

*North Carolina State University Cooperative  
Tree Improvement Program  
53<sup>rd</sup> Annual Report*



*Department of Forestry and Environmental Resources  
College of Natural Resources - May 2009*

**NC STATE UNIVERSITY**

## EXECUTIVE SUMMARY

### SELECTION, BREEDING, AND TESTING

The Arrowhead Breeding Center (ABC) at the Georgia Forestry Commission's Arrowhead Seed Orchard is off to a great start. Funds from our new Contributing Members are the primary source for work that was started in 2008. Breeding on the Sawtimber Elite Population was started at the ABC as well at cooperators' breeding facilities.

Rooted cuttings for the clonal tests Atlantic Coastal Elite (ACE) Population should be ready to go to field trials in the winter of 2009-10. Over 30,000 cuttings were taken this past winter, and another 30,000 cuttings will be stuck in June. Progress has been excellent.

Mainline breeding and testing continues to progress, with 65% of the total PMX breeding and 30% of the full-sib breeding complete. 70 PMX tests have been established throughout the Southeast.

BIG BLUP is finished for the Coastal population and will soon be complete for the Piedmont and Northern data.

### SEED PRODUCTION

Almost 13 tons of loblolly pine seed were harvested by Cooperative members in 2008. Second-generation orchards contributed about 57% of total seed, and coastal orchards accounted for 80% of total seed production. Third-cycle orchards are producing a significant amount of seed, currently 25% of the total.

### RESEARCH

The Cooperative is participating in research projects to study the effects of wood properties on the yield of fermentable sugars for cellulosic ethanol production. Wood cores from 178 clones (1668 sample trees) have been analyzed using NIR to identify interesting clones to measure yields of fermentable sugars. Results are expected by mid 2010.

The Conifer Translational Genomics Network (CTGN) continues to be a major effort for staff and students. To date about 1200 foliage samples have been sent to UC-Davis for DNA isolation. Phenotyping numerous populations for the association genetics project has been a big activity.

A selection index to improve sawtimber potential using volume, rust incidence, stem form and forking has been developed.

The response of seedlings that carry major genes for fusiform rust resistance (r or R alleles) to 10 different bulked fungus inocula were investigated in six families. Significant R type by inoculum source was found in some families, but seedlings with the susceptible r alleles were highly susceptible to rust infection.

Substantial genetic and fertilizer effects on stem form, particularly sinuosity have been found, emphasizing the need to match genetics and silvicultural systems to have both good production and stem quality.

Clonal variation for 2-year height and water use efficiency using  $\delta^{13}\text{C}$  was highly significant. Height was positively correlated with  $\delta^{13}\text{C}$  (0.38+/-0.25) suggesting that as the trees restrict water loss they are limiting growth. Association testing revealed a total of 161 SNPs that were significantly associated ( $p<0.05$ ) with  $\delta^{13}\text{C}$  variation and 79 SNPs associated ( $p<0.05$ ) with height variation before correction for multiple testing

### ASSOCIATED ACTIVITIES, GRADUATE STUDENTS, COOPERATIVE STAFF, MEMBERSHIP

The 12 graduate students funded by the Cooperative all made significant progress in their programs. Jesus Espinoza completed his PhD in 2009. Jesus is a post-doc with CAMCORE.

The staff remained stable this year. Kimberly Dawson joined us for 6 months as a temporary office assistant. J.B. Jett retired as Associate Dean and returned home to Tree Improvement as an Emeritus Professor.

Full membership remained at 12, and there are 9 Contributing Members with 4 joining since the 2008 Annual Report was written. We are optimistic that more will join in the coming months.

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## TREE IMPROVEMENT COOPERATIVE HAPPENINGS

Cellulosic ethanol, SNPs, breeding center, stimulus package, BIG BLUP, biofuel feedstocks, housing starts, translational genomics, logic models, near infrared spectroscopy, association genetics, ARRA, gasification – all words and phrases that a few years ago never entered into tree improvement jargon. Today, we use them in daily conversations about tree breeding and plans for the future. Fortunately words and phrases like genetic gain, seed orchards, sawtimber, breeding strategies, disease resistance, productivity, mating designs, silviculture, clonal forestry, value, and economic impacts still reverberate in those same conversations. Yes, the world has flipped upside down with the horrific economy and the devastating impacts on forestry and the forest products industry, but the old saying “the more things change, the more things stay the same” still holds true. Our tree improvement program is still committed to providing value to our long time members, our new contributing members, to the forest landowners throughout the South, and to stakeholders and interest groups far and wide. Our mission statement:

*To Increase Value to Landowners and Citizens Through Continuous  
Genetic Improvement of Forest Trees*

is still the focus of our daily activities. As you read this Annual Report, I hope you will agree that the Cooperative staff, students, administrators, and members are committed to increasing values to our customers and clients.

Two very significant activities exemplify our commitment to forestry and improving value to landowners. The Sawtimber Elite Population (SEPop) is a major emphasis of the entire Cooperative. The Raleigh staff has spent many hours in the planning and implementation of SEPop (details p. 2 and front cover picture), but this is nothing compared to the effort that members have put forth. The years of breeding, testing, and selection by cooperators allowed us to identify the best of the best for sawtimber potential. These trees are being bred at our new Arrowhead Breeding Center (ABC) and by Cooperators at their own breeding orchards. In addition to the selections that are shared by all members, “internal selections” from individual company breeding programs were also contributed to the SEPop effort. These selections were developed by companies in their own breeding programs, and cooperators were under no obligation or expectation to share this germplasm. They did see the benefit to their own programs as well as to the Cooperative and have contributed these high-value selections. Yes, the cooperative spirit and energy still is strong in our program.

The other significant activity is our clonal testing effort with the Atlantic Coastal Elite (ACE) population (details p. 3 and back cover). Approximately 60,000 rooted cuttings have been or will be produced here in Raleigh by the Cooperative staff this year. This is a major shift in the *modus operandi* of the program. We felt that the benefits to be gained from clonal testing were so great, that we should use our expertise and capabilities to increase genetic gain and value for our customers. We benefitted from our colleagues in the former Rooted Cutting Program who developed the protocols that have been working well. Why do this now and not before? Very simple, funds from our new Contributing Members are to be used to support operational breeding, and we are doing just that with ACE!

The coming months and years will be a challenge with budget cuts from the NC legislature that eventually filter down through the University to our program. Member companies and states are also struggling to meet budgets and maintain programs. We are poised to meet these challenges and emerge as an even stronger program for breeding cycles to come.

*Steve McKeand  
April 2009*



## SELECTION, BREEDING, AND TESTING

### Arrowhead Breeding Center (ABC) and Sawtimber Elite Population (SEPop)

The spring of 2009 marked the second year of activities at the Cooperative's breeding center located at Georgia Forestry Commission's Arrowhead Seed Orchard. Grafting and breeding of the Sawtimber Elite Populations at Arrowhead is the culmination of two major changes in the Cooperative in recent years. First, the Sawtimber Elite Populations (SEPop) are an effort to improve the sawtimber quality of our elite germplasm since a greater emphasis on stem form improvement will lead to an increase in the proportion of sawlogs produced in loblolly pine plantations (see selection index details p. 11). Second, the activities at Arrowhead are a model for the future operating structure within the Cooperative. Since breeding capacity has been reduced, the use of additional funds from the new contributing member class to operate a Cooperative-managed breeding center is a strategic shift in the way the Cooperative will operate for future breeding cycles.

The SEPop are a shift from the typical random mating designs to a combinational breeding idea to increase the probability of producing selections with superior qualities for sawlog production. The most elite selections for sawtimber quality traits were selected for each breeding region. Recent work in the Lower Gulf Elite population revealed that volume, rust resistance, and stem straightness are the most important factors influencing sawtimber grading in standing trees. Rankings in the Performance Rating System were used to select 18 to 30 parents for each breeding zone with various combinations of performance for volume, rust resistance, and straightness.

*Topgrafting & breeding were major efforts at the ABC in 2009.*



Top-grafting of the SEPop, which began in 2008, continued this year, and breeding commenced in all three regional populations. In 2008-2009 a total of 53 selections were top-grafted and 62 bags were installed for 28 full-sib crosses across the 3 breeding zones. Additional crosses are also being added to the SEPop by cooperators.

In addition, approximately five acres of flower-promoting interstock were field-grafted for future topgrafting (see pictures below). We used a selection that is known to graft well and is very effective at promoting both female and male strobili when it is used as an interstock.



*SEPop breeding got off to a start in topgrafts that were made last year. The future breeding orchard is to the right of the current breeding orchard (top photo). Rootstocks were grafted with a flower-promoting selection at the ABC (lower photo).*

GFC has once again been a gracious host to our breeding center efforts. Their collaboration and hospitality will help us accelerate the breeding and testing of this superior germplasm in an efficient and cost-effective way. Once again we want to thank Russ Pohl and his staff for the effort they have put forth to help make this relationship work.



## Atlantic Coastal Elite (ACE) Population

The elite breeding populations in the NC State Tree Improvement Program are intensively managed subsets of the mainline population selected for short-term genetic gain for each of the three geographic regions in the Cooperative. Elite populations are much smaller than the mainline so they can be bred and tested much faster. In each region, these populations are bred as a single population to achieve maximum gain and will be managed mainly to provide improved genetic material for open-pollinated seed orchards, full-sib deployment strategies such as mass controlled pollination, and vegetative propagation for clonal forestry. The elite populations will provide landowners superior trees in as short a time period as possible.

To increase the gain possible from the Coastal Elite Population, clonal testing of the progeny trials will be used to increase the within-family heritabilities and also allow for 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.



In 2008, the Coastal Elite seedlings were screened at the US Forest Service Resistance Screening Center with a very high spore load (50,000 sp/ml) of fusiform rust. Only seedlings that were gall-free are being propagated, increasing the probability of the progeny from these selections being resistant to rust.

Progress on the ACE population has been excellent. This spring, cuttings were collected from ~2400 hedges, representing 51 full-sib families, resulting in an average of 47 hedges per family. Cuttings were also collected from seven checklots and will be used to compare phenotypic traits of the experimental crosses. In all, over 30,000 cuttings were taken and placed in growing medium to induce rooting.

It is still too early to determine the rooting success, but signs thus far are positive. A preliminary screening indicated that rooting percentage will average at least 50%, which will result in over 15,000 trees. In June, cuttings will be taken again from the same crop to produce enough trees for field trials. If all continues to go well, we anticipate establishing clonal trials this coming winter and spring.

*The first cuttings from the ACE population have already started to root (late April 2009). We are cautiously optimistic that rooting will be high and field trials will be established this winter.*



### Third-Cycle Mainline Breeding Progress

Mainline breeding continues to progress, with 65% of the total PMX (pollen mix) breeding complete. An additional 12% is in progress with some seed in hand. Following the reduction in population sizes as described in the Fall 2007 update of the Third Cycle Implementation Plan, the Cooperative is poised to rapidly complete PMX testing.

We are using a complementary design, with pollen mix mating for among family selection and full-sib mating for within-family selection. The numbers of completed crosses are summarized in Table 1. The Coastal region has made the most progress with 80% of PMX breeding and 39% of Mainline Full-sib breeding complete.

Full-Sib progress has continued, but at a slower rate due to the recent changes that placed priority on completing PMX crosses (Table 2). The decision was made to put emphasis on PMX breeding to ensure proper ranking of parents within each subline. These full-sib crosses will generate seeds for within-family selection for the next breeding cycle.

Cooperative members within each region have grafted all parents selected for generating the pollen mix for pollen mix mating. Three third-cycle check seedlots are being created for each of the three regions by using the same 20-parent pollen mix applied to 10 of the selections as females. Checklot crossing has progressed well in all three regions (Table 1), with just a few crosses that have not been completed. Thanks to breeding efforts by full members, checklot seed for the Coastal and Piedmont regions is complete, while the Northern region is approximately 75% complete.

In addition to the third-cycle checklots, several well-characterized families (common family checks) from the first two cycles of breeding are being crossed with the pollen mix. These families will be included in all tests to contribute to estimates of variance components and provide better family comparisons. Nearly all breeding is complete on the common family checks, allowing for more effort to be put towards PMX and Full-Sib breeding.

Progress continues for full-sib controlled crossing made this year although changes in the Fall 2007 Third Cycle Implementation Plan have placed priority on completing PMX crosses (Table 1). Seed production from full-sib cross mating is expected to rise in the coming years as the third-cycle selections produce more flowers. These full-sib crosses will generate seeds for within-family selection for the next breeding cycle.



*Proper field layout of progeny trials has always been a focus of the Cooperative. An excellent example of a well designed test is shown to the left. Severe droughts in 2006 and 2007 caused heavy mortality in this Tennessee Division of Forestry PMX trial – the bad news. The good news is that the mortality was confined to specific blocks in the trial (note the red and orange color on the map that corresponds to the dead trees in the background). This is an excellent example of the benefits of small blocks and the single-tree-plot design being used in PMX tests. We estimate that 9 of the 20 reps can be salvaged and useful data collected.*

Table 1. Summary of 3<sup>rd</sup> Cycle PMX breeding progress, Fall 2008.

Type of crosses	Breeding Region	# Cooperators	# Sublines	Original # Parents	Total Parents w/ Reduction	#Crosses producing Seed	#Crosses of Sufficient Seed	% Done with Reduction
Polycross	Coastal	9	32	390	326	291	260	80%
	Piedmont	8	31	364	294	189	153	52%
	Northern	6	13	182	182	132	107	59%
<b>Polycross</b>	<b>Total</b>	<b>15</b>	<b>76</b>	<b>936</b>	<b>802</b>	<b>612</b>	<b>520</b>	<b>65%</b>
PMX checks	Coastal	9	--	10	--	10	10	100%
	Piedmont	8	--	10	--	10	10	100%
	Northern	6	--	11	--	12	9	75%
PMX CFs	Coastal	9	--	7	--	7	7	100%
	Piedmont	8	--	7	--	7	6	86%
	Northern	6	--	7	--	7	7	100%

#Crosses of Sufficient Seed: A parental cross is counted here if the "total seed requirement" has been met for that cross; i.e. if enough seeds have been produced for that cross to establish a full test series.

PMX checks: Polycrossed checklot mixes

PMX CFs: Polycrossed Common Family checks

Table 2. Summary of 3<sup>rd</sup> Cycle Full-Sib Progress, Fall 2008.

Type of crosses	Breeding Region	# Cooperators	# Sublines	Total # of Crosses	Total Parents w/ Reduction	#Crosses producing Seed	#Crosses of Sufficient Seed	% Done Crosses
Full-Sib	Coastal	9	30	317	--	*	122	39%
	Piedmont	8	28	247	--	*	54	22%
	<u>Northern</u>	<u>5</u>	<u>13</u>	<u>146</u>	--	*	<u>36</u>	<u>25%</u>
<b>Full-Sib</b>	<b>Total</b>	<b>15</b>	<b>71</b>	<b>710</b>	--	*	<b>212</b>	<b>30%</b>

### Third-Cycle Elite Breeding

The expected gains from the Elite Breeding programs make it clear that getting this breeding underway is very important, and the cooperators have made great strides in accomplishing this task. With this tremendous effort, the Coastal region was completed in 2007 and clonal production is now underway. Over the past year, the Piedmont and Northern regions have progressed nicely, with 35% and 38% completed, respectively.

### Third-Cycle Progeny Testing

#### Polycross tests

Since the winter of 2004/2005, Cooperative members have installed 62 PMX tests across all 3 breeding regions. In the fall/winter of 2008-2009, a total of 4 tests were established in Coastal region. In addition, two checklot comparison tests were established to link 2nd Cycle checklots to the 3rd Cycle checklots. The establishment of



these two tests completes the checklot comparisons for the 3rd Cycle. In March 2009, seed was sent out to cooperators for the establishment of PMX tests in 2009-2010. A total of seven tests were sent to Cooperative members. This seed will be sown and seedlings will be established to be put into the field.

Thanks to all the Cooperative members for their efforts to get these tests in the ground!

**Full-Sib controlled cross tests**

Full-Sib controlled cross tests are the source of selections for the Program as it moves forward. By ranking the selections through testing in the PMX tests, the full-sib crosses are designed to optimize the probability of finding superior trees.

As a result of priority being put on PMX tests, full-sib progress has slowed somewhat. However, in March of 2009, enough seed was sent out for the establishment of thirty-six full-sib block plots. Each member is encouraged not to delay the installation of these tests. Once enough seed is collected from a few crosses, block plots can be installed.

Table 3. Third-cycle progeny tests established through 2010.

Region	Test Series	2004-2005	2005-2006	2006-2007	2007-2009	2009-2010	Total
Coastal	CPMX1	15					15
Coastal	CPMX2		5	5			10
Coastal	CPMX3			5	5		10
Coastal	CPMX4				8		8
Piedmont	PPMX1		3	5			8
Piedmont	PPMX2				8		8
Piedmont	PPMX3					3	3
Northern	NPMX1				4		4
Northern	NPMX2					4	4

**BLUP Analysis of Progeny Test Data**

Since late 2007, the Cooperative staff has been working on the ‘Big BLUP’ project. The name derives from the statistical technique used, Best Linear Unbiased Prediction (BLUP), which is at the core of this new analytical protocol for the Cooperative’s progeny test data. The first release of results was completed for the Coastal Plain (CP) region in April 2009. Breeding values are provided for Cooperative members, for parent trees originating and field-tested in the Coastal Plain

areas geographically ranging from the Carolinas to Florida and westward to Mississippi.

The reasons for changing the standard data analyses for Co-op progeny test data were outlined in the 52nd Annual Report (2008). A major deciding factor has been recent developments in analytical software specifically designed for quantitative genetic test analysis. With improvements in computer power and software such as ASReml program, solving large mixed model equations for genetic analysis has become feasible. Some of the technical advantages of the ‘Big BLUP’ method are:

- BLUP can integrate large sets of data from different sources, such as multiple mating designs (open-pollinated, polymix, control-pollinated), test series, and multiple testing regions and generations of genetic selections.
- The information available from all relatives across test series or generations will be utilized in the BLUP analysis. For example, breeding values of 7-56 will be based on all the relatives of that parent tested in different test series and generations.
- Connection between genotypes across test series or generations will be enhanced using the pedigree relationships as well as common checklots.
- The new BLUP method can better analyze categorical traits (e.g. tree form classes, forking, and fusiform rust infection) using generalized linear mixed models.

The first database release for the Coastal Plain region provides breeding values and genetic gain

estimates for 1779 parent trees and 3362 controlled crosses. These values are derived from a robust, wide array of testing designs across a broad range, both geographically and temporally, over a number of years and decades of field-testing. The next steps are to generate BLUP breeding values for selections of the Piedmont and Northern testing regions, and to incorporate information on deployment of material.

With the new protocol, the Cooperative will be reevaluating progeny test data on a regular basis. As new data become available, breeding values can be recalculated and updated each year. These new breeding values and genetic gain estimates will allow us to better compare selections that have been made over the past decades. In addition, they will be very useful for our cooperators, landowners, and foresters as a tool for selecting the most valuable genotypes to deploy in their planting programs. Ultimately, we will incorporate these new breeding values into the Loblolly Pine Performance Rating System (LP-PRS) that is proving to be a useful and practical tool for our cooperators and customers for genetically superior seedling marketing.



*Jim Grissom and Fikret Isik have been the brains of the BIG BLUP operations. Both spent many daylight and nighttime hours working on the analyses. We owe them a big thanks for all their effort.*



## CONE AND SEED YIELDS

Nearly 13 tons of loblolly pine seed were harvested by Cooperative members in 2008, substantially lower than the 2007 harvest of 19 tons (Table 1). The 2008 harvest is the lowest since 1980 when 7.9 tons of seed were collected. A declining seedling market in the South and a late spring freeze in 2007 are likely contributors to the lower harvest. Coastal seed harvests for 2008 were similar to 2007 for first- and third-generation seed but dropped substantially for second-generation seed. For the Piedmont region, there was an increase in the harvest of second- and third-generation seed, and seed collected for the Northern region decreased. The 2008 average seed yields per bushel were slightly lower than 2007 seed yields. Second-generation orchards contributed about 57% of total seed, and coastal orchards accounted for 80% of total seed production. Third-cycle orchard production has increased to 25% of the total amount of seed produced, up from 12% in 2007. As yields from these young orchards continue to increase, substantial improvement in growth and quality will be realized in the coming years.

Table 1. Comparison of 2008 seed and cone yields with previous year.

Provenance	Bushels Of Cones		Pounds Of Seed		Pounds per Bushel	
	2008	2007	2008	2007	2008	2007
Coastal 1.0	3117	2915	4353	4027	1.40	1.38
Coastal 2.0	7281	12839	11314	20291	1.55	1.58
Coastal 3.0	3421	3715	4419	3996	1.29	1.08
Piedmont 1.0	16	21	27	40	1.69	1.90
Piedmont 2.0	2043	1510	2786	2106	1.36	1.39
Piedmont 3.0	1584	391	1958	522	1.24	1.33
Northern 2.0	253	3463	340	6387	1.34	1.84
Totals	17715	24855	25196	37369	1.47	1.51

Annual seed yields have varied over the years due to regeneration needs, changes in membership within the Cooperative, environmental factors, and growth and technology within the industry (Figure 1). For the last 7 or 8 years, cooperators have produced sufficient quantities of improved seed, and therefore have been making selective harvests of about 20 tons of seed per year. From 1968 to 2008, over 2.2 million pounds of improved seed have been produced by Cooperative members. At 12,000 seedlings per pound, this is enough seed to grow over 26 billion improved seedlings. We look forward to greater production from advanced generation seed orchards in the near future!

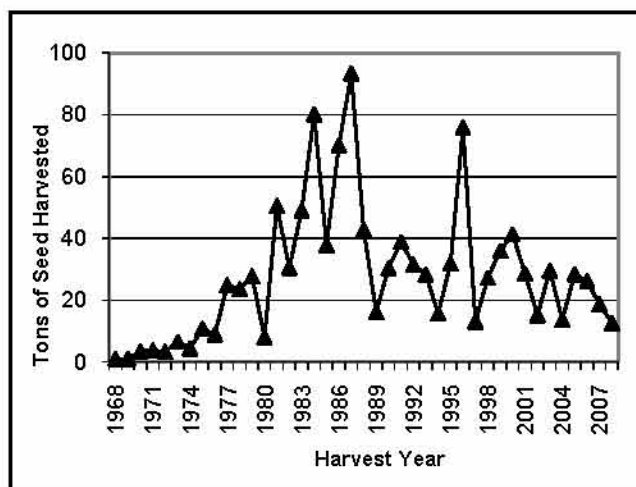


Figure 1. Annual seed yields from 1968 to 2007.



*Orchard managers are routinely using topgrafting for crown replacement to get seed production of the very best selections as early as possible. Chuck Little of the Georgia Forestry Commission hopes for good success with this Coastal selection topgraft.*

## RESEARCH

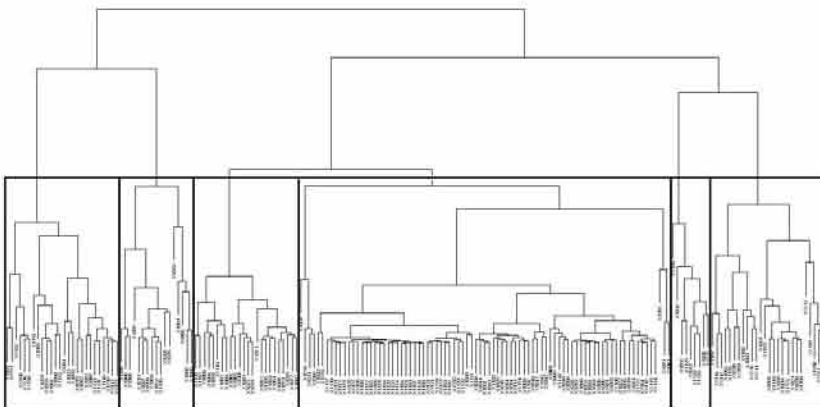
### Biofuels Feedstock Research Initiative

Recent emphasis on development of alternatives to fossil fuels for both generation of electricity and for liquid transportation fuels has created opportunities for landowners interested in alternative markets for pine biomass. Research is needed to learn more about how genetic variation in wood properties might affect the efficiency of converting pine biomass into liquid fuels, and to develop cost-effective strategies for establishment and maintenance of biomass plantations.

**Funded Research** The Cooperative is participating in a research project funded by the College of Natural Resources and the Biofuels Center of North Carolina and led by investigators from the Department of Wood and Paper Science, to study the effects of wood properties on the yield of fermentable sugars for cellulosic ethanol production. The experimental plan will use three different pre-treatments of wood chips to remove lignin and make the cellulose available for enzymatic hydrolysis, and compare the yields of fermentable sugars from twenty wood samples with different wood properties. The wood samples include juvenile wood and mature wood of mixed trees collected from Schenk Forest as examples of typical currently-available biomass, and a set of clonal varieties provided by Plum Creek and CellFor from eight-year-old field tests. David Barker, an MS graduate student with the Cooperative, organized collecting trips last fall to collect wood cores from 1668 trees in three test

locations in South Carolina and Georgia, then ground half of each core in a Wiley mill and carried out NIR spectroscopy to obtain data useful for predicting wood properties. The NIR spectral data (700 wavelengths for each of 1668 samples) were exported as text files and used as phenotypic data in a BLUP analysis carried out by Ross Whetten, to estimate the genetic components of the NIR spectra for more accurate prediction of wood properties.

In parallel, the NIR spectra were also used to predict lignin and cellulose content of each wood sample, with the assistance of Dr. Gary Hodge of CAMCORE. The BLUP values estimated for the 700 wavelength for each of 178 clonal varieties were used to create a correlation matrix showing which trees had similar NIR spectra and which were different, and that matrix was then analyzed by hierarchical clustering to produce a dendrogram showing relationships between the wood properties of the 178 clones. Six clusters were identified (Figure 1), and three clones from each of the six clusters were selected for whole-tree harvesting to provide wood samples for analysis of fermentable sugar yields by our collaborators in the Department of Wood and Paper Science. Three different ramets of each clonal variety were harvested, and the wood samples from different ramets will be kept separate to allow determination of environmental effects on wood properties variation as well as estimation of genetic parameters. The experiments to measure yields of fermentable sugars are now underway, and results are expected by mid 2010.



*Figure 1. Cluster analysis of 178 clones with 6 major clusters outlined in black. The clusters are of different sizes, as would be expected from a normal distribution.*





*Cores from 1668 trees in the Plum Creek / CellFor clonal trials were collected and analyzed for wood properties associated with ethanol production.*

## **Conifer Translational Genomics Network – progress report**

The CTGN project began in September 2007, with the overall goal of moving marker-assisted breeding into practice in the four public forest tree breeding cooperatives in the US. The NC State and Western Gulf breeding programs will be working with loblolly pine, the CFGRP (Florida) program will be working with both loblolly and slash pine, and the PNWTIRC at Oregon State will focus on Douglas fir. Our objective for the first part of the project is to collect foliage samples from a total of 2500 trees in the TIP breeding program and supply them to the UC Davis research group, who will isolate DNA from the samples and carry out analysis of genetic variation at about 7000 loci previously identified as single-nucleotide polymorphisms (SNPs). Phenotypic information will be analyzed together with the SNP genotype information to search for SNP alleles associated with particular phenotypes of interest to TIP.

Other objectives of the project are to develop new methods for integrating such genotype information into the breeding, testing and selection cycle, and to provide opportunities for other members of the tree

breeding community, including graduate students and stakeholders in the four cooperatives, to learn more about the technology and the statistical methods used for these analyses.

To date, about 1200 foliage samples have been sent to UC-Davis for DNA isolation. Unforeseen complications with the tissue storage method were identified early enough to correct the method, but not before about 10% of materials became unusable. These samples will either be re-collected, or if the genotypes involved are not of particularly unique value to the project, simply replaced with other genotypes that will provide comparable or greater value to the experiments.

As part of the efforts underway for the CTGN, Fikret Isik and Ross Whetten are supervising a graduate student, Ms. Funda Ögüt, in a project to carry out simulation of genotype and phenotype data in experimental populations typical of tree breeding programs. These simulated datasets will then be analyzed using various software programs to gain experience with how differences in population structure and genetic parameters affect the ability to detect associations between molecular markers and phenotypes of interest.



## A Selection Index for Improving Sawtimber Quality

The Cooperative has recently emphasized improvements in sawtimber quality due to the increased economic importance of sawlogs in southeastern US timber markets. Earlier work in loblolly pine demonstrated that volume was the primary trait for improving value. However, analyses of the Lower Gulf Elite population (LGEPop) suggested that additional gains in sawtimber quality were possible if a greater emphasis were placed on stem form. Genetic parameters have been estimated in the Lower Gulf Elite population for growth, branching, and form characteristics. Results suggested that the improvements in volume, fusiform rust disease, and stem straightness have improved sawtimber quality in the populations developed within the NCSUCTIP.

The Lower Gulf Elite diallel tests showed favorable correlations among height, diameter, volume, sweep, and rust disease with the sawtimber potential trait (Table 1). Multiple regression was then used to determine the traits that significantly impacted sawtimber potential; volume, rust incidence, sweep, and forking were the most important. This data set offered a unique opportunity to implement a selection index for the improvement of both growth and tree form within an elite population of loblolly pine. The objective of this study was to compare the potential gains in sawtimber quality by selecting on volume and quality traits with different weights.

In the LGEPop, there were six disconnected diallels each with eight parent trees resulting in 128 crosses. Each diallel was comprised of 4 Atlantic Coastal Plain selections, 3 Florida source selections, and 1 Western Gulf selection. Four tests established in Alabama, Florida, and Georgia were used for this study. Each test had 20 blocks and a single-tree-plot design. A total of nine traits were measured at age six in the LGEPop diallel tests: height, dbh, rust incidence, forking, sweep, branch angle, branch diameter, branch frequency, and sawtimber potential (on a 1-4 scale with 1=high sawtimber potential, 4=cull). Genetic analyses of six-year

growth traits and stem form traits were analyzed using the ASReml statistical software package. Phenotypic and additive variances and covariances of traits were used to create a selection index. A selection index is a regression tool that allows the simultaneous improvement of multiple traits in a population. It utilizes the genetic and phenotypic variances of each trait and economic weights to maximize genetic gain in the traits of interest.

The economic weights are typically the economic values of improving a trait by one unit. However, the value of unit increases has not been quantified in loblolly pine, therefore we employed a set of relative weights to compare the response to selection. Three indices were compared:

- 1) Weight volume as the only economically important trait;
- 2) Weight traits according to the coefficients from previous multiple regression efforts: volume = 3, rust = -1, sweep = -1 and forking = -1; and
- 3) Weight sawtimber potential only.





Table 1. Additive Genetic Correlations Among 6-Year Growth and Stem Quality Traits

	Height	DBH	Volume	Sweep	Rust	Fork	BA	BD	BF	Saw
Height	*	0.87	0.88	0.05	-0.03	0.42	-0.05	0.45	0.56	-0.77
DBH		*	0.97	0.23	0.05	0.29	-0.27	0.63	0.23	-0.71
Volume			*	0.18	0.00	0.32	-0.28	0.56	0.33	-0.67
Sweep				*	0.26	0.16	-0.27	0.36	0.14	0.39
Rust					*	0.18	0.13	0.45	0.09	0.41
Fork						*	0.29	-0.07	0.27	0.05
BA							*	-0.46	0.01	0.03
BD								*	0.37	0.03
BF									*	-0.01
Saw										*

Where BA = Branch Angle, BD = Branch Diameter, BF = Branch Frequency, and Saw = Sawtimber Potential

Sawtimber potential was most highly correlated with growth traits suggesting that the overall size of the tree is a major factor in sawtimber quality (Table 1). Sawtimber potential was moderately favorably correlated with rust infection and sweep but weakly correlated with forking and branch traits. Forking was the only quality trait that was unfavorably correlated with growth traits (Table 1), suggesting that if we select for growth, we will increase forking incidence. The relationship among traits is an important consideration when multiple trait improvement is considered. If two traits are unfavorably correlated, the amount of gain that can be made in both traits is limited, whereas if traits are not correlated or favorably correlated, simultaneous gains in traits could be greater. In the LGEpop, the positive (unfavorable) correlation between forking and growth is a limitation if increased growth and less forking is desired.

If volume alone is the selection criterion in the Lower Gulf Elite population (Index 1), sawtimber potential would improve by 5% (lower score is favorable) but rust incidence, forking, and sweep would all shift in the unfavorable direction (Table 2). By adding weights for rust incidence, forking, and sweep, gains in volume and sawtimber potential would not be penalized, but rust incidence would be reduced by 22%. Sweep and forking would increase, but with slightly less magnitude compared

to Index 1 which weighted volume alone. Index 3 demonstrates that if the composite sawtimber potential trait were the only selection criterion, there would be greater improvements in sweep (-14%), rust incidence (-162%) and sawtimber potential (-8%). Forking would increase but substantially less than in Indices 1 and 2. Volume gain would be less compared to the other indices in this experiment, but still a significant improvement of 10% over the population mean.

Interpreting categorical scores can be difficult to translate to meaningful values. However, these indices show that by selecting on sawtimber potential, there would be no change in branch angle, a reduction in branch diameter, and an increase in branch frequency (internode length) (Table 2). Branch angle is essentially unchanged in all indices, but branch diameter increases by 5% in Indices 1 and 2. Branch frequency increases the greatest when volume is weighted, but it is improved in all three indices.

Sawtimber potential is improved in all three indices, but the largest improvement is made when it alone is the economically important trait. A 5 to 8 % reduction in the mean score translated to having a higher proportion of trees scored in sawtimber categories versus being scored as pulpwood or as non-merchantable. The relationship between the

sawtimber score and the partitioning of trees to sawtimber (1 and 2) and non-sawtimber (3 and 4) categories is shown in Table 3. If Sawtimber Potential were used as the only selection criteria (Index 3), there would be an improvement in percent sawtimber from 38% to 58% in the population. Selection on volume, rust incidence, sweep, and forking (Index 2) would also improve percent sawtimber to 50% or higher.

Index 2 represents the improvement in traits that would be possible in growth and form traits using the trait data available in the Cooperative. While the greatest improvement in sawtimber potential would be made by weighting it over other traits, using an index with the available BLUP breeding values for volume, rust incidence, stem form (sweep or straightness) and forking would improve sawtimber potential. Since these are the traits that the Cooperative has used for selection, improvements made in Cooperative populations have increased the likelihood of producing sawtimber quality trees in loblolly pine plantations.

A few caveats are in order. Sawtimber potential was assessed at age 6, thus it is not a true measure of rotation age sawtimber quality, but it is the best approximation to actual sawtimber quality that is available for genetic trials in loblolly pine. The greatest limitation to simultaneous gains in volume and sawtimber potential in the Lower Gulf Elite population is the moderate positive (unfavorable) correlation between volume and forking (0.32). Reducing the incidence of forking while improving growth is not possible in this data set, but the relationship may not be found in all populations.

Utilization of a selection index to improve both growth rate and sawtimber quality is a potentially valuable tool for the Cooperative to consider. As the program progresses in the development of elite breeding populations with specific trait combinations and end products, the proper emphasis on important quality traits will be a critical component of future genetic improvements in loblolly pine.



*Sawtimber quality data from the Lower Gulf Elite Population Trials were instrumental in our decision to aggressively pursue the Sawtimber Elite Population Breeding in 2009 (see summary page 2). Breeding at the Arrowhead Breeding Center got off to a good start this year.*



## Fusiform Rust Resistant Alleles (Fr genes) by Bulk Inocula Interactions in Loblolly Pine

Fusiform rust disease in loblolly pine is an economically important disease in pine plantations across the southeastern United States, causing multi-million dollar annual losses. With the pioneering work of the NC State University Forest Biotechnology Group, fusiform rust disease resistance genes (Fr genes) have been discovered in seven loblolly pine open-pollinated (OP) families. These genes are heterozygous in the OP families where they were discovered, and they segregate in the progeny of those families, so that about half of the seedlings get the resistant form of the gene (R allele) from the mother tree, and the other half get the susceptible form of the gene (r allele). These genes condition the gall vs. no-gall disease phenotype when interacting with a pathogen source that is avirulent to the resistance allele such that R seedlings typically are not galled, and the r seedlings typically are galled. However, if the pathogen has a corresponding virulence allele for the R allele, even plants with R will become galled.

We do not know the DNA sequence of the genes or their exact location on the chromosomes, but we know that they are tightly linked with some DNA markers. Understanding the response of Fr genes to bulked pathogen inocula (Fr gene by inocula interactions) is important to develop sound breeding and deployment strategies for loblolly pine.

The response of seedlings that carry r or R alleles to 10 different bulked fungus inocula were investigated in seven families (six are considered here). The inocula were collected from various regions in the southern US. DNA markers (RAPD) were used to assess whether a seedling had a recessive r allele (susceptible form of gene) or dominant R allele (resistant form of gene). Diseased plants with R and r genotypes were counted for each inoculation-family combination.

We detected significant differences (F-test  $Pr < 0.001$ ) among bulked pathogen inocula for causing disease. The mean level of disease caused by 10 bulked inocula ranged from 0.21 (Central Georgia) to 0.60 (Northern coastal South Carolina). OP

families significantly differed ( $Pr < 0.001$ ) for disease (Figure 1). Family 28 was the most disease susceptible, whereas family 15 was the most resistant. A substantial portion of resistance in families 15, 105 and 29R originated from progeny with R alleles. In families 106 and 28, R genotypes were just as susceptible as r genotypes.

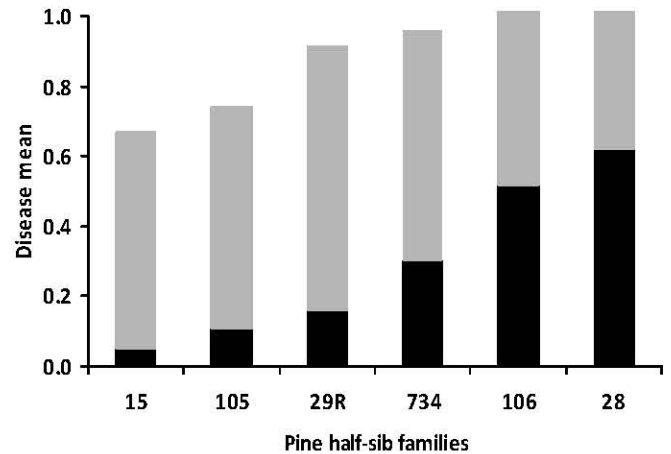


Figure 1. Disease means of seedlings with R (black bars) and r (gray bars) alleles of each OP family across 10 pathogen inocula.

We also found significant Family by Inocula interaction ( $Pr < 0.001$ ). In order to examine this interaction more specifically, we analyzed R and r genotypes separately. The Family  $\times$  Inocula term was highly significant ( $Pr < 0.001$ ) when only R genotypes were analyzed, but that was not the case ( $Pr < 0.378$ ) for r genotypes. This is an important outcome, because it suggests that r genotypes showed a predictable response, regardless of family origin; seedlings with r had a high level of disease incidence. In contrast, the underlying reason for Family  $\times$  Inocula interaction was the unpredictable response of R genotypes. Some R alleles confer complete resistance to some strains of fungal pathogen but little if any resistance to other strains.

We detected significant Inocula by Genotype (R vs. r alleles) interactions when each family was analyzed separately. For example, Inocula  $\times$  Genotype interaction was not significant ( $Pr < 0.326$ )

for family 15, but it was highly significant for family 734 ( $Pr < 0.0001$ ). The reason for the non-significant interaction effect in family 15 was the consistent low disease incidence for R genotypes and high disease incidence for r genotypes. In other words, disease incidence for R and r genotypes was about the same across all inocula; R3 from family 15 being almost always resistant while r3 was consistently susceptible (Figure 2). That was not the case for family 734.

Fusiform rust disease resistance alleles to one particular pathogen inoculum may not confer resistance to another inoculum, as shown for family 734 (Figure 2). Since the virulence levels for

different R alleles under field conditions may be variable across inocula, (because the inoculum in the field may contain fungal pathogens of many different genotypes) the resistance response of various families with known R genes may be unpredictable. The results suggest that in the short run, a stable resistance to fusiform rust disease may be achieved by choosing those (R genes) for which there is little demonstrated virulence, by planting mixed pine families carrying different resistant R alleles, and by stacking multiple R alleles in the pine families. Gene stacking can be enhanced with quantitative resistance to produce more stable disease resistance, and we are pursuing such a long-term strategy for loblolly pine.

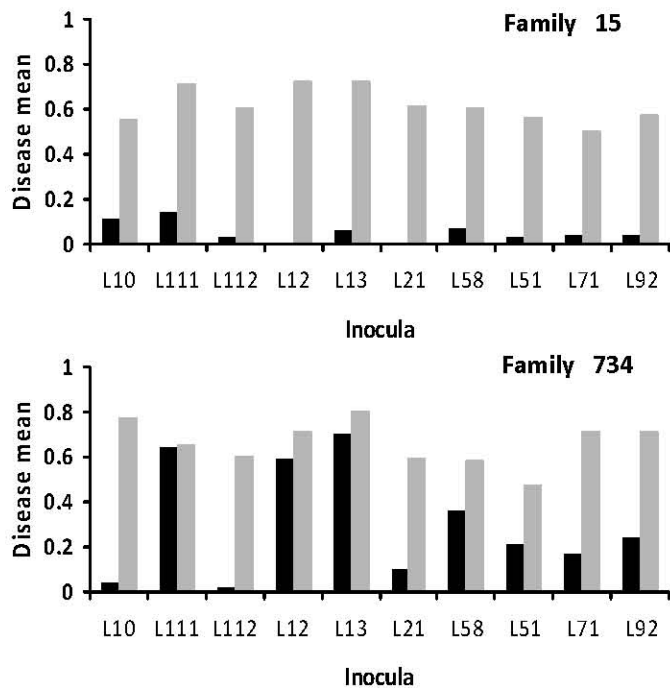


Figure 2. Response of R and r genotypes in two families to 10 different rust inocula. We observe a predictive response of R genotypes (black bars) in family 15 which has Fr3. For family 734 (which has Fr8), the R genotype shows an unpredictable response (interaction) to different pathogen inocula. That is, the R8 seedlings were very resistant to some inocula (e.g., L10 and L112), but they were as susceptible as r seedlings (gray bars) when inoculated with L12 or L13. The different disease rates for the R seedlings across various inocula reflect differences in the pathogen virulence level for the specific R allele in question.

## Genetic and Nutritional Effects on Stem Sinuosity in Loblolly Pine

Dr. Jesus Espinoza recently completed his PhD looking at stem form defects in loblolly pine. Jesus was funded jointly by the Tree Improvement and Forest Nutrition Cooperatives, and part of his field work was supported by MeadWestvaco and ArborGen<sup>1</sup>. Below is a summary of his research.

Genetically improved loblolly pine trees under intensive silviculture have demonstrated dramatic increases in wood production. However, increased input intensity has been associated with a higher propensity for certain

<sup>1</sup> We particularly thank Dr. Phil Dougherty for his contributions to this research and his participation in Jesus's PhD advisory committee.



genotypes to increase stem and branch deformities, as well as increased disease incidence. The responses of several genotypes of loblolly pine to nutrient additions on stem sinuosity were assessed at three and twelve years of age in two different tests located in South Carolina and North Carolina, respectively.

The objectives of the studies were to assess the effect of nutrient additions and genetics on stem form, particularly stem sinuosity, in loblolly pine. Tissue samples from newly expanding shoots at the beginning of growing season were taken and analyzed to assess the association between the nutrient concentrations on the tissues and stem sinuosity in five families from two provenances (Atlantic Coastal Plain “ACP” and Lost Pine Texas “LPT”). The second study was established at the ArborGen research facility near Summerville, SC, with six different genotypes of loblolly pine to determine whether high N and low Ca availability caused sinuous growth. In early spring, eight blocks were fertilized with N as (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> and Ca as CaSO<sub>4</sub>. Nutrient concentrations from flushing shoot tissue were examined and then correlated with measures of stem sinuosity.

Results from the study of repeated nutrient additions in North Carolina showed that the addition of nutrients increased stem sinuosity, branch sinuosity, height, and the levels of N,P, K, Mg, Zn, B and S in the woody tissue of newly expanding shoots and decreased the levels of Mn and Cu. Calcium levels were the same in both treatments. Stem sinuosity was positively correlated with tissue nitrogen (N) concentration, while negatively correlated with manganese (Mn) levels. Negative family-mean correlations between N and Mn were found in both treatments (control and fertilized). There was a negative family-mean correlation between Mn and the height of the trees in the control and fertilized treatments. Differences in stem sinuosity and nutrient uptake were found among families within provenance, indicating a potential to reduce sinuosity by using genetic selection and appropriate nutrient additions (Figure 1).

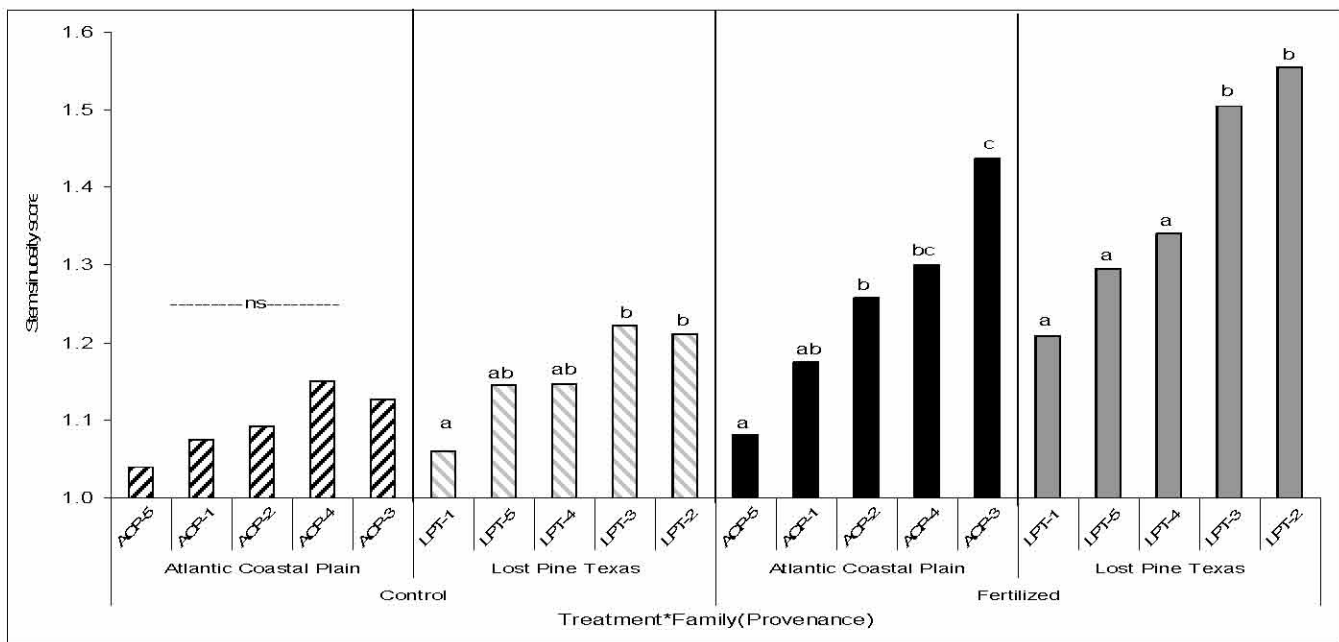


Figure 1. Mean of stem sinuosity scores for families from Atlantic Coastal Plain (ACP) and Lost Pine Texas (LPT) provenances in the control and fertilized plots. Treatment and Family within Provenance effects were highly significant. Provenance effect was not significant. Even though Treatment by family within Provenance interaction effects was significant, families appear to rank very similarly in the two treatments; it could be due to a scale effect. Treatment by Provenance was not significant. Families within provenance are ordered by their sinuosity score in the fertilized plots. Family means with the same letter within each Treatment-Provenance combination were not significantly ( $P < 0.05$ ) different.

The study in South Carolina where only N and Ca were added showed that nitrogen additions caused significant increases in both stem sinuosity and N concentrations. Calcium additions reduced stem sinuosity only when N was added (Figure 2), and did not significantly change in Ca concentrations in the flushing shoot tissue. Manganese (Mn), Ca, N and phosphorus (P) concentrations were all positively correlated with stem sinuosity. The study from North Carolina showed also that in contrast with sweep, stem straightness, forking, and ramicorn branching were all negatively impacted (became worse) by the nutrient addition, especially in the LPT provenance. The ACP provenance showed 34% more susceptibility to fusiform rust than the LPT provenance. Based on our findings, nutrient additions, especially N, increased not only stem and branch sinuosity but also increased the deformations in other stem traits such as straightness, forking, and ramicorn branching, and it also increased the proportion of the trees infected with fusiform rust. However, Ca addition reduced the negative impact of N addition on stem sinuosity. Provenance and family differences were also found.

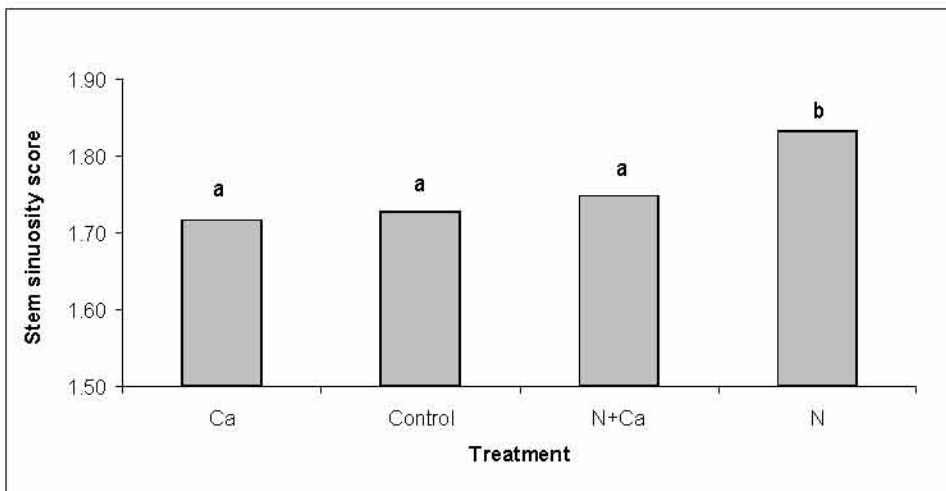


Figure 2. Mean stem sinuosity score (1=no sinuosity, 4=severe sinuosity) by nutrient treatment. Treatment means with the same letter were not significantly ( $P < 0.1$ ) different.

Stem form traits are clearly affected by environmental differences and by genetics. This highlights the importance of matching appropriate site and cultural treatments with suitable genotypes. The choice of these three components has a large impact on the productivity and quality of the plantation.

*Sinuosity, poor-formed trees such as this 10-year-old “beauty” at SETRES2 can result from poor genetics and fertilization. Jesus Espinoza’s PhD results clearly show the need to match genetics and silvicultural systems to have both good production and stem quality.*





## Association Genetics of Early Growth and Water Relations in Loblolly Pine<sup>2</sup>

Understanding the relationship between genotypic and phenotypic variation is of key importance to tree breeders. While traditional methods of quantitative analysis, phenotypic and family level selection have been extremely successful, new methods that incorporate genomic data and marker technologies offer opportunities to make additional gains and enhance our understanding of the underlying genetics that influence the growth and quality of loblolly pine. Association genetics is an analytical method that seeks to find statistical relationships between genetic variation (single nucleotide polymorphisms, or SNPs) and phenotypic variation of traits at the population level. This method was employed to quantify the genetic variation of early growth and water relations in loblolly pine and to discover SNPs associated with these traits.

Water is necessary for plant survival and growth. While water is generally not the most limiting factor for loblolly pine productivity in most of its native range, water availability is important for plantation establishment as well as long-term growth efficiency in plantations. Water use efficiency (WUE) is the amount of biomass accumulated per unit of water transpired in the tree. WUE can be measured directly in controlled experiments or can be measured indirectly using carbon isotope discrimination ( $\delta^{13}\text{C}$ ) which quantifies the amount of  $^{13}\text{C}$  to  $^{12}\text{C}$  in the tissue sample. Variation in  $\delta^{13}\text{C}$  has been correlated with stomatal conductance. A more negative  $\delta^{13}\text{C}$  value indicates a greater deviation from atmospheric carbon isotopic levels meaning the plant is discriminating against  $^{13}\text{C}$  and stomata are likely closed to conserve water loss. A less negative number indicates less discrimination against  $^{13}\text{C}$  and stomata remain open allowing greater transpiration.

In the spring of 2006, 445 unrelated clones of loblolly pine were planted in a raised nursery bed at NCSU in 2 blocks with 1 ramet per clone. The trees were planted at a 6" x 9" spacing and grown for 2 years with no addition of nutrients or water. However, during the summer of 2007 Raleigh experienced an extreme drought that imposed some water stress on the trees. Foliage samples were collected in December of 2007 and analyzed for carbon isotope discrimination ( $\delta^{13}\text{C}$ ) as a measure of water use efficiency. In the spring of 2008, trees were measured for 2-year growth; and above ground biomass was harvested and weighed. An AR1 x AR1 auto-regressive spatial model was used in the ASReml statistical software package to generate predicted clonal genetic values to be used for association testing. Association testing was performed using a GLM model in the TASSEL software package and Storey's Q-value was implemented for multiple testing correction. For this experiment, approximately 4000 SNPs were tested for associations that were available as part of the Allele Discovery of Economic Pine Traits 2 grant (ADEPT2) funded by NSF.

Clonal variation was significant for 2-year height and  $\delta^{13}\text{C}$ . Clonal BLUP values for height ranged from 4.3 feet to 8.1 feet, and values for  $\delta^{13}\text{C}$  ranged from -29.95 to -28.78. Height was positively correlated with  $\delta^{13}\text{C}$  (0.38+/-0.25) suggesting that as the trees restrict water loss they are limiting growth. Clones with greater height had less negative  $\delta^{13}\text{C}$  values suggesting that WUE was lower in faster growing genotypes (Figure 1). Association testing revealed a total of 161 SNPs that were significantly associated ( $p < 0.05$ ) with  $\delta^{13}\text{C}$  variation and 79 SNPs associated ( $p < 0.05$ ) with height variation before correction for multiple testing. Following correction for multiple testing (Storey's Q-value), four SNPs were associated with  $\delta^{13}\text{C}$  and one SNP was associated with 2-year height (Table 1). The SNP associated with height occurs in a locus similar to a Myb transcription factor in *Picea*, and one SNP associated with  $\delta^{13}\text{C}$  occurs in a locus similar to an enzyme in lipid metabolism. The remaining SNPs showed no significant similarity to annotated proteins in blastx database searches.

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<sup>2</sup> This is a summary of part of Patrick Cumbie's PhD research.

Table 1. Preliminary SNP associations with 2-year Height and  $\delta^{13}\text{C}$  of 445 unrelated loblolly pine genotypes

Trait	SNP	q-value	Annotation
Height	0-4517-01-160	0.00287	Myb R2R3 transcription factor
$\delta^{13}\text{C}$	0-7914-01-374	0.00013	No significant BLASTX hit
$\delta^{13}\text{C}$	0-17030-01-94	0.00013	No significant BLASTX hit
$\delta^{13}\text{C}$	2-8731-01-538	0.06495	DGDG synthase I, lipid metabolism
$\delta^{13}\text{C}$	0-10921-01-353	0.08901	No Significant BLASTX hit

Experiments are planned to test for physiological differences among clones in greenhouse experiments, and elite germplasm will be tested for the presence of the SNP alleles associated with desirable traits as part of the CTGN project. If these

preliminary results are confirmed by the greenhouse experiments this summer, we may be able to test the utility of these SNPs in the ACE progeny now being prepared for field testing.

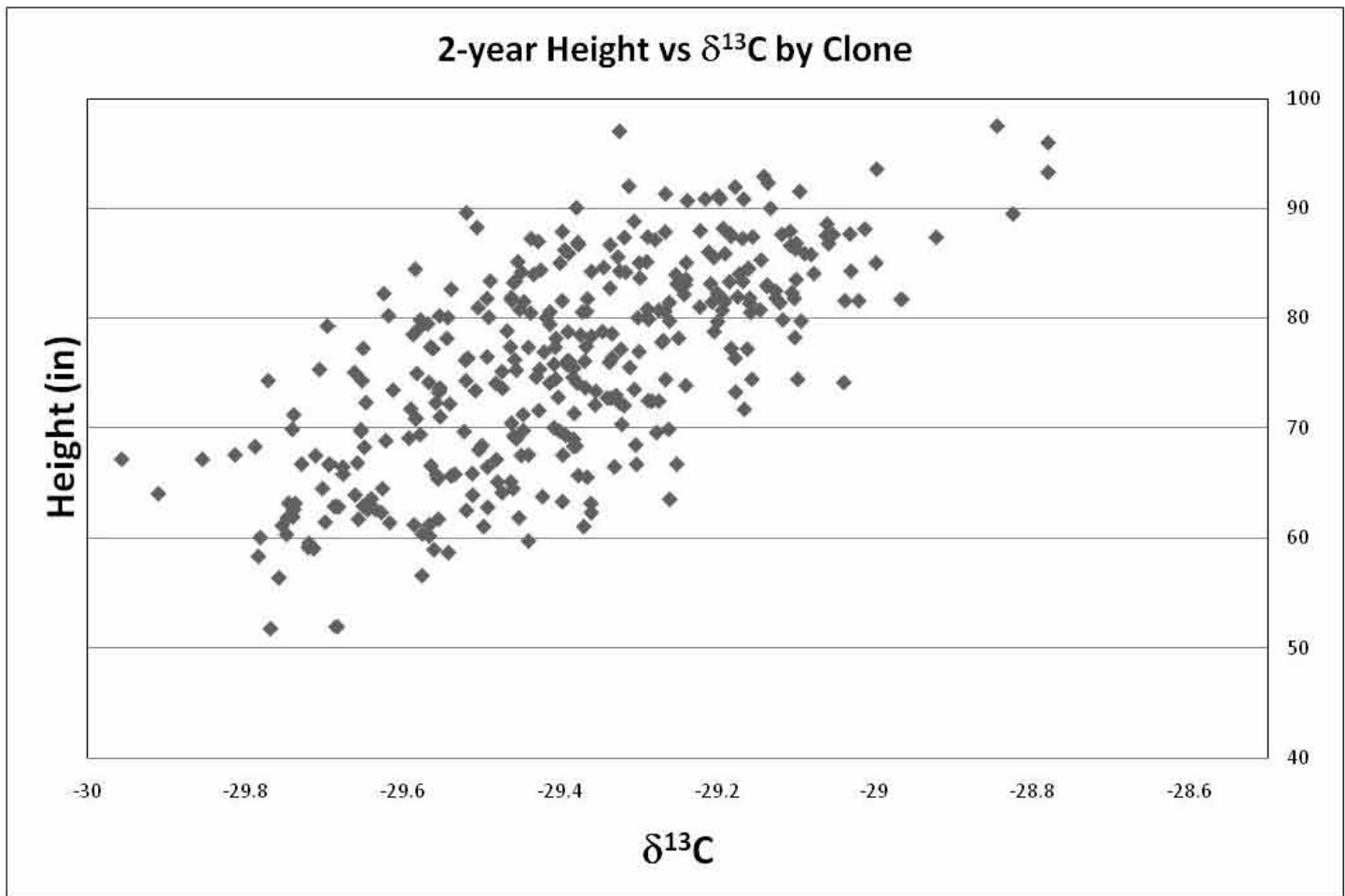


Figure 1. Clonal BLUP values for Two-year height growth (in) and  $\delta^{13}\text{C}$  ( $r^2 = 0.47$ ).



## Grants Received

Steve McKeand, Fikret Isik, Ross Whetten. Subcontract with the University of California – Davis, USDA Coordinated Agricultural Project (CAP). NCSU portion \$548,819. Conifer Translational Genomics Network. 9/07 to 9/11

Sunkyu Park, Ross Whetten, Steve McKeand, Fikret Isik. Economic Analysis of Pine Biomass Feedstocks for Ethanol Production. \$ 162,438. Biofuels Center of North Carolina. 10/09 to 12/10.

Ross Whetten and Fikret Isik. How can high-throughput SNP genotyping be applied to forest tree breeding programs? \$20,000. Forest Biotechnology Research Consortium. 02/09 to 02/10.

Laurie Schimlek, Bailian Li, Fikret Isik. Rapid Techniques for Screening Wood Properties for Genetic Improvement of Loblolly Pine. \$18,357. Subcontract from Univ. Georgia / USDA Forest Service Agenda 2020. 07/06 to 06/09.

## ASSOCIATED ACTIVITIES

### Teaching

*FOR 411 (Forest Tree Genetics and Biology), our undergraduate tree improvement course was taught this spring along with FOR 725 (Forest Genetics – formerly FOR 611). Ross Whetten and Steve McKeand had a great group of students in class, and they enjoyed the tour of the NC Forest Service seed orchard and nursery at Goldsboro. From left→right are Thomas Rudd, Brandon Palmer, Miguel Gonzales, David Barker, Hayley Stevenson, Aaron Chamblee, Ross Whetten, and Funda Ögüt (Steve McKeand – photographer extraordinaire!). As always, Maxie Maynor had a great tour for us.*



### Training

*Part-time employees are essential to the progress and pace of the Program. In addition, they are gaining valuable hands-on training for future careers in forestry. Top row left→right are Jadie Andrews, Matt Allen, and Nick Williams. Bottom row left→right are Maria Wilkes, Mike Cheston, Mike Powell, and Will Armstrong.*



## Cooperative Website ([www.cnr.ncsu.edu/tip](http://www.cnr.ncsu.edu/tip))

Our redesigned website was launched in August 2007. Those familiar with our old site will see many changes and improvements. There are new features such as the [News](#) tab where one can find TIP news, seminars, and a calendar of events of interest. The [Publications](#) tab goes to a list of current publications and all previous TIP annual reports. [Research](#), [Prospective Members](#), and a [FAQ](#) sections are other new additions. For members, access to various programs and data can be downloaded from the web page. Hats off to Saul Garcia for our web page design and his work to keep it current

## Meetings, Workshops, and Short Courses

*The annual Contact Meeting was in Raleigh in December 2008. We took the opportunity to focus on our research at the University and to showcase the Departmental and College facilities such as the new Marker Lab that Saul Garcia is proudly describing.*

*The Tree Improvement labs have been completely refurbished primarily with funds from the University. The 53-year partnership between the Members of the Cooperative and NC State continues to flourish even in these difficult financial times.*



## GRADUATE STUDENTS

Despite the downturn in the economy, we continue to attract graduate students. In 2008-09, the Cooperative supported 11 Masters and PhD students (4 new students joining us in August 2008). Cooperative funds were leveraged with grants, Departmental and College funds, and international agencies to support every one of these students. These graduate students bring fresh ideas and facilitate collaboration with other faculty at NC State and in other institutions. Through the Tree Improvement Program, students are educated and trained to be the tree improvement leaders of tomorrow.



Mike Aspinwall is working on his PhD on a physiology / genetics project focusing on the Hofmann Forest Growth-Genetics-Spacing (GST) Study with John King and Steve McKeand.



David Barker started his Masters degree in August 2008. David is interested in biofuels feedstock production in loblolly pine. He has sampled selections from Plum Creek / CellFor clonal trials, and will be evaluating genetic differences in conversion efficiency for cellulosic ethanol.



Aaron Chamblee is a graduate forester from NC State and started his MS in August 2008. Aaron will be using data from the Plantation Selection Seed Source Study to evaluate geographic variation in resistance to fusiform rust disease.

Jesus Espinoza completed his PhD in spring 2009. His project was jointly funded by the Forest Nutrition and the Tree Improvement Cooperatives, and he looked at genetics and cultural effects on stem quality with emphasis on stem sinuosity in loblolly pine. Jesus is now a post-doc with CAMCORE.



Daniel Gräns's PhD research is looking at solid wood properties in loblolly pine and Norway spruce. Daniel's Nicholson Fellowship supported his work both in Sweden and with the Cooperative. His loblolly pine trial is a Genetics x Culture trial established by International Paper Company in Bainbridge, GA. Daniel is now a lecturer at the Swedish University of Agricultural Sciences (SLU) in Skinnskatteberg and will complete his dissertation this fall.

George Khan is completing his masters degree in tree improvement / biotechnology working with Ross Whetten. George is developing species-specific SNP markers that can be used to distinguish different populations and species of pines.





Funda Ögüt is a PhD student funded by the Turkish Ministry of Education and is working with Fikret Isik and Ross Whetten on molecular aided selection and breeding in loblolly pine.

Ben Smith is wrapping up his PhD research and was funded by a Hofmann Forest Graduate Fellowship and the Cooperative. His project is focusing on incorporating realized gain from genetic improvement into loblolly pine growth and yield models using SETRES2 data and trials established by MeadWestvaco in Walterboro, SC.



Lizzie Wood is a Masters student working with Bronson Bullock in forest biometrics. Lizzie's project is evaluating clonal differences in stem taper using the Plum Creek / CellFor clonal trials.

Sherry Xiong is working on her PhD in tree improvement / biotechnology with Ross Whetten and Steve McKeand looking at the genetic factors of forking in loblolly pine. She is using data from clonal trials of loblolly pine established by MeadWestvaco and now managed by ArborGen.



Jaime Zapata started his PhD in August 2008. Jaime worked for BioForest in Chile and came to NC State to work on the Conifer Translational Genomics Network grant funded by USDA. His interests are to develop breeding strategies that incorporate molecular markers into the selection process.



**COOPERATIVE STAFF** – Fortunately the Cooperative team remained stable this year, except that we gained back an old friend and colleague. Dr. J.B. Jett retired from NC State as Associate Dean for Research and Extension in February and is now Professor Emeritus. J.B. wanted to “stay involved” in University and Cooperative activities and has a small office in our shop. As you might expect, he has already “stayed involved” and has been working with cooperators, students, and staffs on a variety of tree improvement activities. It’s good having J.B. back home, even if it is only part time.

*While Tori was on maternity leave, Kimberly Dawson spent six months with us as an administrative assistant. Kimberly did a great job managing the office, planning meetings (many of you met her at the Contact Meeting in December), and helping to manage the finances during a tough time for all of us. We wish her the best and will miss her friendly face here in the Tree Improvement office.*



**Tree Improvement Staff:** L→R: Ross Whetten, Patrick Cumbie, Josh Steiger, Fikret Isik, Tori Batista-Brooks, Jim Grissom, Saul Garcia, J.B. Jett, and, Steve McKeand

## **MEMBERSHIP in the NCSU Cooperative Tree Improvement Program**

The full members of the program provide financial support to the University and the in-kind support to make tree improvement happen in the field. Their financial contributions to NC State University support the operations, research, teaching, and outreach components of the program. Support from the Contributing Members is used primarily for the on-the-ground breeding, testing, selection, and archiving efforts by the full members and the Cooperative staff. We could not ask for a more dedicated and supportive group of cooperators.

New Contributing Members in FY2009 are International Forest Company, North Carolina Natural Resource Foundation, Scotch Lumber Company, and Z.V. Pate, Inc.

### ***Full Members***

**ArborGen**

**CellFor, Inc.**

**Georgia Forestry Commission**

**Hancock Timber Resources Group**

**North Carolina Division of Forest Resources**

**Plum Creek Timber Company**

**Rayonier, Incorporated**

**Smurfit - Stone Container Corporation**

**Tennessee Department of Agriculture**

**Virginia Department of Forestry**

**Westervelt Company**

**Weyerhaeuser Company**

### ***Contributing Members***

**Dougherty & Dougherty Forestry Services, Inc.**

**International Forest Company**

**Jordan Lumber & Supply Company**

**Molpus Timberlands Management, LLC**

**North Carolina Natural Resource Foundation**

**Resource Management Service, LLC**

**Scotch Lumber Company**

**South Carolina Forestry Commission**

**Z.V. Pate, Inc.**



## PUBLICATIONS OF SPECIAL INTEREST TO MEMBERS (2006-2009)

From our inception in 1956, there have been over 800 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://cnr.ncsu.edu/tip/publications\\_citations.html](http://cnr.ncsu.edu/tip/publications_citations.html)

### 2009

Morse, A.M., D.G. Peterson, M. N. Islam-Faridi, K.E. Smith, Z. Magbanua, S.A. Garcia, T.L. Kubisiak, H.V. Amerson, J.E. Carlson, C.D Nelson, J.M. Davis. 2008. Evolution of genome size and complexity in *Pinus*. PLoS ONE 4(2): e4332. doi:10.1371/journal.pone.0004332

### 2008

Emerson, J.L., L.J. Frampton and S.E. McKeand. 2008. Genetic variation in early growth and bud production among natural populations of Fraser fir. HortScience 43: 589-968.

Isik, F., H.V. Amerson, R.W. Whetten, S. Garcia, B. Li, and S.E. McKeand. 2008. Resistance of *Pinus taeda* families under artificial inoculations with diverse fusiform rust pathogen populations and comparison with field trials. Can. J. For. Res. 38: 2687-2696.

Isik, F., M. Gumpertz, B. Li, B. Goldfarb, X. Sun. 2008. Analysis of cellulose microfibril angle (MFA) using a linear mixed model in *Pinus taeda* clones. Can. J For. Research 38: (in press).

Isik, F, B. Li, B. Goldfarb, and S. McKeand. 2008. Prediction of wood density breeding values of *Pinus taeda* elite parents from unbalanced data: A method for adjustment of site and age effects using common checklots. Ann. For. Sci. 65: 406.

McKeand, S.E., D.M. Gerwig, W. P. Cumbie, and J.B. Jett. 2008. Seed orchard management strategies for deployment of intensively selected loblolly pine families in the southern US. P. 177- 182, In: Lindgren, D. (ed.). Seed orchards, Proceedings from a conference at Umeå, Sweden, September 26-28, 2007 (ISBN: 978-91-85911-28-8)

McKeand, S.E., B. Li, J.E. Grissom, F. Isik, and K.J.S. Jayawickrama. 2008. Genetic parameter estimates for growth traits from diallel tests of loblolly pine throughout the southeastern United States. Silvae Genetica 57:101-110.

Sherrill, J.R., T.J. Mullin, B.P. Bullock, S.E. McKeand, R.C. Purnell, M.L. Gumpertz, and F. Isik. 2008. An evaluation of selection for volume growth in loblolly pine. Silvae Genetica 57(1):22-28.

### 2007

Espinoza, J., S. McKeand, L. Allen, J. Grissom, T. Brooks, and B. Smith. 2007. Response of several genotypes of loblolly pine to environmental stress for stem form traits at age 12. In: Proc. 28th South. For. Tree Impr. Conf., Galveston, TX. (in press).

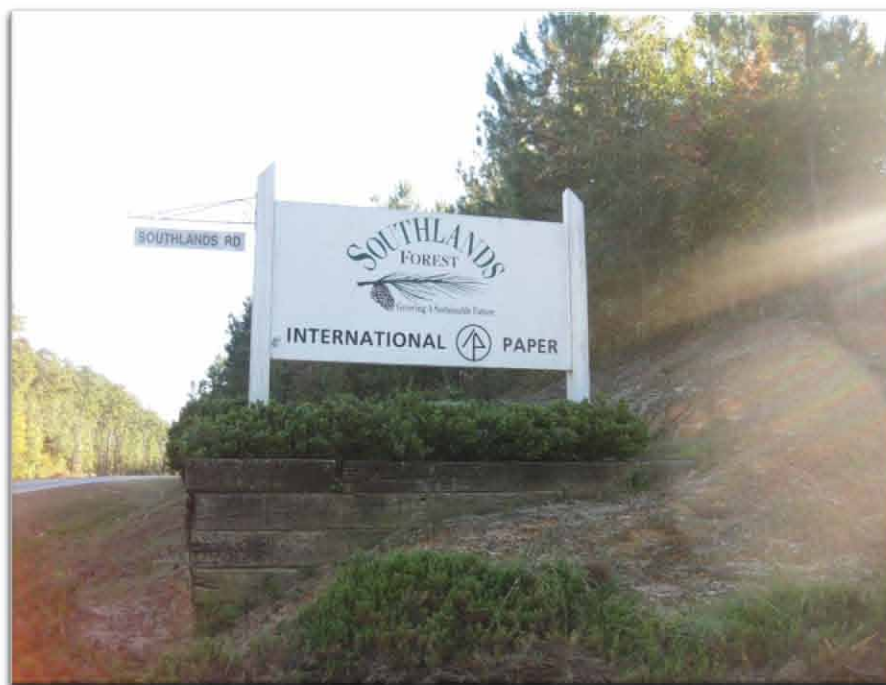
McKeand, S.E., B.J. Zobel, T.D. Byram, and D.A. Huber. 2007. Southern pine tree improvement – a living success story. In: Proc. 28th South. For. Tree Impr. Conf., Galveston, TX. (in press).

### 2006

Alizoti, P., B. Li, and S.E. McKeand. 2006. Early evaluation of intra- and inter-provenance hybrids of loblolly pine for planting in Piedmont regions of the southern US. For. Sci. 52:557-567.

Emerson, J.L., L.J. Frampton, and S.E. McKeand. 2006. Genetic variation of spring frost damage in three-year-old Fraser fir Christmas tree plantations. HortScience 41:1531-1536.

- Hu, X. S., B. Li. 2006. Additive genetic variation and the distribution of QTN effects among sites. *Journal of Theoretical Biology* 243:76-85.
- Li, Hua, H. Amerson, Bailian Li. 2006. Genetic models of host-pathogen gene interaction based on inoculation of loblolly pine seedlings with the fusiform rust fungus. *New Forests* 31: 245-252.
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- Sato, Y. and R.W. Whetten. 2006 Characterization of two laccases of loblolly pine (*Pinus taeda*) expressed in tobacco BY-2 cells. *Journal of Plant Research* 119 (6): 581-588
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*For hundreds of graduate students, faculty, staff, and visitors with the Cooperative, International Paper Company's Southlands Forest was a regular destination for numerous research studies. We spent many spring breaks measuring the Heritability Study and other times working on Masters and PhD student projects.*

*Sadly, Southlands has gone the way of many properties owned by the forest products companies. The Silver Lake section of the property was sold in 2007, and sale of the remaining acres is imminent.*

*Oh the memories!*





For the clonal testing effort with the Atlantic Coastal Elite (ACE) population, over 30,000 cuttings were stuck in containers to root in winter 2009 (top). Another 30,000 cuttings will be stuck this summer. Hedges of the ortets of the clones are managed in the overwintering house (bottom) at the HFL on the NC State Campus.