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NORTH CAROLINA STATE UNIVERSITY
COOPERATIVE TREE IMPROVEMENT PROGRAM

64th Annual Report | May 2020

DEPARTMENT OF FORESTRY & ENVIRONMENTAL RESOURCES - COLLEGE OF NATURAL RESOURCES

NC STATE UNIVERSITY

64th Annual Report

May 2020

Department of Forestry and Environmental Resources

College of Natural Resources

North Carolina State University

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EXECUTIVE SUMMARY

At age 64 years, the Cooperative Tree Improvement Program continues to provide value to the members, to NC State University, to the forestry and scientific communities, and to the landowners and citizens of the region.

SELECTION, BREEDING, AND TESTING

Field measurements have been completed for 17% of the Coastal crosses, 16% of the Piedmont crosses and 6% of the Northern crosses represented in the 4th-Cycle tests.

A total of 193 new parents are being tested in the 4th-Cycle. This past winter, breeding values became available for 28 new parents.

TIP staff, together with the Cooperative members, visited 27 tests and made 92 5th-Cycle selections. This brings the total to 123 5th-Cycle selections made through 2020.

Several new database tools have been developed, including "Annual BLUP" that provides individual tree and parental breeding values based on the annual analysis of 4th-Cycle data; "Progeny Test Data" that can be used to query information pertaining to 4th-Cycle progeny tests; and "CLADIT" a work-tracking tool that will improve clone archiving efficiencies.

RESEARCH

The Pita50K array, comprising 46,439 markers, became available to the community in September 2019. In total, 2482 loblolly pine samples (including additional checks and duplicates) were submitted for genotyping the ACE population. Genomic prediction models are currently being developed.

Three fusiform rust resistance genes were discovered through a combined approach utilizing high-density genotyping (Pita50K array) and RNAseq.

A grant from the North Carolina Department of Agriculture and Consumer Services was awarded for the Loblolly Pine Biomass Cropping Study, at Butner, NC ($80,291; 1/2020-12/2021). In December of 2019, the year eight measurements were completed. Significant family differences were observed for volume and sawtimber potential.

The Cooperative is exploring the use of hyperspectral imaging technology for the assessment of fusiform rust disease resistance and freeze tolerance of loblolly pine seedlings in a nursery environment. The goal is to determine whether this technology provides improved efficiency and objectivity in assessing plant stress compared to more conventional screening protocols.

ASSOCIATED ACTIVITIES, GRADUATE STUDENTS, COOPERATIVE STAFF, MEMBERSHIP

We welcome PRT Growing Services, Ltd. and Tennessee Division of Forestry as new Contributing Members. Unfortunately, we lost PBS International as a Research Associate Member. Graduate students' contributions to the program continue to be critical. We thank April Meeks for her contribution to our Program and wish her well with her career at Rayonier. Chuck Little celebrates his 10th year performing critical work for the Cooperative at Arrowhead. Congratulations to Trevor Walker, Tree Breeding Manager, and his wife Carina on the birth of their son, Isaac.

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A MESSAGE FROM THE DIRECTOR

It would be amiss not to start my message mentioning COVID-19 and the impact it has had on our lives over the last few months. I hope all our friends in the Cooperative and their family members are well and that they continue to remain safe during this unprecedented time. Our committed staff and members have rallied together to ensure the Cooperative has met all the breeding, testing, and selection objectives for the year.

This spring, TIP staff, together with the Cooperative members, made 92 5th-Cycle selections. These selections were grafted at the Arrowhead Breeding Centre in Cochran, GA. We anticipate many of the selections will produce sufficient flowers for 5th-Cycle breeding to be well underway by 2022. With the imminent transition to the next cycle of breeding, staff and members of the Cooperative met at Crossnore, NC to discuss key attributes of the proposed 5th-Cycle breeding strategy. Based on the feedback received from our members, the strategy has been refined, whereby maximum value and long-term sustainability will be achieved for the Cooperative.

Importantly, it is the operational deployment of seedlings with enhanced genetics that provides real value to our members. The continued improvement of breeding strategies and deployment options has resulted in the ever increasing level of genetic gain deployed across the South. Our members have done a fantastic job at efficiently converting gains achieved within our breeding program to gains realized across the landscape. This increase in genetic gain is happening more rapidly now than ever before[1]. This is largely attributable to an increase in the proportion of seed derived from advanced 3rd-Cycle orchards, and a substantial increase in the availability of control-cross seedlings. As measurements of the Cooperative 4th-Cycle progeny tests are accumulated, breeding values of many new 4th-Cycle parents will be obtained. The best of these parents will be grafted into new 4th-Cycle orchards. These highly valued orchards will further increase the genetic quality of loblolly pine seedlings available to organizations and landowners across the South.

Research performed within the Cooperative remains central to our success. Through innovative thinking and the novel application of tools and technology, we seek to improve the efficiency and effectiveness of our tree improvement processes. This year, much excitement has been generated by the release of the Loblolly pine Pita50K Axiom Genotyping Array. The array took approximately four years to develop. Members and staff of the Tree Improvement Cooperative, in partnership with other stakeholders in the forest genetics community, made a significant contribution to the success of the project. The genotyping array is expected to have a large impact on loblolly pine breeding and research outputs. The Cooperative has already genotyped the clones represented in our Atlantic Coastal Elite (ACE) population, and genomic prediction models are currently being developed.

Finally, and most importantly, I would like to acknowledge the fine people who make up the Cooperative. I thank the members for your strong support and continued commitment to the Program. I would also like to thank our great team in Raleigh comprising faculty, staff and students, whose passion for tree improvement is evident through their energy and tireless dedication to achieving our goals.

Kitt Payn, May 2020

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[1]McKeand, Payn, Heine and Abt. Economic Significance of Improved Loblolly Pine Genetics and Its Efficient Deployment to Landowners in the Southern US. Submitted for review, May 2020.

SELECTION, BREEDING, AND TESTING

Breeding Update

The cones from the final cohort of 4th-Cycle crosses were harvested this fall. The seed have been extracted and are presently growing in members' nurseries to be outplanted in 2021. There were an additional 98 4th-Cycle crosses conducted by TIP staff in spring 2019. These crosses will be included in the first 5th-Cycle progeny tests.

In spring 2020, seven crosses were made among Northern Elite population selections that will also be included in 5th-Cycle tests. The Northern Elite population was bred in the 3rd-Cycle using elite Piedmont and Northern parents, and was clonally tested as rooted cuttings planted in 2014. Accelerated breeding was made possible by planting an extra ramet at an orchard spacing at the Arrowhead Breeding Center in 2014. This ensured that flowers for breeding developed at roughly the same time the progeny tests were measured at age 5 years. The Northern Elite tests were measured last year, and crosses were made based on those breeding value estimates and the diversity contribution of their pedigree.

Some members were asked to collect open-pollinated cones from 4th-Cycle selections that were either infrequently used or not used at all in the 4th-Cycle breeding plan. A common reason for 4th-Cycle selections being excluded from the mating list was their close level of relatedness to other parents already included in the breeding plan. The cones came from seven parents, most were selections from the Piedmont Elite population. Open-pollinated progeny testing is a great way to estimate breeding values for potential orchard clones that are not included in the breeding population. The Cooperative plans to use this strategy in the 5th-Cycle to rapidly obtain breeding values for a subset of selections deemed to be potential orchard clones and for close relatives that may be excluded from the mating list.

Fall 2019 saw a productive cone harvest for the second-generation Atlantic Coastal Elite population (ACE2, produced from crosses among the Atlantic Coastal Elite clones). The objective of the ACE2 breeding was to create a population suitable for the validation of genomic prediction models developed using marker-trait associations in the ACE1 population. Seed from 67 crosses among 73 ACE1 clones, harvested in fall of 2018 & 2019, are presently being sown for progeny testing. Surplus seedlings sown for the ACE2 seedling tests will be placed into pots to expedite hedge plant production for a clonal trial series. Seed from an additional 74 crosses bred in spring 2019 will be harvested this fall. This seed may be used for a second sowing for hedge plant production if deemed necessary. The amount of gain expected from the ACE2 crosses compared to the first-generation ACE is shown in Figure 1.

Figure 1. *Distribution of index breeding values (60% volume, 20% straightness, and 20% forking) for clones of the ACE populations: Gray represents the original ACE1 population. Purple shows the mid-parent values for ACE2 crosses sent to stratification in March 2020 in preparation for sowing the ACE2 seedling progeny tests. Blue represents the ACE2 crosses bred in spring 2019 to be harvested in fall 2020 that may contribute to the ACE2 clonal tests.*
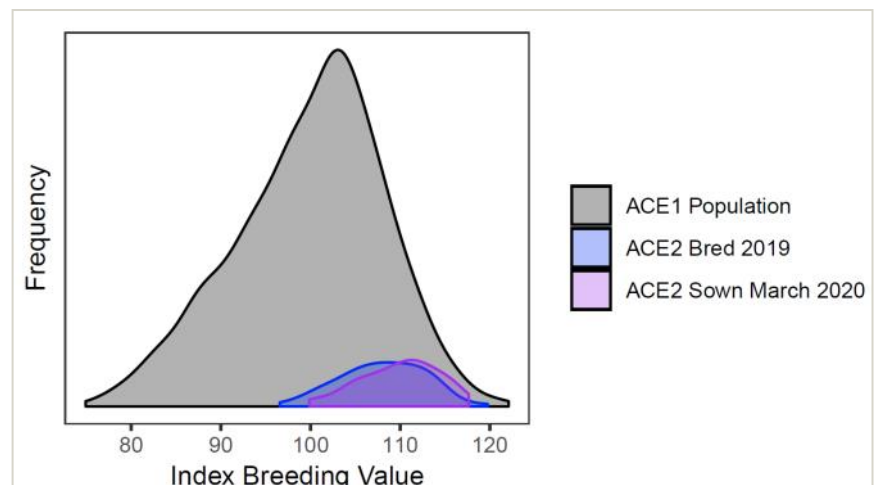
Testing Update

The majority of 4th-Cycle progeny tests have been planted. This upcoming winter will mark the last set of 4th-Cycle tests being outplanted with new parents and crosses, and next winter's tests will be the last trials for these new parents and crosses. Measurements of growth (height, DBH, volume) and stem form (straightness, forking, rust) have been completed for the first three years of 4th-Cycle tests, established in 2014 through 2016. From these data, breeding values for progeny (for 5th-Cycle forward selections) and their parents (4th-Cycle backward selections) have been estimated.

A total of 193 new parents are being tested in the 4th-Cycle. Many of these parents will likely be excellent 4th-Cycle seed orchard clones. This past winter, breeding values became available for 28 new parents, approximately half of which are Coastal source and the others split between Piedmont and Northern (Figure 2). These selections were made during the tail-end of the 3rd-Cycle (including the Piedmont Elite and Lower Gulf Elite population selections). By 2023, approximately two-thirds of the 4th-Cycle selections will have breeding value estimates (Figure 2), and orchard managers will have a large pool of rigorously tested parents to choose among for establishing 4th-Cycle orchards.
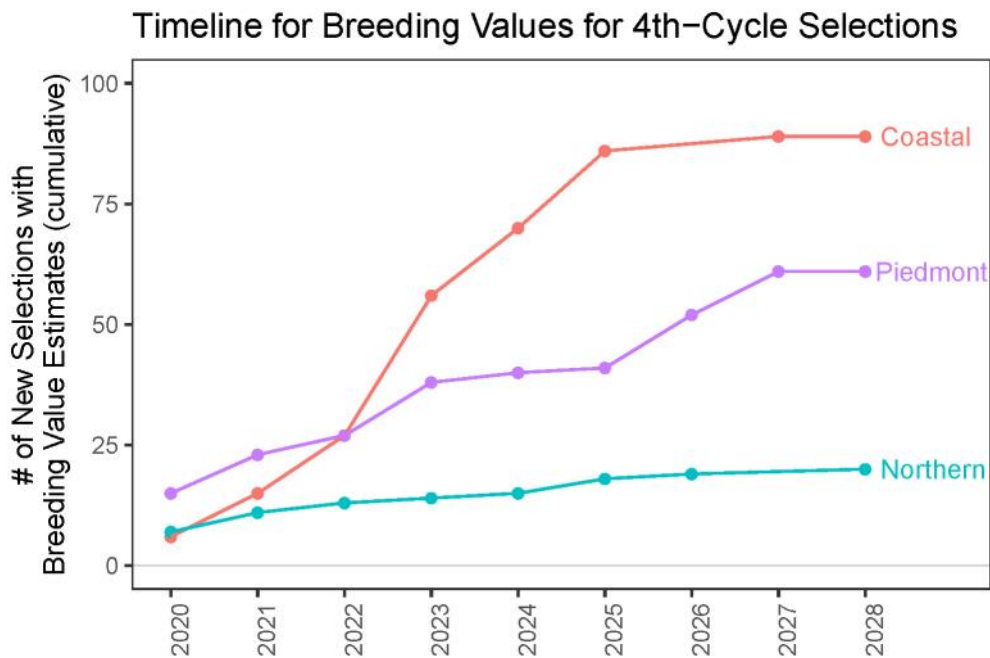

Figure 2. *As 4th-Cycle progeny tests are measured, we will get breeding values for new parents (4th-Cycle Selections), the best of which will go into 4th-Cycle Orchards.*

A challenge for large progeny testing programs is to test many parents and crosses while ensuring comparisons among all pairs of parents/crosses are statistically robust. The total number of parents tested in the 4th Cycle is approximately 980, far too many to plant side by side in a single test. Therefore, the Cooperative staff have carefully selected crosses for progeny testing that will provide adequate connections for comparing trees in different tests. Computer simulation studies suggest that as few as 4-6 common families should link any two trials (Johnson, 2004) or a minimum of 8-12% of the number of families tested (Kerr et al. 2015).

The 4th-Cycle testing strategy has resulted in excellent connections among genotypes tested in separate progeny tests. Figure 3 shows the count of parents in common between any two 4th-Cycle test series. There is a cell for each combination of region and planting year that indicates the number of parents that are shared. Along the diagonal is the number of parents tested in the series. On average, any two

tests share around a third of their parents, and only 5% of possible test comparisons share less than the optimal recommendations from Kerr et al. (2015).

Within a testing region, connections tend to be strongest between adjacent years. This is reflective of the "rolling front" breeding/testing strategy used in the 4th-Cycle, where new crosses were tested as soon as they became available, rather than waiting for all crosses to be harvested before being tested. Given this strategy, an effort was made to ensure that there are sufficient crosses connecting series established in distant years, while avoiding over-testing any particular cross and wasting progeny testing resources. The most disconnected tests within any planting region still have 29 parents in common (Northern population; Figure 3).

The 4th-Cycle strategy was the first case where the Cooperative intentionally tested parents across breeding zones. This was done to allow for the robust comparisons of families when seed source movement is desired (e.g. planting Coastal families in the Piedmont). Because this strategy was not followed in earlier cycles, it is not possible to directly compare breeding values and *PRS*™ scores for Coastal and Piedmont, or Piedmont and Northern parents generated from 2nd- and 3rd-Cycle tests. In the 4th-Cycle tests, the Piedmont and Northern population are particularly well connected, with an average of 76 parents in common and a minimum of 25 parents in common. The least connected tests are among the Coastal and Northern populations, because there are not many Coastal source parents that are deemed adapted to the Northern testing area*. Even with these limitations, the connections implemented in the testing design still meet the recommendations based on Kerr et al. (2015) and Johnson (2004).

Figure 3. Number of parents in common among all 4th-Cycle test series established from 2014 through 2020. The number of parents within a test series is shown along the diagonal. Robust comparison of genotypes from different tests requires at least 4-6 common entries or 8-12% of the genotypes tested. Practially any two 4th-Cycle tests meet these criteria, even across distant testing regions (e.g. Coastal versus Northern).

*Note that the testing of non-adapted material is avoided, and forest managers should still consider the 5°F to 10°F rule for seed source movement described by Schmidtling (2001) (https://www.srs.fs.usda.gov/pubs/gtr/gtr_srs044.pdf).

Johnson, G.R. 2004. Common families across test series—how many do we need? Forest Genetics. 11(2): 103-112

Kerr, R.J., Dutkowski, G.W., Jansson, G., Persson, T. and Westin, J., 2015. Connectedness among test series in mixed linear models of genetic evaluation for forest trees. Tree Genetics & Genomes. 11(4): 67.

4th-Cycle Progeny Test Data Analysis

Objectives

Beginning in 2019 with the first measurements of 4th-Cycle progeny tests, the NCSU Cooperative Tree Improvement Program initiated an annual cycle of test measurements and data analysis. The key purpose was to select individual trees with superior genetic merit to use as parents in the 5th-Cycle breeding population. In order to rank individual trees for selection, the Animal Model was utilized. This model combines the phenotypic records, the experimental design factors, and the pedigree in order to predict the breeding values for progeny, parents, and ancestors. The breeding values for multiple traits are then combined into an index breeding value for the purpose of ranking individual trees for selection.

Datasets

Data analyzed for the 2020 selections included all tests established on or before 2016. Measurements

were analyzed using separate mixed-models by test series. One model was used for the Coastal series and another for the Piedmont/Northern series. Most tests were measured at 4 years, while some were measured at 5 years post-establishment.

The dataset for the Coastal analysis consisted of 18 progeny tests, while the combined Piedmont/Northern dataset comprised a total of 23 tests, including four clonal tests in the Northern Elite series.

Analysis

Narrow-sense heritability estimates were moderate for the majority of test sites (Figures 1, 2). Because the statistical model accounted for different genetic variance at each location, sites with low heritability had very few or no selections because their values were "shrunk" further towards zero.
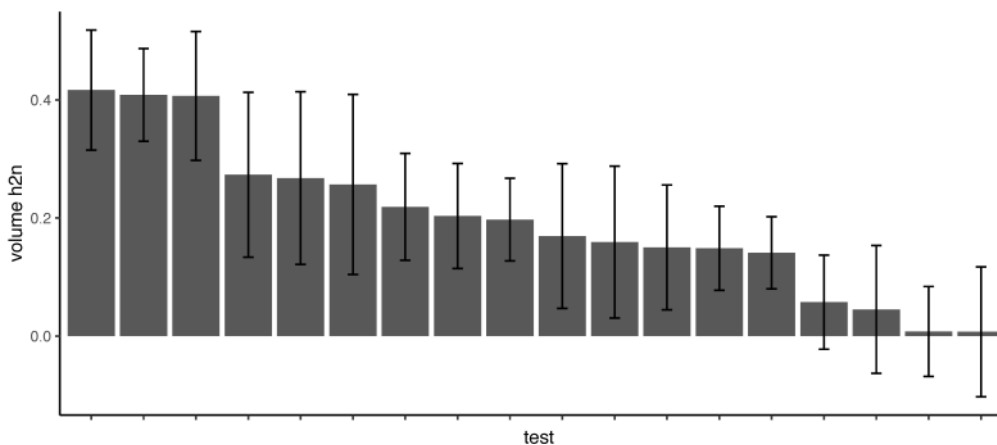
Figure 1. *(left) Narrow-sense heritability for Coastal 4th-Cycle tests analyzed in 2020. Error bars represent ±1 SE. Each bar represents one test site.*

Figure 2. (right) Narrow-sense heritability for volume for the 4th-Cycle tests in the combined Piedmont and Northern dataset. The four tests in the Northern Elite (NE) clonal series are excluded from this figure. Each bar represents one test site.
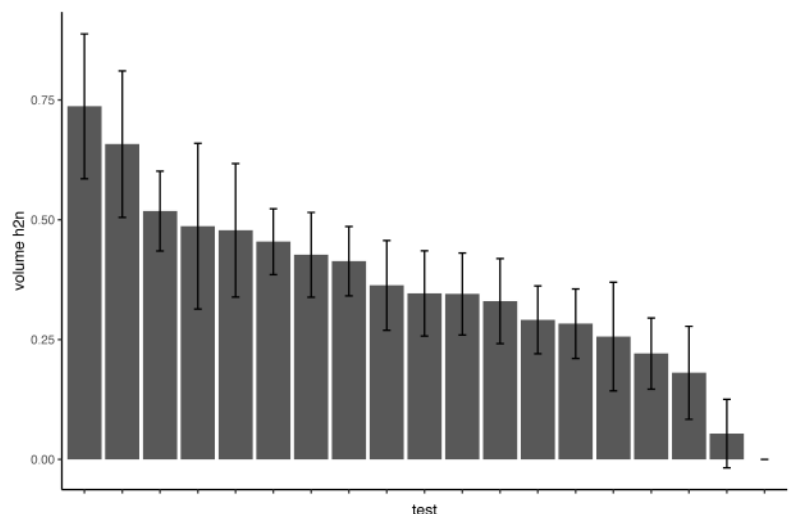
One of the most surprising findings in the 2020 analysis was the high incidence of fusiform rust at some northern locations in the Cold hardy dataset. For example, the average rust incidence at five tests in King and Queen County, Virginia, was 36%. This county is less than 50 miles from the Potomac river, in a region generally considered a low-hazard zone for fusiform rust disease (Figure 3).

Selection candidates

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. To balance gain and diversity in the selections, the MateSelect (Kinghorn 2011) algorithm was used with a target degree of 45 and limits on the number of crosses per selection to develop a candidate list for field evaluation (Figure 4). The algorithm was applied separately for the Coastal and Piedmont/Northern tests.

Note that the MateSelect output generates a list of proposed 5th-Cycle matings, but the algorithm was deemed appropriate to guide selections. To cast a wide net for the in-field evaluation process, the 4th-Cycle crosses with only one tree selected by MateSelect had the next best tree added to the evaluation list.
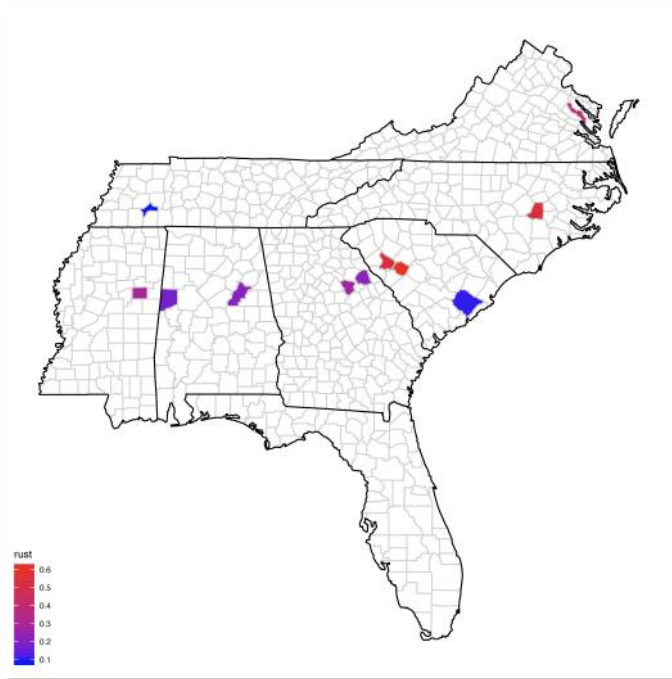
Figure 3. Counties with test sites included in the Piedmont and Northern dataset are shaded according to the average rust incidence of all tests established in that county. Rust incidence was higher than expected in King and Queen County, VA. Wayne County is listed as moderate hazard based on published maps.
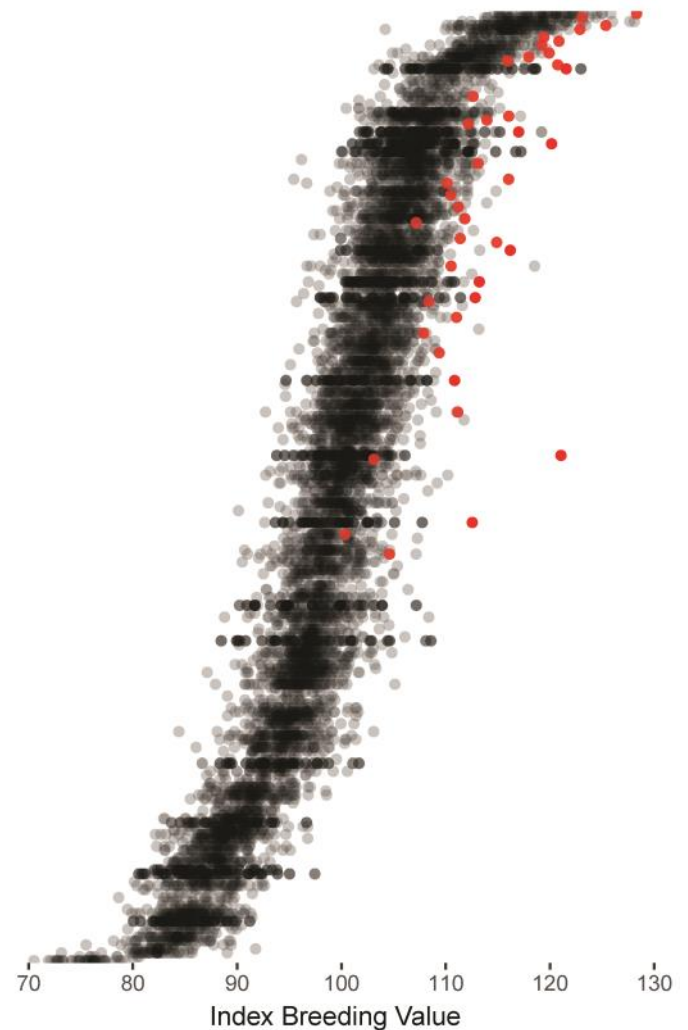
Figure 4. Index breeding values of trees represented in the Piedmont/Northern population, grouped by cross with crosses ordered from top to bottom in decreasing cross mean. Trees identified as candidate selections are highlighted in red.

5th-Cycle Selections 2020

Overview

In 2020, TIP Staff and Cooperative members made individual-tree selections following the analysis of age 4 and 5 year measurements from 4th-Cycle and Northern Elite tests established during the period from 2014-2016. The target number of selections for 2020 was 98.

A two-step selection process was followed. First, the measurement data pertaining to selection candidates were verified in-field by the members. Adjustments to the candidate list were then made to ensure that only the very best trees were included in the final selection evaluation step. In February 2020, TIP staff together with the Cooperative members visited 27 tests (Figure 1) and made 52 selections representing the Coastal population, 24 selections representing the Piedmont population, and 16 selections representing the Northern population. This brought the total number of 5th-Cycle selections to 123, with 29 Coastal selections having been made in 2019, along with two selections from the Northern Elite (NE1) series that had ramets planted at the Arrowhead Breeding Center, GA (Table 1).

Scions were collected at the time the selections were made and were sent to the Arrowhead Breeding Center near Cochran to be grafted for future breeding.

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

Series	2019	2020	Total
CYCLE4C	29	52	81
CYCLE4N		9	9
CYCLE4P		24	24
NE1	2	7	9
Total	**31**	**92**	**123**

Image 1: Scion collection from a 5th-Cycle selection from the Piedmont population (left to right: Trevor Walker, Graham Ford and Austin Quate; photographed by Kitt Payn)
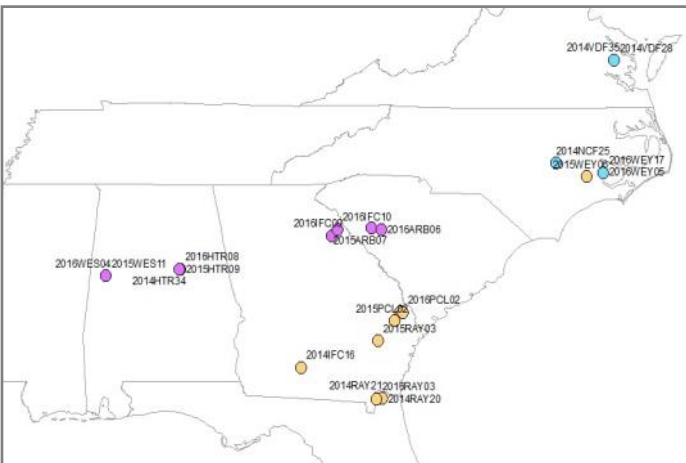
Figure 1. Locations of test sites visited for selections in February 2020.

Pedigree Diversity

Of the 123 5th-Cycle selections made through 2020, 109 selections each represented a unique cross (single selection per cross). The balance of the selections represented seven crosses, where two selections were made per cross.

There were 151 4th-Cycle parents that had a progeny selected for 5th-Cycle breeding. The vast majority of the 5th-Cycle selections were the only progeny selected from their parent (Figure 2). There were 29 4th-Cycle parents that had two progeny selected, and twenty parents with three or more progeny selected. One parent had eleven progeny selected due to its strong index breeding value and relatively low relatedness to the rest of the population. The pedigree of the 123 5th-Cycle selections made through 2020 can be traced back to 182 founders (plus trees from wild natural stands/non-improved plantations).
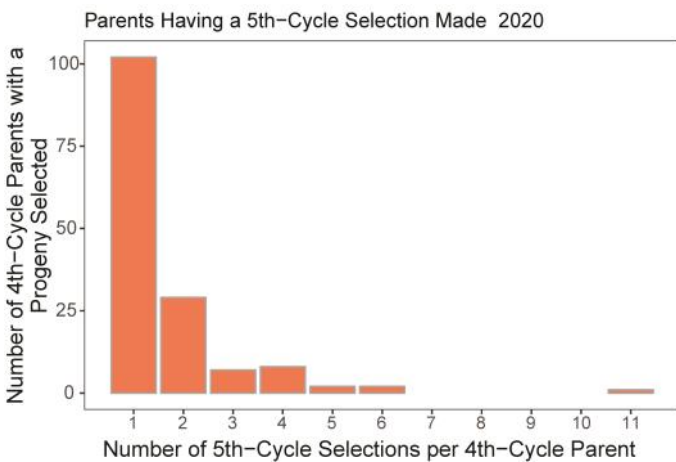
Figure 2. Count of 4th-Cycle parents having one or more 5th-Cycle selections made from their progeny.

Mean Minimum Temperature Origins

Selections were made across the range of mean minimum temperatures of 4th-Cycle crosses represented in tests measured through 2020 (Figure 3). Crosses with Coastal-source temperature origins that were planted in the Piedmont and Northern tests did not have selections made from them to avoid duplicating the Coastal selection effort. Within the Piedmont/Northern tests, there was no need to place temperature constraints on the selection algorithm because top-performing individuals could be found across the range of temperature origins. Notably, the distribution of selections broadly represented the distribution of family mean minimum temperature (Figure 3).
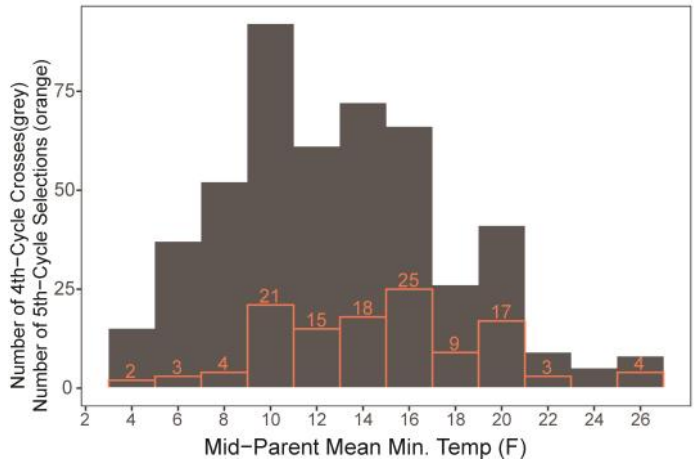

Figure 3. Number of 4th-Cycle crosses (in grey) measured through 2020 and number of 5th-Cycle selections (orange) made from those crosses by mid-parent mean minimum temperature origin.

Inbreeding Coefficients

Of the 123 5th-Cycle selections made through 2020, three 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 one selection came from parents that were more distantly related (F = 0.0625).

Expected Gain

The mean multi-trait index breeding value among the 5th-Cycle selections was 107.7 for the selections from Coastal tests, and 115.0 from Piedmont/Northern tests, compared to a mean of 100 for candidate trees in both populations (Figure 4). These values correspond to 1.25 and 1.76 standard deviations above the mean in terms of index breeding value for the Coastal and Piedmont/Northern tests, respectively. The multi-trait index breeding values includes weightings of 60% on volume, 20% straightness, and 20% rust resistance.
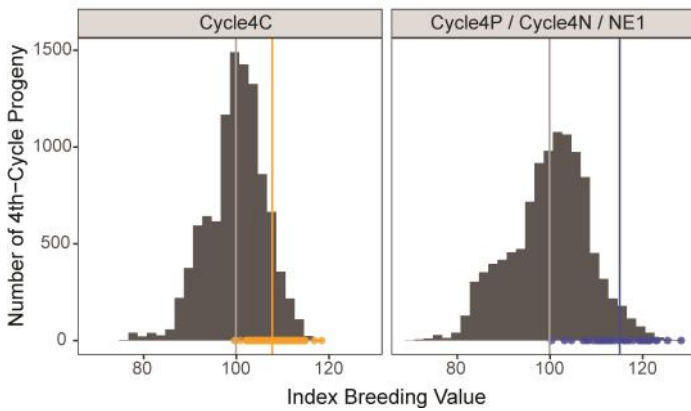
Figure 4. *Multi-trait index breeding values (60% volume, 20% straightness, 20% rust resistance) for trees in 4th-Cycle progeny tests (gray) and those trees that were selected (orange represents Coastal and blue represents Piedmont/Northern selections). Vertical lines indicate the mean of the population (gray) and the mean of the selected (color) trees.*

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.

Next Steps

All selections made through 2020 have been top-grafted at Arrowhead Breeding Center to accelerate flower/pollen development. Breeding among these selections is anticipated to commence in the next two-to-three years. Open-pollinated seed will be collected as soon as possible to expedite breeding value estimation using 5th-Cycle progeny tests.

Image 2: *Ones Bitoki, Virginia Division of Forestry, and Kitt Payn proudly standing alongside a 5th-Cycle selection from the Northern 4th-Cycle population (photographed by Trevor Walker).*

Image 3: *(left) David Barker and April Meeks, Rayonier, along with Kitt Payn and Trevor Walker strategizing over selection locations at a 4th-Cycle coastal test located in Nassau County, FL. (photographed by Tori Brooks).*

Grafting and Topgrafting at the Cooperative's Arrowhead Breeding Center

With the 5th-Cycle selection effort moving into full swing this past year, the 2020 grafting season was the busiest yet at the Arrowhead Breeding Center near Cochran, GA. In January and February, Cooperative members and staff made 92 new selections from 4th-Cycle tests. These selections, as well as selections from 2019, were topgrafted into the "New Breeding Orchard" (a.k.a. the NBO) that is not so "new" anymore. Loblolly pine breeders have been using topgrafting for over 25 years to stimulate early female and male strobilus production to enhance breeding progress. In the NBO, we are topgrafting on to interstocks of selection TIP2201951 that were established in 2009. Selection TIP2201951 is known to graft well and is very effective at promoting both female and male strobili when used as an interstock (see details in the Cooperative's 2009 Annual Report available at http://treeimprovement.org/sites/default/files/annual_reports/2009_Annual_Report_53.pdf.

A total of 778 topgrafts from 109 5th-Cycle selections (92 new selections plus 17 re-grafts from 2019

selections) were made in the NBO. The goal was to graft seven scions into the crown of the TIP2201951 interstocks (Image 1) with the goal of converting the crown to the new selection (Image 2). Using this approach, we have had great success breeding the 4th-Cycle forward selections in the NBO with the first breeding starting in 2016; the last of the 4th-Cycle breeding was "mopped up" last year in 2019. Breeding of the new 5th-Cycle selections could commence as early as next year, but heavy flowering and pollen production should be available in 2022.

The success of the Arrowhead Breeding Center has been due to the financial support by Cooperative members and especially by the Georgia Forestry Commission who have given us access to over 20 acres of land at their Arrowhead Seed Orchard. The other reasons why 4th-Cycle breeding progressed so rapidly are due to the tireless efforts by the Cooperative's staff and students, and the innovations we have employed. In the early years, we were able to initiate topgrafting and breeding in blocks of the GFC seed orchard and clone banks. The years of

Image 1. Seven topgrafts of N395002 were made into the crown of an TIP2201951 interstock (left image). The goal is to convert each crown from TIP2201951 to the new selection. A total of 778 topgrafts from 109 selections were made in February and March of 2020. A few dozen of these are shown in the right image.
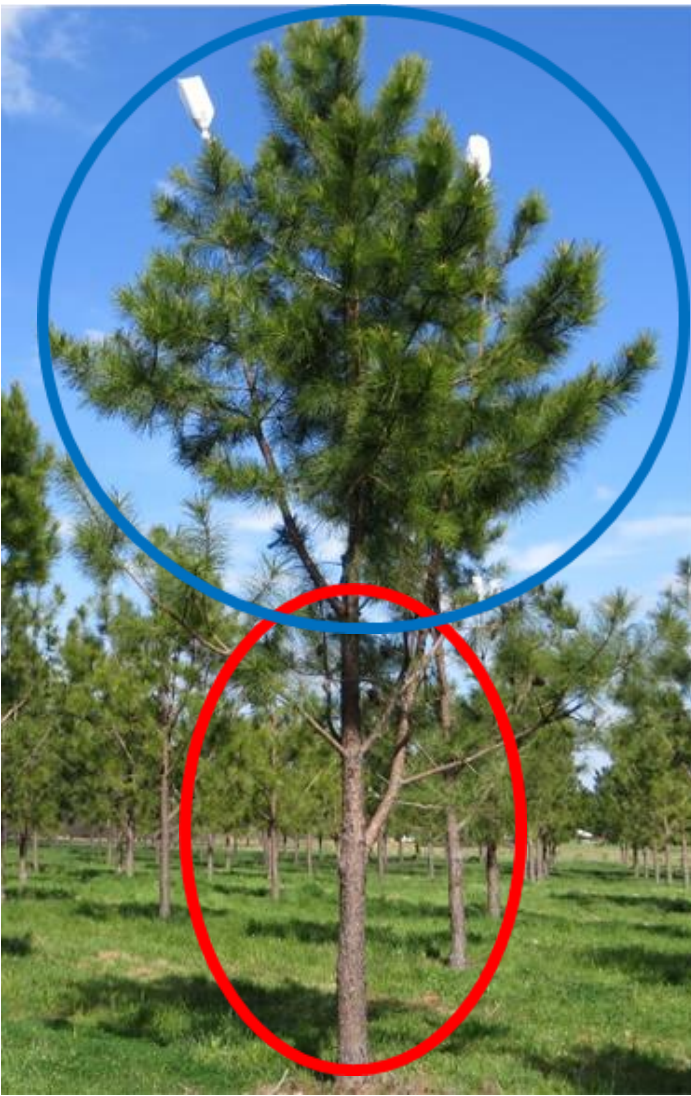
Image 2. *Example of crown conversion in progress in the New Breeding Orchard in March 2016. The top of the TIP2201951 interstock (outlined in red) was grafted in February 2014 with a 4ᵗʰ-Cycle selection (outlined in blue). The bottom branches on the TIP2201951 interstock have since been completely removed, and the crown has been converted to the new selection.*

planning to have clonal blocks of TIP2201951 inter-stocks ready for topgrafting will now pay huge dividends for the 5ᵗʰ Cycle of breeding.

Final "cleanup" grafting of TIP2201951 scions was completed in the Northern Breeding Ramet Orchard (NBRO) this spring (Image 3). The NBRO was established in October 2014 with extra ramets (rooted cuttings) of clones that were tested in the Northern Elite Population in 2014. The idea was to establish

these ramets at Arrowhead so that when the selected clones were identified in the clonal trials, breeding could commence as soon as possible. The ramets of the vast majority of clones that were not selected were used as rootstocks. The first grafts of TIP2201951 were made in 2018, but the grafting success was not 100%, so cleanup grafting was done the last two years. These TIP2201951 interstocks should be an ideal size (20' to 25') for topgrafting of 5ᵗʰ-Cycle selections in 2022 and beyond.

To prepare for the tail end of the 5ᵗʰ-Cycle program and the beginning of the 6ᵗʰ-Cycle program, another new breeding orchard has been established near the graveyard at the Arrowhead Seed Orchard. The Graveyard Orchard was started in October 2017, when GFC gave us access to an additional 10 acres for breeding. Rust-resistant seedlings were planted for rootstock, and in February and March 2019, the first TIP2201951 scions were grafted into the Graveyard1 Orchard (Image 4 right). An additional

Image 3. *Austin Heine did "cleanup" grafting of TIP2201951 into the tops of 16 large rooted cuttings in the Northern Breeding Ramet Orchard (NBRO) at Arrowhead. The ramets from the Northern Elite Population that were not forward selections were used as rootstock to provide more TIP2201951 interstocks for the 5ᵗʰ-Cycle breeding in the next 2-3 years. A total of 191 TIP2201951 interstocks are in the NBRO along with 8 selections from the Northern Elite Population (photographed by Steve McKeand).*

Image 4. *Most grafting in the Graveyard Orchard was completed this year with 205 grafts made in the Graveyard2 (younger) Orchard (left) and 83 re-grafts made in the Graveyard1 (older) Orchard (right). Grafting success in 2019 was only 78.6% due to the drought in central Georgia, so re-grafting was necessary. The TIP2201951 grafts will be grown for the next 5-7 years and should be excellent interstocks for topgrafting future 5ᵗʰ- and 6ᵗʰ-Cycle selections.*

235 seedlings were planted in October 2018 to finish the Graveyard Orchard. In February 2020, 205 grafts of TIP2201951 were made in the younger seedlings (Image 4 left), and 83 re-grafts of TIP2201951 were made in the older seedlings (Image 4 right).

The Arrowhead Breeding Center is well positioned with breeding orchards for topgrafting for the next several years. We estimate that the use of interstock clone TIP2201951 for topgrafting and the crown conversion has sped up the breeding cycle by at least two years compared to our early topgrafting efforts. In the early years, we would topgraft selections into random interstock clones, and put a selection into two different trees. We would typically put two different selections into one crown, since we did not have enough interstock trees to put only one clone into an interstock crown. Sometimes the interstock clone was effective for flower promotion, and sometimes it was not. Additionally, the logistics of managing two different topgraft clones in one crown proved to be very difficult. In the newer breeding orchards, each new selection is topgrafted into only one interstock crown of TIP2201951, since every tree is identical. While we risk the loss of a selection in the breeding orchard, all selections are backed up in at least two other clonal archives.

Image 5. *When the last topgraft was completed on March 16, Steve McKeand was delighted that all 10 digits were still in place with relatively little scaring (photographed by Austin Heine).*

𝙋𝙍𝙎™ Calibration Study Update

The objective of the 𝙋𝙍𝙎™ Calibration study is to develop modeling systems that convert the genetic gain estimates from progeny tests into per-acre growth and yield predictions throughout the rotation. While the experimental designs used for progeny testing are very efficient for ranking families, they utilize *single-tree plots* that contain many different families and cannot be readily used to predict operational gain. To assess operational gain, *block-plots* comprising many trees of the same genotype are required. A block-plot design becomes a challenge when a large number of families are included in the test. The design of the 𝙋𝙍𝙎™ Calibration Study takes a unique approach to this scaling issue by including genotypes from a range of

performance values and correlating the study yields with the progeny test scores rather than the individual family. The resulting model can be applicable to new genotypes without block-plot data as long as they have progeny test data.

Most of the 𝙋𝙍𝙎™ Calibration Study test sites were established in 2013. Six and seven year data are available for the majority of tests (Table 1). A comparison of the age four year measurements of the 𝙋𝙍𝙎™ Calibration Study to the 𝙋𝙍𝙎™ scores indicated strong agreement for growth traits and fusiform rust disease (see 2018 Annual Report, pg. 19-20). The next set of measurements are planned for age ten years and/or immediately before the first thin.

Table 1. *Number of test sites measured by age and series for the 𝙋𝙍𝙎™ Calibration Study (series 555).*

Series	Age 1	Age 2	Age 3	Age 4	Age 5	Age 6	Age 7
555C (Coastal)	11	2	3	9	1	9	1
555P (Piedmont)	6	2	2	3	1	5	
555N (Northern)	4			4		4	

While the tests need to mature before suitable data are available for growth and yield modeling, a preliminary analysis involving site index estimates based on age six measurements was conducted (Figure 1). Site index is a metric of site productivity that uses the average height of dominant trees at a base age (commonly 25 years) to compare the productivity of different stands, regardless of their age or planting density. Site index is a robust measure of site productivity because height growth curves are predictable and relatively independent of stand density. Site index adjustment (height-age curve adjustment) can be considered a first approximation for modeling genetic gain due to its simplicity and effectiveness in most circumstances. Site index adjustment is not appropriate if there are large differences in height-diameter allometry

(allocation of growing resources) or self-thinning (competitive ability) among genotypes. In Figure 1, the site index estimates from the block-plots in the 𝙋𝙍𝙎™ Calibration Study were correlated to the Height scores available from 𝙋𝙍𝙎™. The site index estimates were calculated from the dominant heights observed at age 6-7 years from each block-plot and the site index model published by Diéguez-Aranda et al. (2006)[i]. The regression indicates that some sites are more sensitive to genetic improvement, as the slope of the lines are not constant across sites (Figure 1). The intercepts of the lines also vary because of differences in the productivity of the study sites. If we consider the regression across all sites, a 10 point increase in Height score from the 𝙋𝙍𝙎™ values translates to about an 8.4 foot increase in site index exhibited at age 6 years.

As the tests mature, data from the **PRS**™ Calibration Study will be used to develop modeling systems that convert the genetic gain estimates from progeny tests into per-acre growth and yield predictions. The appropriateness of using a site index adjustment as an approach to model genetic gains will be rigorously tested.
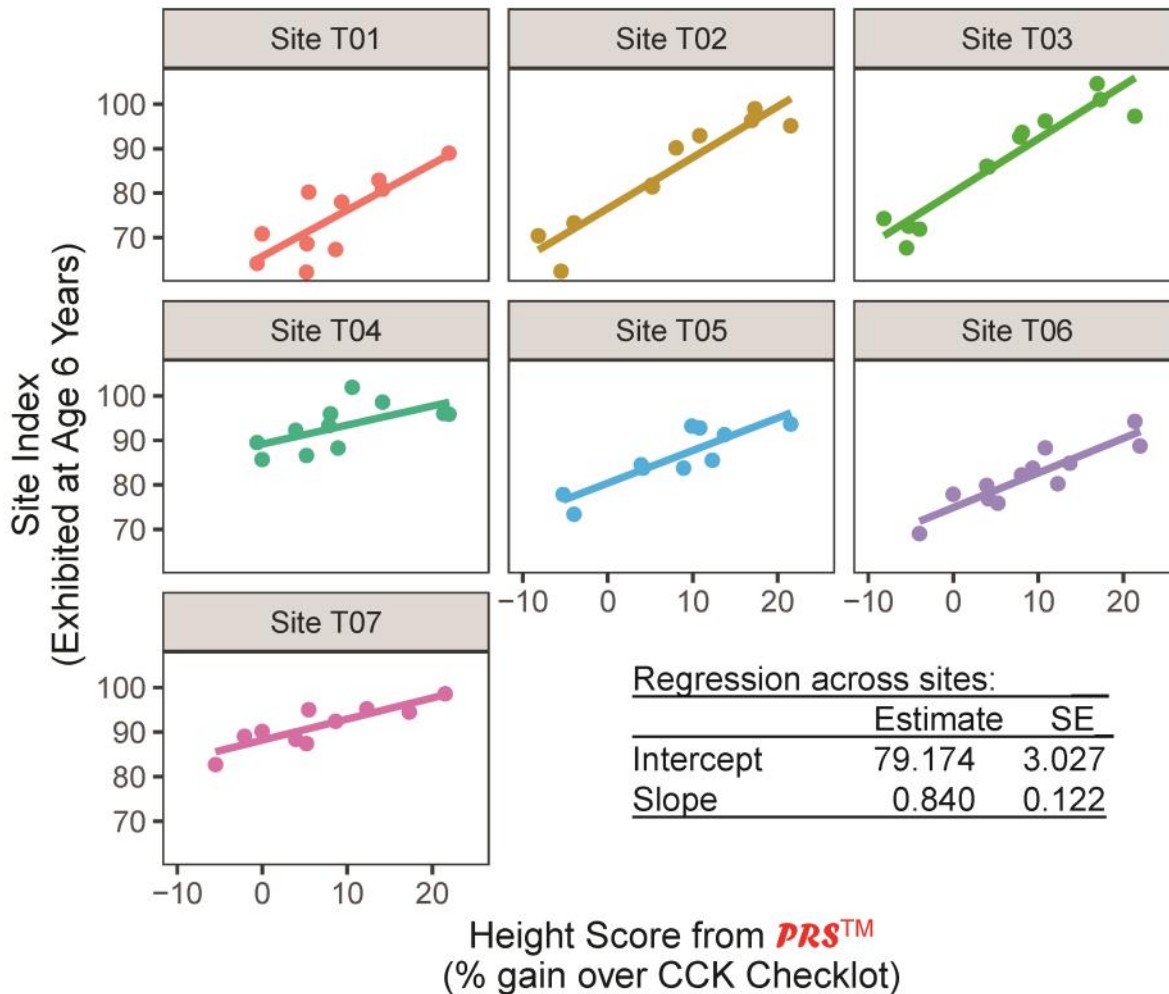

Figure 1. *Regression of exhibited site index (base age 25 years) from age 6 year measurements of the* **PRS**™ *Calibration Study to the Height scores that are published in* **PRS**™ *for Coastal sites. The regression is shown for each site, and parameter estimates for the regression across all sites are given in the table at the bottom right. The regression across all sites inicates that a 10 point gain in Height score from the* **PRS**™ *will result in 8.4 feet increase of exhibited site index at age 6 years.*

[1] Diéguez-Aranda, U., Burkhart, H.E. and Amateis, R.L., 2006. Dynamic site model for loblolly pine (*Pinus taeda* L.) plantations in the United States. Forest Science, 52(3), pp.262-272.

Wood Quality Update

While the Cooperative breeding program has not been selecting for wood properties, members that wish to select families for reforestation with an emphasis on wood properties may do so using breeding values and 𝓟𝓡𝓢™ scores published on the TIPRoot database. The wood quality breeding values are estimated from measurements on standing trees in progeny tests using two advanced rapid-assessment technologies: the Resistograph and TreeSonic. The Resistograph is a drill with a fine needle that records resistance, and is used to drill through trees at breast height to assess wood density. The TreeSonic is a tool comprised of acoustic sensors that are inserted into the tree above and below breast height and tapped with a hammer to record the speed of the sound wave (Image 1). The acoustic velocity is highly correlated with the stiffness of the wood.

Progeny tests are typically measured between ages 7 and 10 years. Table 1 lists the number of tests that have been measured, the number of tests that are scheduled to be measured through 2022, and the number of selections for which wood property breeding values will be available. The locations of progeny tests measured for wood quality through 2019 are illustrated in Figure 1.

Image 1. *The TreeSonic tool contains a probe that is tapped with a hammer and measures the time for the sound wave to pass through the stem. Faster acoustic wave values indicate stiffer wood. Peter McNeary (left) and Ben Maness (right), both undergraduate students pictured here measuring wood properties with the TreeSonic tool (photographed by Austin Heine).*

Table 1. *Progress of wood quality assessments showing the number of tests measured through 2019. Also included are the planned number of tests to be measured through 2022.*

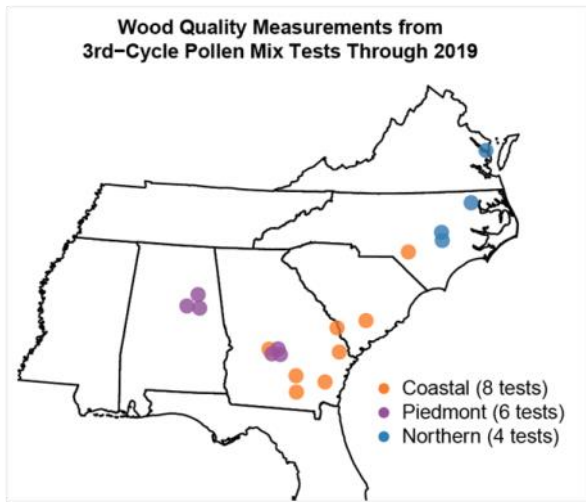| Series	# of Tests Measured Through 2019	# of Parents with Breeding Values on TIPRoot	# of Tests to Measure through 2022	# of New Parents that will have Breeding Values by 2022
Coastal 3rd-Cycle	8	269	4	68
Piedmont 3rd-Cycle	6	183	2	82
Northern 3rd-Cycle	4	127	Measurements Complete	
Atlantic Coastal Elite Population	5		2	21

Figure 1. *Location of progeny tests measured for wood quality through 2019. The drill resistance (density) and acoustic velocity (wood stiffness) breeding values and PRS™ scores are available on TIPRoot.*

The **PRS**™ scores for wood quality can be accessed from **TIPRoot** as follows (Screenshot 1):

- Visit the **TIPRoot** website and select **PRS**™ Values in the menu option on the left of the page.
- Only open pollinated (OP) crosses have wood quality scores published.
- Select your deployment region (Coastal, Piedmont, or Northern)
- Select one of the following checklots: CCK, PCK, or NCK
- Check the box "Show Wood Quality Values"
- Click Go!
- By selecting "Download these data" one can export the data into Excel for convenient filtering.

The column Drill resistance shows the **PRS**™ scores that correspond with wood density, and the column Acoustic Velocity[2] shows the scores that are correlated with wood stiffness. The values represent the percent gain over the checklot. The breeding value scores for parents can be obtained similarly using the Breeding Values page.

Screenshot 1: *Wood quality **PRS**™ scores can be considered when choosing among families for reforestation.*

The following findings have been published by TIP Staff and colleagues in their research on wood quality for loblolly pine:

- There is a substantial amount of genetic variation among families in loblolly pine for their wood density and stiffness[1].
- When choosing among families to plant, there is no correlation between growth rate and wood density or stiffness[1].
- Tree age is the most important determinant of wood quality in plantations[2]. Families with the best juvenile wood quality are approaching the quality of mature wood for an average family[1].
- Families with above-average juvenile wood quality are expected to also have above-average mature wood quality[3].
- Wood density and stiffness are separate traits that are genetically independent [1].
- Variation in proportion of latewood in the annual ring is a large determinant of wood quality when comparing trees that are the same age[4].
- No selection (for or against) wood quality has occurred in the TIP breeding population, as demonstrated by the checklots ranking very near the middle of the population[1].
- Family rankings for wood quality performance is very stable across planting environments (practically no genotype by environment interaction) [1].

The Atlantic Coastal Elite (ACE) population is in the process of being measured for wood properties as part of the Cooperative's genomic selection study. The objective is to develop associations between genomic markers and wood quality. To date, five of the ACE tests have been measured and two still need to be measured. The heritability estimates indicate a large amount of genetic control for these traits are present in the ACE population (Figure 2).

[1]Walker, T.D., Isik, F. and McKeand, S.E., 2019. Genetic variation in acoustic time of flight and drill resistance of juvenile wood in a large loblolly pine breeding population. Forest Science, 65(4), pp.469-482.

[2]Zobel, B. J. and van Buijtenen, J. P. 1989. Wood variation - Its causes and control. Springer-Verlag, Heidelberg, 363 pp.

[3]Williams, C.G., and R.A. Megraw. 1994. Juvenile–mature relationships for wood density in *Pinus taeda*. Can. J. For. Res. 24(4):714–722.

[4]Megraw, R.A. 1985. Wood quality factors in loblolly pine: The influence of tree age, position in tree, and cultural practice on wood specific gravity, fiber length, and fibril angle. TAPPI Press, Atlanta, GA. 89 p.
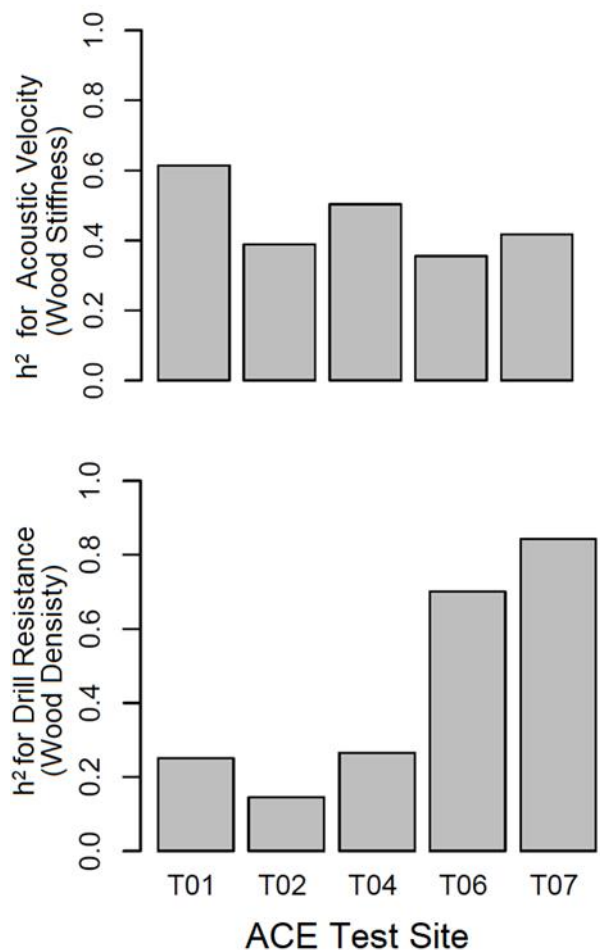
Figure 2. Narrow-sense heritability estimates of acoustic velocity and drill resistance measured on five ACE test sites. The values range from 0.20 to 0.80, indicating moderate to very strong genetic control.

Loblolly Pine PRS™ and TIPRoot Database Updates

4th-Cycle Annual BLUP

The NCSUCTIP is proud to deliver several new database tools this year. The first tool, "Annual BLUP", is a way for us to communicate the results of our annual mixed-model analysis of 4th-Cycle progeny test data to members. We recognize that although we don't have enough 4th-Cycle data for a new PRS™ version quite yet, many members are interested in obtaining breeding values of the 4th-Cycle parents in anticipation of establishing new orchards.

Screenshot 1. The location of "Annual BLUP" on the TIPRoot website. Click the bottom link in the "Publications" section to navigate to the tool.

The Annual BLUP tool is a link repository with each link directing the user to a spreadsheet. These spreadsheets each have two tabs: "TREE" and "PARENT". On the "TREE" tab, the user can see the individual breeding values of each tree retained in the 4th-Cycle data analyses. On the "PARENT" tab, the user can see all parental breeding values. The breeding values may be adjusted from year to year as more measurement data becomes available.

Screenshot 2. Links hosted in the "Annual BLUP" section each point to a different results spreadsheet.
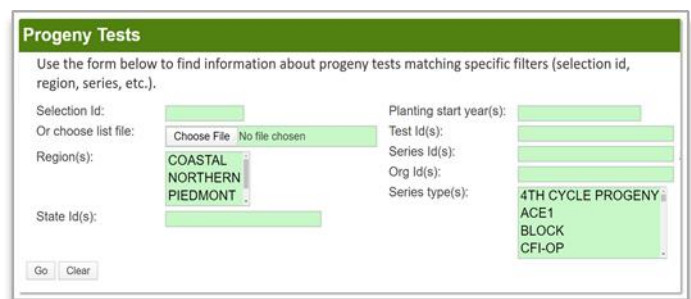
Progeny Test Data

This year we've released a tool which can be used to query all the information stored in TIPRoot pertaining to progeny tests. The tool is called "Progeny Test Data", and is located under "Measurement Data":
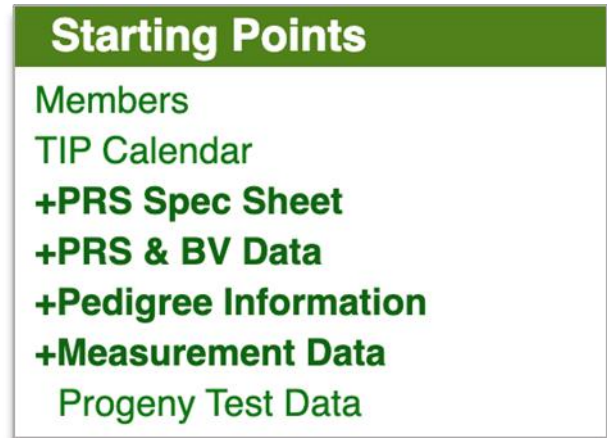
Screenshot 3. Location of "Progeny Test Data" under "Measurement Data" on TIPRoot .

One example of a use-case for this tool is obtaining the establishment report for any 4th-Cycle test. Open the tool by clicking on "Progeny Test Data". The dialogue that pops up will look like this:

Screenshot 4. User interface for "Progeny Test Data" tool. The user can interact with each box to help filter the results of the query.

Click on the "4TH CYCLE PROGENY" entry in the drop-down menu under "Series Type", and then click the "Go" button. Each progeny test in the 4th-Cycle will be displayed, with a clickable link that takes the user to the progeny test.

Screenshot 5. *Results of the query. Clicking on the blue underlined "file" link next to each test under the "Est. Rep." heading will bring up the corresponding establishment report for that test.*

Ever wondered about the raw data the NCSUCTIP is using in their breeding value analysis? Now you can see it as well. Navigate to the "Data Download" tool, found in the "Measurement Data" section:

Screenshot 6. *Location of the "Data Download" tool, in "Measurement Data" section of TIPRoot.*

Clicking on "Data Download" will bring you to an interface that looks similar to "Progeny Test Data" described above. There are a number of boxes and drop-down menus which the user can use to filter the results of the query.

As an example, use the tool to inspect the data from 2014IFC17. Type "2014IFC17" in the "Test ID" box, and select "Go":

Screenshot 7. *The output of the "Data Download" query. The two links in the lower-left corner provide the pedigree and the full test data for the test, "2014IFC17", which was entered in the box.*

Clicking on the blue links in the bottom-left part of the window will allow the user to download the pedigree and data file from the test.

Clone Archive and Database Integrated Tool (CLADIT)

This year, we've released a work-tracking tool to facilitate communication between TIP staff and members regarding clone banking activities. This tool, referred to as "CLADIT" (Clone Archiving Database Integrated Tool), will improve clone archiving efficiencies. It can be found under "Breeding Logistics" in the "Clone Archive" section:

Screenshot 8. *The location of CLADIT underneath "Breeding Logistics".*

Each grafting assignment belongs to a particular "grafting plan". These plans associate the graft with a particular tree improvement effort; for example, one grafting plan is "5th Cycle Selections". The grafting plans can be viewed by clicking the "Archive Plans" link:

Screenshot 9. *The user interface of the "Archive Plans" query. Each plan has a number of selections associated with it. Clicking on the plan report ("pdf") or the "Details" will give you the full list of selections to be grafted for that particular plan.*

Each Full member has their own list of assignments. When logged in to your **TIPRoot** account, only the clones assigned to your organization will be visible in the "Archive Assignments" section. Clicking on this brings up the following window:

Screenshot 10. *Example of an "archive assignments" list. This list is for the Westervelt company. The entire list can be downloaded by clicking the blue hypertext at the top to provide a convenient excel sheet.*

Each selection to be grafted is typically assigned to more than one member, for redundancy purposes. For any given selection, the members assigned to it can be determined using the "Lookup Assignments" tool. For example, if one enters the selection ID "ACE_02X11" into the "Selection" box, the following output is returned, suggesting that this selection is to be grafted by both WES and GFC:

Screenshot 11. *Output of the "Lookup Assignments" query. It can be seen that WES and GFC have been assigned to graft the selection, "ACE_02X11".*

Finally, there is a mechanism to track which clones are grafted in which clone banks. The final link under "Clone Archive", titled "Clone Banks", gives a user-specific overview of all clone banks their organization has registered with NCSUCTIP. The view of this tool is limited to your user account only, so different users have no visibility on one another's clone banks.

Screenshot 12. *User interface for the "Clone Banks" feature. Multiple boxes and drop-downs can be used to explore the data stored in* **TIPRoot** *pertaining to clone banks.*

We believe this centralized system will make it much easier to locate grafts and manage the complicated logistics during breeding season for the Cooperative.

Pictured above is a Tennessee Division of Forestry Clone bank thinned in 2014 in Delano, TN.

Fingerprinting status report

A project to test a workflow for sample collection, processing, and generation of DNA fingerprint data was initiated last year with the goal of sampling multiple ramets of several parents in the breeding program. The project was approved after the May 2019 Advisory Board meeting, and initial sets of samples were collected in the fall of 2019. These first samples did not yield satisfactory results in the fingerprinting step of the process, so the decision was made to pause the sample collection process and pursue an experiment to test an alternative approach to sample collection and processing during the winter and spring of 2020.

The problems observed in the fingerprinting results from the fall 2019 samples seemed to be due to difficulties with DNA purification from dried foliage samples, particularly those collected from seed orchard trees late in the growing season. The ability to collect foliage samples at any time of year, and to dry the foliage using silica desiccant and store it at room temperature, would both be valuable assets for the Cooperative fingerprinting program, because they will permit more flexible and convenient sample collection and storage methods. An experiment was therefore designed to test the suitability of an alternative approach to DNA purification using a set of foliage samples collected in the fall and dried with silica desiccant to determine if changing the DNA purification procedure could overcome the problems with the fingerprinting assay.

A cooperator agreed to provide a set of samples of dried foliage to the service laboratory run by Chris Dervinis at the University of Florida – Gainesville, and pay for the DNA extraction service offered there. The DNA extraction procedure used at that service laboratory was developed for use on either fresh or frozen tissue, and had not been tested on dried samples, but the results of the DNA extraction were encouraging. The yields of DNA seemed sufficient, and the quality of the DNA seemed little different from the dried samples than the results typically obtained by the service lab from fresh or

frozen conifer needle samples. The DNA samples were then shipped to Floodlight Genomics for fingerprinting, where the preliminary results were equally positive. A total of 305 foliage samples were provided for DNA extraction, and of those, 289 samples yielded data at some level, while 16 failed either at the DNA extraction step or at the fingerprinting step. This is a failure rate of about 5.2%, which is not ideal but may be a necessary cost of the convenience of being able to sample foliage any time of the year and store collected samples at room temperature rather than in a freezer. Lower failure rates are certainly possible if samples are collected from young immature needles during the growing season and stored frozen, but the costs of that sample collection and storage procedure are also higher.

A complete analysis of the fingerprinting data is still underway, but simple comparison of the amount of data obtained from each of the 289 samples shows a 275-fold difference between the sample with the most data and the one with the least. The effect of this difference in data yield remains to be seen. It is possible that even the samples with the smallest raw data files will still have relatively complete data from the set of genetic markers assayed, but it may also be that samples with lower yields of raw data will be missing data for a subset of the markers. A more comprehensive analysis of these data will be presented at the next meeting.

Pictured above are needle samples taken for DNA extractions for fingerprinting.

Arrowhead Breeding Center Update

Compared to the past few years at Arrowhead when new breeding orchards were planted, rogued, and hundreds of crosses performed, this was a relatively quiet year. The bulk of the work performed at Arrowhead this spring was comprised of grafting the new 5th-Cycle selections and completing the grafting of TIP2201951 interstock into the Graveyard Orchard (Image 1).

An exciting development this year, is that Georgia Forestry Commission has appointed Anita Johnson (Image 2) as the Arrowhead Seed Orchard Manager. Anita previously worked at Flint River Nursery, and brings a wealth of tree improvement knowledge with her. We are excited to have Anita on board at Arrowhead as she has been working closely this past year with our longtime friend, Chuck Little. Together they have pruned and marked top grafts, collected cones from research crosses, installed pollination bags, and much more. We look forward to working with Anita at Arrowhead in the years to come!

Image 2. Anita Johnson, Arrowhead Seed Orchard Manager, in the lab at Arrowhead this past spring. Anita was a little yellow in this photo, but as you can see, is already getting her hands dirty!

Image 1. Photo taken standing in the older portion of the Graveyard Orchard (planted in October of 2017). These rootstocks had TIP2201951 interstock grafted into them last spring. In the distance is the younger portion of the Graveyard Orchard (planted in October of 2018), TIP2201951 interstock were grafted into it this spring.

The Few. The Proud. The Belleau Wood Oaks

Over the past two years, TIP staff have been working on a project that is quite different from our normal pine tree endeavors. We were approached by a representative of the US Marine Corps (USMC) Foundation about growing some very special trees from an extraordinary place! The USMC Foundation had a project in mind to commemorate the WWI Battle of Belleau Wood, a fierce battle where many Americans died fighting alongside the French against the Germans. They wanted trees from the battle site to be planted in the US to honor the many men who lost their lives in this battle.

In the fall of 2019, approximately 200 acorns of Sessile oak (*Quercus petraea*) were gathered in the cemetery in Belleau Wood, France, by Marines in the Marine Corps Attaché. After the acorns cleared the French Ministry of Agriculture, TIP staff worked with the USDA to ship them through to Raleigh. Following the phytosanitary inspection/treatment stateside, approximately 100 acorns were received in Raleigh (Image 1). They were immediately imbibed in water and sown. Germination was very successful, and we now have approximately 90 oak seedlings up and growing (Image 2)! These trees have been transferred to 7 gallon pots, where they will be grown for the next two years (*Image 3.*). Once the

Image 1. *Acorns inside their USDA inspection station bag after arriving from France.*

trees reach adequate size, the oaks will be planted in various locations around the United States. Future proposed sites/interested parties include the cemetery in Camp Lejeune, NC with the Marine 6th regiment, the Marine 5th regiment in California, the Marine Corps Museum in Quantico, VA, and several other museums around the country. Tree Improvement is proud to be a part of this commemorative project honoring the service members of the USMC!

Image 2. *(above) Seedlings in April 2020.*

Image 3. *(right) Same seedlings repotted for next phase of growth!*

More information can be found in the Camp Lejeune Globe article: https://www.camplejeuneglobe.com/carolina_living/belleau-wood-oaks-to-help-next-generation-remember-famed-battle/article_3fd27f86-1050-11ea-8728-3705be62dc0b.html

SEED AND CONE YIELDS

The 2019 cone crop was much better than the previous year. Table 1 shows that the number of bushels and pounds of seed in 2019 was almost double that of last year's harvest, which was unusually low. Although tons of seed production in 2019 did not get back to the level of seed production seen in 2017 (Figure 1), everyone will agree that it was nice to have another good harvest year after the 2018 season. 2nd-Cycle seed production was 26% of the crop in 2019, while 3rd-Cycle + material made up the majority of the seed produced at 74%.

Table 1. *Fall 2019 cone and seed yields compared with the figures from 2018 harvest*

| Provenance / Orchard Cycle	Bushels of Cones		Pounds of Seed		Pounds of Seed per Bushel	
	2019	2018	2019	2018	2019	2018
Coastal 2.0/2.5	8,058	3,886	10,338	4,435	1.28	1.14
Coastal 3.0	16,213	9,342	20,621	10,394	1.27	1.11
Piedmont 2.0	3,433	1,672	4,776	2,402	1.39	1.44
Piedmont 3.0	11,417	5,716	16,055	6,574	1.41	1.15
Northern 2.0	0	383	0	296	0.00	0.77
Northern 3.0	5,164	3,387	6,078	2,934	1.18	0.87
Totals	44,285	24,385	57,868	27,035	1.31	1.08
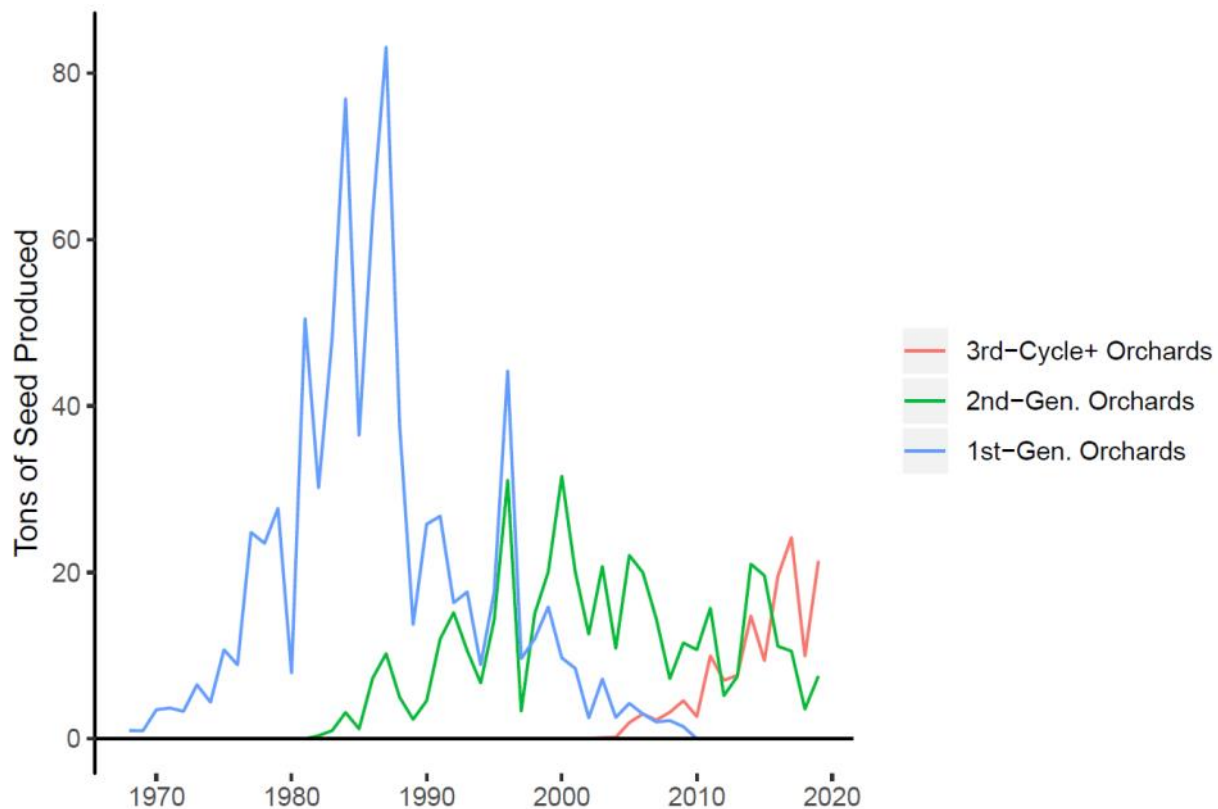
Figure 1. *Since 1968, the Cooperative has tracked annual seed yields from Cooperative members. 1ˢᵗ-Generation seed orchards are no longer being harvested. In 2019, 26% of the seed harvest came from 2ⁿᵈ-generation seed orchards, while the other 74% came from 3ʳᵈ-Cycle + seed orchards.*

RESEARCH

Pita50K: Axiom 50K SNP array for loblolly pine

SNP arrays, or chips, are advantageous genotyping tools in plant and animal breeding because they are repeatable, reliable, and high-throughput. Unlike sequence-based genotyping, each experiment is guaranteed to include the same set of markers. The data can be analyzed and interpreted without specialized bioinformatics skills, and the results from multiple different experiments and research groups can be directly compared.

The Cooperative staff took the lead to develop a SNP array for loblolly pine to jump start genomic applications in breeding. The process took over four years with the following major steps:

2016

SNP marker discovery: With funding from USDA-NIFA, the pineSNP project (Award #: 2016-67013-24469, PI Isik), discovered 642,000 SNP markers from large DNA and RNA sequence data sets. The sequence data sets were produced by previously funded public projects, such as PINEMAP and CTGN at various universities. The project team developed a bioinformatics pipeline (led by Co-PIs Jill Wegrzyn of University of Connecticut and Andrew Eckert, Virginia Commonwealth University) for evaluating and selecting variants for use on the Thermo Fisher Axiom platform for conifer trees. Bioinformatics support was provided to national and international collaborators.

Improvement of loblolly pine genome assembly: For marker discovery, a reference genome assembly is essential. The loblolly pine genome sequence assembly v2.0 was released in August 2016 through the PineRefSeq project (PI David Neale). The pineSNP project Co-PI Wegrzyn used the transcriptome assembly and PacBio Iso-Seq reads to improve the loblolly pine genome assembly in collaboration with Ross Whetten (NCSU). This new assembly (listed as v2.01) was used for SNP calling and marker discovery.

2017-2018

Conifer SNP Consortium (PI Isik) was established in December 2017 to bring the community together to negotiate with technology companies to develop genotyping arrays that will benefit both the domestic and the international tree breeding community. The Conifer SNP Consortium had six different groups (USA-Loblolly pine, USA-Douglas fir, New Zealand/Chile-radiata pine, South Africa-tropical pines, EU-4 tree species, Sweden-Norway spruce), and each group had multiple organizations and companies interested in the SNP array designed for the group. The consortium signed a memorandum of understanding with Thermo Fisher in May 2018 to design six Axiom SNP arrays for the consortium.

2019

Designing the screening array: A list of 642,275 probe sequences was delivered to Thermo Fisher for marker quality analysis and these markers were submitted to Thermo Fisher for quality control in March 2019. Based on bioinformatics quality control metrics, a screening array was fabricated with the best 423,695 probes in May 2019. In order to empirically validate these probes, a diverse panel of 392 diploid loblolly pine samples and 36 haploid megagametophytes were collected by TIP staff and assayed. Data from the screening array were analyzedThe cost of the screening project was paid by the Cooperative Tree Improvement Program members.

Final probe selection for the Pita50K array: As expected, the genetic data analysis revealed a highly skewed minor allele frequency distribution, with a mean below 0.1. We used three main criteria for variant selection: 1) SNP with minimal heterozygosity on the haploid samples, 2) SNP with intermediate minor allele frequency ($q > 0.05$) and displaying three genotype clusters, 3) SNP with genotype ratios in Hardy-Weinberg equilibrium. This SNP selection process resulted in 46,439 markers for inclusion on the Pita50K array. The array became available to the community in September 2019.

The Pita50K Array is an important resource for the Conifer Genetics community. Consortium members have already genotyped over 10,000 trees since the Pita50K array became available. We expect the resources developed by the Conifer SNP Consortium will have a large impact on forest health and productivity research worldwide in the coming years.

How are we using Pita50K so far?

In summer of 2018, TIP staff and students collected needle tissue from each of the 2430 Atlantic Coastal Elite (ACE) population clones and the 21 founders. After visiting three test sites, approximately 98% of the ACE clones were sampled (2382 out of the 2430), along with all 21 ACE founders. In total 2482 ACE samples (including additional checks and duplicates) were submitted for genotyping using the Pita50K SNP array. Genomic prediction models are currently being developed using the ACE1 pheno-

typic data from seven test sites with one ramet per clone per site.

The preliminary results from the ACE1 population revealed a strong correspondence between genetic relationships based on pedigree and genomic relationships based on Pita50K markers (Figure 1). The analysis also detected some pedigree errors in the ACE1 population, with a mismatch rate around 1.76%.

In addition The NCSU Cooperative Tree Improvement Program genotyped 2000 seedling progeny of two full-sib families in January 2020 for fusiform rust gene discovery and development of high-density genetic maps. A high-density genetic map was developed, and major R genes for disease resistance in two loblolly pine families have been discovered (see more details in Eddie Lauer's Ph.D research on pg 32).
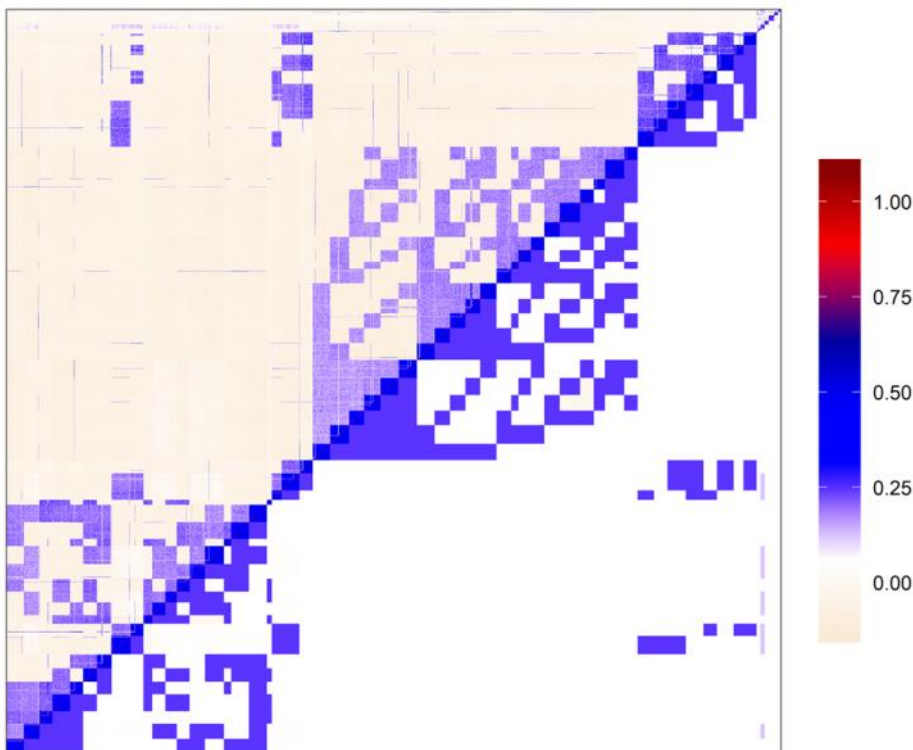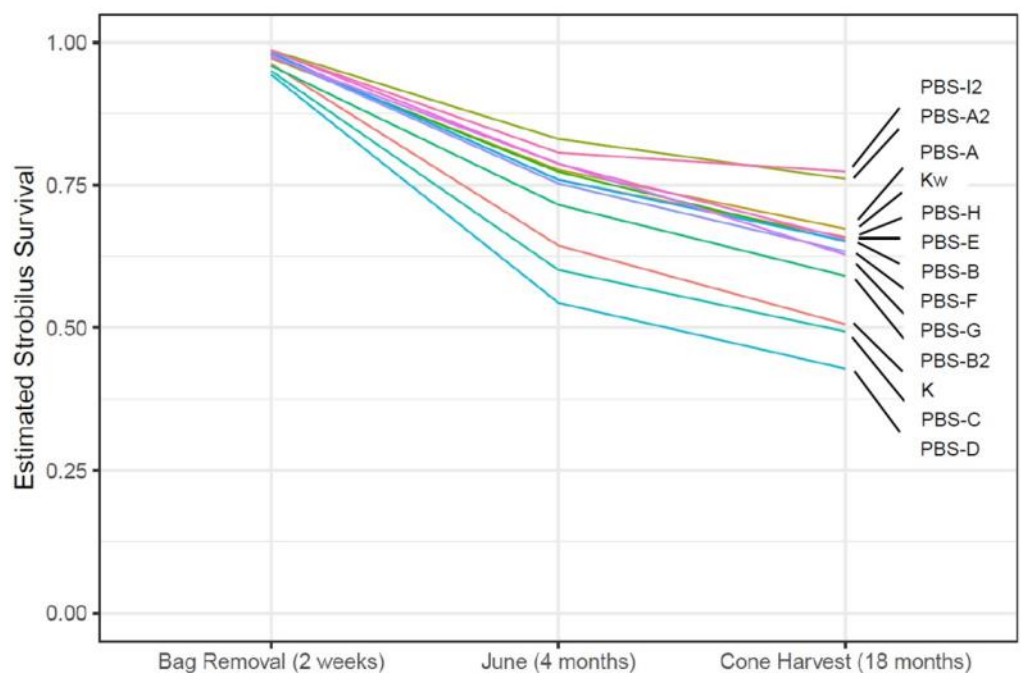

Figure 1. Relationship matrix with each individual on a row and column and their relationships with other individuals indicated by cell color. The realized genomic relationships based on SNP markers are above the diagonal, the expected genetic relationships based on pedigree are below the diagonal. Similarity between relationships from two data sets is apparent, however the realized genetic relationships are more precise as indicated by varied shading. Full-sibling families can be identified as groups of darker squares around the diagonal.

Pollination Bag Study-Summary[1]

Since 2014 Cooperative members, staff, and students have been working on the pollination bag study to find a pollination bag that is superior for female strobilus survival and/or seed yield for mass production of controlled cross seeds in loblolly pine. There were three installations of prototype pollination bags (2014, 2015, and 2017) along with the industry standard Lawson pollination bag, with and without a support wire, and open pollinated controls. Additionally, a time and motion study was performed in the spring of 2017 (results discussed in the NCSUCTIP 62nd Annual Report, pgs 13-14).

Final female strobilus survival for the 2017 study installation was collected in the fall of 2018 at cone harvest and was combined with data from previous years of installation at each assessment period (2 weeks, 4 months, and 18 months). These data were analyzed and estimates plotted (Figure 1). As shown in Figure 1, the two newest bag types (PBS A2 and I2) had the highest strobili survival over all bag types tested. Bag type main effect was highly significant (Pr≤0.0005) at all assessment periods.

At cone harvest for the 2014, 2015, and 2017 installations, one cone was collected and placed into an individual bag to be used for cone analysis from specified reps for each bag type. Cone analysis allowed us to determine the seed efficiency for each cone to see if one bag type was more efficient at producing filled seed. Seed efficiency is the number of filled seed divided by the seed potential (number of fertile scales x2) for that cone. As shown in Figure 2, bag type had a significant effect on seed efficiency (Pr<.0001) with PBS-A2 having the highest seed efficiency per cone.

In the 2015 and 2017 installations, for specified reps, all cones surviving for each bag type were collected and seed extracted to determine the mean filled seed per bag (Figure 3). For bags with surviving cones, bag type did not have a significant effect on filled seed per bag (Pr=0.9848). This was not what we expected given the higher survival of cones and higher seed efficiencies observed in some bag types. The best explanation we can give as to why we did not see filled seed per bag follow the same trend as what was seen with seed efficiency and cone survival is that many cones in each bag

Figure 1. Probability of female strobilus survival by bag type from bag removal (two weeks post pollination) to June drop (four months post pollination) to cone harvest (18 months post pollination). The critical assessment time for survival appears to be at June drop, four months post pollination. The newest prototype bags, PBS-A2 and PBS-I2 had higher conelet survival in June and higher cone survival at time of harvest than other pollination bags.

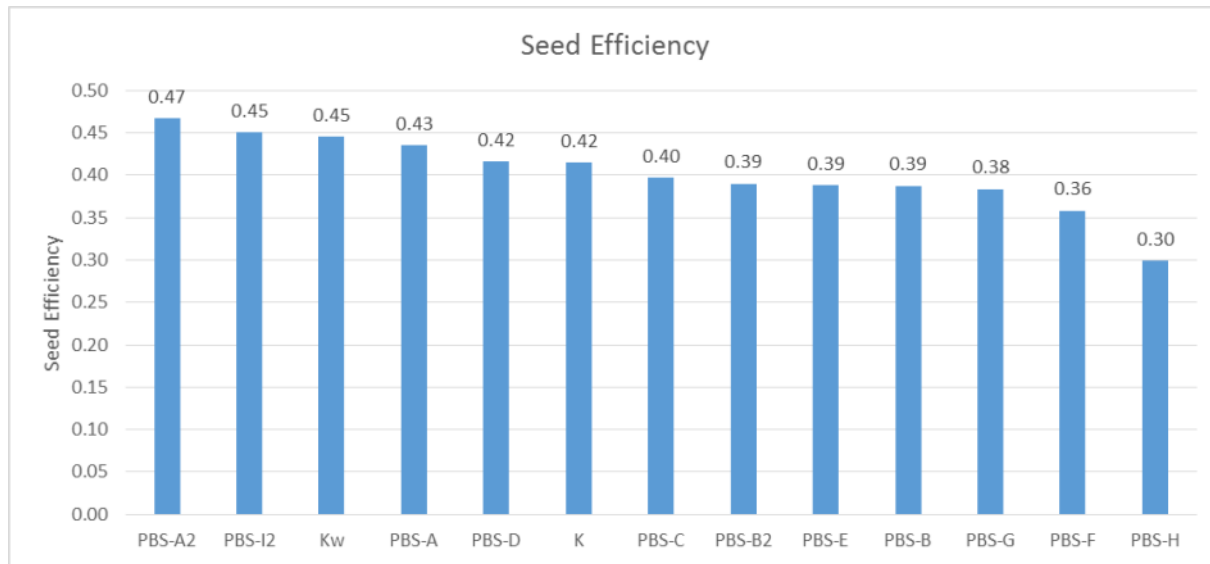[1] *This is a final summary of Austin Heine's MS graduate research*

Figure 2. Estimated mean seed efficiency per cone for each bag type. There was a significant bag type effect (Pr<.0001) indicating that some bag types produced filled seed more efficiently than others.

were suffering from insect damage, and in some orchards, a high number of first year aborted ovules (indicating issues with pollen quality or timing of pollen application). This was not observed as frequently in the cone analysis cones, as these cones were the best cones from each bag.

Completing this study was a huge effort, and we owe many thanks to the orchard managers who installed this study, students that participated in seed extraction and cone analysis, and PBS International for funding this research over the past several years. We have learned a lot from this study, possibly the most important being that rigidity and stiffness of

the bag type material has an important effect on female strobilus survival inside of the bags. We have also been reminded that there are many other important factors such as insect control and pollen quality that can play a much larger role in producing filled seed from mass production of controlled crosses than the pollination bag type used. For a further look at this study, look for the upcoming publication in Forest Science (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. (in press)).
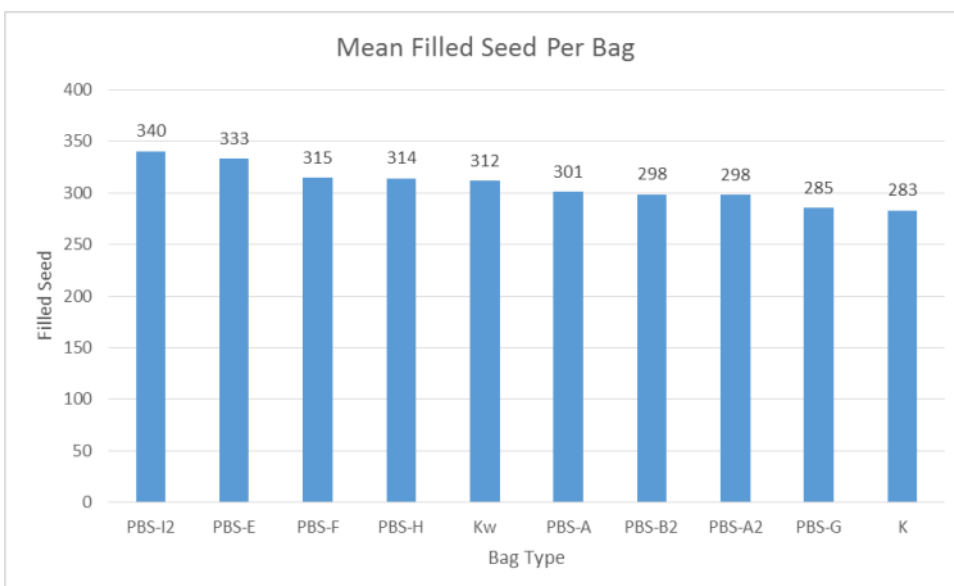

Figure 3. Estimated mean filled seed per bag for bags with surviving cones. Bag type effect was not significant (Pr=0.9848).

Marker-trait association and genomic selection within cloned loblolly full-sib families for fusiform rust disease and stem forking defects[1,2]

Fusiform rust disease and stem forking defects are two important traits in loblolly pine breeding. Selection for these two traits requires field progeny testing and assessment, which may take five years to complete. Molecular breeding for fusiform rust disease and stem forking may replace classical progeny testing for within-family selection in loblolly pine breeding. The objective of this study was to assess SNP markers-trait associations and understand the utility of within-family genomic selection for fusiform rust disease and stem forking defects.
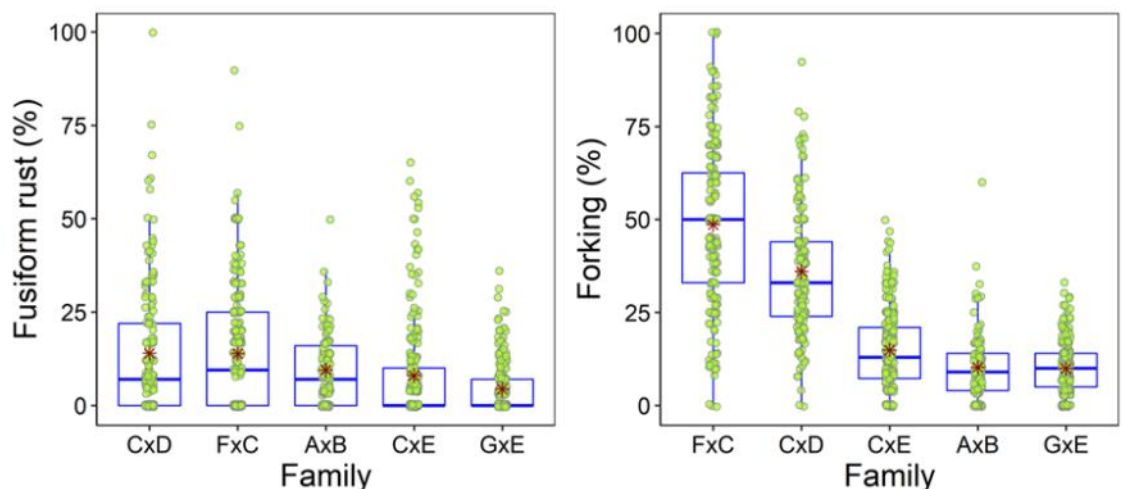
In the study, a total of 1831 clonal varieties from 37 families were used. Cloning was done via somatic embryogenesis. The study was replicated at 26 locations established over 3 years in the southern US. Incomplete row-column design with six replications was used in the field. Test sites were strongly connected within a test series (69 to 396 genotypes in common), whereas the overlap between tests across series was weak, with a mean of 26 genotypes in common. At age six, approximately 31,411 trees (1831 genotypes x 17 copies each) were assessed for incidence of fusiform

rust disease (presence of galls or no galls) and incidence of stem forking (yes or no). A subset of clonal varieties (723) from five full-sib families were genotyped with 17,000 SNP markers.

The incidence of fusiform rust and stem forking of 1831 genotypes were analyzed using generalized linear mixed models to estimate variance components. A separate generalized linear mixed model was fit to obtain best linear unbiased estimates (BLUE) of genotypes. The estimates were used as pseudo phenotypes to estimate marker-trait associations and genomic estimated breeding values. For genomic prediction, Bayesian ridge regression, Bayes B and Bayes Cπ whole-genome regression models were implemented. These models had different assumptions for marker effects.

Three cross-validation (CV) scenarios were used to evaluate across population and within-family prediction accuracies. In scenario one, random CV was implemented using the whole population for training and validation sets. In scenario two, one of the five families at a time was used as the validation set, while the other four families were used as the

Figure 1. Boxplot showing incidence of fusiform rust disease and stem forking for each family. The horizontal line in the middle of the box is the median and the red asterisk inside each box represents the mean. The boxes are the interquartile range (50% of the data). Green circles denote individual genotypes (full-sibs).

training set. In scenario three, within-family CV was performed using each individual family for training and validation. The testing set comprised of 20% randomly selected genotypes within each family. Predictive ability between the training and validation set was estimated as $r_{PA}= r(y_{vs,}\hat{g}_{vs})$, where y denotes the best linear unbiased estimate (pseudo phenotype), $\hat{g}$ is genomic estimated breeding value, and vs indicates the validation set.

The incidence of fusiform rust disease and stem forking was low across most of the test sites with an overall mean of about 10% and 19%, respectively. The mean incidence of fusiform rust and stem forking differed among the five genotyped crosses, ranging between 4-14% and 9-48%, respectively. The incidence of the two traits ranged between 0 and 100% among the clonal varieties within the families (Figure 1). The clone-mean narrow-sense and broad-sense heritability estimates for the two traits were high, ranging between 0.74 and 0.95.

Two SNP loci with large effects were detected for fusiform rust disease incidence, explaining about 62% of variation. Homozygous major genotypes AA showed significantly lower rust disease incidence (2%) compared to the heterozygous AB genotypes (20%). The odds of fusiform rust disease incidence for heterozygous (AB) individuals were 10.7 and 12.1 times greater than the homozygous (AA) individuals for the two SNP loci. Similar results were observed for the individual families and parents having data for the two genotypic classes.

Random cross validation resulted in higher predictive ability estimates for both traits, ranging between 0.69 and 0.74. Using each family as a validation set had low or negative prediction abilities for all the families for stem forking. For fusiform rust disease, the estimates were generally moderate, except for one family (Figure 2).
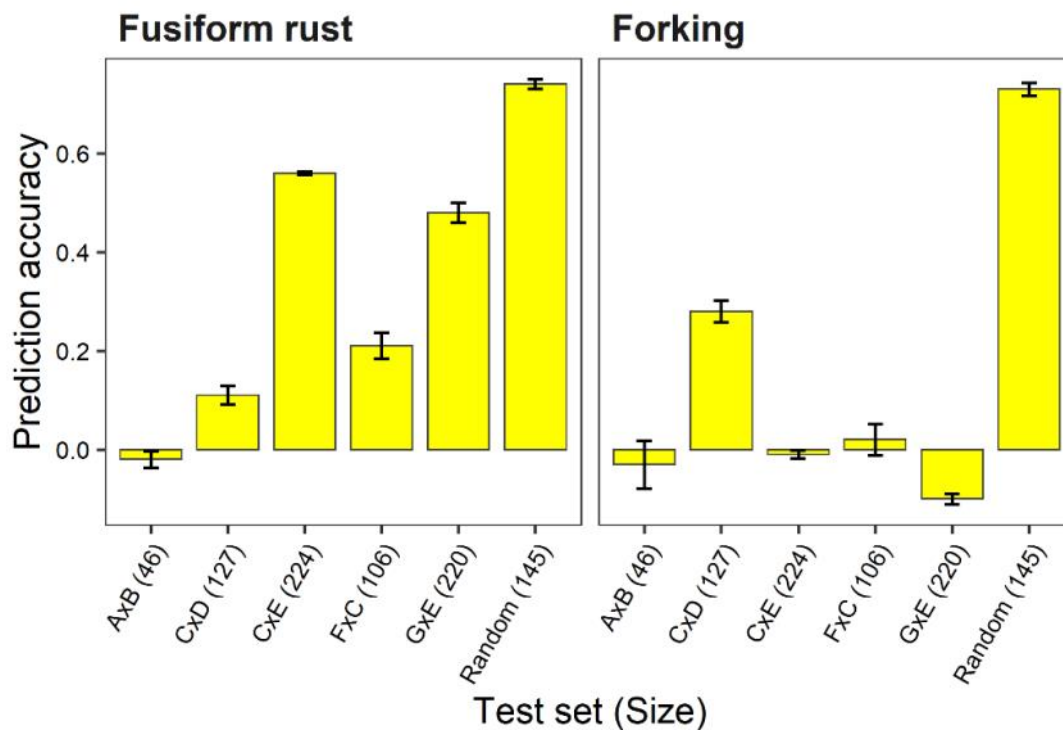

Figure 2: *Prediction accuracy for fusiform rust disease and stem forking from the Bayes B model for random and family cross-validation scenarios. Random cross-validation scenario (splitting the whole population as training and validation sets) had higher prediction accuracies than using a family as the validation set. The validation set size is provided within parenthesis.*

In another scenario, 80% of individuals of a family were randomly selected as a model training set. The remaining 20% of individuals were used as a validation set. Data from other families were not included in the cross-validation. Predictive ability was considerably higher when the same family was sampled for training and validation. Predictive ability estimates for fusiform rust were moderate to high with a range of 0.27 to 0.62, while the estimates for the stem forking defect ranged between -0.03 to 0.39 (Figure 3).
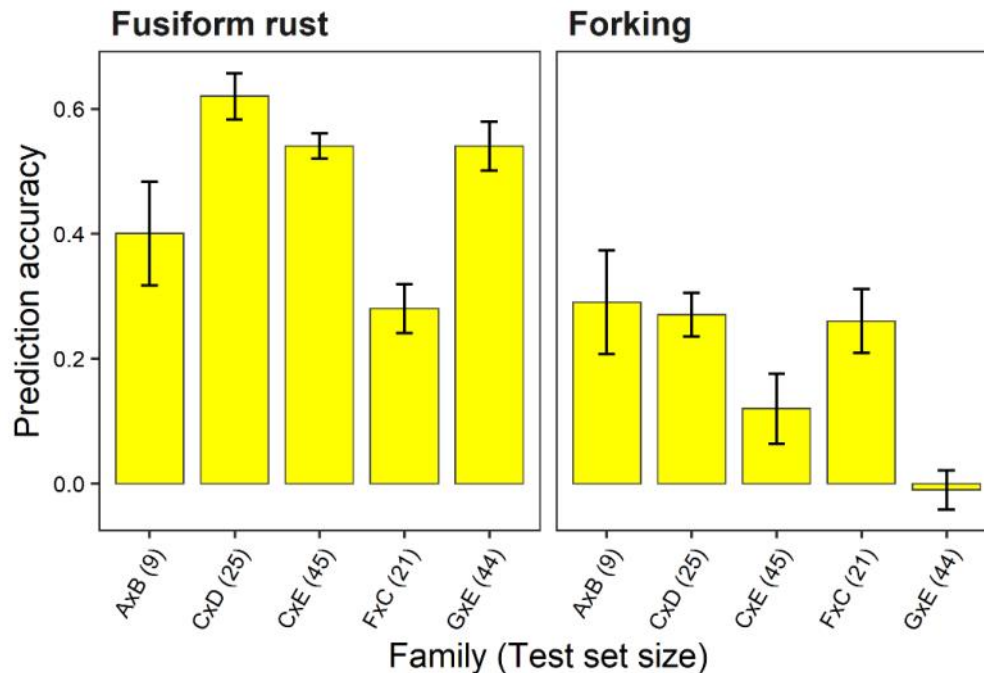

Figure 3. Prediction accuracy for fusiform rust incidence and stem forking from the Bayes B model for within-family cross-validation scenarios. The validation set (20%) for each family is given within parenthesis.

The following conclusions can be drawn from this study:

- The inheritance of fusiform rust incidence appears to be oligogenic, meaning that there are a few loci with large effects and many loci with small effects controlling the disease outcome. The inheritance for stem forking appears more complex, and is likely controlled by environmental factors and many genes with small effects.

- The two SNP loci with large effects discovered in this study should be further explored and validated to be considered for marker-assisted selection for fusiform rust disease in loblolly pine breeding.

- The data suggested that genomic selection within-family for fusiform rust disease could be effective in loblolly pine breeding. In this study, SNP markers traced within family chromosome haplotypes efficiently when the same family was split into training and validation sets. The results for stem forking were inconclusive.

- Using data from a set of families to predict the ranking in another family produced mixed results, suggesting the importance of genetic relatedness between training and validation populations.

- The results in this study are still proof of concept, and the marker-trait phase needs to be tested after a breeding cycle to allow for chromosome recombination.

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". One of the workstreams of this project was focused on the discovery of broad-spectrum[2] fusiform rust resistance genes. Through a combined approach utilizing high-density genotyping (Pita50K array) and RNAseq, at least three such genes were discovered (Figure 1, Figure 2). These genes were found to confer resistance under a high spore load (100,000 spores/mL) of a bulked mixture of 150 different gall isolates at the USDA Forest Service Resistance Screening Center in Asheville, NC. If a gene is effective under this extreme pathogen pressure, it should surely be effective in your deployment area!
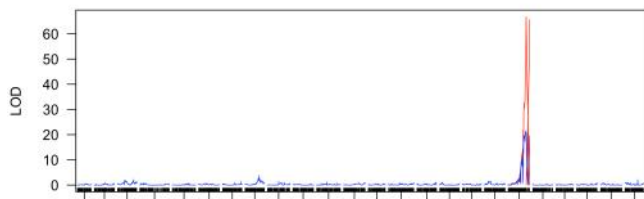

Figure 1. *Genome-wide QTL scan for family TIP330244. The two large peaks in red are separate genes on chromosome 9. Each of these genes re-duces the odds of infection by around 40x.*
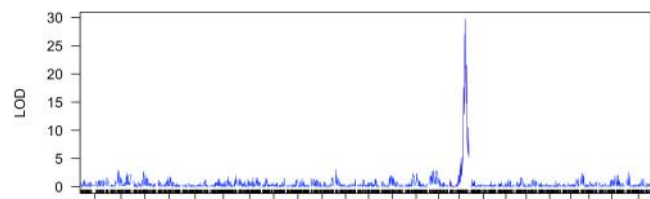

Figure 2. *Genome-wide QTL scan for family TIP1162449. The single large peak is on chromosome 8. This gene is not as effective as the genes in family TIP330244, but still reduces the odds of infection by around 7x.*

Summary

The large population size employed in this study (2000) enabled high-resolution mapping of rust resistance genes. We have pinpointed the location[2] of these genes at a resolution of less than 0.5cM. This large population size had the added benefit of producing a very high-quality map, due to the large number of meioses sampled. The genetic map produced in this study is the highest-quality map ever produced for loblolly pine and will be a valuable research tool for the conifer genetics community (Figure 3).

Sequence-Level Confirmation

In this study, allelic variation within the expressed genes was directly analyzed via RNASeq. Loblolly pine has one of the largest genomes ever assembled (~22Gbp), so methods of reducing this to a smaller size have the potential of getting us even closer to the gene or perhaps to the gene itself.

A bulked-segregant analysis (BSA) was used to determine the "skew" in allele frequency between diseased and non-diseased samples in each family. Any locus with an extremely high frequency skew has the potential to be near or within a rust resistance gene. Briefly, eight "bulks" were collected from each population: three diseased bulks, three resistant bulks, and two random bulks. RNA was extracted from each bulk and sequenced with PacBio ISOSeq as well as the Illumina NovaSeq6000 instrument. Short-read data from the Illumina instrument were aligned against the long-read PacBio ISOSeq reference transcriptome produced for each family (Figure 6). After variant calling, the read-count ratios between diseased and resistant samples were used to generate odds ratios to determine the relationship between the rows and columns of the matrix. Fisher's Exact Test was then used to determine if the odds ratio at each site was significantly different from the null model.

The null model was extrapolated from the allele frequencies estimated in the "random" bulks for each family. A small number of significant "hits" were found. Five of these were within NBS-LRR genes, which are common disease resistance genes found in all species of plant (Figure 4).
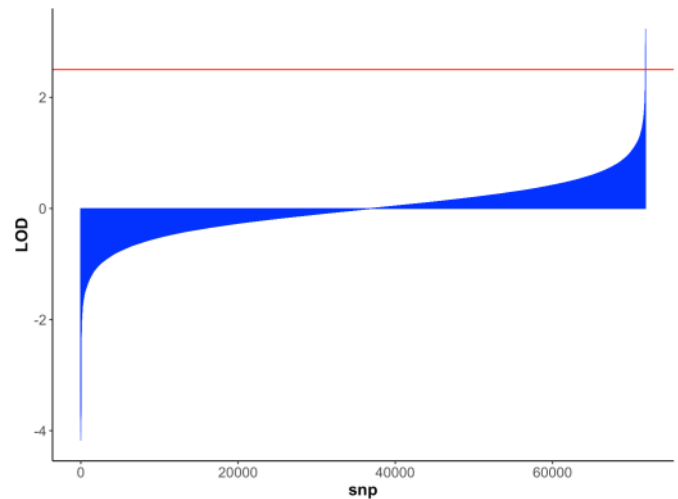
Figure 4. *Log of the odds ratios (LOD) for all high-quality variants detected in family TIP330244. The candidacy cutoff is shown as a horizontal red line. Only 10 SNPs fell into this category; three of which were within NB-LRR resistance genes.*

A small number of SNPs immediately stood out in this analysis. These were completely monomorphic in the diseased bulks but polymorphic in the resistant bulks. This is the expectation of completely dominant resistance genes. An example of one of these SNPs is shown below (Figure 5).
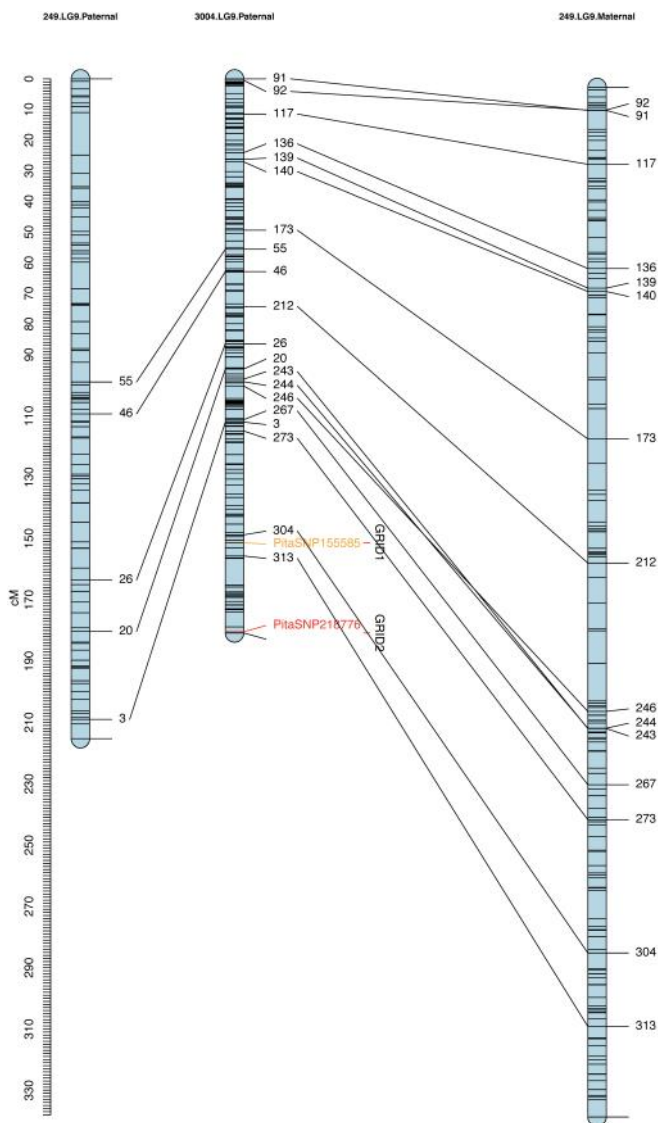

Figure 3. *The paternal complement of chromosome 9 from TIP330244 is shown in the middle, with the paternal and maternal complements of the same chromosome from TIP1162449 shown on either side. Markers mapped in both families are shown as solid lines to demonstrate the collinearity of the maps produced for both families. The rust resistance genes found in TIP330244, coined "GRID1" and "GRID2" (for General Rust Immunity Determinant), are shown as red boxes next to the colored type. These boxes represent the 95% Bayesian Credible Intervals for the QTL. The peak markers from the Pita50K array cosegregating with GRID1 and GRID2 are shown in orange and red, respectively.*
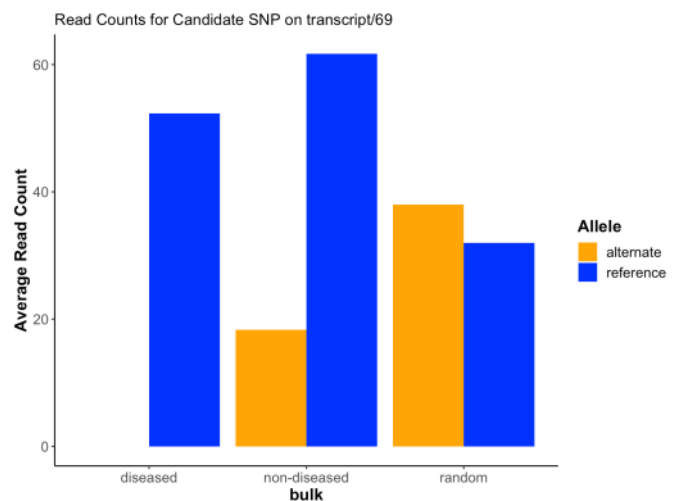

Figure 5. *Read counts at a candidate SNP in "Transcript 69", a TIR-NBS-LRR gene.*

The SNPs that were highly associated with disease resistance from BSA were converted into sequence-based markers via the AgriSeq platform from Thermo Fisher Scientific. A pilot project is currently underway to determine which of these markers co-localize with the QTL identified using the Pita50K chip. These markers can be used as an inexpensive marker tool to identify seedlings carrying one, the other, or both resistance genes.

What's Next?

The two full-sib populations used to discover these genes were planted at two locations in South Carolina and Georgia, with the help of ArborGen and IFCO. These trees can be used directly to introgress the resistance genes into the mainline breeding population. They can also be crossed with each other to potentially generate "true breeding" disease-resistant pine varieties. This will potentially have a huge impact on landowners across the Southeast.
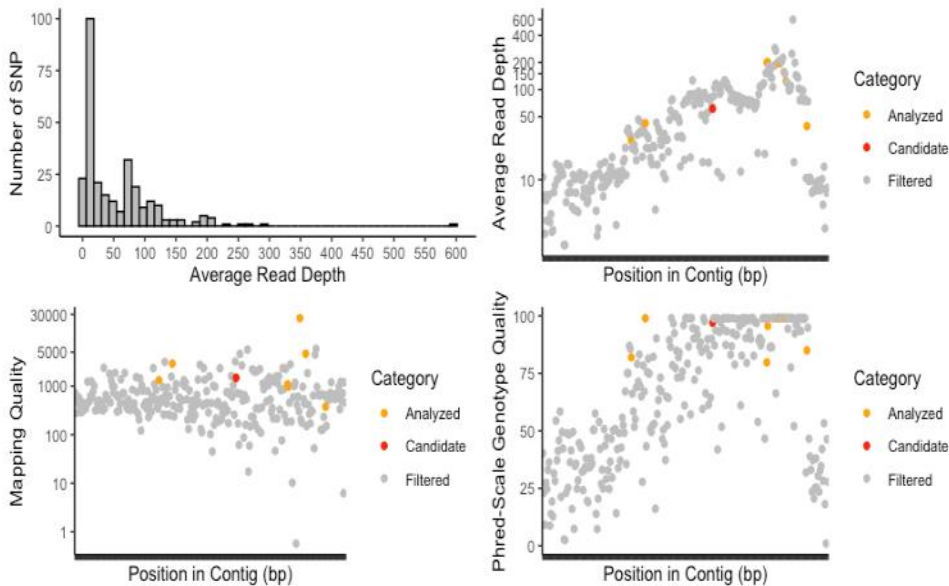

Figure 6. Read depth and mapping statistics on one candidate gene, Transcript 69. A linear increase in read depth from the 5' to 3' portion of the transcript suggest this gene may be part of a duplicated gene family.

Optimal Mating for Monoecious Species: An update of *PineBreed*[1]

In the 2019 annual report, we introduced the first phase of development of the software package called *PineBreed*. It was developed to address the pressing issues associated with designing optimal mating lists for monoecious species. Maximizing the genetic gain while managing the inbreeding levels is always a challenge for monoecious species, like *Pinus taeda*, that suffer from a high genetic load. *PineBreed* successfully balances these two important but contrasting goals.

To briefly summarize, *PineBreed* is a one-stop solution to create an optimal mating list that maximizes genetic gain while constraining the increase in average group coancestry. The input comprises a list of candidates with their breeding values. The TIPRoot database is used to autocomplete the potential candidates' pedigree. Using the numerator relationship matrix, inbreeding coefficient and average coancestry coefficient, a permission matrix is created that defines whether mating between two candidates is allowed. The modified differential evolution (DE) algorithm (Storn and Price, 1995) then analyzes the entire search space to create numerous possible mating lists. An objective function (Kinghorn, 1998 and NCSUCTIP annual report 2019) is used to test the performance of each of the possible mating lists. The output is an optimal mating list that outlines the

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

mate-pairs, estimated progeny mid-parent breeding values, and relatedness coefficient of mate-pairs and the entire pedigree.

Numerous improvements were made to the *PineBreed* algorithm during the past year. One notable addition is the use of a new optimization algorithm called the compact second-order cone programming algorithm, *compactSOCP* (Yamashita *et al.*, 2018). This algorithm optimizes the candidates' selection and contributions by applying a modified second-order cone programming optimization algorithm to solve the optimal contribution selection problem (Meuwissen, 1997). The optimal contribution selection problem is a mathematical optimization problem that outlines the requirements of selection problems efficiently. Algorithms aimed at solving this problem try to maximize the genetic gain from selecting a group of individuals under a constraint on genetic diversity. Unlike other previously developed algorithms, *compactSOCP* has been shown to attain exact optimal solutions of the selection problem at a faster computation speed and with more efficient memory utilization (Yamashita *et al.*, 2018).

We tested the long-term effectiveness of *PineBreed* over multiple generations. We compared the *PineBreed* differential evolution (DE) optimal mating designs to the positive assortative mating (PAM) designs and random mating (RM) designs in a long-term setting of 10 breeding cycles. A data set from the third cycle of the Cooperative loblolly pine breeding population was used to select 962 candidates for mating. This represented the initial starting population. The entire pedigree of 962 candidates consisted of 1071 individuals. An index breeding value (Volume-Straightness-Rust) for each candidate was available. A recursive strategy was designed to simulate breeding cycles over 10 generations to evaluate the long-term effect of optimal mate-pair designing. With 962 candidates as the starting point, 1000 crosses were designed for each cycle and 30 progenies were simulated for each of the crossed families. The best preforming progeny from each family was used as a candidate for the next breeding cycle. This strategy was applied to mimic the natural selection process where truncation selection of best-performing candidates results in the better-performing offspring. This process was repeated for 10 cycles. No overlapping generations were allowed.

The DE algorithm outperformed both the PAM and RM algorithms in meeting the overall goal of maximizing genetic gain while managing inbreeding levels effectively. The DE algorithm was able to constrain the full-pedigree average coancestry after

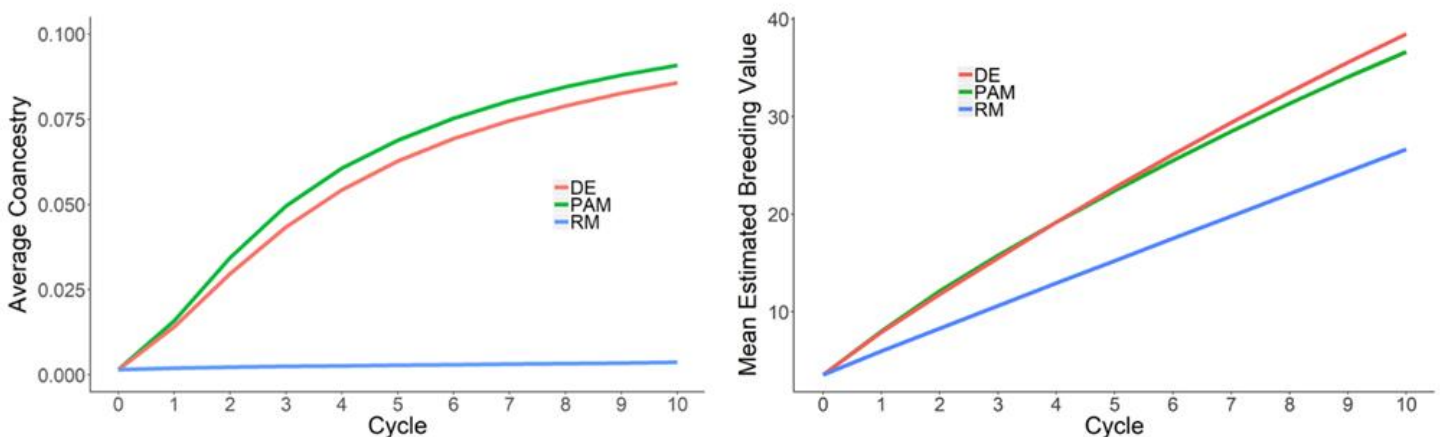***Figure 1.*** *Comparison of average coancestry and average estimated breeding values of the full pedigrees in each of the 10 cycles. The differential evolution (DE) algorithm, adapted for PineBreed, outperformed the positive assortative mating (PAM) and random mating (RM) algorithms in the overall goal of maximizing genetic gain from the selected candidates, while managing the increase in average coancestry.*

10 cycles to 0.086, while the PAM algorithm was higher at 0.091 (Figure 1). The DE algorithm managed to design crosses without breaking the average coancestry mate-pair constraint of 0.125. However, the PAM algorithm failed to enforce the average coancestry constraint with numerous crosses every cycle (Figure 2). In the long term, failing to adhere to the constraints would result in detrimental issues, including loss of genetic diversity and lower genetic gain. Failing to strictly follow constraints is the biggest shortfall for the PAM algorithm. The DE algorithm performed 5% better than the PAM algorithm over 10 cycles in increasing the genetic gain (Figure 1). The increase in breeding value per generation was 27%, with the mean breeding value for the base population increasing from 3.5 to 38.5 at the end of the 10th cycle. The similarity in the performance of both the DE and PAM algorithms can be attributed to the same initial population of candidates being used for determining the mating list. Additionally, the *compactSOCP* algorithm determines the selection and contribution of the candidates, and thus the same pool of candidates is used to create mating lists using different optimization methods of DE, PAM and RM. With the candidates and their contributions being kept constant, the differences observed are subject to the method of optimization applied to create the optimal mating lists. Though RM was able to minimize the increase in inbreeding and coancestry levels, it was unable to maximize genetic gain, and it failed to allow any form of control over the mate-pair designing process. With a more robust selection process and new candidates added every cycle, *PineBreed* will be able to outperform other methods to create an optimal mating list.

The completion of this study will see the development of a suite of software that can utilize not only genetic relationships from the pedigree but also utilize genomic relationships derived from SNP markers. The framework and methods adapted for loblolly pine breeding also have relevance to the breeding of other monoecious species.
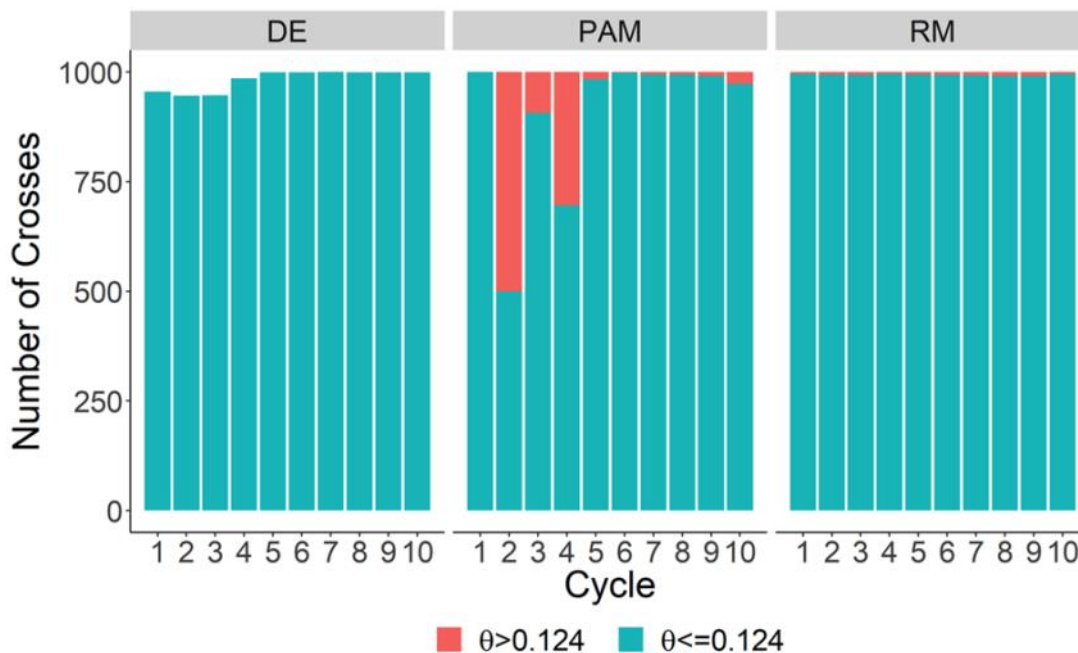

Figure 2. *The total number of crosses and the selected parents average coancestry for each cycle of mating list designed by the Pinebreed differential evolution (DE), positive assortative mating (PAM) and random mating (RM) algorithms. For the long-term test, the average group coancestry of the selected parents was constrained to be less than 0.124 for PineBreed and positive assortative mating algorithms. No constraints were placed on mate-pair selection in random mating. Crosses suggested by the mating lists that broke the constraint are shown in red. Optimization through PineBreed, resulted in no crosses designed that broke average coancestry constraints. The PAM algorithm repeatedly failed to adhere to average constraints.*

Loblolly Biomass Genetics/Cropping Study – Eight Year Summary[1]

The Loblolly Pine Biomass Genetics/Cropping Study was planted in the spring of 2012 and is now in its 9th growing season. The purpose of the study is to evaluate different families in combination with different thinning regimes in order to inform forest landowners how best to maximize their returns when supplying both the bioenergy and sawtimber markets. Ten of the fastest growing loblolly pine families from two provenances, Atlantic Coastal Plain and Piedmont, were established on a Piedmont site in North Carolina at a high planting density (1037 stems per acre). Provenance and family differences in biomass and sawtimber potential were assessed based on year six measurements. These results were presented in the 2018 Annual Report.

In December of 2019, TIP staff and students returned to the site to complete year eight measurements. Status, diameter at breast height (DBH), total height, forking, and sawtimber potential were recorded. Stem lean (presence/absence of excessive lean) and stem breakage (presence/absence of major stem breakage) were also re-measured because of the winter storm events that have occurred at this site over the past several years.

Based on the age 6 year assessment, the Piedmont provenance was not significantly higher proportion of trees with sawtimber potential than the Coastal provenance. This is believed to be a result of the significantly higher proportion of stems that suffered stem breakage in the Coastal provenance compared to the Piedmont provenance at year three. After 8 growing seasons, the difference between provenance was only marginally significant for sawtimber potential (p-value=0.0904). At both ages 6 and 8 years, the proportion of trees with sawtimber potential varied significantly among families (p-values both <0.0001). Age 8 results of family differences in sawtimber potential can be seen in Figure 1, emphasizing the importance of knowing which family is being planted when landowners are selecting families for future sawtimber production.

Height and diameter measurements were used to calculate the green tons per acre for each family (Figure 2). The change in green tons per acre over time is illustrated in Figure 3. Family rankings for volume have been stable through time, with differences among families increasing through age 8 years. When comparing the top families for Figures 1 and 2, families C9, P1, and P9 appear to be top
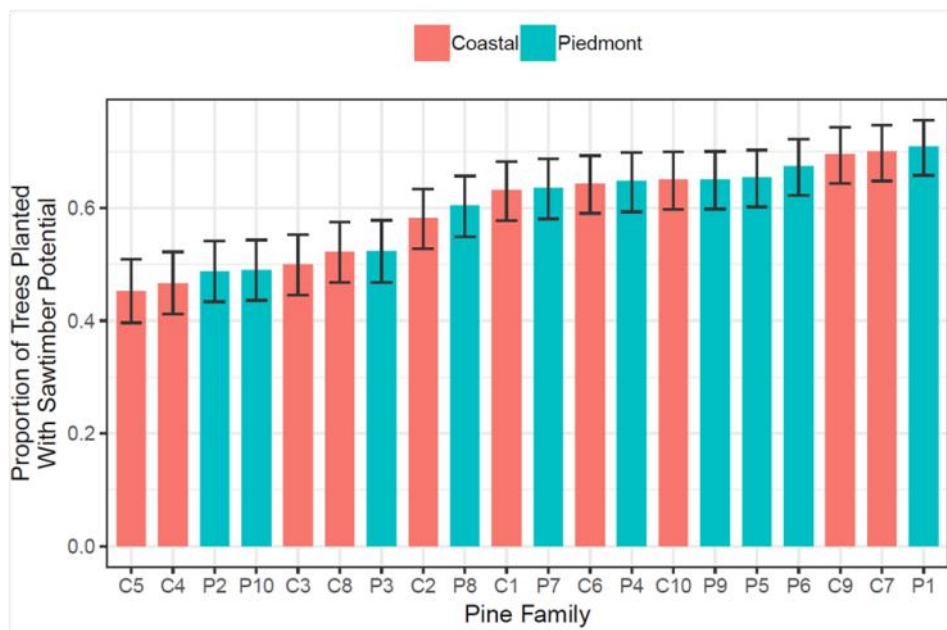

Figure 1. *Proportion of trees planted with sawtimber potential for each family at year 8. The provenance effect on sawtimber potential was marginally significant (p-value=0.0904) but highly significant family differences were observed.*

producers for both volume and sawtimber potential. Although it is still early in the study to draw conclusions about final sawtimber yields, selecting families such as C9, P1, and P9 could be the best alterna-

tive for forest landowners in an ever changing market where pulpwood prices and sawtimber prices fluctuate quarterly.

Figure 2. Green tons per acre at age 8 years for each family. Differences among families were greater than among provenances.
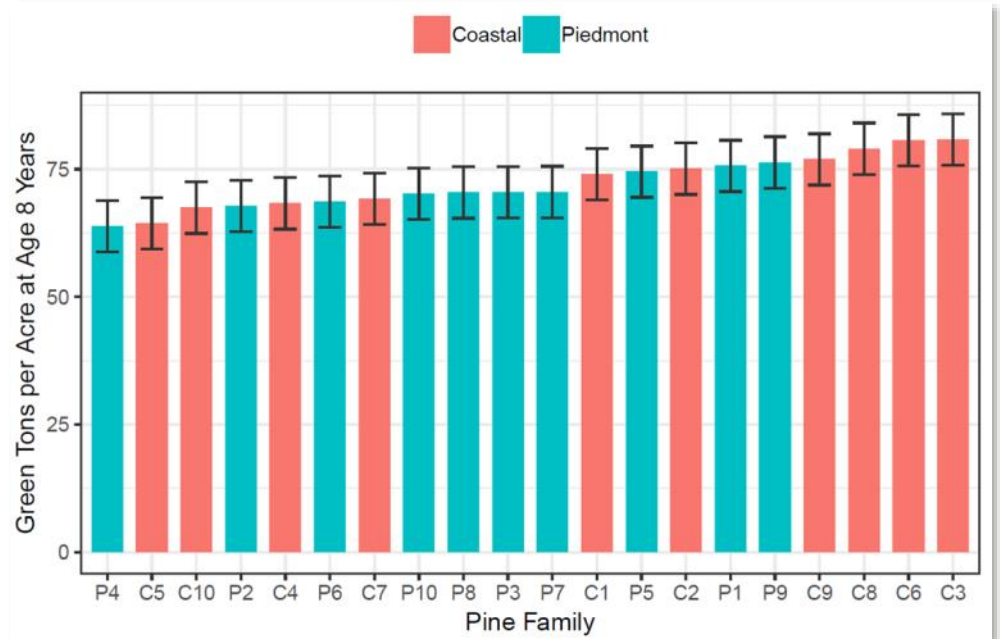
The original study plan called for half of each rep to be harvested for biomass production after 8 years of growth. Given the difficulties of converting pine biomass to lignocellulosic ethanol, the pine biofuels market has not developed at the rate that was anticipated, and harvesting for this reason is no longer a possibility. Therefore, TIP staff have shifted the focus of the study away from forestry-based feedstock

for ethanol production and toward biomass feedstock for pellet production. There may be opportunities for landowners to generate income from thinnings that are processed as a renewable energy feedstock. The market for woody feedstock for export is firmly established, and domestic consumption appears to be growing.
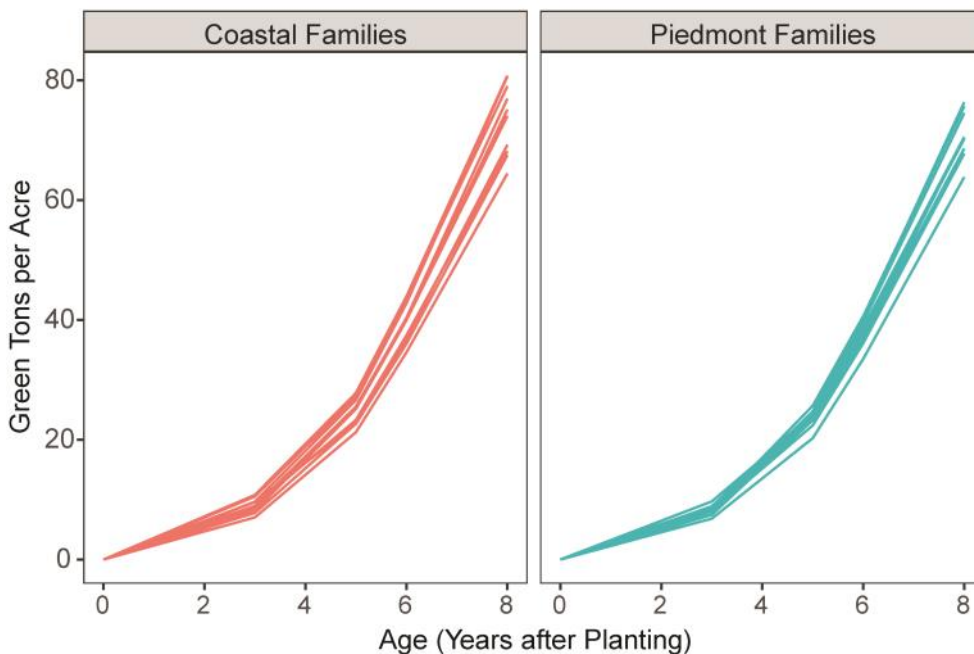

Figure 3. Green tons per acre over time for each family based on measurements recorded at years 3, 5, 6, and 8. The growth curves rarely cross each other, suggesting that family performance has been stable through age 8 years.

We are very grateful to announce that in February of 2020, we received a two year grant to support this future research. Year 8 data will be used to guide a thinning during the 2020 growing season (age 9). With the high planting density of 1,037 stems per acre, the trees are already at crown closure and competing with each other (Image 1). Alternative thinning regimes will be performed on the split-split plot design in replace of the original planned clearcut. The results will inform landowners in how best to maximize their returns when supplying wood for both pellet production and sawtimber markets. The effect of the thinning treatments on volume production and stem quality will be assessed through subsequent measurements in the years that follow.
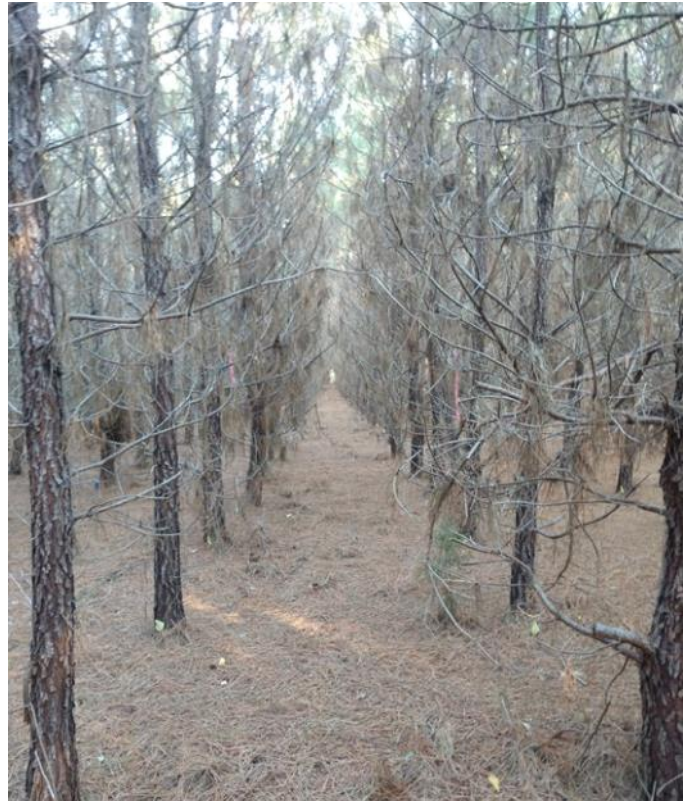

Image 1. *At a 6' x 7' spacing (1037 stems per acre), this study has certainly reached canopy closure and is now ready to be thinned.*

Development of a Tree Architecture Phenotyping Sensor

The size and distribution of knots formed in the tree stem affects lumber and veneer recovery. The size and shape of a knot depends on the branch diameter and branch angle. The challenge with incorporating branch characteristics in a routine field assessment is that visually graded traits, in the absence of direct measurements, are prone to human errors. Moving from subjective scoring to a repeatable data-driven assessment of branch characteristics across scoring teams and test locations will increase measurement precision and ultimately genetic gain. The development of a tree architecture phenotyping sensor that leads to the efficient measurement of branch characteristics may enable the Cooperative to routinely identify families with desirable branch characteristics for operational deployment.

The NCSU Cooperative has initiated a collaborative research project with Dr. Yin Bao, an assistant professor in the Department of Biosystems Engineering at Auburn University, to develop a high throughput

tree architecture phenotyping sensor. Dr. Bao envisions using a smart camera to scan a tree and automatically extract the tree architecture traits. His lab has assembled a prototype that consists of a 3D stereo camera and an edge computer. Like the human vision system, the 3D stereo camera captures high-resolution color images and depth images obtained from stereo matching. Many 3D sensing technologies exist today. Terrestrial LiDAR likely provides the highest 3D reconstruction quality for this application. However, there are several drawbacks when using LiDAR technology, including cost, which is in the range of tens of thousands of US dollars, and subsequent data processing requiring expertise in the analysis of massive 3D point cloud data. In contrast, the commercial stereo camera used in this project costs $450 and records 2K resolution stereo video at 15 frames per second, which is believed to be suitable for the intended purpose. The depth accuracy depends on richness of texture in the image, which the bark can provide. The recent rapid advancement

in artificial intelligence has significantly improved computer vision and visual recognition. Machine/deep learning will be used to detect trunks and branches in the color images. Based on the 2D detection results, 3D architecture traits can be extracted from the depth maps including branch size, branch angle, and stem diameter.

High resolution imagery and ground truth measurements were collected at a 4th-Cycle progeny test in Moultrie, Georgia (Image 1). A total of 10 trees were selected. Each tree was divided into five 3' intervals along the stem, with the first interval starting at a height of 3'. Three branches were selected for each interval, and their diameter and angle from the stem were measured with a digital caliper and a digital angle finder, respectively. In addition, one stem diameter measurement was taken for each interval for estimating stem taper. The measured branches were tagged with color-coded flagging so that they could be identified in the imagery. Each tree was then scanned with the stereo camera from four diagonal directions with respect to the planting grid. The scan for each tree was completed within 2 minutes. The manual measurements were a lot more time-consuming, with each tree taking an average of 30 minutes to measure.

The imagery and direct measurement data will be used for the sensor development phase. The branches and stems in the images will be annotated, and the annotation will be used to train a deep neural network that performs object detection. The ground truth measurements will be used to evaluate the accuracy of the image-derived branch diameters and branch angles. Both the object detector and the stereo 3D reconstruction contribute to the final measurement accuracy. We are currently assessing the system accuracy and robustness concerning the stereo 3D reconstruction. The 3D branch characteristics will be estimated using the annotated image data and the depth information. A correlation analysis will reveal the upper limit of the sensor performance given the stereo 3D sensing method that was selected. The performance of the object detector is expected to improve as more training data are collected.

The initial focus of this study is on branch diameter, branch angle and stem diameter. However, the long-term goal is to develop an imaging protocol with the sensor that enables the extraction of as many traits as possible in one scan. Breeders will be able to quickly quantify common architecture traits such as volume, height, stem taper, straightness, branch angle and branch diameter. Additionally, defects such as ramicorn branching, forking, and possibly fusiform rust galls may be quantified as well. It is our hope that such high-throughput phenotyping technology will improve the efficiency and objectivity of forest tree measurements in the near future.

Image 1. Data collection at a 4th-Cycle progeny test. Left: a sensor prototype for tree architecture phenotyping. The tablet is showing a video stream from the sensor and a simple user interface in a web browser. Middle: manual collection of ground truth measurements (Payn and Bao). Right: scanning a tree using the vision sensor (Bidese and Bao).

High Throughput Phenotyping of Loblolly Pine Seedlings Using Hyperspectral Imaging[1]

Digital imaging technologies have been widely studied for the phenotyping of crops in plant breeding programs. Digital images have been used for the non-destructive assessment of yield, plant architecture, and physico-chemical traits. Compared to manual measurement, these methods are more efficient and reduce the subjectivity inherent in human evaluation of variables such as plant health. These methods are yet to be incorporated in loblolly pine breeding programs. This study investigates the use of hyperspectral imaging for the assessment of fusiform rust disease resistance and freeze tolerance of loblolly pine seedlings in a nursery environment.

Fusiform Rust Resistance

Fusiform rust disease is recognized as the most economically important disease of loblolly pine in the southern United States. Therefore the identification of families with enhanced resistance to fusiform rust is a key objective for the Cooperative breeding program. A routine method for screening families involves the controlled inoculation of seedlings at the USDA Forest Service Resistance Screening Center (RSC) in Asheville, NC. Approximately six months following inoculation, seedlings are visually assessed for the presence of rust galls. The objective of this study is to determine whether hyperspectral imaging technology can be used to efficiently and accurately assess fusiform rust disease incidence within a shorter time period following inoculation.

The experiment comprises 82 families, plus two standard check lots, representing a range of breeding values for resistance to fusiform rust in progeny field trials. Hyperspectral images using a visible near infra-red spectrometer were collected prior to inoculation and then on a monthly interval for six months following inoculation. In addition to imaging, the disease incidence for all plants was assessed based on visible galls that appeared on the stems.

The hyperspectral images were segmented from the background using spectral methods, and algorithms were developed for discriminating stem pixels from needle pixels using both spectral and spatial features. Statistical discrimination models will be developed for classifying seedling scans to different levels of disease incidence. Once optimized, the efficiency and objectivity of these imaging methods will be evaluated relative to conventional disease scoring protocols.

Image 1. This image shows the setup used for collecting hyperspectral images. A motorized conveyor moves the board on which the tray with seedlings is placed. The line-scanning hyperspectral imager collects data one line at a time. Halogen lamps are used to illuminate the scene, and a piece of gray cloth is used as a plain backdrop for the images. In the background, some of the seedlings from the experiment are visible, all in line to go through the scanning.
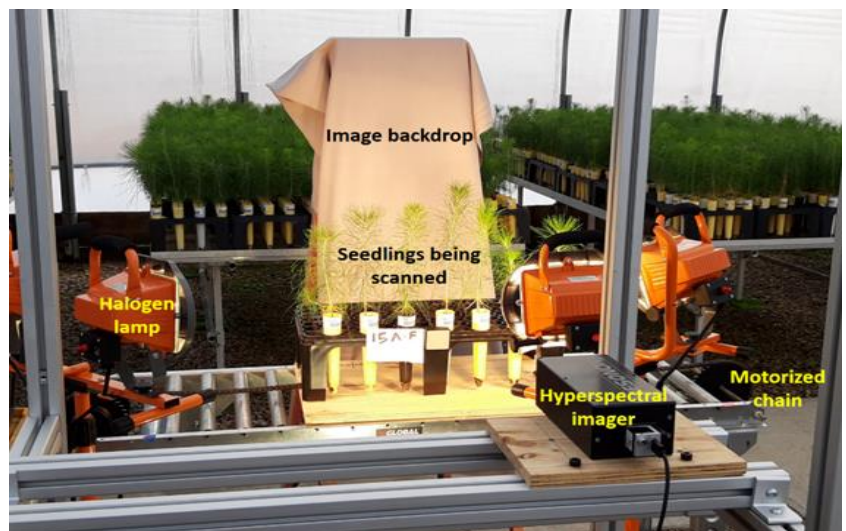
[1] *Summary of Piyush Pandey's graduate research*

Rapid Assessment of freeze tolerance

Loblolly pine is planted across a wide temperature gradient in the southeastern United States. Ensuring the planting stock is suitably adapted to the growing environment is critical to achieve high productivity. Average minimum temperature is an important climatic variable related to growth and survival, and is used to guide the transfer of improved seed throughout the species distribution.

Advanced generation families are assigned to cold hardiness zones based on the average minimum temperature of the region from which their founding ancestors (first generation selections) were adapted. This method has been successful but as the number of cycles in the breeding program increases, so too will the number of founding ancestors increase for each selection. The precise assignment of the correct cold hardiness zone for a new selection will become ever more challenging, particularly when the founding ancestors represent a larger geographic region with a range in average minimum temperatures.

Long-term field studies are considered the most reliable method for identifying cold-tolerant families because the extended testing period increases the likelihood of exposure to extreme cold events. This process is resource intensive and extremely 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 highly productive and well-adapted loblolly pine families across the landscape.

This study presents a novel approach to assess freeze damage of loblolly pine seedlings using hyperspectral imaging. A seedling population of 98 families, representing a wide range of mean minimum temperature at geographical origin, was raised in the nursery. Using a freeze chamber, a portion of the population (16 replications, each family represented by a single seedling per replication) was subjected to an artificial winter freeze. Hyperspectral images were acquired before and after the freeze event. The freeze-induced seedling damage was scored categorically by visual inspection after image acquisition. Computer vision algorithms are being developed to extract spatial, spectral and temporal features pertaining to freeze tolerance. Classification algorithms will be used to detect subtle differences between the spectral patterns of stressed and non-stressed plants. Finally, the correlation between freeze damage, quantified using the spectral data, and the mean minimum temperature at the geographic origin of each family will be determined.
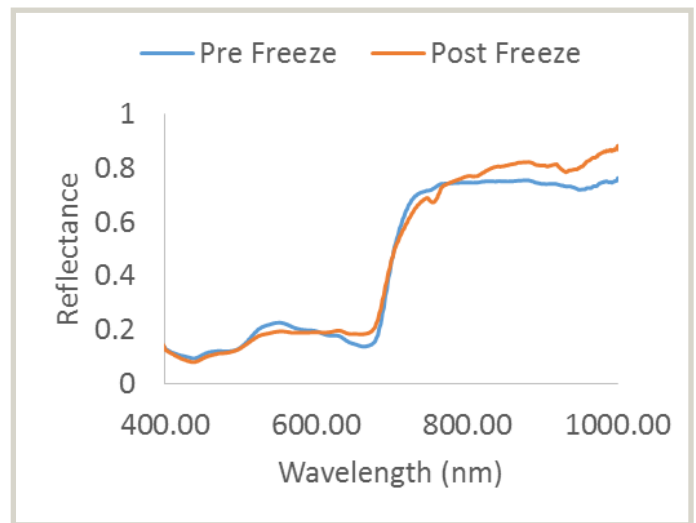
Figure 1. Spectra derived from the needles of a seedling that was frozen for the experiment on cold-hardiness. The pre-freeze spectrum and the post-freeze spectrum are visibly different, particularly in wavelength regions that have distinct vegetation signatures. Classification algorithms detect subtle differences between the spectral patterns to discriminate between stressed and non-stressed plants, as well as between plants of different physico-chemical properties.

Prediction of Breeding Values of *Pinus taeda* in the Fourth Cycle[1]

Progeny testing in forest tree breeding is an important step to evaluate the genetic merit of trees for selection. Forest tree breeders often employ specific statistical models to predict the genetic merit of individuals. Model selection depends on the objectives, mating design employed, and the availability of information, such as pedigree data.

In this study, the efficiency of three linear mixed models were compared for the prediction of breeding values in the 4th-Cycle of the Coastal Breeding Population of loblolly pine. The models included the general combining ability or family model (GCA), animal model (AM), and reduced animal model (RAM). The GCA model and RAM solve the mixed model equations for the parents only. While the AM solves mixed models for the entire pedigree, including the progeny.

For the study, data comprising measurements from a total of 10,720 trees, representing 266 full-sib families in the 4th-Cycle progeny tests, were used. The tests were established at 18 sites in the Southern US in 2014, 2015 and 2016. An alpha cyclic row-column design was used in the field to account for the environmental noise in two directions (rows and columns). Tree height (m), stem volume (dm^3), and stem straightness (1 to 6) were assessed at ages 4 or 5 years.

Perfect positive linear relationships ($r = 1.0$) between the parental breeding values from the GCA model and the AM for all three traits were observed when the same covariance structures were used (Figure 1).
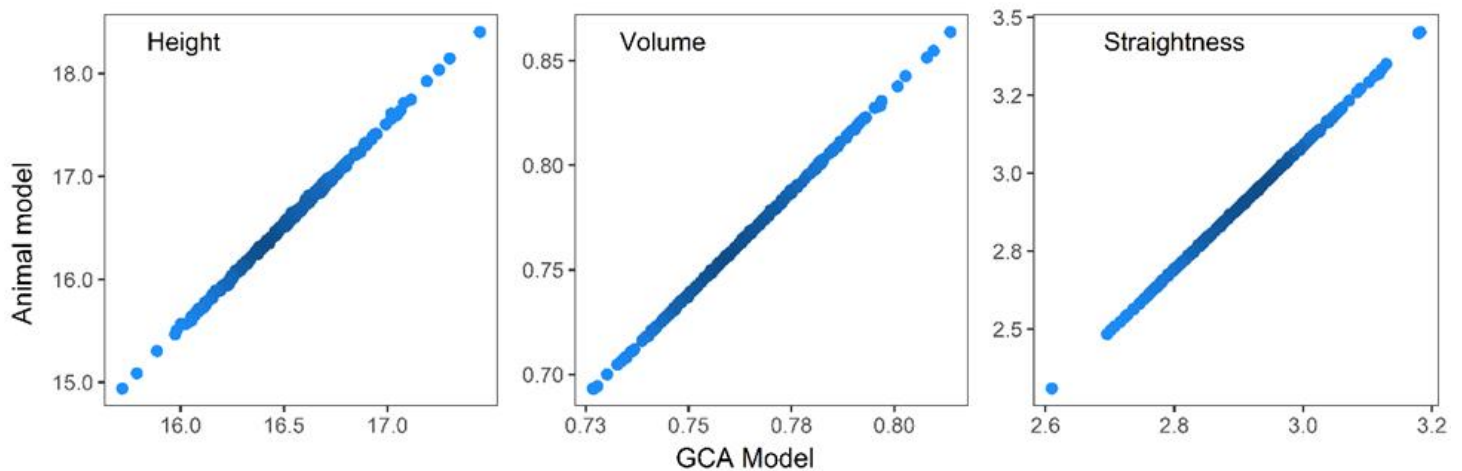

Figure 1. Correspondence of parental breeding values from the AM and GCA model for height, stem volume, and stem straightness. The correlation coefficients were perfect for all three traits.

In order to obtain breeding values of full pedigree (progeny, parents and grandparents etc.) on the same scale, animal models with pedigree are preferred since GCA models cannot produce progeny breeding values directly. However, the AM can be computer demanding, and the model log likelihood may not converge. The reduced animal model (RAM) developed by Quaas and Pollak (1980) is an alternative to the AM. Like the GCA model, the RAM solves the mixed model equations for the parents only. The progeny breeding values are obtained by

back-solving the equations from parental predictions and using weights from the GCA and AM models.

The correlations of predictions for individual trees from the AM and RAM are presented in Figure 2. When the same covariance structures were employed in both models, the correlation was perfect (2a). However, when more complex covariance structures were fit for the residuals and peripheral

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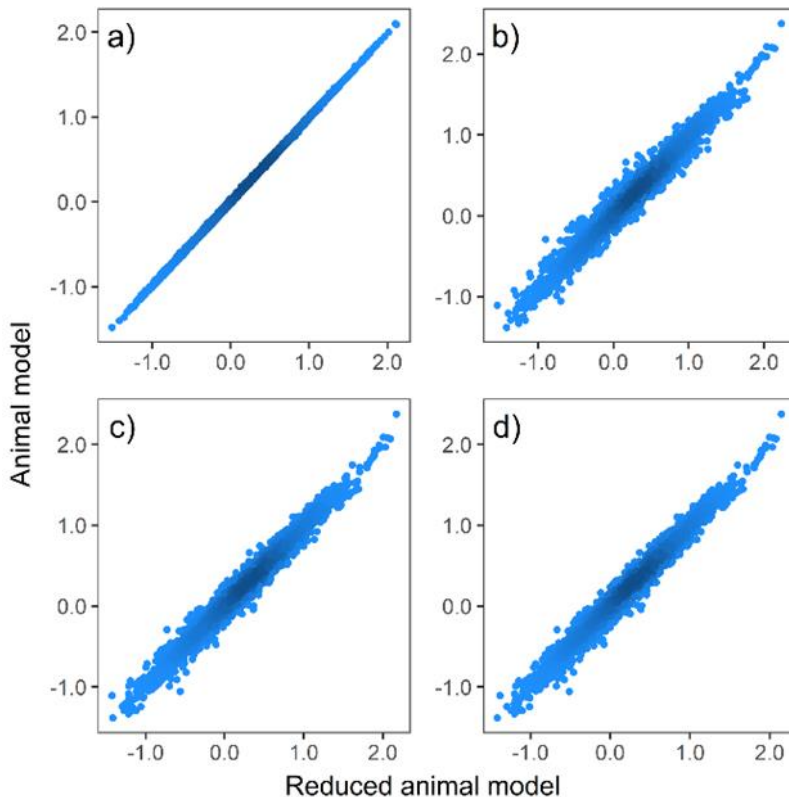[1] *This is a summary of Syamsudin Ahmad Slamet's MS graduate research.*

Figure 2. *Correspondence of breeding values of progeny height breeding values from the AM and RAM models. a) Same scaled identity covariance structure was used in the AM and RAM for the peripheral effects (e.g., block, row, column effects) and residuals (r =1.00). Figures b, c, and d show when a block-diagonal covariance structure was used for the peripheral effects and residuals (r = 0.99). The difference between models is caused by different weights used for the RAM models.*

effects in the RAM, the correlation coefficients were slightly lower (Fig 2b, 2c, 2d).

Computation time for the AM was approximately double the time to run either the RAM or GCA model. For large progeny data sets, the RAM should be the preferred model to obtain the predictions on the same scale for the full pedigree because these models allow fitting complex covariance structures to random terms. The shrinkage estimator to estimate weight coefficients for RAM models can be easily obtained from the GCA models with complex variance-covariance structures. Results suggested that the RAM is an attractive alternative to fit more complex linear models to account for the heterogeneity in the data with less computational time and resources.

Grants

New 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.

Continuing Grants

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

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

Richard Venditti, et al. "Preparing Diverse and Rural Students and Teachers to Meet the Challenges in the Bioenergy and Bioproducts Industry". TIP portion: $33,588. 2017-2021.

ASSOCIATED ACTIVITIES

Meetings, Workshops, and Short Courses

In preparation for 5th-Cycle breeding and testing, Cooperative members met at Crossnore, NC in August 2019 for the 5th-Cycle planning meeting. The objectives of the meeting were to review the advances made in the 4th-Cycle strategy, and discuss proposed improvements for the 5th-Cycle. Key attributes of the breeding strategy, including population size, population composition and breeding logistics were presented. Through discussion and feedback from our members, further refinement of the strategy was achieved to ensure maximum value is attained for our members in the next breeding cycle.

Pictured above: *TIP members and invited speakers that gathered to discuss 5th-Cycle breeding objectives at the TIP Indaba.*

The next interaction with members was the annual TIP Contact Meeting that was held in Tuscaloosa, AL and hosted by The Westervelt Company on November 5-7, 2019. Indoor presentations were led by the TIP staff and a visiting professor from Auburn University, Dr. Yin Bao. The group bundled up to head out in the field to stops that included a ***PRS***™ Calibration site and progeny tests to discuss establishment and selection activities. Tree breeders really love to see and hear about where all of their hard work ends up, a mill! Attendees were given a tour of two Westervelt sawmills. The capacity and technology these mills utilize was impressive to see in action. We would like to thank Brandon Loomis and all the Westervelt staff for all they did in preparing for and assisting with the meeting. Brandon was able to juggle his job, planning this meeting, and a National Guard duty assignment; thank you for your service Brandon!

The faculty and staff members associated with the Cooperative are busy throughout the year with extension activities, both domestic and abroad. Some highlights include Ross Whetten successfully executing the IUFRO Tree Biotechnology meeting that was held in Raleigh, NC in June 2019. Steve McKeand taught a 10-day short course at Nanjing Forestry University on Applied Genetics and Tree

Pictured above: *Attendees happily smiling after a BBQ meal during the 2019 Contact Meeting.*

Breeding from October 21-November 1, 2019. Fikret Isik co-taught a workshop organized by the University of Florence and NC State University on genetic data analysis for plant and animal breeding programs in Montepulciano, Italy. Fikret also helped to establish the North American Forest Genetics Society and currently serves as the vice president for the organization.

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

- Workshop lecturer at the Instituto Tecnologico de Costa Rica on marker applications in tree breeding, in Costa Rica, July 16 to 20, 2019 - Whetten

- Lecturer at National Taiwan University (NTU), Department of Forestry and Environmental Resources and Taiwan Forestry Research Institute in Taipei city, Taiwan, November 2019 - Isik

- Lecturer at Department of Forestry, National Chung-Hsing University, Taiwan, October 2019 - Isik

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

- SFTIC, Genetics, and Improvement of Forest Health and Productivity. June 3-6, 2019, Lexington, Kentucky - TIP Staff and graduate students.

- Organized the North American Quantitative Forest Genetics Workshop during the SFTIC, Lexington, Kentucky, June 3-6, 2019 - Isik

- IUFRO Forest Tree Biotechnology Conference, Raleigh, NC, June 23-28, 2019- TIP staff and students.

- TN Division of Forestry Forester's Academy, Bell Buckle, TN, July 24, 2019 - Heine

- IUFRO Breeding and Genetics of Five-Needle Pines and Rusts of Forest Trees Conference, Invermere, BC, Canada, July 22-26, 2019 - Isik

- Georgia Forestry Association Annual Conference, Jekyll Island, GA, July, 2019 - Payn, Walker, Heine

- National Association of Plant Breeders meeting, Pine Mountain Georgia Aug 2019 - Whetten, Isik, Lauer

- Auburn Nursery Cooperative Annual Meeting, Auburn, AL, September 10-12, 2019 - Heine

- NCFS Ranger Training, Rocky Mount, NC, January 29, 2020 - Heine

- The NCSU Genetics and Genomics Initiative, co-lead the Plant and Animal Breeding Research Interest Group and organized two workshops for the group, FY 2020 - Isik

Staff

Dr. Kitt Payn celebrated a year as director in November 2019. This busy first year included a short course, a breeding strategy meeting, two contact meetings and an advisory meeting. In addition to engaging with Cooperative members, the new director has enjoyed fulfilling his teaching and graduate student advisory role as part of the faculty. He has been a great addition to the team and excels in bringing the various staff together on difficult projects to make them ultimately successful.

Dr. Isik also had some exciting news with the approval of a university consortium he championed with other faculty members here at NCSU. The Advanced Tree Genomics Consortium (ATGC) will expand genomic research in tree crops, and faculty will work with industry to assist in research and development focused on the utilization of genomic tools in breeding populations.

Otherwise, it was a pretty typical year for staff here in Raleigh, other than a global pandemic! It was smooth sailing until the Coronavirus, COVID-19, tried to stop us in our tracks. 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.

Visitors

NCSU and the Tree Improvement Program continue to be a hub for scholars who want to learn more about tree improvement and benefit from the skills our staff and faculty possess. Last year we reported on Jikai Ma, from Nanjing Forestry University in China. He completed the remaining half of his assignment in December 2019 with the group, and we enjoyed his time with us.

Our next visitor is Dr. Murat Alan. Dr. Alan is on sabbatical and joins us from Karabuk University in Turkey. Dr. Alan arrived in July 2019 and jumped right into helping out with projects in the greenhouse, field measurements, and breeding activities down at Arrowhead. He is also sitting in on the Tree Improvement courses, taught by Steve (FOR 727) and Kitt (FOR 725), as well as Fikret's Introduction to Data Analysis in Natural Resources course. Dr. Alan is scheduled to return to Turkey in June and has been a wonderful addition to the TIP crew.

Pictured above: *Dr. Alan working on pollinations at Arrowhead Breeding Center this spring.*

Lastly, we had two short-term visitors from South China Agricultural University, Xi Wang and Xin Xin Zhang. They arrived in October and stayed until December 2019. They came to learn more about forest genetics and tree breeding and analyzing data using quantitative genetics principals. We always appreciate having folks visit and learn from us and enjoy the interaction and learning new ideas from them as well!

Pictured left: Our visitors, Xi Wang and Xin Xin Zhang, from South China Agricultural University shortly after they arrive.

Teaching

The Cooperative faculty and staff continue molding future forestry and natural resource professionals by teaching courses at both the graduate and undergraduate level. In summer 2019, Austin Heine taught Silviculture at the NCSU Forestry Summer Camp at the Hill Forest, NC to undergraduate students. Fikret Isik taught Advanced Quantitative Genetics and Breeding (FOR/CS/ANS 726) in the fall. This spring, Fikret also taught his course NR554, Introduction to Data Analysis in Natural Resources, which covers general statistical procedures using SAS and R programing. In the spring, Kitt Payn, taught FOR 411 (Forest Tree Genetics and Biology), our undergraduate tree improvement course, and the graduate level FOR 725 (Forest Genetics). Each spring semester, Ross Whetten teaches FOR 350 (Ethics in Natural Resource Management), BIT 815 (Analysis of Deep Sequencing Data Analysis), and SMT 483 (Capstone course in sustainable materials and technology). This year FOR 727 (Tree Improvement Techniques) was taught by Steve McKeand and Austin Heine, which included a week of hands on training at the Arrowhead Breeding Center for students.

All of the spring courses were interrupted by COVID-19 stay home orders in mid-March, which

left our faculty quickly reorganizing the remaining lectures and classwork. Each class was quickly adapted, and lectures were provided online for students. Kudos to our faculty for the quick response and continuing the level of education to prepare students for future forestry careers.

Pictured above: Another great group of students from FOR 727/493 this spring at the Arrowhead Breeding Center. Over the week of spring break, they worked closely with staff and faculty learning tree improvement and tree breeding techniques.
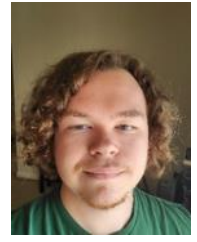
Graduate Students

It was another great year for our hard working graduate students. Colin Jackson defended his Master's thesis in 2019 and decided to stay on for a Ph.D. Sam Slamet also defended and completed his MS degree in May 2020. We welcomed three new graduate students in Fall 2019, Piyush Pandey, Sinem Satiroglu, and Ugur Memis.

Austin Heine   Colin Jackson   Doug Dobson

Austin Heine, Ph.D. – Austin began his Ph.D. research following his MS defense in 2018. His interest is in pollen viability and vigor to make the process for full-sib seed production more efficient and effective.

Colin Jackson, Ph.D. – Colin began his joint Ph.D. with Camcore and TIP upon completion of his MS program in 2019. His research will delve into utilization of genomic tools and marker data in pine breeding. At SFTIC in June 2019, he won the Zobel award for best oral presentation.

Doug Dobson, MS – Doug started in Fall 2018 and will complete his research this summer. His research is titled "Genetic homogeneity and stand growth uniformity of *Pinus taeda* planted at different stand densities."

Eddie Lauer, Ph.D. – Eddie continues his Ph.D. program that started in January 2018 and is titled, "Discovery of Fr Genes for Fusiform Rust Disease in *Pinus taeda*".

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

Nasir Shalizi, Ph.D. candidate– Nasir's research is titled "Best linear unbiased predictions of breeding values in *Pinus taeda* using expected and realized genomic relationships". He presented GVBV research results at SFTIC 2019. Nasir has been writing and publishing and is expecting to finish his program this summer.

Piyush Pandey, Ph.D. – Piyush began his joint research with TIP and Biological and Agricultural Engineering. His research is titled "High throughput phenotyping of loblolly pine using hyperspectral imaging".

Sinem Satiroglu, MS – Sinem joined us this fall from Turkey. The project she is working on is **"**Prediction of genetic merit in loblolly pine using spatial and multivariate models".

Syamsudin Slamet, MS – Sam is a Fulbright student pursuing a dual-master's degree in Forestry and Statistical Genetics. His research title is "*Prediction of Breeding Values from Row-Column Designs in Pinus taeda Progeny Tests in the Southern US*" .

Trevor Walker, Ph.D. – Trevor continues his research focuses on 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".

Eddie Lauer   Khushi Goda   Nasir Shalizi   Piyush Pandey   Sinem Satiroglu   Sam Slamet   Trevor Walker   Ugur Memis

Undergraduate Army

Undergraduate students continue to be a vital part of our program. They assist us in completing our annual research activities on campus and in the field. This year was no exception. Undergraduates helped measure wood quality in the ACE tests, and assess 8 year growth and stem quality of the bioenergy trial at Butner, NC. They were also involved in data entry, seedling cultivation, and cone processing from the largest breeding year to date at Arrowhead Breeding Center. One student, Peter McNeary, even took on an undergraduate research project involving the analysis of 2nd-Cycle progeny test data to quantify the degree of uniformity for heights of OP and FS families. Below are some action shots of our current students!

Clockwise starting from top left: *Peter McNeary (left) and Ben Maness (right) both juniors in Forest Management helping measure wood properties in an ACE test in SC over fall break. Seth Lamoreaux is a sophomore in Forest Management. Jikai Mai (left), visiting student from Nanjing University in China, and Krista Wooten (right) helping with seed sowing this past spring. Krista is a senior in Forest Management. Trevor Franklin is a Junior in Forest Management.*

Membership in the NCSU Cooperative Tree Improvement Program

In summer of 2019, we welcomed PRT Growing Services, Ltd. as a new Contributing Member. The New Year began with a new/old member! Tennessee Division of Forestry (TDF) originally left the Cooperative as a full member in 2017, but continuing discussions have brought TDF back as a Contributing Member, and we are excited to have them back on board! Unfortunately, we lost PBS International as a Research Associate Member in January 2020. As always, we value the members and their support of our research and breeding activities. A list of our Cooperative partners is presented below.

Full Members

ArborGen, Inc.	Rayonier, Inc.
Georgia Forestry Commission	South Carolina Forestry Commission
Hancock Timber Resources Group	Virginia Department of Forestry
International Forest Company	The Westervelt Company, Inc.
North Carolina Forest Service	Weyerhaeuser Company

Contributing Members

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

Research Associate Members

Arauco - Bioforest, S.A.	New Brunswick Tree Improvement Council
GenoVerde, Inc.	Nova Scotia Department of Natural Resources
J.D. Irving, Ltd	USDA Forest Service

Publications of Special Interest To Members (2016 - 2019)

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

2020

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. (in press).

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. (in press).

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.

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.

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 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

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

2018

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Front cover: The loblolly pine Pita50K Axiom Genotyping Array was released in September 2019. The staff and members of the NCSU Cooperative Tree Improvement Program made a significant contribution to the project. The genotyping array is expected to have a large impact on loblolly pine breeding and research. The array has been successfully used to genotype the Atlantic Coastal Elite (ACE) population, and genomic prediction models are currently being developed. In this image, Tree Improvement Manager Trevor Walker is evaluating an ACE clone in the field.

Back cover: *(top)* It was a busy grafting season at our Arrowhead Breeding Center near Cochran, GA. Breeding of the new 5th-Cycle selections could commence as early as next year, but heavy flowering and pollen production should be available in 2022. *(bottom left)* Numerous 5th-Cycle selections were made this year throughout the southeast. Pictured is David Wilkinson with Hancock Forest Management admiring a beauty! *(bottom right)* The Cooperative is exploring hyper-spectral imaging technology for the assessment of fusiform rust disease resistance and freeze tolerance of loblolly pine seedlings in a nursery environment.