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EXECUTIVE SUMMARY

2012-2013 was an eventful year for the Cooperative. By far, the most exciting development in the program this past year was the initiation of our fourth cycle of breeding. Our best projection is that we will be able to increase the rate of gain getting to landowners by up to 25% or more in the 4th cycle of breeding. The present value per acre planted from increasing the rate of gain from 1% per year to 1.25% per year is conservatively estimated to be about \$600/per acre planted or over \$500 million across the region.

SELECTION, BREEDING AND TESTING

Comparing mating algorithms developed by animal breeders to our mating system for the 3rd cycle of breeding, we elected to modify our breeding strategy and progress to a new approach to managing the Cooperative's breeding populations for the 4th cycle and beyond. We used the MateSelect algorithm to generate around 1600 crosses that balance gain and diversity. The first of the 4th-cycle selections were made in full-sib block plots this past winter and topgrafted at the Arrowhead Breeding Center for breeding.

We plan to use a rolling front progeny testing strategy to establish progeny tests every year and have ~20% of families overlap across years. The Cooperative will implement alpha-cyclic incomplete block designs with row-column configuration in the 4th cycle progeny testing. Tests in the Coastal Plain and Piedmont breeding populations will be measured at age 4 years for most traits; tests in the Northern region where growth is slower, will be measured at age 5 years. Stem and wood quality traits will be measured at later ages.

The Cooperative wrapped up the 3rd-cycle polymix trials and final assessments have been made in most trials.

The testing phase of the Sawtimber Elite Population for the Northern region commenced with over 22,000 cuttings being stuck in 2013. Clonal field trials of 16 elite crosses are planned for the winter of 2013-14.

SEED PRODUCTION

Cooperative members collected just over 24,000 pounds of loblolly pine seed in 2012; 58% of the seed was from 3rd-cycle orchards.

RESEARCH

- Additional samples of clonal varieties of loblolly pine were tested to characterize genetic variation in sugar yields. Cores were dried and powdered and the samples were then subjected to py-MBMS at the National Renewable Energy Laboratory in Golden, CO.
- In a 23-year-old trial comparing different levels of inbreeding in loblolly pine, wood specific gravity was not significantly affected by inbreeding. Bending strength (measured by acoustic stress-wave velocity) decreased slightly.
- The efficiency of factor analytic linear mixed models for a large, multi-environmental diallel trial of loblolly pine was superior to other traditional analytical models.
- In the Genetics-Spacing-Thinning trial at the Hofmann Forest, assessments of 6-year growth and quality traits, results showed that the economic value of stands can change dramatically by incorporating more accurate product class allocation and sawtimber quality measures.
- Data from the 1994 Plantation Selection Seed Source Study were used to model growth response to various climate scenarios. This integrates both genetic and environmental effects and is meant to overcome the critical limitations of population response function and transfer function methods by making full use of data from seed source trials.
- We have worked with other PINEMAP investigators to develop a cost effective, easily implemented, and high-throughput genotyping method, which we are now using to test the hypothesis that genomic regions controlling climate adaptation can be identified. Preliminary results suggest that 8,000 to 10,000 unique genetic loci can be genotyped per individual tree in a cost-effective, high-throughput system.

ASSOCIATED ACTIVITIES, GRADUATE STUDENTS, COOPERATIVE STAFF, MEMBERSHIP

We gained new members including a new full member to contribute to this breeding effort. There are 11 Full Members, 16 Contributing Members, and four Research Associate Members in the Cooperative. Students were taught in numerous classes. Three visitors spent extended periods with us in Raleigh. We recruited four new graduate students, and we graduated three excellent graduate students.

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INNOVATION AND HARD WORK

A MESSAGE FROM THE DIRECTOR

Working hard and being innovative are good descriptors for the Cooperative staff and the members. 2012-2013 was an eventful year for the Cooperative. We made our first 4th-cycle selections. We initiated a new breeding strategy to utilize these selections as well as other selections in the breeding populations. We gained new members including a new full member to contribute to this breeding effort. New studies were established. Students were taught in numerous classes. Several visitors spent extended periods with us in Raleigh. Great accomplishments were made on the existing NIFA grants, and new competitive research grants were submitted. We recruited some excellent new graduate students, and we graduated some excellent old graduate students. It's exhausting just thinking about what's been going on.

By far, the most exciting development in the program this past year was the initiation of our fourth cycle of breeding. I've actually been here since the beginning of the second-generation breeding, so I've seen dramatic changes over the years in how tree improvement is managed, but nothing comes close to what is about to take off. Of course, each cycle of breeding is always different, and we feel that significant improvements are made each generation. We have new technologies available to accelerate breeding. We are much more efficient in our field testing designs. Analytical capabilities allow for breeding values to be calculated with greater precision and accuracy than ever before. New generations of molecular markers using the loblolly pine genome sequence promise to open doors for genomic selection. New algorithms allow for the most appropriate matings to be done to increase genetic gain while maintaining genetic diversity for long-term improvements to be made. All of these technologies and more are being brought to bear on our 4th-cycle breeding program.

Details of the new breeding strategy are described starting on page 2 of this annual report. What I want to focus on here is my favorite question – *so what?* What impact will the increased genetic gain have on our members, landowners in the southern US, and citizens of the region? The story I like to emphasize is the economic impact that tree improvement has by delivering gain to the forest and to the people who benefit from it. As scientists, it's gratifying to do research and get our information published to impact research and development. As breeders, we get the chance to extend far beyond the academic exercises and put our research and innovations into practice. My best projection is that we will be able to increase the rate of gain getting to landowners by up to 25% or more in the 4th cycle of breeding. Based on our estimates of operational gain delivered to the forest, the Cooperative has delivered a rate of gain of about 0.5% gain per year over the last 40 years. Over the last decade, we estimate that this has increased to 1% gain per year. With more emphasis on breeding the best genotypes to increase both volume and value to landowners and with better delivery systems (e.g. increased operational production of full-sib seedlings and clones), I believe we can increase this annual rate of improvement to 1.25% or higher. The present value per acre planted from increasing the rate of gain from 1% per year to 1.25% per year is conservatively estimated to be about \$600 (this is \$600 per acre planted). Historically, about 900,000 acres have been planted each year with loblolly pine seedlings derived from The Cooperative's breeding effort. If this continues, the present value of getting more genetic gain to landowners is over \$500 million. Even if the assumptions are drastically reduced, the economic consequence of our work is staggering.

It is both gratifying and a bit staggering to think about the impact that southern pine tree improvement has had and will continue to have on the region. The Cooperative's staff and each member of the program should be very proud of the work we are doing, putting innovations to work for the benefit of landowners for decades and centuries to come.

Steve McKeand- May 2013

SELECTION, BREEDING, AND TESTING

Fourth Cycle Breeding Plan for the NCSU Tree Improvement Program¹

Since March 2011, the Cooperative has been working to revise third-cycle plans and transition to the fourth cycle of breeding for loblolly pine. A task force was established to develop research documents and strategies in four main areas: 1) Breeding strategies, 2) Progeny testing, 3) Research needs, 4) Logistics. The Task Force members are: Paul Belonger (Plum Creek), Ones Bitoki (Virginia Department of Forestry), Tommy Conwell (Westervelt), Patrick Cumbie (ArborGen), Russ Cox (Tennessee Division of Forestry), Early McCall and Josh Sherrill (Rayonier), Nick Muir (International Forest Company), Russ Pohl (Georgia Forestry Commission), and Bob Purnell (Weyerhaeuser). The input and contributions from the task force have been invaluable, and we will continue to work to modify and improve the plan as it is implemented. The following are the results summarized from several task force meetings and technical documents from the last two years.

Moving from the 3rd- to the 4th-Cycle breeding

The Cooperative's third-cycle breeding effort commenced in the mid 1990s and used a complementary mating design (McKeand and Bridgwater 1998). Pollen mix breeding was used to estimate parental breeding values, and forward selections were to be made in block plot plantings of full-sib families that were crosses among selections with sublimes. Pollen mix breeding progressed reasonably well, but full-sib breeding and forward selection lagged behind.

We also recognized some additional issues. Some of the selections that were planned for breeding did not rank high enough to keep in the population for the fourth cycle. When low-ranking parents were removed, some of the sublimes were left with very few individuals, so we had to combine some sublimes. And finally, the subline system was introduced to increase genetic diversity at the population level, but it

does not allow crosses between superior individuals across sublimes to increase genetic gain.

The MateSelect algorithm developed by Kinghorn (2011) for animal breeding (details below) allowed us to model various scenarios for the fourth cycle and to evaluate different genetic gain and relatedness options. After comparing these modeling efforts to the subline breeding system planned to complete the 3rd-cycle breeding, we asked some simple questions: Why not start the 4th-Cycle breeding now? Is there a compelling reason to finish the 3rd-cycle subline breeding? If the algorithms developed for animal breeding programs are efficient to design breeding for the next generation, then why wait until we have all the forward selections made from the 3rd-cycle breeding? Why not use both the soon-to-be made forward selections and the backward selections that have yet to be crossed in the 3rd cycle and start the 4th-cycle breeding now? Do we need/want the sublimes?

These were easy questions to ask, but difficult questions to answer. In summary, we elected to modify our breeding strategy and progress to a new approach to managing the Cooperative's breeding populations for the 4th cycle and beyond. Below is a summary of the approach.

Pedigree analysis

The recording and management of pedigrees are important for any breeding effort. Tracking the pedigree can help to maintain genetic diversity and avoid inbreeding issues and maximize genetic gain in the breeding population. Pedigree analysis of current mainline breeding populations (selections in polymix tests) showed that inbreeding and thus diversity is not currently a problem for the mainline breeding populations. The average inbreeding coefficient in three breeding populations (BP) was 0. When we analyzed Coastal mainline BP (426 selections from polymix

¹ We want to acknowledge the efforts of David Barker, PhD student with the Cooperative, Without his modeling efforts, progress would have been slower and breeding efforts in 2013 would likely have been delayed.

test series 1 to 4), the total number of founders (f) was 435 and the effective number of founders (f_e) was 265. The f_e is the number of founders needed to produce the same diversity as presently exists in the gene pool. The reason that f_e is smaller than f is clear; some parents have a larger contribution to the gene pool than others. For example, the marginal genetic contribution of one parent to the gene pool was

2.43% (it had 18 offspring in the Coastal BP). However, the contribution of the rest of the selections to the gene pool was smaller, e.g. mainly 1 progeny. FAO (1998) recommends a minimum effective population size of 50 for the purpose of minimizing inbreeding in cattle breeding. This effective population size of 50 corresponds to an inbreeding rate of 1% per cycle.

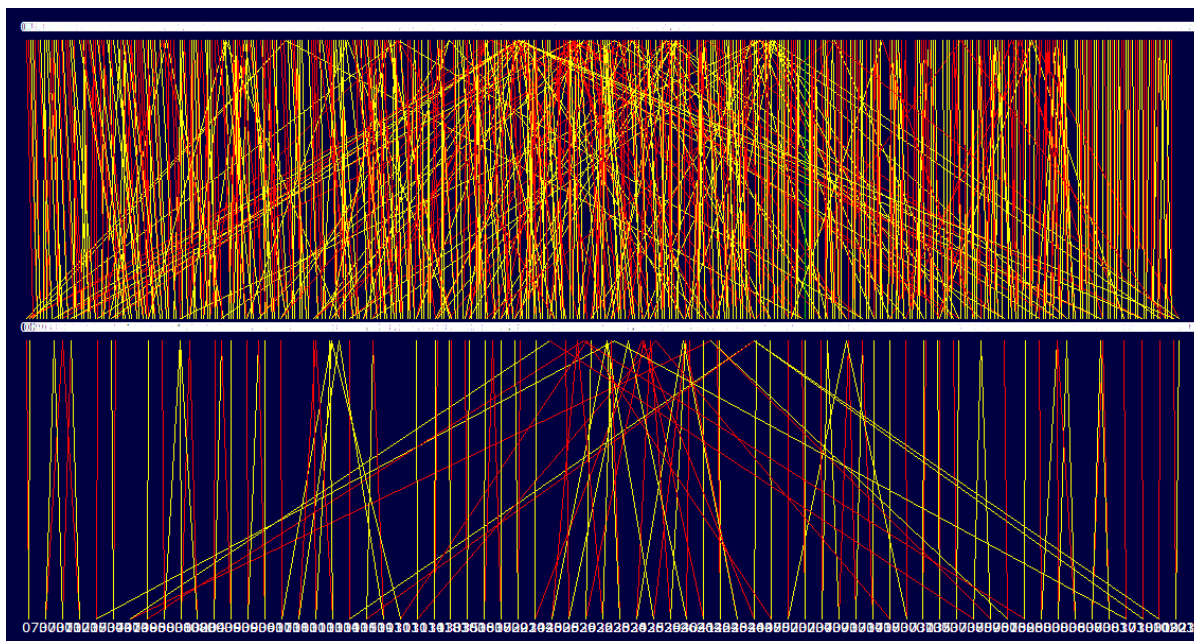


Figure 1. Pedigree structure of 3rd third cycle Coastal mainline breeding population is shown. Except for a few selections, the contribution of founders to the 3rd cycle mainline population (the bottom row in the figure) is minimal. The average inbreeding in the population is zero, suggesting a genetically diverse population.

Fourth cycle breeding strategy

Index values were constructed for selections using parental breeding values. In construction of the indices, a higher weight was given to volume ($V=60\%$) followed by straightness ($S=20\%$) and rust disease incidence ($R=20\%$) – e.g. a VSR622 index. All the existing selections as well as selections coming from full-sib family blocks were considered as candidates for mating as long as their index value exceeded a cut off point ($VSR622 > 1$). For the Northern breeding population, rust was not included in the index (VS73), because the rust incidence was low, and breeding values for rust were not calculated.

A Differential Evolution (DE) algorithm developed for animal breeding programs (Kinghorn 2011) was used to optimize genetic gain and manage inbreeding

in the mainline populations. Constraint on target degrees = 45 was used to design the mating for the fourth cycle. This constraint limits the algorithm's search for the optimal mating design using a vector that goes from the origin to the boundary (Figure 2), which indicates the limit on gain. The horizontal (X) axis represents parental coancestry and the vertical (Y) axis represents the index value. The 10 degrees increments are indicated using light gray lines. The most extreme response possible when all emphasis is shared by just two component objectives: Progeny Index (xG) and Parental Coancestry ($xAx/2$) from the MateSelect tool. The more vertical the target degrees vector, the more parental coancestry is allowed and the more gain is possible.

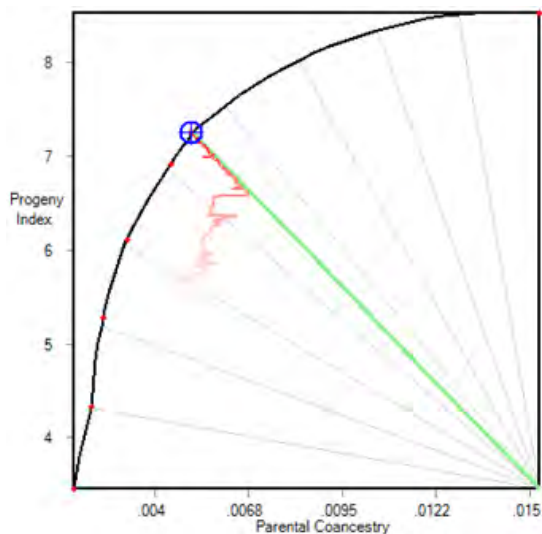


Figure 2. The figure illustrates use of the relatedness and index values (breeding value) to optimize breeding (a list of crosses for fourth cycle). The x-axis is the parental coancestry and y-axis is the index value. A hard constraint is set where the algorithm is prevented from seeking a solution beyond a vector (highlighted in green). In this specific scenario a constraint is set at 45 degrees from vertical starting from the origin. As the target degree increases the expected breeding value of new population increase at the expense of less diversity (higher coancestry).

In designing the breeding, an input file for maximum crosses per selection was generated. A maximum of 5 crosses was allowed for the algorithm. The number of allowable crosses per parent also impacts gain and coancestry in the progeny population. The higher the number of crosses allowed per parent, the greater the gain, but coancestry will also increase. We also used candidate lists for three breeding populations. The candidate lists are the selections with index value >1 and with known pedigree. The mating design generated for the Coastal BP is given in Figure 3. The final mating designs are composed of three sub populations. The large sub population was generated by a hard constraint on coancestry (TD=45) to allow more diversity and less gain. In other words, any solutions containing half-sib matings or closer are weighted against (progeny F threshold < 0.125). For the Coastal BP, the algorithm generated 493 crosses from 319 unique selections (green color in diallel presentation given in Figure 2). The blue color dots are the substitute crosses. Those are the crosses that already had been made by Cooperative members in internal breeding programs or in elite populations. The number of substitute crosses (seed in hand) was 215. To emphasize short-term genetic gain, we generated crosses among the top 35 selections in the population. A hard constraint on coancestry again was used, but this time the target degree was set to TD=25 for more emphasis on genetic gain compared to diversity. The algorithm generated 85 crosses (red dots in the diallel diagram, Figure 2). In total, we obtained **793** crosses for the fourth cycle Coastal Popu-

lation (Figure 4). Since the Coastal population is the largest in the Cooperative, more crosses and hence more effort is being devoted to this region as compared to the Piedmont and Northern populations. For the Piedmont, **492** crosses were generated from 281 unique selections (elites=55, TD45=294, substitutes=143). The Northern population targets Virginia, North Carolina and Tennessee and is smaller in scale. We generated **301** crosses for the Northern region from 209 unique selections (elites=35, TD45=146, substitutes=120). In total the Cooperative plans to test about 1600 crosses by 2022.



Above: The fourth-cycle breeding effort commenced at the Cooperative's Arrowhead Breeding Center in 2013. The Georgia Forestry Commission's contributions to management of the Breeding Center continue to be invaluable to the program.

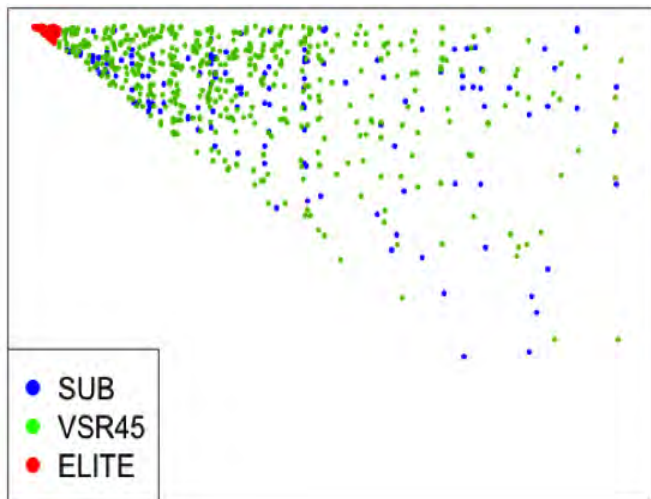
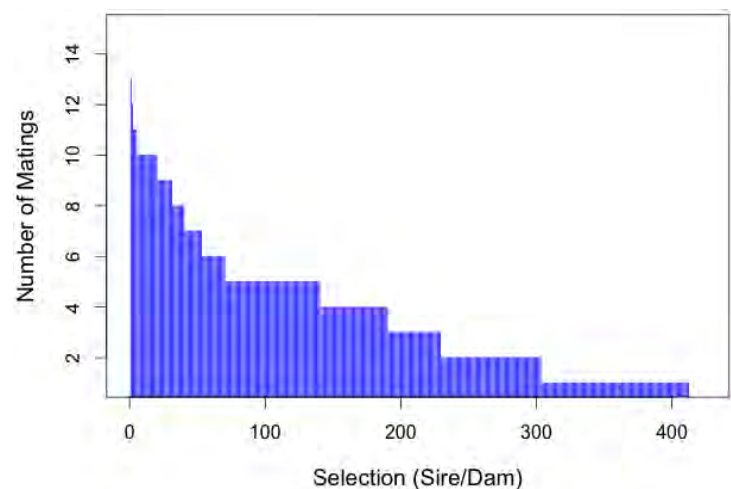


Figure 3 (left). Mating design of the Coastal BP. The population was formed by combining the elite crosses (red dots in the corner), crosses generated based on TD=45 (green dots) and crosses that were available from members and from specialty populations (blue dots or substitutes). In total 397 unique selections were used to generate 793 crosses. Crosses are distributed according to their expected breeding values. Crosses with higher expected BV, such as elites are towards the left upper corner.

Figure 4 (right). Number of matings per selection. The counts of matings per selection were sorted from the most to the least (reading left to right). In the Coastal breeding population the best selection (with high index value) had 13 crosses, the second best had 12 crosses suggested by the DE algorithm. A very high majority of crosses had five or fewer crosses.



Progeny testing

Connecting progeny tests established in different years and in different locations is crucial for reliable comparisons of thousands of genetic entries for selection. For the 4th cycle, we plan to use a **rolling front progeny testing** strategy. The idea is to establish progeny tests when enough genetic entries are available every year and have ~20% of families overlap across years. This way, progeny test data would be better connected using overlapped families and pedigree instead of relying on checklots. Checklots will be primarily used to measure genetic improvement.

The Cooperative will implement alpha-cyclic incomplete block designs with row-column configuration in the fourth cycle progeny testing. This is a departure from the randomized complete block design for prediction of parental GCA values and block plots for within full-sib family selection implemented in the third cycle plan. The new design breaks up a block into much smaller units. The experimental designs for

each location will be generated using the *CycDesignN v4.1* software (John and Williams 1987). The objective function makes sure, to the extent possible, that each family appears equally in the rows and columns. The efficiency (E) of the design is measured by the F statistic with a range of 0 to 1 (the most efficient). Each row and column is a replication unit (two-dimensional blocking structure) with the following strata: Replicates (site), Rows within replicate, Columns within replicates and Plots. Such design controls microsite heterogeneity better than other incomplete block or complete block designs.

We target approximately 1100 trees per progeny test in one location (~2 acres) and have 60 full-sib progeny per cross over two to four years of progeny testing. Individual tree models will be used to predict breeding values across generations and for different genetic entries (parents, full-sibs, and progeny) simultaneously. Such a testing strategy and analysis

method allows simultaneous predictions of full-sib family (cross), parental and individual tree genetic merit on the same scale. Predictions for parents, full-sib crosses and progeny will be more reliable because of information coming from relatives and also because of overlap of genetic entries among the tests. The strategy reduces the testing efforts and the cost of test establishment and test maintenance. The drawback of this new experimental design and testing strategy is that the design is more complex. Rigorous record keeping will be necessary in order to track pedigrees and facilitate proper analysis of progeny test data. All tests must be correctly installed and documented for each tree ID with standard row and column positions for spatial analysis.

Measurement / Selection age

In the Cooperative's third cycle, progeny tests were measured at age six years. In order to speed up the breeding cycle and increase genetic gain per time, the Task force also examined the possibility of early selection. Simulations were carried out using empirical data from Coastal mainline polymix progeny tests and the Early Diallel Measurement Series progeny test data. Literature was summarized about age-age genetic correlations and early selection efficiency for loblolly pine and other conifer species.

Selection for growth traits and straightness at age 3 instead of age 6 can be effective on a gain/year basis. Selection at ages 4 and 5 was found to be highly efficient for selecting for height and volume, respectively, at age 20. Correlations between sweep and straightness measurements were also studied, and these two traits are highly correlated ($r=0.96$) at age 6, so that measuring both is unnecessary. Analysis of rust at ages 3 and 6 in the Coastal mainline population was also undertaken. Breeding values for each family for rust at ages 3 to 4 and 6 are very well correlated ($r = 0.94$), so early selection for rust resistance should be successful. For forking and ramicorn branching traits, age 4 and 6 are moderately correlated ($r = 0.68$). Selection efficiencies greater than 1 were found for early selection of height, volume and straightness when time was taken into account. The Task Force accepted the following recommenda-

tions for the fourth cycle: Measure fourth-cycle tests at 4 years for the Coastal and Piedmont regions. However, given low expression of some phenotypes, such as wood density, stiffness and saw timber quality, a subset of progeny tests will be measured at age 6 for the final selection. For the Northern region, tests will be measured one year later since growth is slower, and expression of phenotypic traits is delayed.



Above: Paul Belonger with Plum Creek Timberlands making a 4th cycle selection this past winter.

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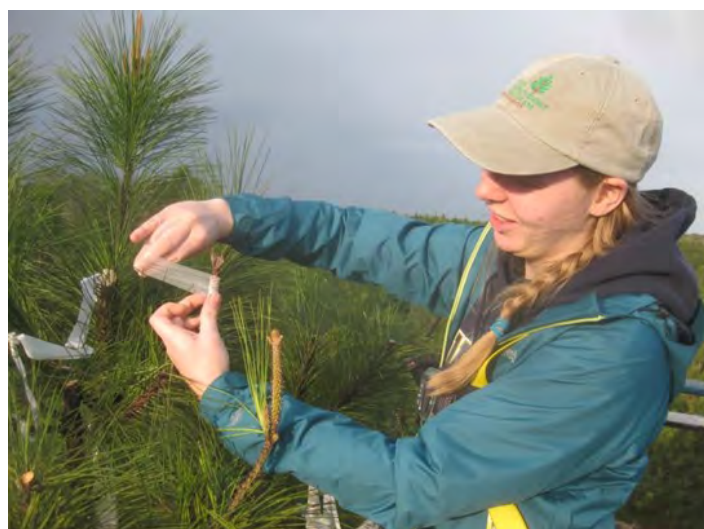
The Beginning of Another Round of Breeding: 4th-Cycle Selections Made in 2013

The first of the 4th-cycle selections were made in the full-sib block plots this past winter. Data from the 3rd-cycle pollen-mix (PMX) trials were used to calculate breeding values for the parents of the full-sib families. Families that had acceptable breeding values for the volume, straightness, rust (VSR) index were considered for selection. Individual-tree breeding values were then calculated for trees in each full-sib family block plot that was considered for selection. The individuals in each family were ranked for the VSR index, and field screening and selection of the best individual in each cross commenced in January 2013.

The ranking based on the family and individual-tree breeding values worked extremely well in the field. With few exceptions, trees at the top of the list were the superior phenotypes when we looked at them on the ground. All totaled, 105 fourth-cycle selections were made this year: 70 in the Coastal, 29 in the Piedmont, and 6 in the Northern population.

These trees were topgrafted at the Arrowhead Breeding Center at the Georgia Forestry Commission's Arrowhead Seed Orchard in Cochran, GA in February 2013. They were also grafted into archives by the Cooperative members. With the new breeding strategy, we are optimistic that breeding can be completed on these selections within the next 3 years.

Pictured below: Scions were collected from the selections and topgrafted at the Arrowhead Breeding Center in Cochran, GA. Laura Townsend (graduate student) had excellent grafting technique even 40 feet up in the air.



Above left: The first of the 4th-cycle selections was made by ArborGen in Lumberton, NC. The team effort and happy faces on the crew include: L→R: Patrick Cumbie (ArborGen), Jim Grissom & Jadie Andrews (NCSU), Chris Judy (ArborGen), Steve McKeand (NCSU), David Brown (ArborGen), and Josh Steiger (NCSU). Not pictured is Graham Ford, graduate student, who did much of the data analysis work and calculated individual-tree breeding values for all the candidate trees. And of course, Fikret Isik who is the brains behind all the data analyses and selection effort was instrumental in getting these trees selected.



Left: Six selections were made for the Northern breeding population. Ones Bitoki with the Virginia Department of Forestry is proud of his 4th-cycle find! Right: International Forest Company became a full member of the Cooperative on January 1, 2013. IFCO hit the ground running making contributions to the breeding, testing, and selection effort. By the third week in January, Nick Muir had his first 4th-cycle selection for the Piedmont breeding region.

Third-cycle Mainline Breeding and Testing

The Cooperative breeding program has seen much change over the past 18 months. During this time, third-cycle PMX breeding was completed, and the last of the third-cycle tests were established. In all, a total of 320 selections from the Cooperative's Coastal provenance were bred and have been put into progeny tests. In the Piedmont region, 262 selections were bred and are undergoing testing across four test series. Finally, the Northern provenance saw breeding completed for 164 selections. However, only 117 of these selections were put into third-cycle progeny tests. For selections that have been bred but not tested, they will be incorporated into the 4th-cycle testing strategy (see 4th-Cycle Progeny Testing , p5).

In addition to breeding the nearly 800 individual selections across the three provenances, Cooperative

members also conducted extensive breeding and testing of full-sib crosses. These crosses were made to serve as the selection pool for much of the 4th cycle's breeding genetic stock. In all, 452 full-sib crosses were made, and approximately 300 of these crosses have been put into field tests, to date. Again, any breeding completed on crosses that were not tested in the third cycle will instead be tested as part of the 4th-cycle testing strategy.

Among other activities in the third-cycle progeny testing, in Fall 2012, the third PMX series for the Coastal region tests and the first Piedmont PMX test series were measured at age 5 years. These measurements are vital for generating the information that goes into generating PRS™ scores; the more data we have the better!

Clonal Testing of Northern and Piedmont Elite Populations

To increase the gain possible from the Northern Sawtimber Elite Population (SEPop) and the Piedmont Elite Population, clonal testing of the progeny trials will be used to increase within-family heritabilities and also allow among family selections to be made. The increased within-family selection power gives higher likelihood that the forward selections themselves can be confidently grafted directly to production orchards.

After cone collection and seed extraction in Fall 2011, the Cooperative had sufficient seed from a majority of the Northern SEPop crosses as well as from some of the Elite Northern Population (EN-1, EN-2, EN-3). As a result, the decision was made to clonally replicate individual genotypes within each full-sib family using rooted cuttings from the top 16 crosses. These 16 full-sib families were chosen based upon straightness, forking characteristic and overall growth (height and dbh). We also generated a sawtimber score for each family to aid in the selection process. This scoring system combined volume, straightness and forking in a 2:1.5:1.5 ratio. Since fusiform rust is not a major concern in the Northern region, no attempt was made to screen the crosses for disease resistance. For those crosses not being included in this

round of testing, we plan to include them in the 4th-cycle testing effort. Furthermore, after reviewing the Piedmont Elite Population more carefully, we determined that many of the crosses originally selected for clonal testing did not have the genetic potential worthy of clonally testing. As a result, only three Piedmont crosses will be included in this testing effort. To aid in planning and logistics, we combined the Northern and Piedmont populations. In addition to clonally testing the experimental crosses, we will also include several checklots from both the Northern and Piedmont regions.

Collection of cuttings commenced in late February 2013, and by mid-March all cuttings had been stuck and were placed in the mist house. In all, approximately 22,000 experimental cuttings were collected and stuck in rooting media. Many families have begun to show signs of successfully rooting, but it is still too early to determine what the overall rooting numbers will be. Once rooting percentages for the families are known, an experimental design will be developed to test the clones. These trees will be established this coming winter in tests at sites located in the Piedmont and Northern regions.



Pictured left: Jadie Andrews, Ross Whetten, Fikret Isik, and Barry Goldfarb evaluating cuttings on April 22, 2013 from the Northern Sawtimber Elite Population.

SEED AND CONE YIELDS

Cooperative members collected just over 24,000 pounds of loblolly pine seed in 2012 (Table 1). Compared to last year, overall seed yields were down significantly, most likely due to a poor flower crop in spring 2011 compared the bumper crop the year before. Average seed yield per bushel remained stable, just slightly increasing over last year's yield.

Annual seed yields have varied over the years due to several factors such as regeneration needs, changes in

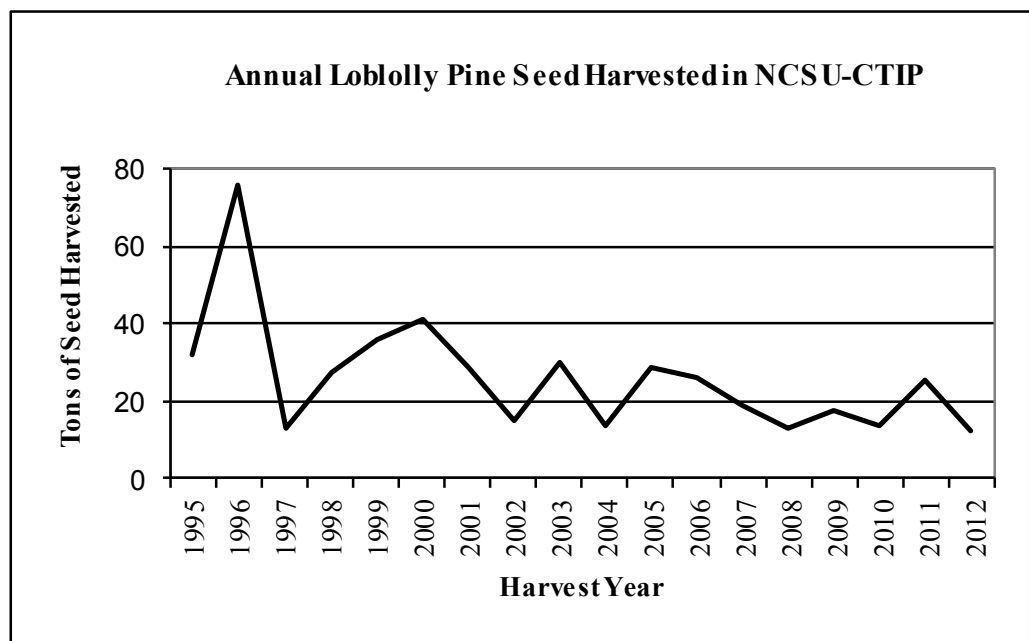
membership within the Cooperative, environmental factors (Figure 1). For the last 10 years, cooperators have produced sufficient quantities of improved seed, and therefore have been able to be increasingly selective at cone harvest.

From 1968 to 2012, nearly 2.4 million pounds of improved seed have been produced by Cooperative members. At 12,000 seedlings per pound, this is enough seed to grow 29 billion improved seedlings!

Table 1. Comparison of 2012 seed and cone yields with previous year.

Provenance/ Generation	Bushels Of Cones		Pounds Of Seed		Pounds per Bushel	
	2012	2011	2012	2011	2012	2011
Coastal 2.0/2.5	5132	12352	7339	18131	1.43	1.47
Coastal 3.0	7782	10333	10631	14572	1.37	1.41
Piedmont 2.0	1509	9395	2314	11113	1.53	1.18
Piedmont 3.0	2241	5277	3007	5040	1.34	0.95
Northern 2.0	494	1487	715	2190	1.45	1.47
Northern 3.0	409	441	425	332	1.04	0.75
Totals	17567	39285	24430	51378	1.36	1.21

Figure 1. Annual seed yields from 1995 to 2012.

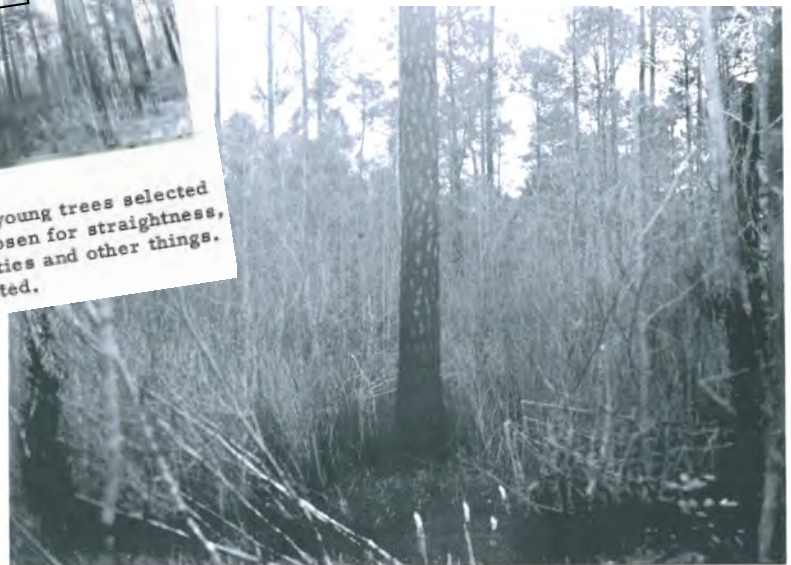


A look back...

In honor of completing the first year of 4th-cycle selections and breeding, we bring you excerpts from the very first Tree Improvement Annual Report (1957). A look at the first generation selections and breeding preparations being made, how far we have come!



The above tree on the left is one of the relatively young trees selected to be used in the seed orchards. Such trees are chosen for straightness, fast growth, good pruning, good crown, wood properties and other things. The tree on the right is one of the older trees selected.



Selections must sometimes be made for special conditions. The stand of loblolly pine pictured above is growing on 9.5 feet of peat before mineral soil is encountered. Growth rate is rapid, and some of the selected trees were nearly 100 feet tall at 50 years of age.



Field grafting will be used in the establishment of the seed orchards. Here Zobel and McElwee examine some preliminary tests on field grafting. This type of grafting is difficult to do but has many advantages over greenhouse grafting.

To see all of the NCSU Tree Improvement Annual reports, go to:

www.TreeImprovement.org

RESEARCH**Clonal Variation in Loblolly Pine for Conversion to Ethanol¹**

A previous experiment tested clonal varieties of loblolly pine to characterize variation in yields of fermentable sugars from enzymatic hydrolysis. Alkaline pretreatments with mechanical beating produced more sugars than either alkaline pretreatments without mechanical beating or a dilute acid pretreatment. An additional sample set of wood cores was collected in order to better characterize genetic variation in sugar yields. These new wood samples were taken from 150 loblolly pine trees in a Plum Creek Timber Company

progeny test in Oliver, GA (same test sampled for the previous experiment). These 150 trees are composed of 30 different loblolly pine genotypes (clonal varieties), and 5 trees were sampled per clone. Two cores were taken per tree, each core was dried and powdered. The samples were then subjected to pyrolysis molecular beam mass spectrometry (py-MBMS) at the National Renewable Energy Laboratory (NREL).

¹This is a summary of David Barker's PhD research to be completed in the fall of 2013.

Py-MBMS provides compositional analysis of the biomass in the form of ion counts at mass/charge (m/z) ratios from 50 to 450. Many m/z ratios (also referred to as ‘peaks’) are associated with one or more specific molecules. Principle component analysis (PCA) was used to identify those peaks with relatively higher amounts of variability. PCA was conducted on the mean of the ion counts of the two samples from each of the 150 trees sampled. Principal components 1 and 2 account for 41% and 12% of the variation in the population, respectively (Figure 1). The plot of PC1 vs. PC2 shows outliers in three regions of the plot. The higher loadings on the outliers indicate that they are responsible for a relatively higher proportion of sample variation than those m/z peaks clustered near the middle of the plot. These outliers fall into three general groupings: carbohydrates (red), extractives (black), and lignin precursors (blue).

Some of the variation in these outlier m/z peaks can be attributed to genetic effects, especially for those peaks related to carbohydrate and lignin components. Additionally, estimates of % extractive and lignin composition have been calculated from the MBMS data for each of the sample trees, and these estimates have been tested to see if genotype plays a major role in these gross measures of biomass composition (Table 1). Also available are estimates of lignin and cellulose contents from analysis of Near Infrared (NIR) spectra taken from these samples. Clones were highly significant for all of these characteristics, though it is interesting that clonal mean repeatability is much higher for the NIR-based estimate of lignin

PC1 vs PC2 for Pine py-MBMS

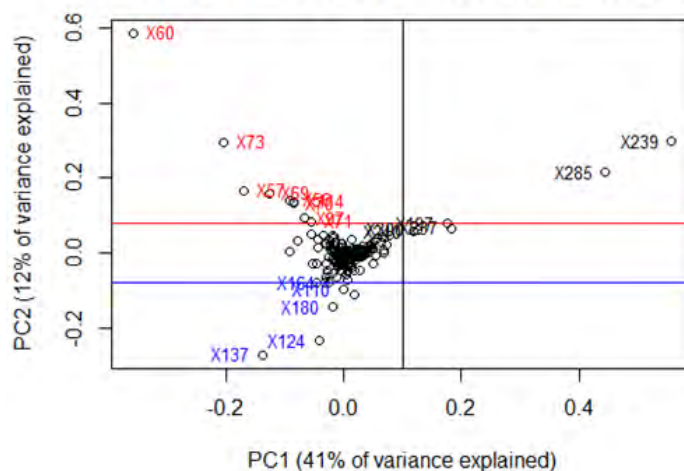


Figure 1. Loadings plot from principle component analysis of MBMS data. Outliers (labeled with a text ID) indicate the ion count ‘ m/z peaks’ most responsible for variation within the sample set.

content than for the MBMS-based estimate. This may be due to the fact that the NIR model is based only on data from loblolly pine, whereas the MBMS model is based on multiple species, including grasses and hardwoods.

Data on sugar yields from enzymatic hydrolysis have also been obtained for these samples through a cooperative effort with NREL. Analysis of these data is underway. Additionally, data from thermochemical conversion of these samples are anticipated within the next few months. These data are being obtained in cooperation with a separate group at NREL.

Table 1. Genetic parameters for compositional estimates from MBMS and NIR data.

	Component	Clone Mean Repeatability	Std. Err.	P-value (clone)
NIR Model	Lignin	0.69	0.23	0.001
	Cellulose	0.69	0.24	0.002
MBMS Model	Lignin	0.30	0.18	0.050
	Cellulose	0.35	0.28	0.102

This research is part of Integrated Biomass Supply Systems project funded by USDA-NIFA, for more information on this project please visit www.se-ibss.org

Effect of Inbreeding on Growth and Wood Properties in Loblolly Pine¹

With advanced generations of tree improvement on the horizon, it is necessary for forest tree breeders to address how inbreeding shapes breeding strategies. Inbreeding can increase rapidly in intensively selected breeding populations and is detrimental in outcrossing species such as loblolly pine. Managing inbreeding and the deleterious effects on metric traits is a primary objective in forest tree breeding programs. For these strategies to be successful, reliable estimates of the degree of inbreeding depression are necessary.

In these studies, the effects of inbreeding were quantified for 10 Coastal and 10 Piedmont loblolly pine lines. Each of 10 selected parents was bred to other related and non-related selections to provide a gradient of inbreeding coefficients (F); F = 0, 0.125, 0.25, 0.5. Progeny from each cross were planted in replicated field trials, and measurements were collected. At age 9, there was generally a decrease in metric traits with increase in inbreeding coefficient (Table 1), but the probability of fusiform rust incidence and straightness were not affected by inbreeding. A more detailed summary of the age 9 results can be found in the Cooperative's 42nd Annual Report from 1998.

Following early measurements, test sites were either lost or abandoned, except for one Coastal installation established and managed by Weyerhaeuser Company in Beaufort County, NC. Height, diameter, survival, and wood properties were measured at age 23. Table 2 gives means of the traits measured at age 23 for each of the four inbreeding levels.

Relative to progeny of outcrosses, height showed significant inbreeding depression. Progeny of full-sibs and selfs were 5.44%, and 4.14% shorter than outcrossed progeny. Probability of survival also significantly declined to 0.52 and 0.40 in progeny of full-sibs and progeny of selfs. Diameter at breast height for halfsibs = 0.125 and fullsibs = 0.25, respectively, was 1.92% and 7.3% smaller than outcrossed progeny, but progeny of selfs had the greatest DBH of all mating types; 2.48% larger than outcrossed progeny.

This increase was due to low survival and greater growing space in the selfed plots in the split-plot design.

Wood specific gravity was not significantly affected by inbreeding. Bending strength (measured by acoustic stress-wave velocity) decreased slightly with inbreeding. Stress-wave velocity was 2.75%, 2.50%, and 2.71% less in progeny of half-sibs, full-sibs, and selfs relative to outcrosses.



Pictured above: 12 mm cores from study trees saturating in buckets for specific gravity measurements.

Significant inbreeding depression was seen in growth traits at both ages, but not in form, quality, or wood properties. This is because growth and survival traits are more closely tied to fitness than resistance to fusiform rust, straightness, and wood properties. Growth and survival have a selective advantage on fitness.

For all traits measured, at ages 9 and 23, there was significant variation in response to inbreeding among the 10 lines. Some lines show greater susceptibility to inbreeding than others. The presence of this variability implies that a single approach to managing inbreeding in a breeding population is not optimal. A multifaceted strategy that accounts for or utilizes this variability is more appropriate.

¹ This is a summary of Graham Ford's MS thesis research. Graham completed his Master's degree last fall and started his PhD program with the Cooperative in November 2012.

Table 1. Means at age 9, 95% confidence intervals, number of observations (N), and percent inbreeding depression, are given at each of the four inbreeding levels for height and volume traits. Inbreeding levels with same letter are not significantly different. (*%ID is inbreeding depression and calculated as percent reduction in mean relative to progeny of outcross.)

Provenance	Mating Type	Height (m)	95% CI		N	% ID*
Coastal Plain	Outcross (F = 0)	10.8 a	10.7	10.9	585	--
	Half-Sib (F = 0.125)	10.2 b	10.1	10.3	590	5.5
	Full-Sib (F = 0.25)	9.4 c	9.3	9.6	576	12.7
	Self (F = 0.5)	8.6 d	8.3	8.8	468	20.6
Piedmont	Outcross (F = 0)	10.2 a	10.2	10.3	1079	--
	Half-Sib (F = 0.125)	9.8 ab	9.8	10.0	999	3.7
	Full-Sib (F = 0.25)	9.6 b	9.5	9.7	979	5.9
	Self (F = 0.5)	8.4 c	8.3	8.6	751	17.8

Provenance	Mating Type	Volume (dm ³)	95% CI		N	% ID
Coastal Plain	Outcross (F = 0)	100.6 a	97.3	104.0	585	--
	Half-Sib (F = 0.125)	88.1 b	85.0	91.3	590	12.4
	Full-Sib (F = 0.25)	74.5 c	71.5	77.6	576	26.0
	Self (F = 0.5)	67.2 c	62.3	72.0	468	33.2
Piedmont	Outcross (F = 0)	104.6 a	102.4	106.7	1079	--
	Half-Sib (F = 0.125)	95.2 a	92.8	97.5	999	9.0
	Full-Sib (F = 0.25)	93.2 a	90.3	96.1	979	10.9
	Self (F = 0.5)	70.4 b	66.6	74.1	751	32.7

Table 2. Means at age 23 are given at each of the four inbreeding levels for growth, survival, and wood quality traits in the Weyerhaeuser trial in Beaufort County, NC. Inbreeding levels with the same letter are not significantly different.

Mating Type	Height (m)	DBH (cm)	Survival freq.	Specific Gravity	Stress Wave Velocity (m/s)
Unrelated (F = 0)	22.7 a	24.5 ab	0.63 a	0.454 a	4213.7 a
Half-Sib (F = 0.125)	22.5 ab	24.0 b	0.63 a	0.458 a	4097.8 b
Full-Sib (F = 0.25)	21.5 c	22.7 c	0.52 b	0.457 a	4108.3 b
Self (F = 0.5)	21.8 bc	25.1 a	0.40 c	0.452 a	4099.3 b

Predictions of Genetic Merit in Tree Breeding Using Factor Analytic Linear Mixed Models and Blended Genomic Relationship Matrices¹

Increases in computer power and in the efficiency of DNA sequencing technologies are providing new opportunities to plant and animal breeders to fit more complex statistical models for predictions of genetic merit of individuals. Such models can be powerful to account for heterogeneity in the data and as a result, can increase the accuracy of predictions and genetic gains from breeding programs.

This study evaluated the efficiency of factor analytic (FA) linear mixed models for a large, multi-environmental trial of loblolly pine. Height was assessed on 37,269 trees at age six years in a diallel experiment established by Plum Creek Timber Company. Among models fit, Factor Analytic (FA) mixed models produced the smallest AIC model fit statistics. FA models captured both the variance and covariance at the genetic level better than models with simpler covariance structures, and they provided more accurate predictions of genotypes. The mean narrow-sense heritability estimates for height was about 0.20 when more complex variance structures were used, compared to 0.13 when simpler variance structures were employed. FA models were parsimonious compared to unstructured covariances. The FA models provided a natural framework for modeling genotype by environment interaction. Genotype by environment interactions were non-significant as suggested by high genetic correlations both for additive (0.83) and dominance (0.91) effects.

Molecular marker data, especially single-nucleotide polymorphic (SNP) markers have been commonly used to predict genetic merit in plant and animal breeding programs. However, marker data usually have missing genotypes and they need to be imputed. The effects of percent (level) and pattern (random or structured) of missing data, and mating designs on the accuracy of imputation of genotypes were investigated. Linkage based BLUP was used to impute missing genotypes for an empirical (unbalanced) data set for loblolly pine. For simulated (balanced) data sets, both BLUP and Hidden Markov Model (HMM) approach-

es were used. The actual data had 178 clones that were genotyped at 3,461 biallelic SNP markers. The simulated data consisted of double-pair and half-diallel mating designs with 2880 and 2940 individuals, respectively. The accuracy of imputation for the empirical data was higher for the structured pattern of missing data at any level of missing data percentages. Regardless of the pattern of the missing data, imputation accuracy was less than 0.70 when the data had greater than 40% missing values. Imputation accuracy for the simulated data with structured missing data (as obtained from genotyping with a low-density array of SNPs) was not affected by mating design for either the BLUP or the HMM approaches.

In breeding programs marker data might not be available for all the individuals. Combining information from pedigree and DNA markers might improve prediction accuracies in tree breeding programs. The matrix showing blended genetic relationships derived from both pedigree and DNA markers is called an **H** matrix. Using the inverse of the **H** matrix in predictions of breeding values is called HBLUP. A cloned population of loblolly pine and simulated data sets were used to examine the efficiency of blended additive genetic relationships derived from pedigree and realized genomic relationships derived from SNP markers. Genotypes at 3,461 SNP loci were available for 166 genotypes out of a total of 354 clones. Two simulated datasets were created, with 300 or 600 trees genotyped at 1000 markers out of a total of 1200 trees. For the empirical data, the accuracy of predictions based on pedigree (ABLUP) was 0.79. Predictions based on HBLUP had accuracy of 0.72 to 0.76. For the simulated data set, the accuracy values for

¹This is a summary of Funda Ögüt's PhD dissertation. Funda studied with Fikret Isik and Ross Whetten and successfully defended her PhD in November 2012. She has a post-doctoral position in the Crop Science Department at NC State. We are particularly thankful to Plum Creek Timber Company, especially Paul Belonger, for providing a valuable data set from their diallel experiments.

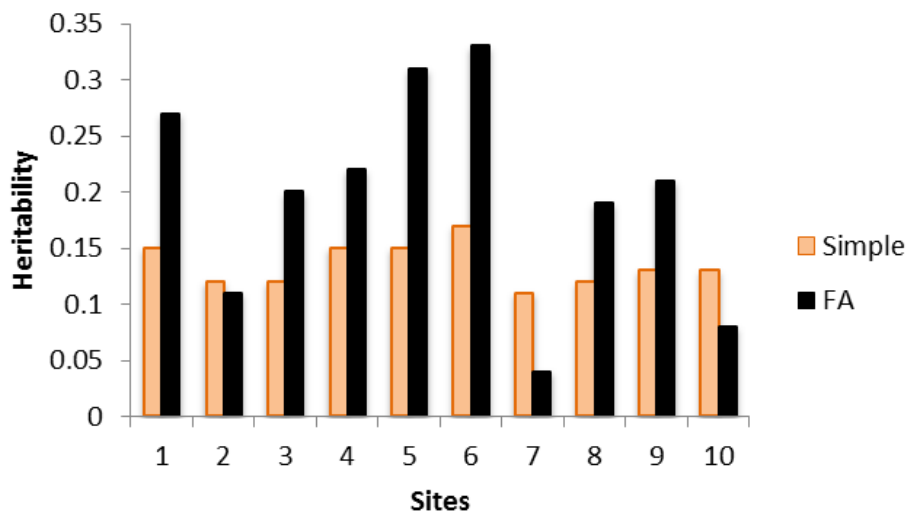
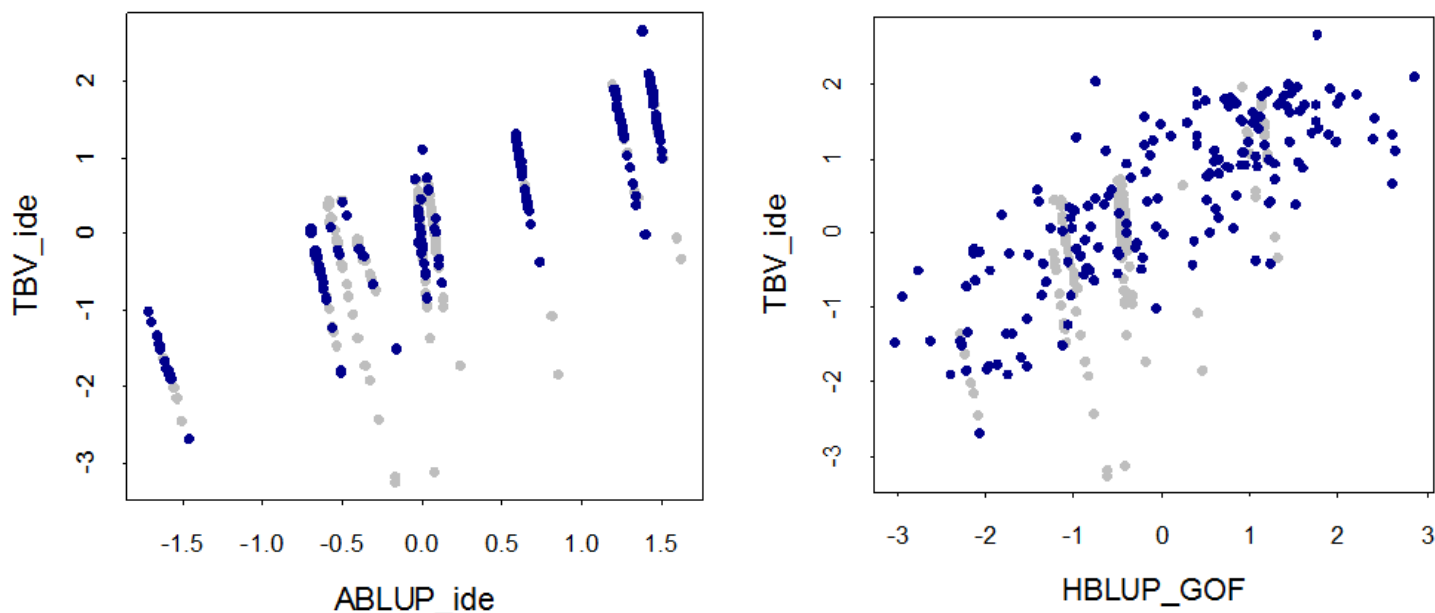


Figure 1. Individual tree narrow sense heritabilities for all 10 progeny test sites using the simple versus FA models. The simple model assumes that additive genetic variance is the same across sites and no genotype by environment interaction. FA models assume sites have different additive genetic variance and account for genotype by environment interactions. In both models, a different residual variance covariance structure (Diagonal) was fit.

HBLUP models were higher compared to ABLUP model. Also, as the genotyped population size increased the accuracies increased. HBLUP uses all the available phenotype, pedigree, and genotype data in a single step and is easy to implement for genomic

based selection. The major advantage of using genomic relationships matrices is that markers can capture the Mendelian sampling effect within full-sib families for selection.

Figure 2. Scatter plots between true breeding values (TBVs) and estimated breeding values (EBVs) obtained from ABLUP (left) and HBLUP (right) methods using actual data. TBVs were predicted from complete data and EBVs were obtained using leave-one-out data set (354 trees: 166 genotyped and 188 non-genotyped). Genotyped individuals are in dark blue and non-genotyped individuals are in gray.



Impacts of Different Levels of Genetic Homogeneity on Juvenile Stem Characteristics and Potential Stand-level Value in Loblolly Pine¹

The availability of improved genetics in loblolly pine coupled with advancement in silvicultural techniques provides foresters many options to optimally managing forest land in the Southeast. However, the interaction of varying families and clones with different levels of genetic diversity across the landscape is not well understood. The recent, wide-spread deployment of more genetically homogenous genotypes such as clones and full-sib families drives the demand for better understanding growth and uniformity in stands with varying levels of genetic diversity.

We hypothesized that stands planted with more genetically homogenous genotypes would be more uniform for growth traits and sawtimber potential in juvenile loblolly pine. Our results indicate that less genetically diverse genotypes are not more uniform than the more genetically diverse genotypes, and in most cases genetic homogeneity actually led to more variation in growth traits.

Additionally, we wanted to test for block, spacing, genotype and interaction effects on growth. At ages 3, 4 and 5 total height growth was significantly different among genotypes, and compared to other effects in the model, genotype had the largest effect on total height at all ages. At age 3, spacing did not have a significant effect on height among genotypes. However, at ages 4 and 5, spacing was highly significant ($P < 0.0001$). Furthermore, the interaction effects of block x spacing and genotype x spacing were highly significant at ages 3, 4, and 5. Diameter-at-breast height (DBH) was significantly affected by all effects at ages 3, 4, and 5. Genotype effect, spacing effect, and the interaction between the two became increasingly significant over time. In contrast, the block effect and the interaction of genotype x blocking became less significant over time.

For economic assessment, we compared the predicted values of 10 genotypes using three scenarios based on growth and yield projections using the LobDSS software package. The first scenario was based solely on

LobDSS yields, generated by including dominant height, TPA, and basal area for each family at age 6 (Table 1). The second scenario combined LobDSS yields with empirical data collected from the same general location. These data were tallied from Hofmann Forest mill tickets over nearly a decade of plantation harvesting. Harvest data were used to generate percent yields by product class (pulpwood, chip-saw, sawtimber) by regressing the total green weight in tons at harvest for each class by age. Only harvest data from stands that were harvested between the ages of 20 and 30 were included, as to minimize the influence of the more mature stands on product class distribution. Finally, we adjusted scenario 2 with sawtimber potential scores (score of 1-4) assessed for each genotype at both densities (Table 2). In scenario 3, sawtimber scores from each genotype were compared to those of the Seed Orchard Mix (SOM), which was used as a baseline because it is comprised of many different genotypes and also had the lowest seedling purchase price of the four genetic types. In an effort to measure potential economic differences, families that received more sawtimber scores of 1 compared to SOM, received a price increase of 25% for sawlogs, which was applied to the percent increase over the SOM. Additionally, the proportion of sawtimber potential scores of 4 in the SOM was compared to the proportion in the other genotypes. Since sawtimber scores of 4 were deemed to be non-merchantable trees, we removed or added the percentage change from SOM to the pulpwood tonnage class.

Our results show that by incorporating more accurate product class allocation and sawtimber quality measures, the soil expectation value of stands can change dramatically (Figure 1). These results suggest

¹This is a summary of Josh Steiger's MS Thesis work. Josh completed his Masters of Science degree under the direction of Bronson Bullock in the Spring of 2013. Josh worked on the Genetics-Spacing-Thinning (GST) Trial at the Hofmann Forest in his "spare time" as the Tree Improvement Program Manager for the Cooperative.

Family*	Survival %	TPA	DBH	BA/Tree	BA/Ac	Median Height	Dominant Height
C1	91.8	200	4.33	0.102	20.4	19.0	21.9
C2	95.3	208	5.20	0.148	30.7	26.6	29.0
C3	97.7	213	5.24	0.150	31.9	28.7	32.3
FS1	93.8	205	6.17	0.208	42.4	29.2	31.4
FS2	97.2	212	6.67	0.239	50.6	28.6	30.9
FS3	97.9	214	6.25	0.213	45.5	28.5	30.6
HS1	95.0	207	6.10	0.203	42.1	29.1	31.3
HS2	98.5	215	6.30	0.217	46.5	28.4	30.4
HS3	97.9	214	6.23	0.212	45.2	27.4	29.2
SOM	96.5	210	5.86	0.187	39.3	28.7	30.6

Table 1. Summary statistics for each genotype at age 6 in the 436 TPA treatment with a sample size 534.

*Family ID's are: C= clone; FS= full-sib, HS= half-sib or open-pollinated ; SOM = Seed Orchard Mix

that if the landowner is paid for the quality of their timber, not just the volume, then some genotypes are better options than others. Furthermore, it appears that the proportion of non-merchantable trees can

vary considerably across genotypes, implying that close attention be paid not only to the proportion of high quality sawlogs in any particular genotype, but also to the proportion that is non-merchantable.

Table 2. Percent sawtimber potential score by genotype and planting density at age 5.

Family	Score 1		Score 2		Score 3		Score 4	
	436 TPA	218 TPA	436 TPA	218 TPA	436 TPA	218 TPA	436 TPA	218 TPA
C1	1.2	0.6	22.2	6.8	40.7	34.8	35.8	57.8
C2	21.4	15.5	44.0	44.3	19.6	27.6	14.9	12.6
C3	19.9	19.9	43.4	43.9	23.5	21.1	13.3	15.2
FS1	20.5	14.6	54.4	48.5	19.9	29.8	5.3	7.0
FS2	25.3	9.8	46.0	48.9	24.7	39.7	4.0	1.7
FS3	13.6	12.5	54.0	52.3	23.9	30.1	8.5	5.1
HS1	10.3	9.0	58.2	44.0	24.8	38.0	6.7	9.0
HS2	11.2	7.4	50.0	52.3	33.5	37.5	5.3	2.8
HS3	7.6	9.6	62.2	58.8	24.4	29.4	5.8	2.3
SOM	12.2	13.3	48.8	50.9	29.1	31.2	9.9	4.6

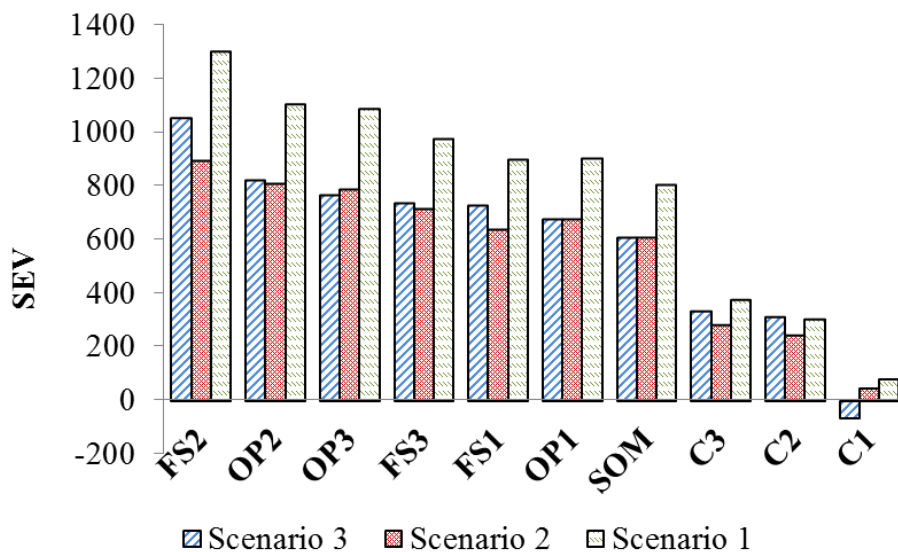


Figure 1. Maximum SEV (Soil Expectation Value) for each scenario at 436 TPA for all genotypes at 5% interest rate.

Modeling the Growth of Loblolly Pine Seed Sources in the Southeastern United States¹

The establishment and analysis of provenance tests for investigating the genetic variation among forest trees has a long tradition in forestry. Such tests, which were meant to identify superior seed sources for planting at specific locations, might provide valuable information for assessing the response of populations to climate change.

The Plantation Selection Seed Source Study (PSSSS) was initiated in 1994 by the North Carolina State University Cooperative Tree Improvement Program to determine the patterns of geographic variation in plantation selections and to understand pine genotype interactions with the environment. Loblolly pine phenotypes were initially selected from plantations based on superiority of phenotypic indicators such as height, stem form, and disease resistance. Pollen mix seedlings from 140 of these selections, sampled from 7 seed sources, were planted at 20 test locations throughout the southeastern United States, and growth, stem quality and fusiform rust incidence were measured at tree ages 4 and 8 years.

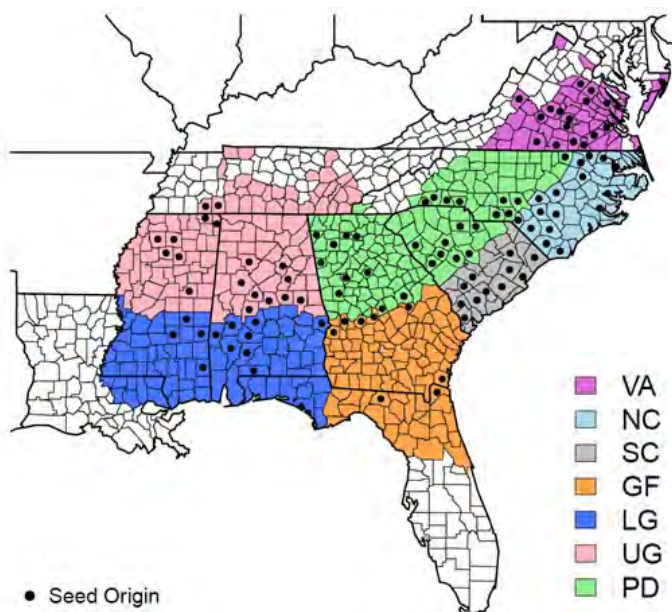


Figure 1. Seven seed sources sampled (colored regions) and locations of the plantations where seed were collected (black dots).

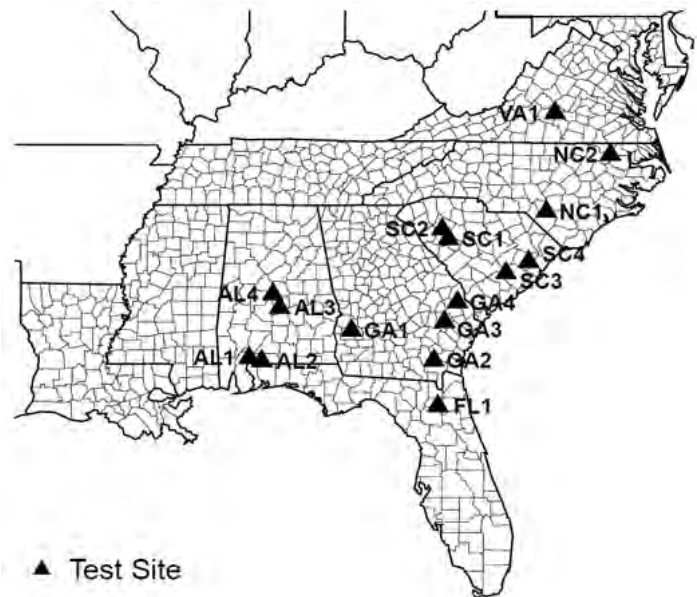


Figure 2. Location of Plantation Selection Seed Source Study (PSSSS) test sites across the Southeastern United States.

In this work, a statistical model to predict the responses of different seed sources to climate change using climate variables as predictors is proposed. The approach integrates both genetic and environmental effects and is meant to overcome the critical limitations of population response function and transfer function methods by making full use of data from provenance trials. The model was developed and tested using data from the Plantation Selection Seed Source Study.

The statistical model can be used as a quantitative tool to model the effect of climatic variables on the performance of loblolly pine seed sources. Furthermore, the model could also be used to identify superior performing families through simulation studies, and to estimate their growth potential for a given planting site under a given future climate.

¹This is a summary of part of Alfredo Farjat's PhD research. Alfredo is funded by the PINEMAP project, a Coordinated Agricultural Project funded by the USDA National Institute of Food and Agriculture.

Two examples are given here to illustrate possible applications of the proposed model. A hypothetical climate scenario was created from historical data, assuming a 5% decrease in precipitation, 2% increase in maximum temperatures, and an increase of 2°C in minimum temperatures. This hypothetical scenario was used for predictions in the following application examples.

The first example (Figure 3) estimates the 8 year height growth for the climatic conditions previously described, using Coastal South Carolina seed sources. From the estimated values, it can be inferred that South Carolina seeds perform very well in the Geor-

gia-Florida coastal plain region, and their estimated growth decreases as we move north.

The second example (Figure 4) predicts the relative performance of loblolly pine seed sources from Virginia compared to all the other seed sources under consideration, throughout the southeastern United States. The blue colors in the predicted deviations plot indicate that this seed source will only be superior in the most northern regions under the modeled climate change scenario. Notice that the relative performance of Virginia seed sources in southern regions is very poor, which is in agreement with experience.

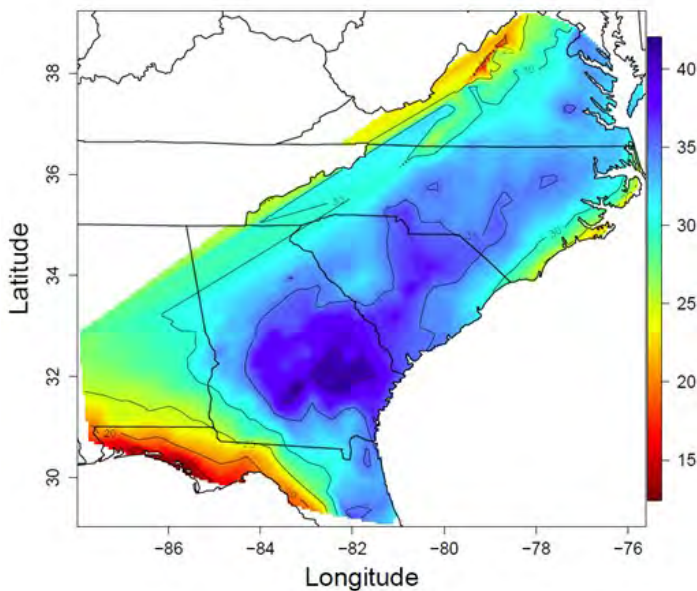


Figure 3. Predicted 8 year height (feet) of Coastal South Carolina seed source across the Southeast under a hypothetical climate change scenario. Notice that Coastal SC seed source is expected to perform well across the Southeast, but highest growth (dark blue) is expected to be in southern GA.

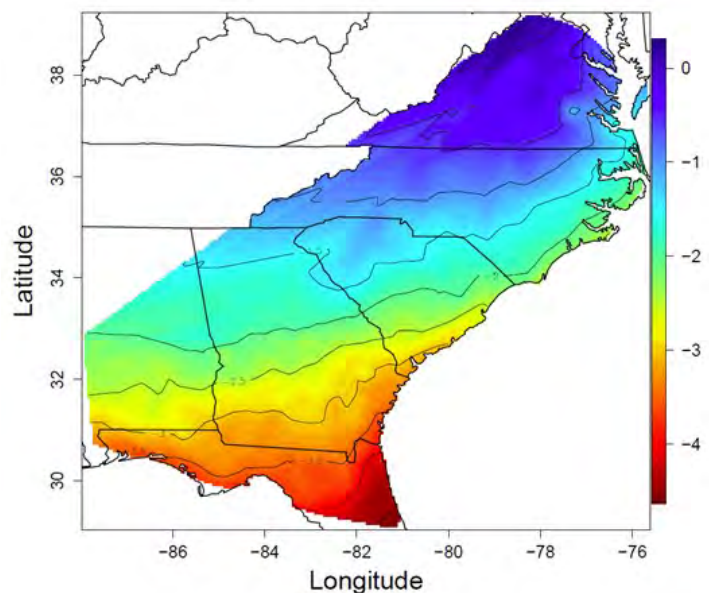


Figure 4. Predicted performance (feet) of Coastal Plain Virginia seed source (deviation from site means relative to all other seed sources). The blue colors indicate that this seed source will only be superior in the most northern regions under the modeled climate change scenario.

For more information on PINEMAP project funded by USDA-NIFA, please visit www.pinemap.org.

Genomic Analysis of Loblolly Pine Adaptability to Climate Conditions¹

The projected increase in severe weather events due to climate change has potential to affect the productivity of pine plantation stands and impact the forest industry. Loblolly pine has a very diverse gene pool and is adapted to various environments, and is thus a good candidate to breed for adaptation to climate change. The PINEMAP project, funded by USDA in 2010, proposed to accomplish this by testing for associations of individual genetic marker loci with growth and quality characteristics using climate factors as covariates. Existing high-throughput genotyping methods for pine are too expensive to allow routine application in breeding populations. Over the past two years, we have worked with other PINEMAP investigators to develop a cost effective, easily implemented, and high-throughput genotyping method which we are now using to test the hypothesis that genomic regions controlling climate adaptation can be identified.

The genetic marker system will be used to collect genotype information from trees in two different sites of the Plantation Selection Seed Source Study (PSSSS). The PSSSS consists of 140 pollen mix families planted in 20 test locations representing much of the loblolly pine range. Phenotypic data from measurements at ages 4 and 8 years are available to be used together with genotypes from the genetic marker analysis.

A method known as genotyping-by-sequencing (GBS), previously described for maize and barley by Elshire et al. (PLoS ONE 6: e19379, 2011) and for wheat and oats by Poland et al. (PLoS ONE 7: e32253, 2012) is currently being adapted for use with pine. This method is carried out in 96-well format, and employs methylation-sensitive restriction enzymes to enrich for gene regions in the pine genome. To reduce costs, the method uses “barcoded” adapters and an index primer set to allow combining individual samples in a single sequencing lane while maintaining individual sample identities. DNA sequencing is carried out using Illumina HiSeq2000 or other comparable high-throughput instruments; the HiSeq2000 is the current platform of choice due to the number of

reads and the read quality obtained. The resulting sequence data are analyzed using Java-based, platform-independent software (TASSEL) obtained from the Institute for Genomic Diversity at Cornell University.

Progress and Results

Tissue samples were collected as 3/4-inch bark disks from 1600 trees in the PSSSS site in Screven County, GA, in October 2012. The phloem tissue was peeled from the underside of each bark disk in the field, and stored at ambient temperature in a barcoded vial containing an anti-oxidant preservative solution until DNA was extracted using a procedure adapted from a published protocol (Ivanova et al, 2008, *Pl Mol Biol Rep* 26:186). Methods for grinding tissue samples in 96-well format have been optimized for the phloem tissue samples, and over 3600 DNA extractions have been completed in the past six months. The yield of DNA is somewhat variable from these samples, as the amount of phloem tissue obtained from a bark disk is inconsistent, so we have incorporated a 96-well assay for DNA quantitation into the sample-processing workflow. Sequencing libraries can be prepared from pairs of 96-well plates (192 samples at a time), and the first 480 samples are expected to be submitted for DNA sequencing soon. The estimated cost of consumable supplies and reagents for DNA extraction and library preparation is about \$1.50 per sample. A preliminary experiment was conducted over the summer of 2012 to test the recovery of marker sequences from DNA samples of a single loblolly pine parent tree and a set of 90 haploid megagametophytes from seeds of that parent. Promising results were obtained; 15% of single-end sequence tags (64-base DNA sequences from one end of a genomic DNA fragment) showed the expected 1:1 ratio of presence and absence in the haploid DNA samples and mapped to a single location in the v 0.6 draft assembly of the pine genome sequence. These results suggest that 8,000 to 10,000 unique genetic loci can be genotyped per individual tree in a cost-effective, high-throughput sys-

¹ *This is a summary of Laura Townsend's MS research project being funded by the PINEMAP.*

tem. Further analysis is underway using new software that allows joint analysis of sequences from both ends of the DNA fragments, and the most recent (v0.9) draft assembly of the pine genome sequence.

Analysis of the phenotypic data from the Screven County, GA site with genotype data will allow testing of the hypothesis that segments of the genome that affect growth in the 140 PSSSS families at that site can be identified using BLUP estimation of genomic effects. Tissue samples from a second site will be col-

lected during the summer of 2013 and genotyping carried out as soon as possible after DNA extraction and library preparation are completed. A parallel analysis on the second dataset will detect genomic regions involved in control of phenotypes in a different environment, and the concordance between the allelic effects observed in one environment versus the other will be compared to test the hypothesis that genotype-by-environment interactions can be observed at the level of individual genes or genomic regions.

Right: Snapshots of the phloem tissue sampling procedure used at the PSSSS site in Georgia.



Grants received

We have been busy keeping up with all of the research initiatives that were sponsored in the last few years. The proposal and the list below is of current projects being funded:

Continuing Grants

Fikret Isik is a co-principal investigator with Vincent Chiang and Ron Sederoff on a NSF Plant Genome grant, Regulation and Modeling of Lignin Biosynthesis. TIP portion: \$43,548. 09/2009 to 08/2013.

Ross Whetten, Steve McKeand, Fikret Isik. Subcontract with the University of Florida, USDA Coordinated Agricultural Project (CAP) “Integrating research, education and extension for enhancing southern pine climate change mitigation and adaptation”. TIP portion: \$867,665. 3/2011 to 2/2016.

Steve McKeand, Bronson Bullock, Ross Whetten, and Fikret Isik. Biofuels Center of North Carolina. “Loblolly Pine Biomass Genetics/Cropping Study”. \$148,419. 7/2011 to 9/2014.

Ross Whetten, Steve McKeand, and Fikret Isik. USDA via subcontract with the University of Tennessee. Integrated Biomass Supply Systems (IBSS). TIP portion: \$652,369. 8/2011-7/2016.

Fikret Isik is a co-principal investigator with Vincent Chiang on a U.S. Department of Energy grant, Modeling of Cellulose, Hemicellulose and Lignin-Carbohydrate Complex Formation and Regulation to Understand Plant Cell Wall Structure. TIP Portion: \$20,223. 9/2011-8/2014.

ASSOCIATED ACTIVITIES

Meetings, Workshops, and Short Courses

It has been an active year for TIP but a departure from the last year's various workshops and short courses. We spent a lot of time with Cooperative members getting the 4th-cycle breeding plan started. We have found seclusion often breeds great ideas, so we sequestered members in cabins in Crossnore, NC and dubbed the meeting the "Crossnore Conclave". We made them eat, drink and breathe breeding...sounds great right?? Well it was great, and from there the ball continued rolling all the way back to Raleigh where feedback and questions from members were worked through and evaluated by TIP staff and students. The ideas generated in Crossnore helped the TIP staff develop and "touch up" the strategy. Staff and members were confident in the plan, and timelines were created with the start date being....THIS YEAR!! After picking our jaws up off the floor, we got busy planning, selecting, grafting, and breeding. This did not leave us much time to do a lot of outreach activities, but don't worry, we were able to squeeze a few in before we knew what was going to hit us!



Above: "Crossnore Conclave" attendees which includes TIP Cooperative members, staff and students.



Pictured Above: Instructors and attendees of The Genetic Data Analysis Workshop held in Prague, Czech Republic from June 11-15, 2012. The workshop attracted 25 attendees from industry, research and academia from 11 countries. The workshop was hosted by the Czech University of Life Sciences -Prague.

The genetic data analysis workshops continue to be a big attraction. This year Fikret Isik, was invited to teach a 'Summer Course in Genetic Data Analysis - Applications for plant and animal breeding' by the Czech University of Life Sciences. The course took place on June 11-15, 2012 and was hosted by Dr. Milan Lstibůrek at Czech University of Life Sciences and NCSU-TIP alumnus. Dr. Jim Holland, Professor of Crop Science and Research Geneticist (USDA) and Dr. Chris Maltecca, Animal geneticist with the Department of Animal Science at NCSU joined Fikret to teach the summer course. The course covered advanced quantitative genetics for analysis of genomic and phenotypic data for plant and animal breeding. 25 professionals, graduate students and faculty from 11 different countries attended.

Both Steve and Fikret were invited speakers at the IUFRO meeting, "Seed Orchards and Breeding Theory", in Antalya, Turkey from May 19-25th, 2012. Fikret happens to be a great tour guide when it comes to his home country, so if you ever get the opportunity to visit Turkey make sure Fikret is with you!

IEG-40 took a hiatus in 2010, but after a “fun run” during the 2011 SFTIC meeting, delirious runners Dana Nelson (USFS), John Davis (UFL), and Steve McKeand decided to resurrect IEG-40 for 2012. With help from Josh Bronson (USFS), the IEG-40 meeting “Integrating Fusiform Rust Research, Screening, and Breeding” was held in Asheville, NC at the USFS Resistance Screening Center on June 14-15. Invited speakers were Dr. Richard Hamelin (UBC-Canada FS), Dr. Jim Kolmer (USDA-ARS), and Dr. Richard Sniezko (USFS). The meeting took a close look at fusiform rust disease pathogen, screening and interactions, and breeding initiatives to control the disease. The meeting involved industry, research and state/federal agencies working to tackle these issues.



Above: IEG-40 speakers and attendees at the USFS Resistance Screening Center in Asheville, NC

The annual TIP Contact meeting was hosted by NCSU Tree Improvement in Asheville, NC. The Co-op used the information and ideas generated at the IEG-40 meeting as a springboard for more work in resistance screening for operational breeding practices. Attendees took a tour of the facilities at the screening center and got an overview of the collaboration that USFS RSC and NCSU have had in pre-screening families for fusiform rust as an early selection tool. Due to the adventure factor over the past few years, we kept the group at the location the whole time, sorry folks no bus stories this year. But there was enough adventure on the golf course before the meeting started. Yes, Fikret had a club, enough said :)!



Above: Big crowd enjoying the TIP Contact Meeting, we had wonderful Fall weather and color show!

Our new annual ritual and TIP staff/student bonding has come in the form of NCSU Arbor Day and NCSU Earth Day celebrations here in Raleigh, NC. We all dress in the TIP uniform, spread the word on Tree Improvement, and give out baby pine trees. We usually have a game in tow, but this year we went with props like breeding bags, cone bags full of cones and bottles of pollen (the crowd favorite). It is fun to see the crowds react to what we do and ask a lot of really great questions about the research. A lot of pines went home to good families but no real takers on the bottles of pollen...wonder why???



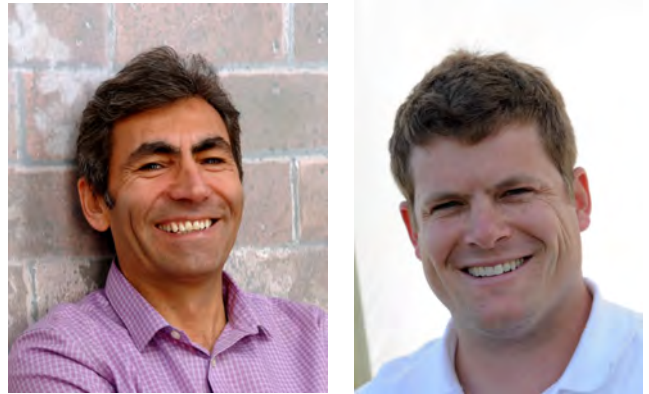
Right: TIP grad students Greg Albert, Jesse Spitzer, Graham Ford, and Laura Townsend were REALLY happy to be out on a Saturday morning teaching the masses about Tree Improvement at the Arbor Day celebration.

Staff

This year proved to hold big changes in the staff. Saul Garcia left the program back in September 2012 and Jim Grissom followed in May 2013. We wish them the best in future endeavors and appreciate their contribution to the program over the years. We also lost a long time temporary employee that kept the boys in check and the baby trees happy and growing. In April, Maria Wilkes also left to join her husband in another part of the state, we will miss her hard work and her wonderful baking! The remaining staff have stepped in to fill the gaps for those folks we lost over the year, but a search for a permanent Data Analyst is underway. We also continue to have a great crop of undergraduate students to help assist the staff during the year, several new faces among familiar ones.

We have two big university award winners on our team this year in Fikret and Josh. Dr. Fikret Isik was part of the inaugural group of University Faculty Scholars, a recognition and reward program for top NC State early- and mid-career faculty who will receive university donor funding for each of the next five years to support their academic endeavors. Josh Steiger was awarded the University Award for Excellence in the College of Natural Resources. His nomination will move to the university level for university wide awardees, and potentially all the way to the Governor's mansion for the Governors award. We are super proud of these folks and are thrilled they have been recognized for their contributions to the university over the years.

*Left to right: Big TIP winners,
Dr. Fikret Isik and Josh Steiger*



Visitors

This has been a busy year for visitors here in Tree Improvement. We were able to utilize their expertise for help with data analysis and in turn teach them about Tree Improvement here in the southeast US. Three particular visitors stayed for an extended period with us throughout the year. Dr. Lianfang Li came from Southwest Forestry University in China for a 3 month tour; she helped assist on data analysis on the Interplanting Study at the Hofmann Forest. Dr. Fatih Temel from Coruh University in Turkey visited for 3 months as well, and assisted on analyzing PMX trials for the Cooperative. Samuel Egbäck joined us in January for 5 months on the Nicholson Exchange Program. He is working on his PhD at the Swedish University of Agricultural Sciences and had the honor of helping the staff select the first 4th-cycle selection and got the full Arrowhead breeding experience this Spring. We really enjoyed hosting all of these folks throughout the year!



*From L to R: Dr. **Fatih Temel** (Coruh University, Turkey)*

***Dr. Lianfang Li** (Southwest Forestry University, China)*

***Samuel Egbäck** (Swedish University of Agricultural Sciences, Sweden)*

Teaching

Faculty associated with the Cooperative teach a wide array of courses. Steve McKeand and Ross Whetten teach FOR 411 (Forest Tree Genetics and Biology), our undergraduate tree improvement course, and FOR 725 (Forest Genetics). Ross also teaches FOR 350, Professional Development III: Ethical Dilemmas in Natural Resource Management, and BIT 815, Deep Sequencing Data Analysis. Fikret Isik's and John Frampton's class, NR554 - Introduction to Data Analysis, continues to be popular and a "must take" course for grad students. Fikret developed and taught FOR 726 Advanced Quantitative Genetics and Breeding in 2012. The course is interdepartmental (Forestry, Crop science, Animal science). Dr. Jim Holland from Crop Science joined Fikret to teach in fall 2012. Ross Whetten, Chris Maltecca (Animal science) and Gary Hodge were guest lecturers.

Graduate Students

Tree Improvement students Funda Ögüt, Graham Ford, and Josh Steiger finished up their research and degrees this year. Funda has a postdoc with Dr. Jim Holland here at NC State in Crop Science, and Graham is continuing with Tree Improvement to get his PhD. Josh of course is continuing his vital role with the Cooperative as a research assistant. We are proud of all these folks and their outstanding accomplishments. We also welcomed several new students this year to continue the tree improvement research.



Jesse Spitzer

Greg Albert

David Barker

Laura Townsend

Alfredo Farjat

Graham Ford

Greg Albert, MS - Greg joined the group in Spring of 2012 and will be working at SETRES2. His research is, "Effects of genetic provenance and nutrition on fine root biomass and turnover in loblolly pine".

David Barker, PhD Candidate – David is currently employed with Rayonier, Inc. as the Seed Orchard manager in Georgia. He is fervently writing and plans to defend in the fall. His project is titled, "Genetic differences in loblolly pine for conversion efficiency for cellulosic ethanol".

Alfredo Farjat, PhD - Alfredo is continuing his efforts on the PINEMAP project titled "The effect of climatic variables on pine growth". He and his wife welcomed a baby girl, Violeta, in February 2013.

Graham Ford, PhD - Graham defended his Masters thesis in October and graduated in December 2012. He decided to continue with TIP and began his PhD in November and is pushing through classes. He is currently undecided on research so stay tuned.

Jesse Spitzer, MS – Jesse joined us in the Fall of 2012. His research is titled "Broad-Based Testing Compared to Local Testing of Families of Loblolly Pine for Resistance to Fusiform Rust".

Laura Townsend, MS - Laura signed on in June of 2012 and is working in the lab on PINEMAP objectives. Her research is titled "Genomic analysis of loblolly pine adaptability to climate conditions".

Membership in the NCSU Cooperative Tree Improvement Program

This year has proven to be another busy year for membership. We welcomed The American Chestnut Foundation and Arauco-Bioforest SA in as Research Associate Members. The big news came in the form of a new Full Member; International Forest Company moved from the Contributing Membership class to become a Full Member. IFCo hit the ground running, taking on full in-kind breeding and testing responsibilities and just in time for starting the 4th cycle. With the push on the 4th-cycle strategy, recruiting efforts were limited, but we hope that potential new members will be just as excited as we are about the future of the program and opportunities to contribute to and benefit from our work. As always, we appreciate the support of our research and breeding activities by our Cooperative partners listed below.

Full Members

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

Research Associate Members

American Chestnut Foundation
 Arauco-Bioforest, SA
 Biofuels Center of North Carolina
 US Forest Service

Contributing Members

American Forest Management, Inc.
 Dougherty & Dougherty Forestry Services, Inc.
 F&W Forestry Services, Inc.
 Jordan Lumber & Supply Company
 Milliken Forestry Company, Inc.
 Molpus Woodlands Group, LLC
 North Carolina Natural Resources Foundation
 Pacolet Milliken Enterprises, Inc.
 ProFOR Consulting
 Resource Management Service, LLC
 Scotch Land Management, LLC
 South Carolina Forestry Commission
 The Campbell Group, LLC
 Timberland Investment Resources, LLC
 White City Nursery, LLC
 Z.V. Pate, Inc.

Publications of Special Interest To Members (2010-2013)

From our inception in 1956, there have been over 900 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>

2013

- Alberto, F., S. Aitken, R. Alia, S.C. González-Martínez, H. Hanninen, A. Kremer, F. Lefèvre, T. Lenormand, S. Yeaman, R.W. Whetten, O. Savolainen. 2013. Evolutionary response to climate change - evidence from tree populations. *Global Change Biology*. In press.
- Frampton, L.J., F. Isik, D.M. Benson. 2013. Genetic variation in resistance to *Phytophthora cinnamomi* in seedlings of two Turkish *Abies* species. *Tree Genetics & Genomes*. 9:53-63, DOI 10.1007/s11295-012-0529-0.
- Nelson, C.D., G.F. Peter, S.E. McKeand, E.J. Jokela, R.B. Rummer, L.H. Groom, and K.H. Johnsen. 2013. Pines. P.427-459, In: B.P. Singh (ed.) *Biofuel Crops: Production, Physiology and Genetics*. In press.
- Ögüt, F., C. Maltecca, R.W. Whetten, S.E. McKeand, F. Isik. 2013. Genetic analysis of diallel progeny test data using factor analytic linear mixed models. *For. Sci.* In press.
- Xiong, S.J., S.E. McKeand, R.W. Whetten, and F.T. Isik. 2013. Genetics of stem forking and ramicorn branches in a cloned loblolly pine family. *For. Sci.* In press.
- Zapata-Valenzuela, J., R.W. Whetten, D.B. Neale, S.E. McKeand, F. Isik. 2013. Genomic estimated breeding values using genomic relationship matrices in a cloned population of loblolly pine. *G3:Genes, Genomes, Genetics*. doi:10.1534/g3.113.005975.

2012

- Aspinwall, M.J., J.S. King, and S.E. McKeand. 2012. Productivity differences among loblolly pine genotypes are independent of individual-tree biomass partitioning and growth efficiency. *Trees - Structure and Function*. DOI 10.1007/s00468-012-0806-4.
- Aspinwall, M.J., S.E. McKeand, and J.S. King. 2012. Carbon sequestration from 40 years of planting genetically improved loblolly pine across the southeast United States *For. Sci.* (published online April 12, 2012; <http://dx.doi.org/10.5849/forsci.11-058>).
- Cumbie, W.P., F. Isik, and S.E. McKeand. 2012. Genetic improvement of sawtimber potential in loblolly pine. *For. Sci.* 58:168–177.
- Espinoza, J.A., H.L. Allen, S.E. McKeand, and P.M. Dougherty. 2012. Stem sinuosity in loblolly pine with nitrogen and calcium additions. *For. Ecol. and Manag.* 265: 55–61.
- Isik, F., H.V. Amerson, R.W. Whetten, S.A. Garcia, and S.E. McKeand. 2012. Interactions of Fr genes and mixed-pathogen inocula in the loblolly pine-fusiform rust pathosystem. *Tree Genetics & Genomes*. 8(1):15-25.
- McKeand, S. and R. Pohl. 2012. The Arrowhead Tree Breeding Center, \$483 million present value to the citizens of Georgia and the South. *Georgia Forestry Today*. 8(1): 24-29.
- Zapata-Valenzuela, J., F. Isik, C. Maltecca, J. Wegrzyn, D. Neale, S. McKeand, and R. Whetten. 2012. SNP markers trace familial linkages in a cloned population of *Pinus taeda* – prospects for genomic selection. *Tree Genetics and Genomes* DOI 10.1007/s11295-012-0516-5.

2011

- Aspinwall, M.J., J.S. King, F.L. Booker, and S.E. McKeand. 2011. Genetic effects on total phenolics, condensed tannins and non-structural carbohydrates in loblolly pine (*Pinus taeda* L.) needles. *Tree Physiology* 31: 831-842, doi: 10.1093/treephys/tpr073.

- Aspinwall, M.J., J.S. King, J-C. Domec, S.E. McKeand and F. Isik. 2011. Genetic effects on transpiration, canopy conductance, stomatal sensitivity to vapor pressure deficit, and cavitation resistance in loblolly pine. *Ecohydrology*. Published online in Wiley Online Library (wileyonlinelibrary.com) DOI: 10.1002/eco.197.
- Aspinwall, M.J., J.S. King, S.E. McKeand, and B.P. Bullock. 2011. Genetic effects on stand-level uniformity, and above - and belowground biomass production in juvenile loblolly pine. *Forest Ecology and Management*. 262: 609–619.
- Aspinwall, M.J., J.S. King, S.E. McKeand, and J-C. Domec. 2011. Leaf-level gas-exchange uniformity and photosynthetic capacity among loblolly pine (*Pinus taeda* L.) genotypes of contrasting inherent genetic variation. *Tree Physiology*. 31: 78-91.
- Cumbie, W.P., F. Isik, B. Li, and B. Goldfarb. 2011. Effects of propagule type on genetic parameters of wood density and growth in a loblolly pine progeny test at ages 10 and 11 years. *Tree Genetics & Genomes*, 7:1147–1158, DOI 10.1007/s11295-011-0402-6.
- Cumbie, W.P., A.J. Eckert, J.L. Wegrzyn, R.W. Whetten, D.B. Neale, and B.Goldfarb. 2011. Association genetics of carbon isotope discrimination, height, and foliar nitrogen in a natural population of *Pinus taeda* L. *Heredity* advance online publication 19 January 2011; doi: 10.1038/hdy.2010.168.
- El-Kassaby, Y.A., S. Mansfield, F. Isik, and M. Stoehr. 2011. In situ wood quality assessment in Douglas-fir. *Tree Genetics & Genomes*, 7:553-561. DOI: 10.1007 /s11295 - 010-0355-1
- Grissom, J.E., J.H. Roberds, C.D. Nelson, F.T. Isik, S.E. McKeand, and R. Rousseau. 2011. Genetic variation in a long-leaf pine population: a long-term field study of a 13-parent diallel. P. 51-53. In: Proc. 31th Southern Forest Tree Improvement Conf., Biloxi, MS. <http://www.sftic.org>.
- Isik, F., C.R. Mora and L.R. Schimleck. 2011. Genetic variation in *Pinus taeda* wood properties predicted using non-destructive techniques. *Annals of Forest Science* 68:283–293, DOI 10.1007/s13595-011-0035-9
- Isik, F., R. Whetten, J. Zapata-Valenzuela, F. Ogut, and S. McKeand. 2011. Genomic selection in loblolly pine - from lab to field. *BMC Proceedings*, 5(Suppl 7):I8 <http://www.biomedcentral.com/1753-6561/5/S7/I8>
- Mullin, T.J., Andersson, B., Bastien, J.-C., Beaulieu, J., Burdon, R.D., Dvorak, W.S., King, J.N., Kondo, T., Krakowski, J., Lee, S.D., McKeand, S.E., Pâques, L., Raffin, A., Russell, J., Skråppa, T., Stoehr, M., and Yanchuk, A.D. 2012. Economic importance, breeding objectives and achievements. Chapter 2 In: *Genomics of Conifers*. Edited by: C. Plomion, J. Bousquet, and C. Kole. Volume in *Genetics, Genomics and Breeding of Crops Plants*. Science Publishers, an imprint of Edenbridge Ltd., Enfield, NH, USA. 456p.
- Sherrill, J.R., B.P. Bullock, T.J. Mullin, S.E. McKeand, and R.P. Purnell. 2011. Total and merchantable stem volume equations for mid-rotation loblolly pine (*Pinus taeda* L.). *South. J. App. For.* 35:105-108.

2010

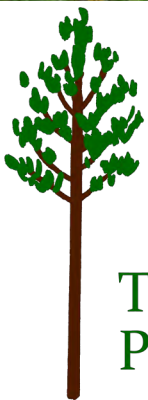
- Aspinwall, M.J., B. Li, S.E. McKeand, F. Isik, and M.L. Gumpertz. 2010. Prediction of whole-stem α -cellulose yield, lignin content, and wood density in juvenile and mature loblolly pine. *South. J. Appl. For.* 34:84-90.
- Dougherty, D., R. Bryant, H. Burkhart, P. Dougherty, S. Jones, S. McKeand. 2010. Valuing tomorrow's loblolly pine plantations today. *Forest Landowner*. Jan./Feb. 2010: 19-21.
- Eckard, J.T., F. Isik, B. Bullock, B. Li, and M. Gumpertz. 2010. Selection efficiency for solid wood traits in *Pinus taeda* using time-of-flight acoustic and micro-drill resistance methods. *For. Sci.* 56(3), 233-241.
- McKeand, S., P. Cumbie, and B. Abt. 2010. Investment in genetically improved loblolly pine – landowner benefits today and for generations to come. *Forest Landowner*. Jan./Feb. 2010: 27-29.
- Nelson, C.D., T. L. Kubisiak, and H. V. Amerson. 2010. Unravelling and managing fusiform rust disease: a model approach for coevolved forest tree pathosystems. *For. Path.* 40: 64–72.
- Whetten, R.W. and R. Kellison. 2010. Research gap analysis for application of biotechnology to sustaining US forests. *J. Forestry* 108(4):193-201.
- Xiong, S.J., F. Isik, S.E. McKeand and R.W. Whetten. 2010. Genetic variation of stem forking in loblolly pine. *For. Sci.* 56: 429-436.



The most exciting development in the program this past year was the initiation of our fourth cycle of breeding. Included in this report are the plans and details of the new breeding strategy, but the cover captures the action shots that made this first year of the 4th cycle a successful one.

Front Cover: Steve McKeand (TIP) and Josh Sherrill (Rayonier) making a 4th-cycle selection.

Back: (Clockwise) Chuck Little topgrafting, TIP Staff and students (and 4th-cycle mascot, Bear) at Arrowhead Breeding Center, Jadie Andrews (TIP) fishing for scion from a new selection, TIP staff topgrafting into evening hours.



TREE IMPROVEMENT PROGRAM

NC STATE UNIVERSITY

