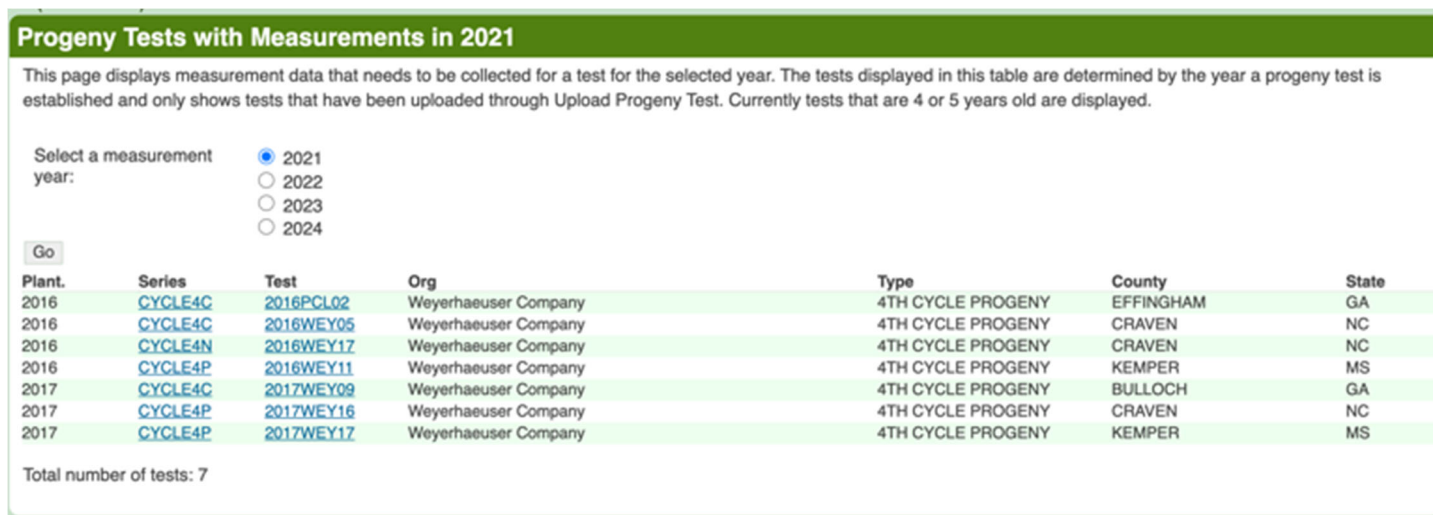

Figure 6. The Soon to be Measured tool provides an easy view of upcoming test measurement assignments. Each test is shown as a hyperlink; clicking on this link takes the user to the Progeny Test Data table.

Arrowhead Breeding Center Update

It was another busy year at the Arrowhead Breeding Center that began in late June of 2020 with a huge effort in pruning the lower limbs of the New Breeding Orchard (NBO). The earliest rows of the NBO were planted in 2008 at a 25' x 25' spacing, but twelve years of growth resulted in tight crowns making it difficult to operate lift equipment during breeding season (Images 1a and 1b). After considering the option of thinning every other tree, a decision was made to lift the crowns as high as possible without damaging the trees. Our target was to leave 40-50% of the total tree height with live crown after pruning. By pruning the trees and keeping the crowns high and tight, we were able to maximize the number of rootstock available for topgrafting 5ᵗʰ-Cycle selections; and the pruning created plenty of room for moving around the crowns for future breeding efforts (Image 2). Additional pruning work included the removal of lower limbs from the rootstock in the Northern Breeding Ramet Orchard (NBRO). The NBRO is expected to hold approximately 170 5ᵗʰ-Cycle selections with topgrafting scheduled for spring 2022. Special thanks to Chuck Little, Anita Johnson, and Dick Young for their help in this effort!

Management of soil fertility has been an important reason for the success of the Arrowhead Breeding Center. From the time an orchard block is cleared and slated for planting with rootstock, annual soil and foliage samples are collected to determine the fertilization needs. We have made substantial improvements in the nutrition status of the orchards over the past several years (Figure 1), and the Cooperative has benefited from the orchards

displaying substantial growth and development, as well as excellent flowering on the 1-year-old 5ᵗʰ-Cycle topgrafts (discussed in another article of this report). We firmly believe that good soil fertility is playing an important role in this production. Grafting in spring of 2021 filled up the rest of the rootstocks in the NBO, so we are counting on the NBRO and Graveyard Orchards developing rapidly to accommodate topgrafting the remaining 5ᵗʰ-Cycle selections.
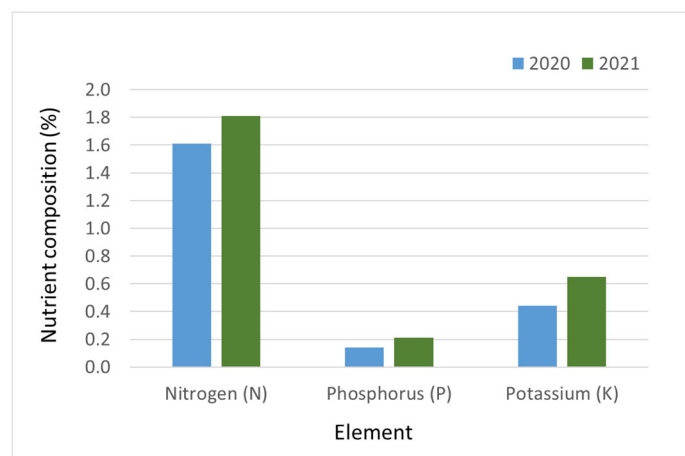
Figure 1. *Summary of key macronutrients obtained from foliage nutrient reports taken in March of 2020 and March of 2021 from the oldest block of the Graveyard Orchard. Fertilization has led to consistent increases in N, P, and K. This is promoting the rapid growth that has been observed in the Graveyard Orchard and will ensure the trees reach an appropriate height prior to the topgrafting of 5ᵗʰ-Cycle selections in spring 2024.*

Image 1a.(l) *The NBO in June prior to the pruning of the lower branches. Tight crowns let little light reach the ground.* **Image 1b.(r)** *The NBO after pruning. Crowns are no longer touching and an abundance of light is reaching the ground. There is now easy access to the crowns with bucket lifts for 5ᵗʰ-Cycle breeding (Photos provided by Austin Heine).*

SEED AND CONE YIELDS

The 2020 cone crop appeared to be another great year (Table 1). As one of the members put it, the 2020 cone crop was, "a really good cone harvest with excellent seed yields". That's what we like to hear! In fact, the 2020 seed harvest was the best the Cooperative has had in 20 years, a little shy of the ~41 tons of seed produced in 2000 (Figure 1). This year, 2nd-Cycle seed production was 28% of the crop, while 3rd-Cycle+ material made up the majority of the seed produced at 72% (Figure 2).

Table 1: *Fall 2020 cone and seed yields compared to the 2019 harvest. Cones collected from 4th-Cycle orchards were included in the 3rd-Cycle data, hence the "3rd-Cycle+" label in the text.*

Provenance / Orchard Cycle	Bushels of Cones		Pounds of Seed		Pounds of Seed per Bushel	
	2020	2019	2020	2019	2020	2019
Coastal 2.0/2.5	10,897	8,058	16,453	10,338	1.51	1.28
Coastal 3.0+	25,642	16,213	37,540	20,621	1.46	1.27
Piedmont 2.0	3,662	3,433	5,677	4,776	1.55	1.39
Piedmont 3.0+	11,319	11,417	16,341	16,055	1.44	1.41
Northern 2.0	294	0	445	0	1.51	0.00
Northern 3.0+	3,955	5,164	5,015	6,078	1.27	1.18
Totals	**55,769**	**44,285**	**81,471**	**57,868**	**1.46 Avg.**	**1.09 Avg.**
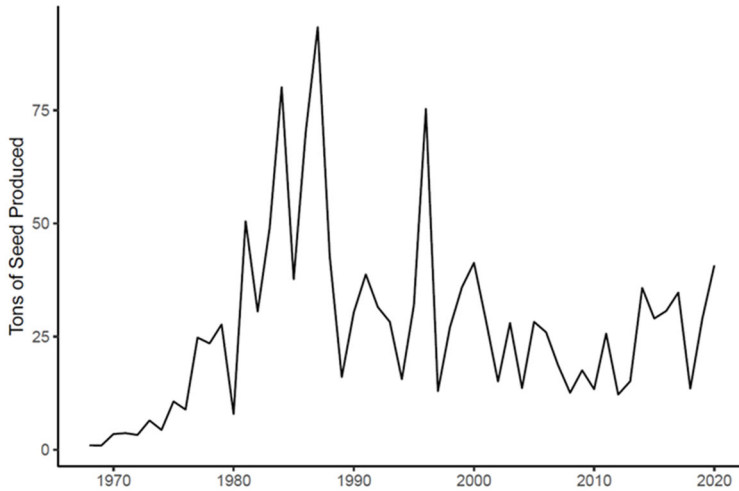
Figure 1. *Total tons of seed produced yearly by the Cooperative members since record keeping began in 1968. The 2020 cone harvest had the highest total tons of seed produced in 20 years!*

Figure 2. *Since 1968, the Cooperative has tracked annual seed yields from Cooperative members. 1st-Generation seed orchards are no longer being harvested. In 2020, 28% of the seed harvest came from 2nd-Generation seed orchards, while the other 72% came from 3rd-Cycle+ seed orchards.*
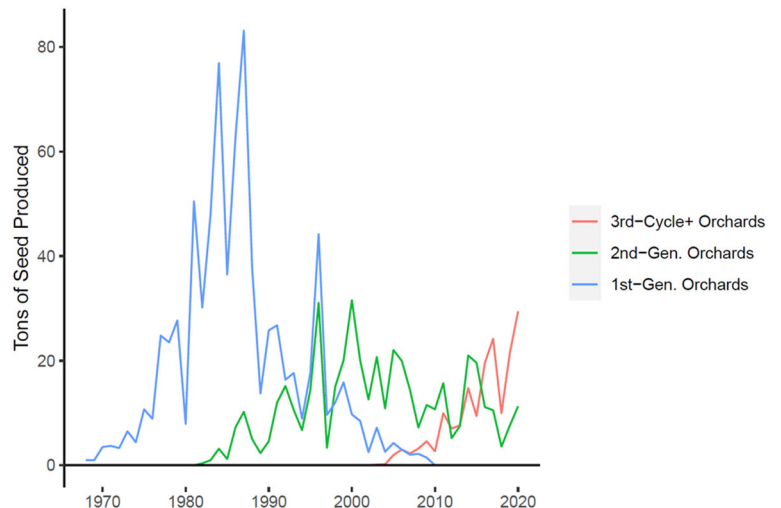
RESEARCH

Expanding our Knowledge about Loblolly Pine Pollen to Increase Filled Seed per Cone from Controlled Pollinations.[1]

Open-pollinated cones typically outperform control-pollinated cones with respect to the amount of filled seed per cone. Based on cone analysis from the Tree Improvement Program multi-year pollination bag study, open-pollinated cones produced an average of 30 more filled seed per cone than the cones obtained from the best pollination bag type. This disparity was primarily attributed to the difference in first year aborted ovules (see Figure 2 of "PBS Pollination Bag Study Update" in the 2017 Annual Report). According to Bramlett et al. (1977), seedbug feeding or a lack of viable pollen are the main cause of first year aborted ovules. Since open-pollinated cones were on the same branches as the control-pollinated cones in the pollination bag study and had minor issues with first year aborted ovules, seedbug feeding was ruled out as the cause of the differences in first year aborted ovules, leaving pollen as the likely culprit. With this in mind, several research projects have been initiated to further investigate loblolly pine pollen viability and vigor with the aim of increasing the number of filled seed per cone from controlled pollinations.

The first research project investigates new technology that can be used to test loblolly pine pollen viability. In the summer of 2020, testing began on 20 unique pollen lots that were collected in the spring of 2019. Each pollen lot was tested using our standard in-vitro pollen germination test by incubating pollen grains on agar plates and counting the number of germinated pollen grains per plate. The same pollen lots were then assessed using a new technology that involves impedance flow cytometry (IFC). IFC measures the resistance of pollen grains as they pass through an alternating current electrical field. In November of 2020, we were able to start testing IFC using the Ampha Z32 provided by Amphasys, a company located in Switzerland. The use of impedance flow cytometry for viability testing of loblolly pine pollen is currently under investigation. A key objective of the study is to correlate the pollen viability results to final seed yields. In the spring of 2020, five unique pollen lots were applied to 7 different clones at the Arrowhead Breeding Center in Cochran, GA. Following pollination and bag removal, insect cages composed of wire mesh were placed around the cones to protect them from insect damage (Image 1). Cone analysis results from these cones will be used to determine whether IFC or in-vitro germination testing is better at predicting the amount of filled seed per cone.

Respiration testing of these same pollen lots is also scheduled to be performed. A machine capable of rapidly assessing respiration on a bulk sample of pollen lots at once has been identified.

Image 1. Insect cages around cones that will be used in the fall 2021 cone analysis. Five unique pollen lots were applied to 7 different clones. Left is an image of a single replication of one clone having been pollinated by 5 unique pollens, and an open-pollinated control. Insect cages were installed shortly after bag removal to protect cones from insect feeding. The image on the right shows conelets protected inside of the wire mesh with aluminum wire supports (photos by Austin Heine).

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

[2] *Amphasys is a company focused on chip-based single-cell analysis and can be found at https://amphasys.com/ .*

The second research project aims to quantify the impact that pollen development stage at time of collection has on filled seed yields in loblolly pine. In late February 2021, Austin Heine traveled to Rayonier's Yulee Seed Orchard to collect pollen for this study. Pollen was collected from five different clones. While collecting pollen from each clone, five different stages of pollen development were captured along with a control. The five stages are classified in Bramlett and Bridgwater (1989) as 3.3, 3.6, 3.9, 4.0, and 5.0. Stage 3.3 is equivalent to pollen that is still developing when catkins are green in color and squeeze yellow when pressed between fingers (Image 2). Stage 5.0 is a pollen catkin where over 10% of the pollen contained in that catkin has already released (Image 2). Individual catkins were removed from a cluster and placed in designated collection bags according to their development stage. The control was collected using the conventional method of pollen collection where an entire cluster of catkins was clipped from the tree with a range of stages of development included in the sample.

Immediately following pollen extraction, the pollen lots were taken to Arrowhead Breeding Center where the Pollen Development Classification Study was installed. A total of 10 trees (females) were bagged with 30 pollination bags per tree (5 male clones x 6 pollen stages) giving a total of 300 pollination bags (Image 3). All cones in the pollination bags were pollinated at stage 5.0 when female strobili were fully receptive. All bagged strobili that were not at stage 5.0 at time of the 2nd pollination were removed.

During bagging, study trees were injected with ARBORjet Tree-age G4 insecticide to protect the cones from insect damage. Cones will be harvested in October 2022. Cone analysis will determine whether the pollen development stage at time of collection significantly effects filled seed yields. Furthermore, cone analysis should determine whether pollen collected at an early stage leads to a higher proportion of 1st-year aborts or empty seeds (pops).

Pollen testing using in-vitro germination, respiration, and impedance flow cytometry is currently taking place in the TIP lab in Raleigh, NC. We are grateful to LaShaun Sanders and Jessica Maynor with Arborgen, and Dan Morrow with Weyerhaeuser for their help in in-vitro pollen germination testing. We are also grateful to Jake Rogers and Hogan Robinson with IFCO for helping with the tree injections. David Barker with Rayonier for provided the seed orchard and lift for fresh pollen collection as well as pollen extraction facilities. Chuck Little (TIP) and Anita Johnson (Georgia Forestry Commission) for helped install and remove pollination bags in this study. This research project is a true testament to the power of the cooperative members working together to achieve a common goal!

Image 2. *Left is an image of catkins at Stage 3.3, the earliest stage of pollen collection when pollen catkin is still green and squeezes yellow when pressed between fingers. Right is Stage 5.0 where over 10% of the pollen has already shed from the catkins and is fully mature. Pollen was dried in a forced air drying system. (photos by Austin Heine).*

[3]ARBORjet is a company that specializes in tree health protection and tree injections and can be found at https://arborjet.com/ .

Image 3. *Pollination bags installed for the Pollen Development Classification Study at Arrowhead Breeding Center in March of 2021. Over 300 pollination bags were installed and carefully monitored during the 2021 breeding season to ensure pollen was applied while female strobili were at maximum receptivity so that we can test if the stage of pollen development at time of collection has an impact on filled seed per cone (photo by Austin Heine).*

References

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Bramlett, D.L. and F. Bridgwater. 1989. Pollen development classification system for loblolly pine. In Proc 20th Southern Forest Tree Improvement Conference Charleston SC. pp. 116–121.

Development of Pita50K Array

Introduction

With funding from USDA-NIFA project (Award number: 2016-67013-24469, PI Isik), a collaborative effort began in 2016 to develop a SNP array for loblolly pine. The first high density genotyping array was completed in October 2019. Since then, over 12,000 loblolly and a number of slash pine trees have been genotyped. This project was part of the Conifer SNP Consortium efforts that also developed SNP arrays for *Pinus radiata*, tropical pines, *Picea abies*, and a multiple species array for EU/France.

The Pita50K array consists of 46,439 probes that have been validated through a screening array. Current and future applications for the array include production of linkage maps, genome wide association studies, DNA fingerprinting, and genomic selection applications that have the potential to fundamentally shift the process of loblolly pine tree improvement in coming years.

Genetic Material & SNP Discovery

Sequencing data used for SNP discovery for Pita50K originated from five separate studies and utilized both high throughput and reduced representation sequencing methods in an effort to capture as much allelic diversity as possible on the array. The five studies included three from the Pinemap project, utilizing the ADPET2 and PSSSS populations, two exome capture studies originating from the University of Florida, and additional CTGN probes that had been confirmed as useful in prior genotyping efforts. A total of 2,698 unique trees were sequenced between the studies and represented genetic material from across the natural range of loblolly pine. This composite set of sequencing data served as the basis for variant discovery for markers to be included on the array. Marker discovery was facilitated through the creation of a custom bioinformatics pipeline that included stringent quality control, variant calling, and probe design steps (Caballero et al. 2021). A total of ~8.1 million variants were discovered via the pipeline. These 8.1 million variants yielded 642K probes that successfully met Thermo Fisher's technical specifications to be included on the screening array for assessment.

Screening Array

Successfully designed probes were submitted to Thermo Fisher for assessment using their informatics scoring criteria, assigning probes into recommendation categories. Since the number of probes scored exceeded the amount of space present on the screening array, probes were ranked based on the combination of their Thermo Fisher recommendation and score from a custom index that prioritized uniqueness of the probe sequence and distance from other SNP markers. This was done in an effort to increase the chance of selecting probes that will successfully convert to a marker on the array. A total of 423,695 variants were selected and successfully tiled on the screening array and their quality was assessed using a diversity panel of 424 DNA samples, including 36 haploid samples, made up of NCSU Cooperative Tree Improvement genetic material.

Selection of Pita50K Markers

Assessment of markers on the screening array revealed that 84,845 variants were placed into preferred categories by Thermo Fisher. The number of markers once again exceeded the space available on the commercial Pita50K array, so additional filtering steps were performed to remove very rare markers and those that were likely due to sequencing or genotyping errors. Post filtering and tiling, the Pita50K design included 46,439 high-quality, validated probes. Ultimately, through the use of the screening array, metrics for marker utility substantially increased when comparing selected versus non-selected markers. Of the selected probes included on Pita50K, 33.9% of probes were annotated in or near 5,688 unique genic regions, and 2.9% were annotated as having medium or high impact on protein product. The screening array procedure resulted in an array enriched for high-frequency markers that will be useful for genomic prediction, QTL analysis, and genome-wide association studies. Since *Pinus taeda* is a wild outcrossing species with little breeding history, most SNPs are expected to be low-frequency, and this was apparent in the site frequency spectrum of markers on the screening array (Figure 1). This observation reiterates the need to use population-based metrics for SNP selection as opposed to bioinformatic scoring criteria. The probes on Pita50K are located over 22K scaffolds across the reference genome assembly.
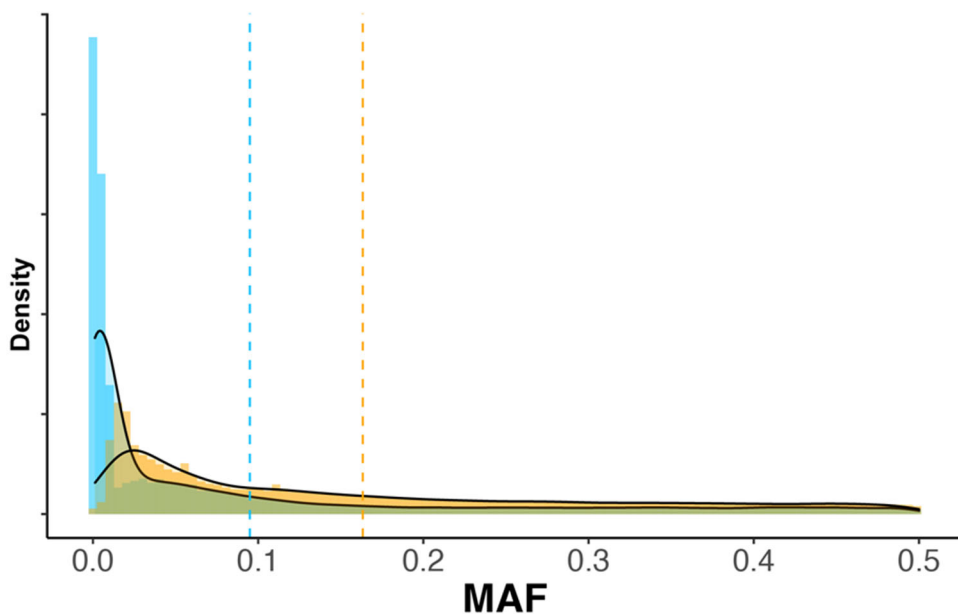
Figure 1. Site frequency spectrum for SNPs selected (orange) and not selected (blue) for inclusion on the Pita50K Affymetrix chip. On the X axis is the minor allele frequency (MAF) for each marker. The flat distribution of the selected markers shows the impact of SNP selection using population-based metrics.

How has the array been used?

To date, Pita50K has been utilized by the Cooperative in a number of different projects. The 2063 clones from 21 parents in the Atlantic Coastal Elite (ACE) population were genotyped by Pita50K to evaluate genomic selection methodologies. These markers were also used to calculate genomic relationship matrices for improved genetic parameter estimation and for pedigree error corrections. The moderate to high predictive abilities shown from the study indicate that the performance of individuals with no phenotypic data could be predicted within the same population. The results also indicated considerable gains could be made from genomic selection compared to traditional BLUP methodologies. More recently, additional phenotypic data for wood stiffness and density have been obtained and are now being evaluated to determine if genomic markers will improve prediction accuracies and predict breeding values for individuals with no phenotypic information.

About 480 individual trees in 4th-Cycle progeny tests were genotyped for the purpose of prediction of genomic breeding values. While this research is still underway, preliminary results look promising, especially for tree height and stem straightness. Prediction abilities for height and stem straightness were 0.29 and 0.57, respectively. This suggests that genomic models trained using a clonal population such as Atlantic Coastal Elite can be successfully applied to genomic prediction of seedling progeny.

Construction of a high-density consensus linkage map using Pita50K was funded via the USDA-NIFA project "Genomic Selection in Forest Trees: Beyond Proof of Concept" (Proposal Number: 2018-06309) (Figure 2, p.25). A total of 10,204 markers were successfully mapped through the use of two large QTL discovery populations. Results indicate that the linkage map from this study provides a much higher genetic resolution when compared to previous consensus maps. This linkage map was used to identify three large-effect non-specific resistance QTL for fusiform rust disease resistance. The high genetic resolution from this study resulted in tight confidence intervals for the QTL positions which is important for marker assisted breeding. In the future, this map will be a valuable tool for genome sequencing and assembly of the loblolly pine genome. A publication detailing this study has been submitted to the journal Heredity and is currently under review.

In addition to the studies outlined above, Pita50K has also shown value in identifying and correcting points of pedigree contamination. Recently, through genotyping, it was discovered that a ramet of an ACE clone that was grafted at Arrowhead as a part of the 4th Cycle actually originated from a filler tree that was not updated as such on the maps. This tree was bred as a female, pollen collected and used on other females, seed extracted, and progeny sown into 4th-Cycle progeny tests. With the use of the array, we were able to identify this error and make the necessary corrections within the pedigree and database.
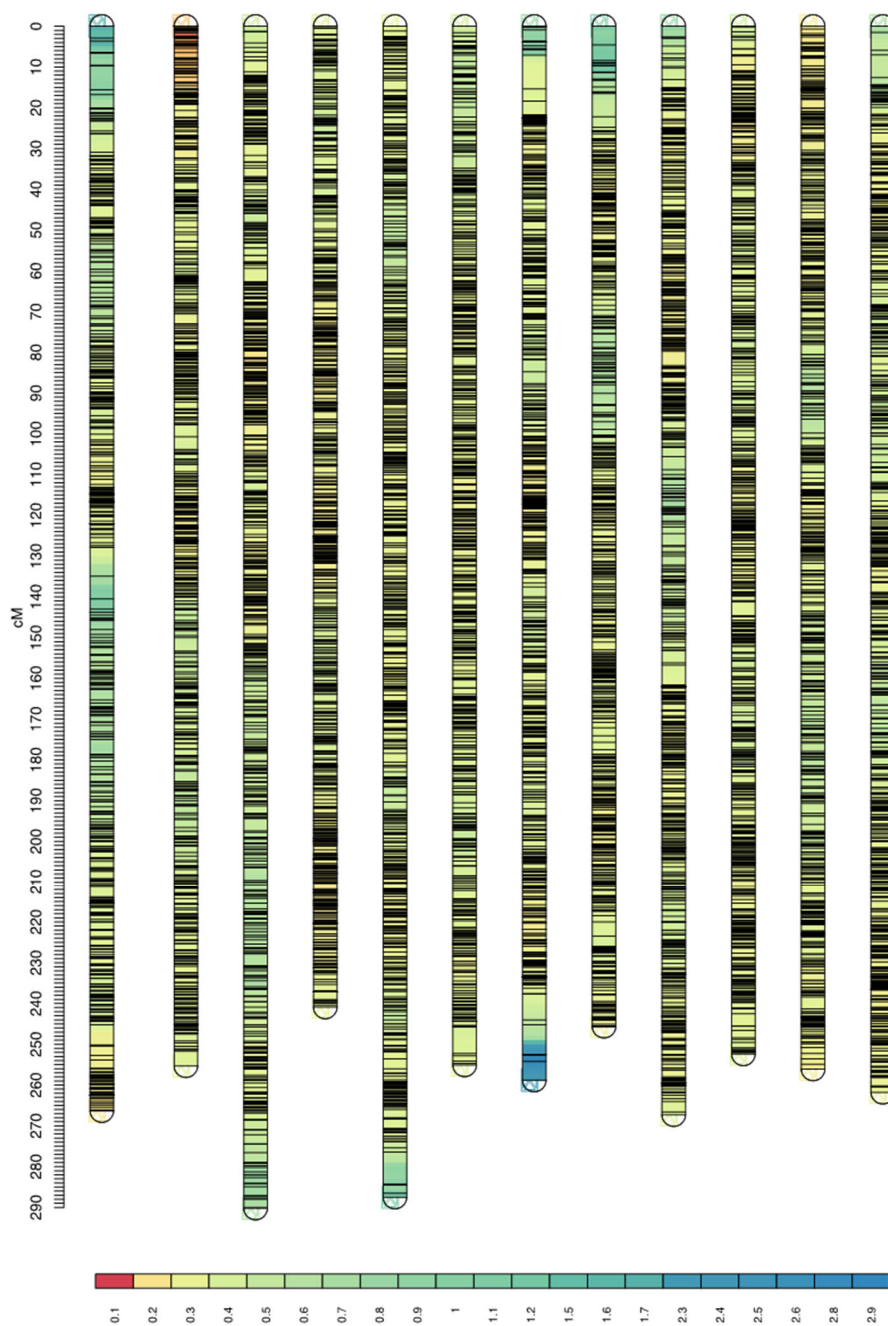
Figure 2. *Density heatmap of the consensus map from Lauer and Isik (2021, in review) for the twelve chromosomes of* Pinus taeda. *Regions of each chromosome are colored according to marker density, with red regions indicating higher density and blue regions indicating lower density. Color legend shows the colors corresponding to average intermarker distances, the black lines are the individual markers.*

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Caballero, M., E. Lauer, J. Bennett, S. Zaman, S. McEvoy, J. Acosta, C. Jackson, L. Townsend, A. Eckert, R. W. Whetten, C. Loopstra, J. Holliday, M. Mandal, J. L. Wegrzyn, F. Isik. 2021. Towards genomic selection in *Pinus taeda* (Pinaceae): Integrating resources to support array design in a complex conifer genome. Applications in Plant Sciences (in review).

Edwin Lauer and Fikret Isik. 2021. "Major QTL confer race-nonspecific resistance in the coevolved *Cronartium quercuum* f. sp. *fusiforme - Pinus taeda* pathosystem". Heredity (in review)

Genotyping Update of 4th-Cycle Progeny

This year, the Cooperative initiated a new project for testing the ability of genome-wide markers to predict breeding values for individual trees in 4th-Cycle progeny tests. With the help of Cooperative members, needle samples from a total of 480 individual trees were sampled and sent to the TIP laboratory in Raleigh. Samples needles were cut into small ~4-5mm segments and were placed into 96-well sampling blocks that were then shipped to the University of Florida Forest Genomics Laboratory for DNA extraction. Although tissue processing took a significant amount of time, it paid dividends in terms of a low sample drop-out rate. In fact, only 6 samples had call rates below 10%, indicating a 99.98% success rate in tissue processing and DNA extraction (Figure 1). This was encouraging since the samples were of mixed origin, with some being freshly sampled from progeny tests and others coming from long-term silica storage. The fact that DNA extraction was successful for nearly all samples indicates that proper sample processing may be more important than tissue age or storage conditions.

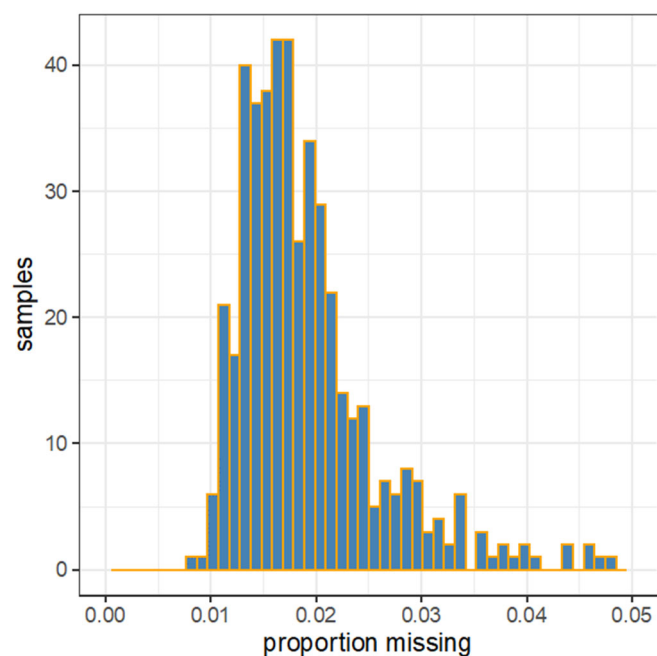Although the genomic prediction study is still in early phases of model design and validation, some interesting findings about the genome structure for loblolly pine have already been made. For example, we were able to estimate the rate of linkage disequilibrium (LD) decay over physical distance. LD represents the non-random association among alleles at different loci; generally, it is a measure of independence between genotyped markers. If LD decays rapidly, markers that are spaced relatively close together are independent and provide different information; if LD decays slowly, then markers that are spaced close together are not independent, and they provide similar information. Using the Pita v.2.01 reference genome as a guide, markers appearing on the same contigs were used to estimate the decay of LD over physical distance. Using a total of 2777 marker pairs appearing on the same contigs, it was observed that the median LD (R^2) between pairs of markers drops by 50% within 20kb, and drops another 50% between 20kb and 1Mb (Figure 2). For a genetically diverse species such as loblolly pine, with a genome more than 22Gb in size, many markers will be required to capture all the genetic variation in our breeding population.

Figure 1. Distribution of missing genotypes for the 480 samples across 30K Pita50K markers being used on the combined ACE1/4th-Cycle genomic prediction dataset. No sample had more than 5% missing data, which minimizes potential errors caused by genotype imputation.
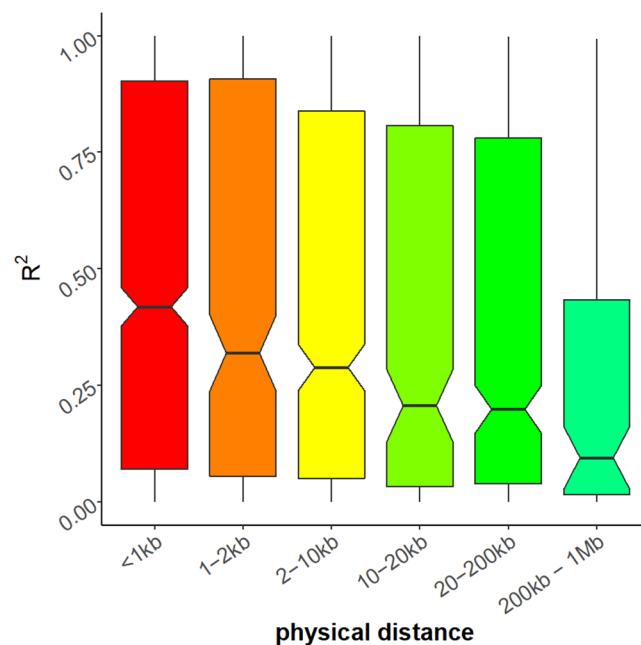

Figure 2. LD decays rapidly for loblolly pine. In this figure, 2777 marker pairs appearing on the same contigs of the Pita v.2.01 reference genome were used to estimate LD decay over physical distance. On the X axis, pairs of markers are binned by physical distance. On the Y axis, interallelic R^2 values are shown for all pairs of markers.

Genetic Mapping of Fusiform Rust Resistance Genes[1]

Overview

An important milestone was met this year in the project funded by USDA-NIFA grant 2018-06309 (PD: Isik): "Genomic Selection in Forest Trees: Beyond Proof of Concept". The major Quantitative Trait Loci (QTL) underlying non-race-specific fusiform rust disease resistance described in the 2020 Annual Report were validated in field trials. The clonally propagated Atlantic Coastal Elite (ACE) population was genotyped with the Pita50K SNP array, and markers that were mapped in the genetic map of Lauer and Isik (2021) were used in a genome-wide association analysis. The same regions associated with QTL in the mapping populations were associated with rust resistance in the ACE field trials (Figure 1).

This validation represents a major proof of function for the resistance genes discovered in the mapping populations. It shows that these genes are not only effective in controlled inoculation experiments, but also in the field. Secondly, the broad specificity of these genes is apparent in their efficacy across eight environments ranging from western Alabama to the coast of North Carolina.

Comparative genomic mapping using the consensus map published in De La Torre (2019) placed the GRID1 and GRID2 resistance genes on Linkage Group 2, which had been previously associated with resistance genes discovered in Wilcox et al. (1996) and Amerson et al. (2015) (Figure 2). Specifically, Linkage Group 2 was associated with *Fr1*, *Fr3*, *Fr4*, *Fr6*, *Fr7*, and *Fr9*. Additionally, the updated posi-
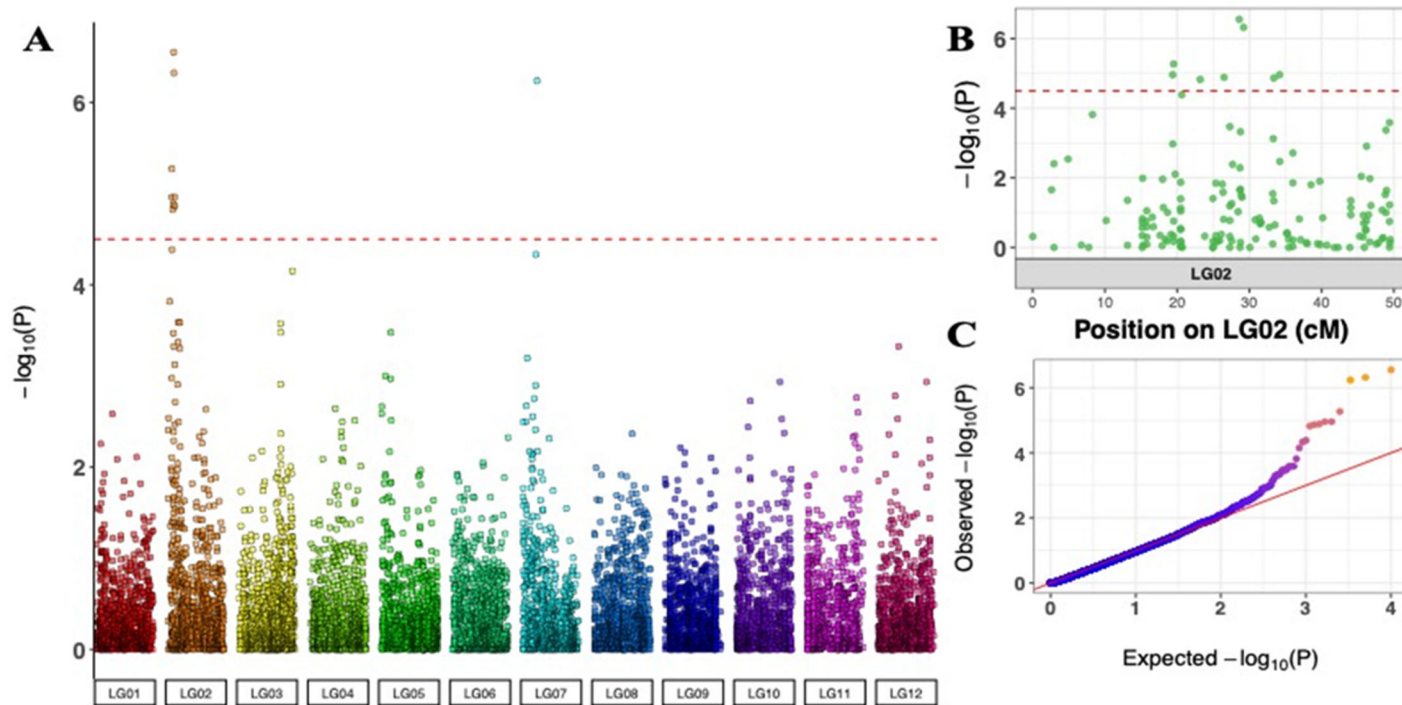
Figure 1. *Genome-wide association study (GWAS) using the clonally propagated ACE population, established across eight environments in the southeastern United States (Shalizi and Isik 2019). Chromosome names and orientations were adjusted to agree with the genetic map published by De La Torre et al. (2019). Linkage group assignments were established by cross-referencing contigs of the Pita2.01 reference genome represented in both genetic maps. The two resistance genes from TIP330244 were placed on Linkage Group 2, and the resistance gene from TIP1162449 was placed on Linkage Group 7. A: Linkage groups 2 and 7 were associated with field-level rust resistance in the ACE population. B: Two distinct regions on Linkage Group 2 contained significant GWAS hits, corresponding to GRID1 and GRID2. C: QQ-plot showed tight agreement between expected and observed P values, indicating minimal influence of long-range LD or population structure.*

[1] *This is a summary of Eddie Lauer's PhD graduate research.*

tion for GRID3 is now 47cM on Linkage Group 7 (Figure 2), around 73cM from another locus associated with rust resistance (Cumbie et al. 2020). Most of the parents used in the Wilcox and Amerson studies were progeny tested by the North Carolina State University Cooperative Tree Improvement Program (Table 1). It was immediately apparent that the resistance donors of *Fr1*, *Fr2*, *Fr5,Fr6, Fr7, Fr8,* and

Fr9 were mediocre or below average for rust resistance in multi-environmental trials compared to the resistance donors of the GRID genes. The enhanced efficacy of the GRID genes relative to the previously identified *Fr* genes is likely due to lower specificity (non-race-specific broad-spectrum resistance). More research will be required to address this hypothesis.
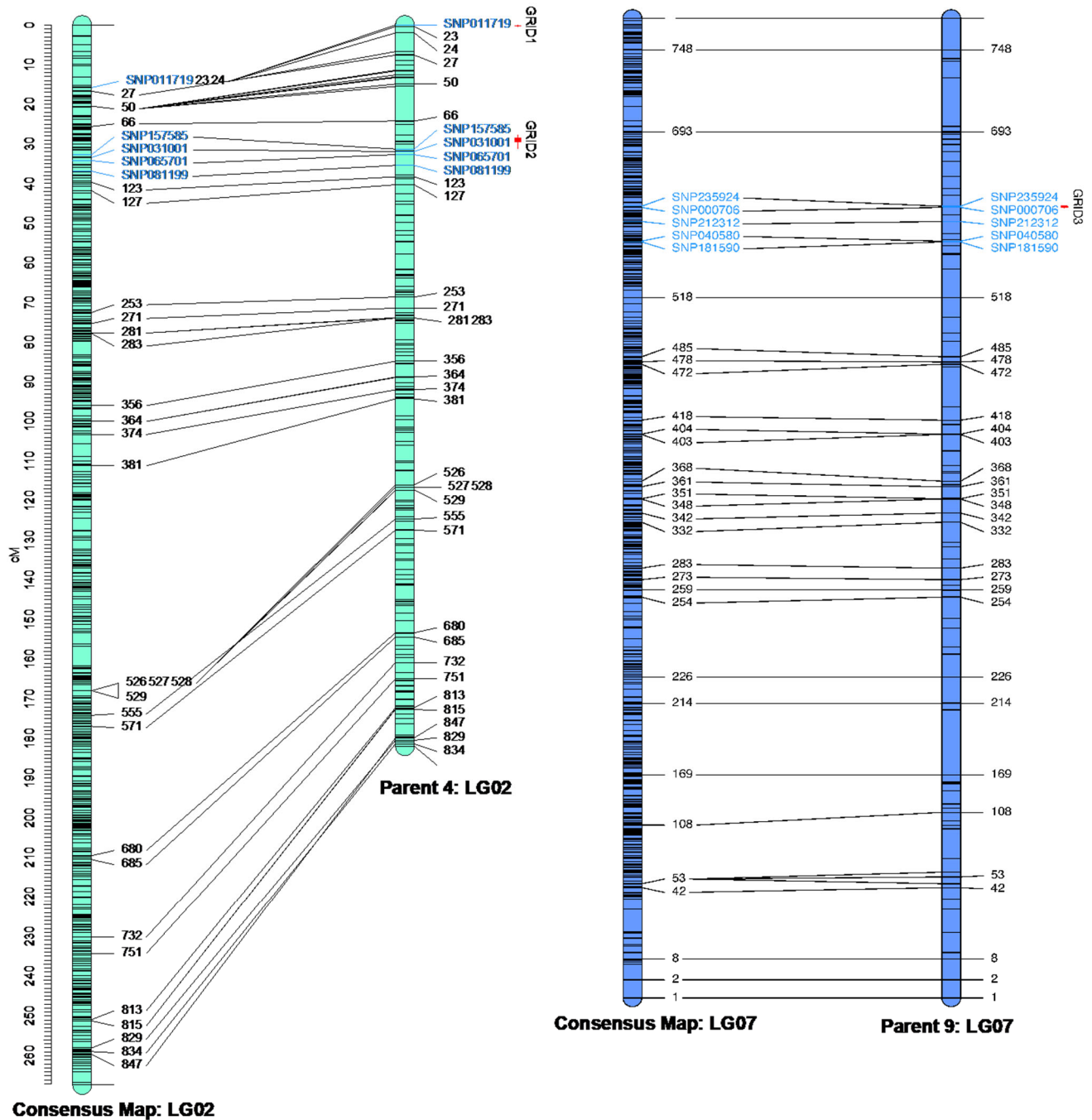

Figure 2. *Updated map positions for GRID resistance genes. For each linkage group, the parent-specific back-cross map is shown to the right of the consensus map published by Lauer and Isik (2021).*

Summary

The findings from this study are described in a manuscript that was submitted to the journal Heredity. The discovery of single loci that confer general resistance against a large sample of pathogen isolates that holds up across a wide geographic range is a significant addition to our body of knowledge regarding genetic disease resistance in forest ecosystems. The highly contrasting performance between the resistance donors used in this study and the resistance donors from previous studies suggests these genes have different modes of action or different resistance specificities, and some resistance genes may be more specific than others. We believe the high mapping resolution in each population, combined with the high-depth coverage of the expressed gene content via RNAseq, indicates that the resistance identified in our mapping populations may be derived from the action of single genes. However, these data do not strictly preclude the possibility that a cluster of resistance genes could be acting in concert to provide resistance against a large group of pathogen isolates. More work is needed to determine the identity of the causal genes underlying non-race-specific broad-spectrum resistance in these families.

Table 1. *Performance of putative resistance donors in multi environmental trial analysis. The overall disease incidence in the population (683,000 trees from 2,350 parents) was 0.35. Parent ID corresponds to donors of Fr genes that were published in Amerson et al. (2015). Parents TIP330244 and TIP1162449, donors of GRID genes discovered in this study, are superior in multi environmental trials.*

Parent	Progeny	Test sites	Rank	Mean	Gene
TIP330244	236	15	11	0.06	GRID1,GRID2
TIP1162449	2281	48	19	0.15	GRID3
10-5	5384	67	413	0.26	*Fr1*
A	681	10	28	0.20	*Fr2*
B	479	11	114	0.22	*Fr5, Fr9*
C	693	4	1286	0.21	*Fr6, Fr7*
D	1602	30	795	0.39	*Fr8*

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Genomic selection in the Atlantic Coastal Elite Population[1]

Genomic selection (GS) is a method that fits a large number of DNA markers simultaneously to predict breeding values of individuals without phenotypic records. The development of rapid and cost-effective genotyping technologies has enabled the practical application of GS to become widely used in livestock and plant breeding. In loblolly pine breeding, GS could be efficient for the prediction of genetic merit of genotypes within the same or closely related population.

In this study, the Pita50K array (Caballero et al. 2021, under review) developed for loblolly pine was used to genotype the Atlantic Coastal Elite population. After data filtering and quality control procedures, about 31,000 SNP markers were available for 2,023 clonal varieties. The clonal varieties were field tested at eight sites across the southeastern USA (Shalizi and Isik 2019). One genetically identical copy (ramet) of each clonal variety was planted in a row-column position at each test site.

The genetic parameters and efficiency of genomic selection were investigated for age six stem volume, stem straightness, and fusiform rust disease incidence. The SNP markers were first used to construct a realized additive relationship matrix, which was fit in GBLUP models to estimate variance components. Clone-mean heritabilities from GBLUP models for stem volume, stem straightness, and fusiform rust disease were 0.55, 0.60, and 0.66, respectively.

Whole genome regression models (ridge regression, BayesA and BayesB) were fit to evaluate predictive ability of SNP markers using *Random*, *Family*, and prediction for *New-environment* cross-validation (CV) scenarios. In the *Random* CV scenario, the population was randomly split into model training (80%) and validation (20%) sets. For the *Family* CV scenario, families were randomly selected for training (80%) and validation (20%) sets. In the prediction of a *New-environment* scenario, a training model was developed using the data from seven sites (training set) to predict the same varieties in a new environment (eighth site).

Average prediction accuracy for stem volume dropped from 0.43 in *Random* CV to 0.36 when a set of randomly sampled families were used as training model to predict clonal varieties in other families (*Family* CV) (Table 1).

Table 1. *Mean (range in parentheses) predictive ability for volume, stem straightness, and fusiform rust disease incidence from GS models for the three cross-validation (CV) scenarios. Sampling whole families produced lower prediction accuracies because of weak genetic relatedness between the training and validation sets.*

Cross-validation	Volume	Straightness	Fusiform rust
Random	0.43 (0.30 - 0.53)	0.57 (0.51 - 0.63)	0.26 (0.18 - 0.36)
Family	0.36 (0.24 - 0.54)	0.55 (0.41 - 0.64)	0.25 (0.14 - 0.35)
New environment	0.32 (0.23 - 0.47)	0.40 (0.25 - 0.47)	0.18 (0.07 - 0.31)

The predictive ability of stem straightness was high in the *Random* (0.57) and *Family* (0.55) CV scenarios, while the predictive ability for fusiform rust disease was low (~0.26) in the two scenarios. The ranking of clonal varieties within individual families was considerably lower in Family CV compared with Random CV (Figure 1). Lower within-family rank correlations were due to weak genetic related-ness between the training and validation sets in the Family CV scenario. Predictive ability of clones in a new environment were moderate for volume (0.32) and straightness (0.40) but lower for fusiform rust disease (0.18) (Table 1). This might likely be affected by the low overall rust disease incidence (5.6%) in the experimental population.
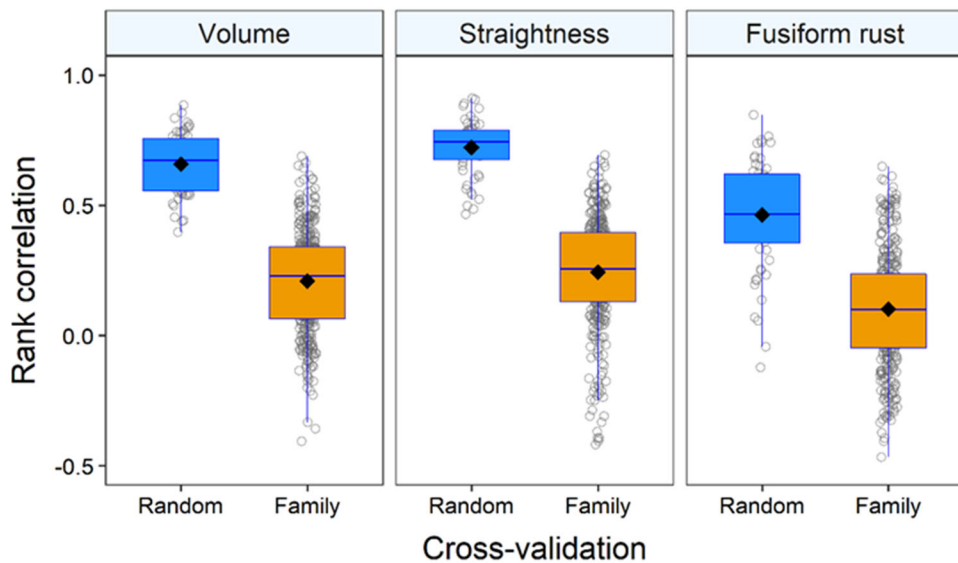
Figure 1. *Boxplots showing the distribution of **within-family** rank correlations for stem volume, straightness, and fusiform rust disease incidence for the across-population Random and Family cross-validation (CV) scenarios. Ranking of clonal varieties were assessed within individual families. The circles correspond to individual families. The black diamonds within boxes represent the mean rank correlations. Within-family ranking of genotypes was considerably lower when a set of families were randomly selected for model training and validation (Family CV scenario).*

Compared to a traditional loblolly pine breeding strategy, the incorporation of GS increased expected genetic gain per year for the traits analyzed. The expected genetic gain from GS was approximately twice the expected genetic gain achieved by the traditional breeding strategy (Figure 2). Higher gain was mainly due to 50% shorter breeding cycles with genomic selection.

This study indicates the importance of genetic connectedness between the training and validation data sets, which is the key for higher predictive abilities. The results for fusiform rust disease were not con-clusive, mainly due to a very low incidence of the disease within the experimental population. The real impact of GS in forest trees will be the prediction of phenotypes of juvenile material (e.g. seedlings in the nursery) in a breeding program, thereby eliminating field-testing. Therefore, the results from this study should be validated through another cycle of breeding to determine whether the marker-QTL phase holds from one generation to the next. Research addressing this important aspect of GS is currently underway.
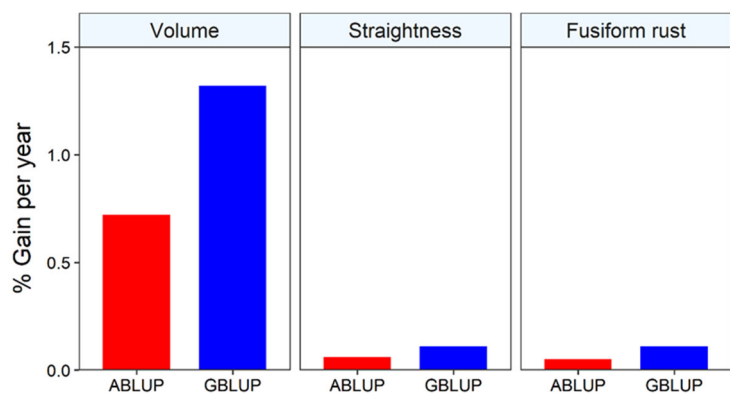
Figure 2. *Expected genetic gain per year (%) for volume, stem straightness, and fusiform rust disease incidence from ABLUP and GBLUP models. The selection intensity was 2.34, where 50 out of 2,023 clonal varieties were selected for each trait. The breeding cycle **L** was assumed to be 7 and 12 years for the GBLUP and ABLUP, respectively. Expected genetic gain per year almost doubled when genomic selection was applied, primarily because of a shorter breeding cycle but also because of the higher accuracy of predictions.*

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Biomass Genetics/Cropping Study at Butner – Growth and Thinning at Age 9 Years

The Biomass Genetics/Cropping Study near Butner, NC was planted in the spring of 2012 (funded by NCDA Grant #17-072-4031) and is now in its tenth growing season. The purpose of the study is to evaluate different families under various silviculture regimes to inform forest landowners how to maximize their financial returns in both the bioenergy and sawtimber markets. Ten of the fastest growing loblolly pine families from the Atlantic Coastal Plain provenance and ten of the fastest growing families from the Piedmont provenance were established on a Piedmont site in North Carolina at a high planting density (1,037 stems per acre). An additional objective of the study was to evaluate the risk and gain associated with planting fast-growing Coastal families where the risk of cold damage is high, but the gains in growth may outweigh the risk in a short-rotation bioenergy market (as compared to a traditional sawtimber rotation where stem defects are highly undesirable). Analysis through age six years have been published by Maynor et al. (see publication list on pg 44), and the 8-year results were described briefly in the 2020 Annual Report.

The original study plan called for a clearcut harvest treatment for biomass production after 8 years, followed by laboratory measurements of ethanol production. Due to difficulties of converting pine biomass to lignocellulosic ethanol, the pine biofuels market has not developed at the rate that was anticipated in the original grant proposal. Therefore, TIP staff shifted the focus of the study toward biomass feedstock for pellet production, since the market for woody feedstock for export is firmly established, and domestic consumption appears to be growing. There may be opportunities for landowners to generate income from pine plantation thinnings that are processed as biomass pellets. For these reasons, the clearcut harvest treatment was abandoned in favor of various thinning intensity treatments. In February 2020, we received a two-year grant to support this research, with the new objective being to provide information to guide landowners on how to profitably plant loblolly pine for biomass production through thinning while having the flexibility to manage for traditional products like sawtimber.

A thinning plan was developed by TIP staff that included three treatments: no thin (planting density of 1,037 trees per acre minus mortality from competition), moderate thin (residual of 519 trees per acre), and heavy thin (residual of 288 trees per acre) (Image 1). The 8-year measurements were used to guide which trees to remove, preferring the largest, defect-free trees to remain. After the list of trees to remove was developed, students and staff returned to the field in July 2020 to mark the thinning (Image 2). Shortly after marking, a request for proposal was sent out to local contractors that could thin the study according strict guidelines to ensure that the site was not rutted and that damage to residual trees was min-

Image 1. *A map of the Butner Trial with the individual thinning treatments applied to each rep (1-5). Non-thinned (NT) plots have ~1,037 trees per acre (the original planting density minus mortality from competition), moderate thin (MT) plots have a residual of 519 trees per acre, and heavy thin (HT) plots have 288 trees per acre. Reps are incomplete blocks.*

imal. The contractor that implemented the thinning used a Kobelco excavator on tracks with a Fecon mulching head. The operator was able to mulch trees from the top down without causing damage to residual trees or their crowns. Thinning began in early October 2020 and was completed the first week of November 2020 (Image 3).

After the thinning treatments were complete, aerial drone and ground based LiDAR measurements were taken throughout the trial. The objective of the LiDAR measurements include identification of crown shape and size metrics that may better explain variation in productivity, defect, and value of the different provenances, families, and thinning treatments. The ground-based LiDAR point clouds have extremely high resolution (Image 4).

We are hopeful that the results will better inform landowners how best to maximize their returns when supplying wood for both pellet production and saw-timber markets, while also answering more fundamental research questions concerning the thinning response of different genotypes with respect to volume production and stem quality. The LiDAR processing algorithms also have special promise to increase efficiency in tree improvement progeny test-

ing. These measurements were taken to establish the baseline (post-thinning) so that future LiDAR measurements can assess the changes that occur for each genotype in response to the thinnings.

Image 2. *Trees to be harvested during the thinning were marked with blue paint. Residual trees were the larger and had less defect. The high planting density of the trial (1,037 stems per acre) has already induced several years of self-pruning, which is visible in this photo at age 9 years (photo by A. Heine).*

Image 3. *Residual trees in one of the heavy thin treatment plots (288 trees per acre). Note the lack of damage to trees after the thinning operation was complete (Austin Heine).*
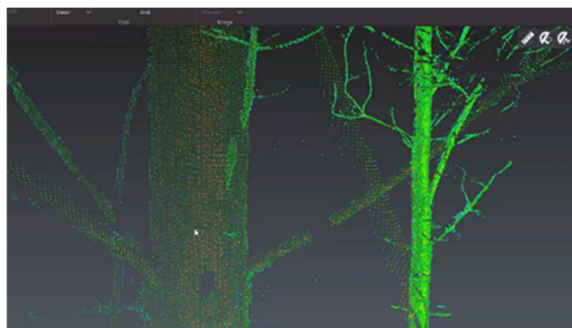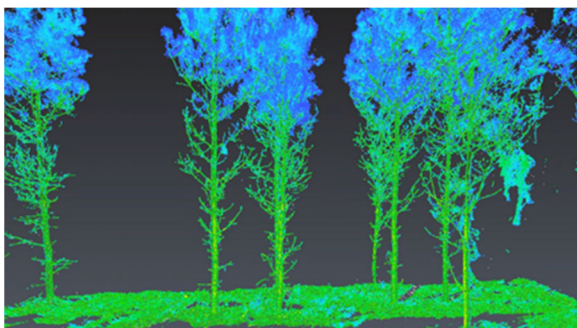

Image 4. *Examples of the ground-based LiDAR point cloud collected in the heavy thin treatment plots.*

Hyperspectral Imaging for the Prediction of Freeze Damage and Minimum Winter Temperature at Seed Source Origin of Loblolly Pine Seedlings

Freeze tolerance is the most important adaptability trait for deployment of loblolly pine in the southeastern U.S., and average annual minimum winter temperature (MWT) at the seed source is the standard indicator of cold adaptability. The Cooperative Tree Improvement Program assigns advanced generation families to cold hardiness zones based on the MWT of the location from which their founding ancestors originated (that is, following their pedigree back to the first generation selections). This method has been very successful in mitigating risk of freeze damage but as the number of cycles in the breeding program increase, so too will the number of founding ancestors increase for a given selection. Consequently, the precise assignment of the cold hardiness zone for a new selection will become more challenging, particularly when the founding ancestors originate from locations with a wide range of MWTs.

Long-term field studies are considered the most reliable method for identifying cold-tolerant families, but they are resource intensive and time consuming. The development of a high-throughput screening tool to characterize and quantify freeze tolerance among different genetic entries of seedlings will facilitate the accurate deployment of advanced generation loblolly pine families across the landscape.

The Tree Improvement Program, in collaboration with the department of Biological & Agricultural Engineering at NC State University, investigated a novel approach for the assessment of freeze-induced damage and prediction of MWT at seed source origin using hyperspectral imaging of seedlings. A population comprising 98 seedlots representing a wide range of MWT at seed source origin (Figure 1) was subjected to an artificial freeze event in January of 2020 to simulate a mid-winter freeze within a containerized nursery environment. A custom-assembled hyperspectral imaging system was used for scanning the seedlings prior to the freeze event and on four occasions during the days and weeks after the freeze.

The seedlings had accumulated 946 chilling hours (calculated as hours below 46 °F) before being placed in a freeze chamber. The temperature within the chamber was reduced from 32 °F to a target temperature of 10 °F, at a rate of 3.6 °F per hour. The seedlings remained at the target temperature for 1 hour before raising the temperature to 50 °F at a rate of 5.4 °F per hour. Below is a summary of the results recently published in Lu et al. (2021).

Seedling freeze damage was visually scored on a scale of 1–4, 44 days after the freeze event. Since the majority of the seedlings were scored as nonsymptomatic (51.9%), the four-point scores were condensed into binary categories corresponding to the visible absence or presence of freeze damage. The logit link function was used to model the probability of freeze damage. The hyperspectral image data that comprised spectra of each seedling were then averaged for each family, and used to develop family-level predictive models for each scanning date.
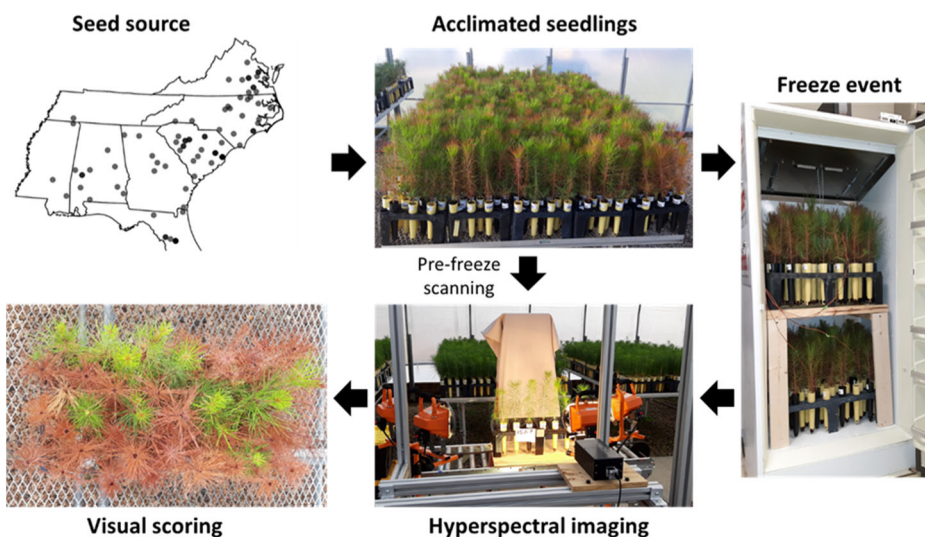

Figure 1. Process flow of the study involving hyperspectral imaging and visual scoring for the prediction of seedling freeze tolerance and Minimum Winter Temperature at seed source origin.

As expected, there was a significant positive relationship ($R^2 = 0.28$; $p < .001$) between the family MWT and logit scores for freeze damage was observed (Figure 2). Prediction accuracies of freeze damage and MWT based on hyperspectral data varied among seedling portions (full-length, top, middle, and bottom portion of aboveground material) and scanning dates. The highest prediction accuracy of freeze damage ($R^2 = 0.79$) was achieved using hyperspectral data obtained 41 days after the freeze event (Figure 3). The highest prediction accuracy of MWT ($R^2 = 0.78$) was achieved using hyperspectral data obtained prior to the freeze event (Figure 4).

As a guideline, the transfer of loblolly pine seed sources 5 °F colder is considered low risk, but moving as far as 10 °F is not recommended because of the risk of cold damage (Schmidtling 2001[1]). In our study, the best hyperspectral model gave predictions of MWT with a root mean squared error of 2.45 °F, which suggests sufficient precision to rate families for seed source transfer well within a 10 °F plant hardiness zone. This research demonstrates that hyperspectral imaging has the potential to serve as a rapid, nondestructive and objective tool for the prediction of MWT of origin of loblolly pine seedlings.
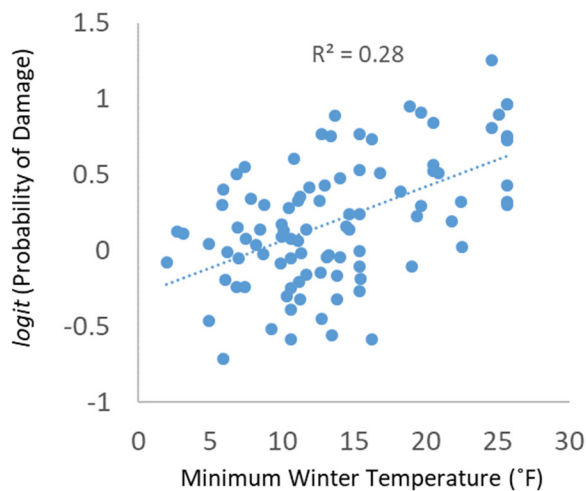

Figure 3. Scatter plots of the cross-validation prediction (holding out one family at a time) of freeze damage family logit values assessed at Day 44 after freezing based on models using hyperspectral data from seedlings scanned 41 days after the freeze event.
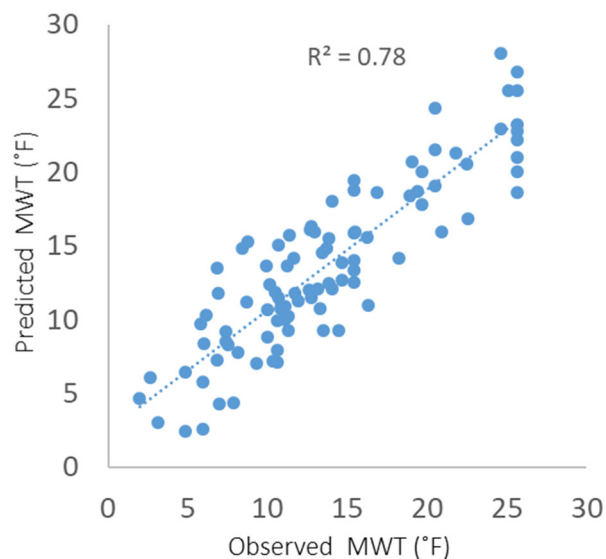
Figure 4. Scatter plots of cross validation prediction (holding one family out at a time) of minimum winter temperature (MWT) based on models using hyperspectral data from seedlings scanned six days prior to the freeze event.

[1]Schmidtling, R.C. 2001. *Southern Pine Seed Sources.* Gen. Tech. Rep. SRS-44. U.S. Department of Agriculture, Forest Service, Southern Research Station. Asheville, NC, 25 p.

Figure 2. Scatter plot between logit scores for family-level freeze damage (44 days after freeze event) and the family minimum winter temperature of origin. As expected, the minimum winter temperature of seed source origin was a significant indicator of cold damage probability.

Reference

Lu Y, Walker TD, Acosta JJ, Young S, Pandey P, Heine AJ, Payn KG (2021) Prediction of freeze damage and minimum winter temperature of the seed source of loblolly pine seedlings using hyperspectral imaging. Forest Science, in press.
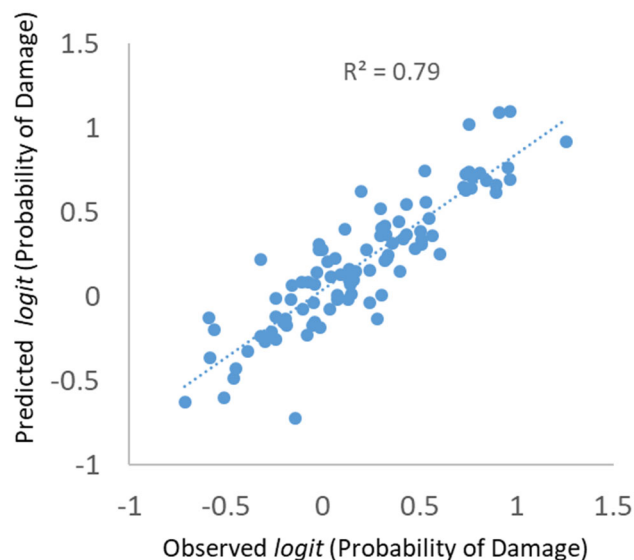
Identification of Fusiform Rust Disease Incidence in Loblolly Pine Seedlings Using Hyperspectral Imaging

In our project aimed at using hyperspectral imaging for the phenotyping of fusiform rust disease incidence in a greenhouse environment, recent progress has been made in the classification of diseased and non-diseased seedlings based on sensor data. Multiple steps were carried out for this analysis. These include the acquisition of seedling images, image processing for the extraction of relevant image features that can be used for creating machine learning models, and the creation of discrimination models for classifying the seedlings into diseased and non-diseased classes based on spectral data.

Various deep learning models were used to separate the seedling images from each other, and also segment out relevant regions of interest of each seedling image. The stem was of primary interest due to the presence or absence of visible stem galls. We also investigated the information contained in the images of the whole plant as well as selectively in the foliage pixels. Figure 1 shows the result of this image processing step where the different regions of interest are highlighted in different colors.

The spectral data extracted from the pixels were averaged for each region of interest, and the modeling step involved the classification of spectral data based on the source of the data: visually scored diseased or non-diseased seedlings at six months post inoculation.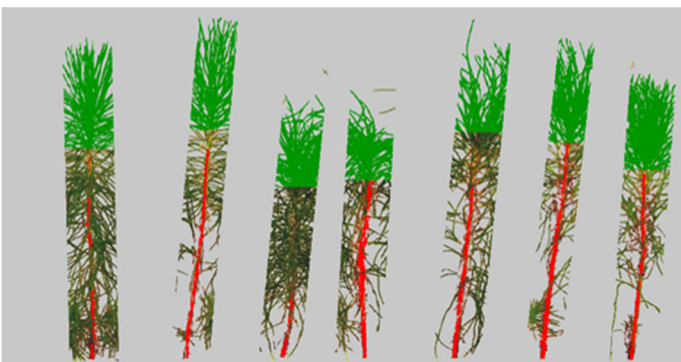 The first objective was to determine whether the classification of diseased or non-diseased seedlings would be possible using this imaging methodology. The second objective was to determine which part of the plant provides the optimum data for the highest accuracy of disease classification. The choice of classification model was also a variable in the analysis. We found that Support Vector Machine (SVM) models using the spectral data from the top portion of stem foliage provided the best accuracies in classification.

Figure 2 shows the accuracies derived from the classification models based on data from the different parts of the seedlings. The metric used is balanced accuracy, which is the mean of the rate of correctly classified seedlings in the diseased class (sensitivity) and the non-diseased class (specificity). This study represents the first instance of using hyperspectral imaging for the identification of fusiform rust disease incidence in loblolly pine seedlings. One of the conclusions that can be derived from this study is that a more controlled environment for data acquisition, with a targeted imaging of the top portion of the stem, would increase the potential for creating more accurate models.

Figure 1. *This figure illustrates the separation of different regions of interest in the seedling images acquired at six months after inoculation. The pixels detected as stem are shown in red. The green needles at the top of each seedling are shown in a different color because they were used as separate regions of interest during the discrimination analysis.*
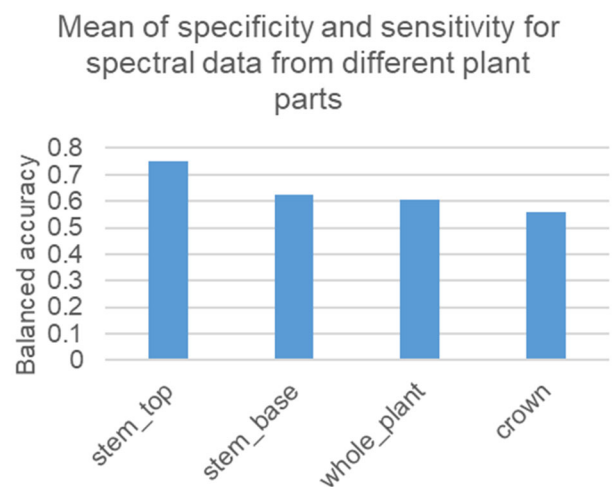

Figure 2. *The accuracy of classification obtained using spectral data from different regions of the seedling images acquired at six months after inoculation. The metric is balanced accuracy which is the mean of the rate of correct classification calculated separately for the diseased and non-diseased classes.*

[1] *Summary of Piyush Pandey's graduate research*

Fusiform Rust Disease Incidence Screening in the Northern Population – An Update

Fusiform rust is recognized as the most damaging disease of southern pine forests. The most effective means for reducing damage has been to plant disease resistant seedlings. Families of loblolly pine that are currently used for reforestation vary considerably in their resistance to fusiform rust disease. This variation is attributed to differing levels of selection effectiveness for disease resistance made by tree improvement programs and the level of genetic variation present in the recruitment population. In high rust hazard regions such as the upper Coastal Plain and lower Piedmont of Georgia, tree breeders have successfully applied a high selection intensity for rust disease resistance. Conversely, in low rust hazard regions such as the upper Piedmont and northern regions, including Virginia, a relatively low selection intensity for this trait has been applied. The future level of fusiform rust disease incidence in traditionally low rust hazard regions remains to be determined. In the event of elevated incidence levels, the identification and deployment of disease resistant families will be beneficial to the Cooperative and the region as a whole.

The evaluation of fusiform rust disease resistance in loblolly pine breeding programs is traditionally performed in field tests. Rust disease incidence is measured in 4th-Cycle tests in all three populations (Coastal, Piedmont and Northern). The Northern region is characterized as having low rust disease incidence, although certain sites stand out as having higher incidence levels such as five recently measured tests in King and Queen County, Virginia, that had an average rust disease incidence of 36%. Information from these sites will be valuable in quantifying the level or rust disease resistance among Northern families. Data generated from artificial inoculations of Northern families performed at the USDA Forest Service Resistance Screening Center (RSC) in Asheville, NC will provide further insight into the variation and range of rust disease resistance across the Northern breeding population.

The current study comprises 78 seedlots of Northern pollen-mix (NPMX) families that were chosen based on seed availability and frequent representation of parents tested in both 3rd and 4th-Cycle progeny tests. In addition, seven open pollinated seedlots that were sourced from the Virginia Division of Forestry were included to represent orchard clones located in a Northern pollen cloud. The checklots include two routine RSC checklots, seven non-improved regional checklots, a checklot from Livingston Parish, four 3rd-Cycle Piedmont pollen-mix (PPMX) families representing a range of rust disease resistance, and similarly four 3rd-Cycle Coastal (CPMX) pollen-mix families. Seedlots were recently sown at the RSC (Image 1) and the trial will be designed based on the germination counts. The inoculation is scheduled for mid-June and we look forward to scoring the presence or absence of rust galls in December.

Image 1. *Subset of the seedlots sown at the Resistance Screening Center this spring. Seedlings will be transplanted to trays following germination, and inoculations are scheduled for mid-June 2021.*

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

Grants

New Grants

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

Continuing Grants

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

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

Ross Whetten is the principal investigator on a USDA-National Institute of Food and Agriculture grant, "Identifying Functional Variation in Complex Plant Genomes", $490,000, 2/15/2018-8/14/2021.

Richard Venditti, Kitt Payn, et al. on a USDA-National Institute of Food and Agriculture grant, "Preparing Diverse and Rural Students and Teachers to Meet the Challenges in the Bioenergy and Bioproducts Industry". TIP portion: $33,588. 2017-2021.

ASSOCIATED ACTIVITIES

Meetings, Workshops, and Short Courses

This year we had to interact with members in a new way, virtually. While we really look forward to seeing all of the members again and getting back in the field to look at trees, we are also happy to have found a new way to connect. We managed to complete several member visits as well as hold successful workshops, all online. We found that a virtual format allowed many folks from member organizations to attend meetings and workshops that would not typically be able to attend in person. Austin Heine utilized camera and video technologies that allowed virtual visits to the *PRS*™ Calibration trials to discuss the different families and how they perform. It was a challenging year for sure, but those challenges allowed us to expand our reach and has us also thinking outside the forest!

In late January, we hosted a virtual workshop on grafting for Cooperative members and some guests from around the US and Canada. We had hoped to have the presentation live and in person in Georgia, but COVID-19 had other ideas. Using Zoom, the workshop was filmed on campus and delivered to a live audience, a first for some of the old-timers in the group. J.B. Jett presented the science and art of grafting (Image 1). Steve McKeand spent time talking about the ins and outs of top grafting. Austin Heine relayed his experiences on growing quality rootstocks for grafting. The workshop was well attended with 50 participants and about 15 others who watched the recording later.

Image 1: Dr. J.B. Jett lecturing the group during the Grafting Workshop in January 2021.

The faculty and staff members associated with the Cooperative are typical busy throughout the year with extension activities, both domestic and abroad. This year was far from typical, but we were able to fit some extension work in where there were opportunities.

Below are records of faculty and staff invited as instructors/speakers:

- Short Course: Developed an online short course for PepsiCo plant breeders on genetic Data Analysis from the International Oat Breeding Program. July 21-23, 2020- Isik

- Workshop: MateSel software for mating designs in forest trees: NCSUCTIP members August 7, 2020- Isik

- Guest Lecture: Tree Improvement 101. Stephen F. Austin State University, Summer Field Camp. Summer 2020- Walker

- Graduate Course: Linear Model, Breeding value, GxE interaction, and Genomic Selection. Virtual with Swedish University of Agricultural Sciences, Umea, Sweden. (26 students attended) September 28-October 5, 2020-Isik, Walker, Shalizi

- Guest lecture: "ENV 705L Silviculture: The Ecological Management of Forest Systems". Nicholas School of the Environment, Duke University. February 10, 2020- Isik

- Invited Speaker: Forest Tree Genomics, Biodiversity, and Sustainability. The National Congress of Life Sciences, Lima, Peru April 28-30, 2021- Whetten

Cooperative faculty and staff also interact with members, landowner groups, and forestry groups throughout the year. Here is a list of some of the extension and outreach activities in addition to annual member visits:

- Woodland lunch and learn webinar with NCSU Extension: Seedling Genetics, Why it matters. September 16, 2020- Payn

- Master Tree Farmer Program (Clemson University): Loblolly Pine Genetic Improvement in the Southeastern USA. December 7, 2020- Payn

Teaching

The Cooperative faculty and staff continue to lead the way in training future forestry and natural resource professionals by teaching courses at both the graduate and undergraduate level. This year the university closed the campus to almost all face-to-face classes, and most classes were taught online with any labs/field trips carried out with spacing restrictions. Fikret Isik taught Advanced Quantitative Genetics and Breeding (FOR/CS/ANS 726) in the fall and Introduction to Data Analysis in Natural Resources (NR 554), which covers general statistical procedures using SAS and R programing. In the spring. Ross Whetten always has a busy spring semester of teaching which includes FOR 350 (Ethics in Natural Resource Management), BIT 815 (Analysis of Deep Sequencing Data Analysis).

Kitt Payn taught FOR 411 (Forest Tree Genetics and Biology), our undergraduate tree improvement course, and the graduate level FOR 725 (Forest Genetics) this spring with our graduate student, Colin Jackson, serving as the Teaching Assistant. Both forest genetics classes took a field trip to the NC Forest Service Claridge Nursery and seed orchard in Goldsboro, NC in March 2021. The students toured the facilities and got a glance at the day to day workings of North Carolina's operational tree improvement program. We appreciate Bobby Smith and Jim Slye with NCFS for the opportunity and field tour for the students!

Staff

We left last year's staff update with the following and despite our best efforts these thoughts still read true today: *The evolving situation had us changing courses constantly as orders from the university and the government were pushed out. The staff reacted quickly and completed the crucial on-campus activities, including seed processing for the sowing of next year's progeny tests. We acknowledge and appreciate all the work our members have put in during this unprecedented time. As a Cooperative, we have ensured that there have been no setbacks regarding our testing objectives.*

It has been quite a year to navigate, and we are looking forward to seeing it in hindsight! We still completed all of the research and BTS (breeding, testing & selection) objectives and more, but it was like running an obstacle course each time to determine how to get them done. The pandemic caused routine activities to become extensive acts of paperwork, weekly COVID testing, layers of approvals, separated travel scenarios, staggered work times with spacing, and various PPE. It required a lot of extra time and effort from faculty and staff every day, especially in the early days when restrictions were tight and tensions high. Like everyone else, we held meetings and collaborations that looked like the intro to the Brady Bunch sitcom.

We miss seeing and working with each other and, we miss connecting with members. While the year was long and tough, we have a newfound appreciation for each other and the work we do!

In the midst of the pandemic, we welcomed Dr. Nasir Shalizi to the fold as Post-Doctoral scholar in November 2020 after he successfully defended his PhD program with the group. His skill-set offered the unique chance to evaluate some Cooperative research data sets in a new light. He has also stepped up and assisted in the lab and field as efforts were shuffled and staggered. We appreciate all of his expertise and his contribution to Cooperative research objectives.

Pictured right: Dr. Nasir Shalizi

Pictured below: the "TIP Bunch" during any given meeting...we even have nametags :)!
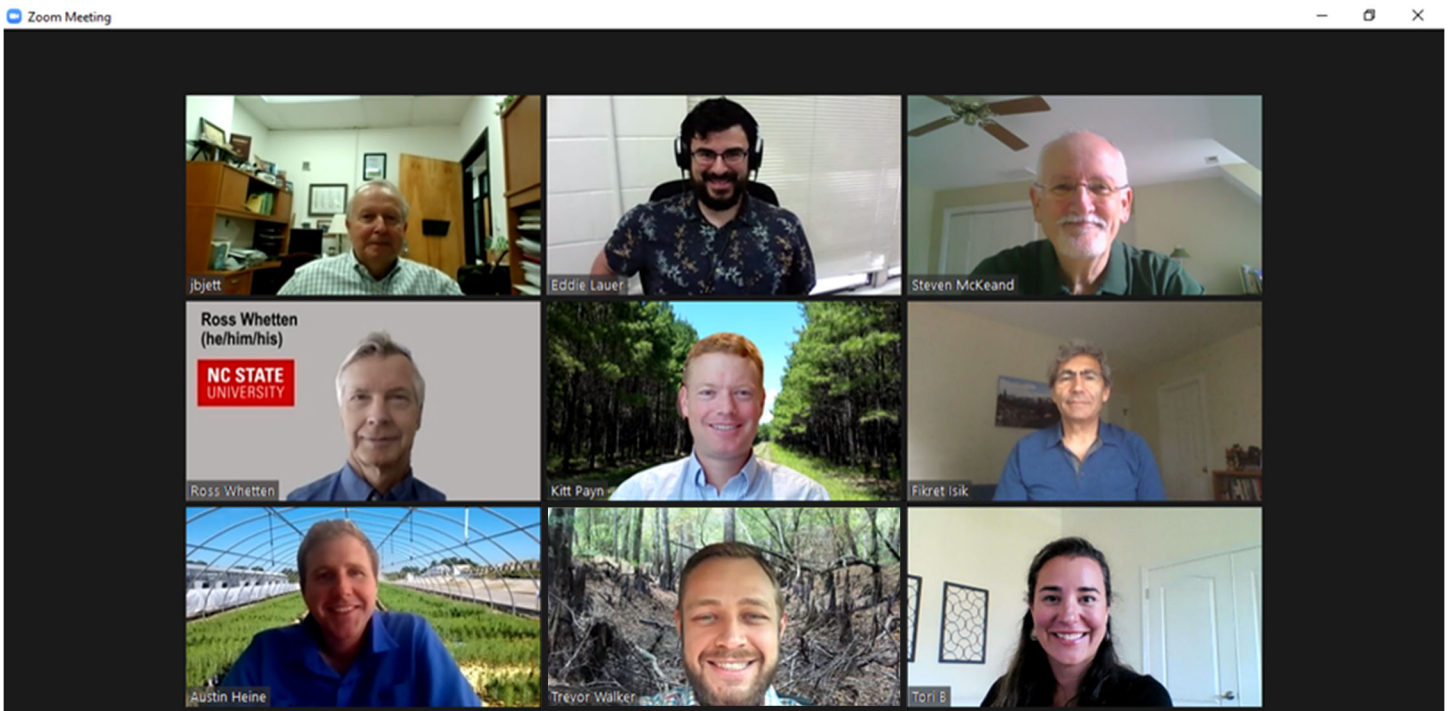
Graduate Students

We had another great year for our hard working grad students. Doug Dobson successfully defended his Master's thesis in fall of 2020. Nasir Shalizi also completed his PhD program in the fall and began the Post-doc position with Cooperative. Despite the challenges of bringing on a student during the pandemic, we also welcomed Sarah Conner who will be working on a Masters degree with the program. Below is the list of current students and their research:

Austin Heine, PhD – Austin has been busy with the pollen this year! The title of his research is "Expanding our Knowledge about Loblolly Pine Pollen to Increase Filled Seed per Cone from Controlled Pollinations."

Colin Jackson, PhD – Colin began his joint research with TIP and Camcore upon completion of his MS program in 2019. His research title is "Comparison of Genome Wide Association and Whole Genome Regression Methodologies in Loblolly Pine".

Eddie Lauer, PhD candidate – Eddie plans to conclude his PhD program this summer that is titled, "Discovery of Fr Genes for Fusiform Rust Disease in *Pinus taeda*".

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

Piyush Pandey, PhD – Piyush has a joint research project with TIP and Biological and Agricultural Engineering. His research is titled "High Throughput Phenotyping of Loblolly Pine Using Hyperspectral Imaging".

Sarah Conner, MS – Sarah joined the group in January after completing her undergraduate degree here at NCSU. The title of her research is "Genetic Variation in Fusiform Rust Disease Resistance among Families Represented in the Northern Breeding Population of Loblolly Pine in the southeastern U.S."

Sinem Satiroglu, MS – Sinem started her program in fall of 2019. Her project is "Prediction of genetic merit in loblolly pine using spatial and multivariate models".

Trevor Walker, PhD candidate – Trevor continues his research titled, "Quantitative genetics and the efficiency of SNP markers for within family selection in *Pinus taeda*".

Ugur Memis, MS – Ugur began his program in fall 2019, and his research is titled "Genetic parameter estimates from row/column experimental designs in loblolly pine".

Austin Heine　　　　Colin Jackson　　　　Eddie Lauer　　　　Khushi Goda
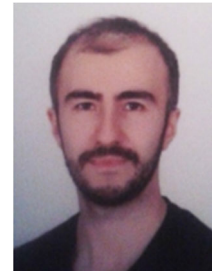
Piyush Pandey　　　Sarah Conner　　　Sinem Satiroglu　　　Trevor Walker　　　Ugur Memis

Membership in the NCSU Cooperative Tree Improvement Program

In an unusual year, we continued to have some bright spots, and one of them was in membership! In spring of 2021, we welcomed Burgin Timberlands, LLC as a new Contributing Member. We also added a new category for folks who see value in our research and want to support the program. These organization will be listed as "Benefactors", and we are happy to list Canfor as a Benefactor. We hope that others who find value in our research see this as a new opportunity to contribute. A list of our Cooperative partners is presented below.

Full Members

ArborGen, Inc.

Georgia Forestry Commission

Hancock Timber Resources Group

IFCO Seedlings

North Carolina Forest Service

Rayonier, Inc.

South Carolina Forestry Commission

Virginia Department of Forestry

The Westervelt Company, Inc.

Weyerhaeuser Company

Contributing Members

American Forest Management

Blanton's Longleaf Container Nursery

Burgin Timberland Management, LLC

Campbell Global, LLC

Charles Ingram Lumber Co.

Dougherty & Dougherty Forestry Services

F&W Forestry Services, Inc.

Four Rivers Land & Timber Company, LLC

Gelbert, Fullbright & Randolph Forestry Consultants

Green Diamond Resource Company

GreenWood Resources

Jordan Lumber & Supply Company

Larson and McGowin, Inc.

Meeks Farms & Nursery, Inc.

Milliken Forestry Company

Molpus Woodlands Group, LLC

ProFOR Consulting

PRT Growing Services, LLC

Resource Management Service, LLC

Roseburg Resources

Scotch Land Management, LLC

Shoeheel Land Management (Z.V. Pate, Inc.)

Tennessee Division of Forestry

Timberland Investment Resources, LLC

Research Associate Members

Arauco - Bioforest, S.A.

GenoVerde, Inc.

J.D. Irving, Ltd

New Brunswick Tree Improvement Council

Nova Scotia Department of Natural Resources

USDA Forest Service

Benefactors

Canfor Corporation

Publications of Special Interest To Members (2016 - 2019)

From our inception in 1956, there have been over 360 refereed publications written by Cooperative Scientists, Students, and Associates. Below is a list of publications from the last 3+ years. The entire list is available at our web site: https://www.treeimprovement.org/tip-tech-transfer

2021

Matallana-Ramirez, L.P., R.W. Whetten, G.M. Sanchez, and K.G. Payn. 2021. Breeding for climate change resilience: a case study of loblolly pine (*Pinus taeda* L.) in North America. Frontiers in Plant Science (in press)

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

Lu, Y., T.D. Walker, J.J. Acosta, S. Young, P. Pandey, A.J. Heine, and K.G. Payn. 2021. Prediction of freeze damage and minimum winter temperature of the seed source of loblolly pine seedlings using hyperspectral imaging. For. Sci. (in press, available online at https://doi.org/10.1093/forsci/fxab003)

Maynor, J.A., F. Isik, T.D. Walker, R.W. Whetten, A.J. Heine, K.G. Payn, and S.E. McKeand. 2021. Provenance and family variation in biomass potential of loblolly pine in the Piedmont of North Carolina. For. Sci. (in press, available online https://doi.org/10.1093/forsci/fxaa056)

McKeand, S.E., K.G. Payn, A.J. Heine, R.C. Abt. 2021. Economic significance of continued improvement of loblolly pine genetics and its efficient deployment to landowners in the southern United States. J. For. 119 (1):62-72. https://doi.org/10.1093/jofore/fvaa044

2020

Calleja-Rodriguez, A., J. Pan, T. Funda, Z. Chen, J. Baison, F. Isik, S. Abrahamsson, and H.X. Wu. 2020. Evaluation of the efficiency of genomic versus pedigree predictions for growth and wood quality traits in Scots pine. BMC Genomics 21:796. https://doi.org/10.1186/s12864-020-07188-4

Frey, G.E., T. Durmus, E.O. Sills, F. Isik, and M.M. Comer. 2020. Potential alternative tree species as substrates for forest farming of log-grown shiitake mushrooms in the southeastern United States. HortTechnology 30 (6):741-744. https://doi.org/10.21273/HORTTECH04721-20

Hansen O.K., K. Lamour, R. Whetten, J. Xu, A. Cuenca, M.S. Islam, and U.B. Nielsen. 2020. Ad hoc breeding of a genetically depauperate landrace of noble fir (*Abies procera* Rehder) using SNP genotyping via high-throughput targeted sequencing. Tree Genetics & Genomes 16(5):63. https://doi.org/10.1007/s11295-020-01460-0

Heine, A.J, T.D. Walker, S.E. McKeand, J.B. Jett, and F. Isik. 2020. Pollination bag type has a significant impact on cone survival in mass production of controlled pollinated seeds in loblolly pine. For. Sci. 66(5):589–599. https://doi.org/10.1093/forsci/fxaa013

Kurt, Y., L. Matallana-Ramirez, W. Kohlway, R. Whetten, and L.J. Frampton. 2020. A fast, flexible and inexpensive protocol for DNA and RNA extraction for forest trees. Forest Systems 29(2):e018. https://doi.org/10.5424/fs/2020292-16730

Mphahlele, M. M., F. Isik, M.M. Mostert-O'Neill, S.M. Reynolds, G.R. Hodge, and A.A. Myburg. 2020 Expected benefits of genomic selection for growth and wood quality traits in *Eucalyptus grandis*. Tree Genetics & Genomes 16(4):49. https://doi.org/10.1007/s11295-020-01443-1

Pais A.L., R.W. Whetten, and Q-Y. Xiang. 2020. Population structure, landscape genomics, and genetic signatures of adaptation to exotic disease pressure in *Cornus florida* L. – insights from GWAS and GBS data. Journal of Systematics and Evolution, 58(5):546-570 https://doi.org/10.1111/jse.12592

Shalizi, M.N., S.A. Gezan, S.E. McKeand, J. Sherrill, W.P. Cumbie, R. Whetten, and F. Isik. 2020. Correspondence between breeding values of the same *Pinus taeda* L. genotypes from clonal trials and half-sib seedling progeny trials. For. Sci. 66(5):600–611. https://doi.org/10.1093/forsci/fxaa016

Walker, T.D., B.P. Bullock, B.C. Smith, and S.E. McKeand. 2020. Modeling self-thinning patterns in loblolly pine with provenance and family effects. For. Sci. 66(6):712-725. https://doi.org/10.1093/forsci/fxaa030

2019

Chan, J. M. and F. Isik. 2019. Genetic variation in resistance to *Uromycladium acaciae* fungus, growth, gummosis and stem form in *Acacia mearnsii* populations. Tree Genetics & Genomes 15(3): 35. https://doi.org/10.1007/s11295-019-1341-x

DeWitt, N., M. Guedira, E. Lauer, M. Sarinelli, P. Tyagi, D. Fu, Q. Hao, J.P. Murphy, D. Marshall, A. Akhunova, K. Jordan, E. Akhunov, and G. Brown-Guedira. 2019. Sequence-based mapping identifies a candidate transcription repressor underlying awn suppression at the *B1* locus in wheat. New Phytologist 225(1):326-339. https://doi.org/10.1111/nph.16152

Isik, F. and S.E. McKeand. 2019. Fourth cycle breeding and testing strategy for Pinus taeda in the NC State University Cooperative Tree Improvement Program. Tree Genetics & Genomes 15:70 https://doi.org/10.1007/s11295-019-1377-y

McKeand, S.E. 2019. The evolution of a seedling market for genetically improved loblolly pine in the southern United States. J. For. 117 (3): 293-301. https://doi.org/10.1093/jofore/fvz006

Shalizi, M.N. and F. Isik. 2019. Genetic parameter estimates and GxE interaction in a large cloned population of *Pinus taeda* L. Tree Genetics & Genomes (2019) 15: 46. https://doi.org/10.1007/s11295-019-1352-7

Walker, T.D., F. Isik, and S.E. McKeand. 2019. Genetic variation in acoustic time of flight and drill resistance of juvenile wood in a large loblolly pine breeding population. For. Sci. 65(4):469–482 https://doi.org/10.1093/forsci/fxz002

2018

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

Grattapaglia, D., O.B. Silva-Junior, R.T. Resende, E.P. Cappa, B.S. Müller, B. Tan, F. Isik, B. Ratcliffe, and Y.A. El-Kassaby. 2018. Quantitative genetics and genomics converge to accelerate forest tree breeding. Frontiers in Plant Science 9:1693. https://doi.org/10.3389/fpls.2018.01693

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Front Cover: Our favorite kind of flower bouquet and so pretty in pink! Unfortunately you have to be in the tops of trees to admire them, but Dr. Kitt Payn was able to capture them at just the right moment!

Back cover: Top- Dr. Steve McKeand (L) and Dr. Kitt Payn (R) happy to be up in the trees, at an acceptable distance of course! They are holding up their hands (high fives) to signify the first season of breeding among 5th-Cycle selections.

Bottom left: Nothing to see here, just scanning the forest! We are always looking for new ways to utilize technology to quantify the genetic response to thinning, and to guide forest landowners on how to maximize their financial returns. Read about the NCDA funded Loblolly Pine Biomass Genetics/Cropping Study on page 33.

Bottom right: The 5th-Cycle is well on it's way with an increasing number of new selections being topgrafted into the orchard at Arrowhead Breeding Center in Cochran, GA. These 5th-Cycle selections are also genotyped by the PITA 50k Array, so we will know more about these selections than any population before. The implications are very exciting!