

# North Carolina State University Cooperative Tree Improvement Program 54<sup>th</sup> Annual Report



Department of Forestry and Environmental Resources  
College of Natural Resources - May 2010

**NC STATE UNIVERSITY**

## EXECUTIVE SUMMARY

### SELECTION, BREEDING, AND TESTING

Mainline breeding has progressed since last year, with 70% of the total PMX (pollen mix) breeding complete. Full-Sib breeding progress has continued but has slowed some due to the recent changes that placed priority on completing PMX crosses.

To date, a total of 71 PMX progeny tests have been established in the third-cycle. As a result, there are only one Northern, two Coastal, and two Piedmont test series remaining. Full-sib block plots are progressing with 200 family block plots established to date.

In the spring of 2010, good progress was made on the Sawtimber Elite Population at the Arrowhead Breeding Center. Nearly 250 bags were pollinated, representing 102 of the 163 crosses. In addition, approximately 300 grafts were made to bulk up existing selections as well as to add new selections to the population.

This past winter, the Cooperative established six of the nine clonal tests across four different states for the Atlantic Coastal Elite (ACE) Population.

### SEED PRODUCTION

Cooperative members collected 17.6 tons of loblolly pine seed in 2009. Third-cycle orchards produced 26% of the harvest. Second-generation orchards made up 66% of the 2009 harvest, while first-generation orchards decreased from 12% in 2008 to 8% of the harvest in 2009. Coastal orchard seed made up 73% of the total harvest followed by Piedmont (26%) and Northern (1%).

### RESEARCH

The genetic basis of forking in loblolly pine was tested by assessing trees in a large number of diallel tests (268 test series, ~ 1000 tests in the 2nd-generation testing program) and in a clonal test of MeadWestvaco in South Carolina. By using family selection with a selection differential of 20%, forking could be reduced 12% to 23% across the different regions of the Cooperative. A single-marker analysis of 1257 loci identified 11 and 9 markers that were significantly associated with stem forking and ramicorn branching, respectively.

In a study looking at physiological variation in different loblolly pine genotypes, results provided little support of the hypothesis that more genetically homogeneous individuals would show greater uniformity in a plantation setting, at least not in the first three years. Also, differences in biomass partitioning may be partially related to genetic differences in productivity, but actual height and ground-line diameter growth rate over time was the best indicator of productivity.

Genetic analyses of the Plantation Selection Seed Source Study are underway to determine the performance of different sources of loblolly pine across a wide range of sites and to further investigate GxE.

After two and a half years of supported research, the CTGN project has yielded genotypes for about 4800 useful SNP loci in a set of about 1500 trees from the NCSUCTIP breeding population. Association studies are underway.

Association testing revealed 278 SNPs associated with the sawtimber index and slightly fewer SNPs for individual trait breeding values prior to multiple test correction. After correction for multiple testing, there were 14 significant SNP-phenotype associations for sawtimber index, volume, and straightness.

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

Full membership remained at 12, and there are 14 Contributing Members with 4 joining since the 2009 Annual Report was written. We are optimistic that more will join in the coming months.

## TABLE OF CONTENTS

	Page
<b>EXECUTIVE SUMMARY</b> .....	<b>i</b>
<b>TABLE OF CONTENTS</b> .....	<b>ii</b>
<b>TREE IMPROVEMENT COOPERATIVE HAPPENINGS</b> .....	<b>1</b>
<b>SELECTION, BREEDING, AND TESTING</b> .....	<b>2</b>
Third-Cycle Mainline Breeding Progress .....	2
Third-Cycle Progeny Testing .....	3
Third-Cycle Elite Breeding .....	4
The Atlantic Coastal Elite Population: Progress and Plans .....	5
<b>CONE AND SEED YIELDS</b> .....	<b>6</b>
<b>RESEARCH</b> .....	<b>8</b>
Genetic Analysis of Forking Defects in Loblolly Pine .....	8
Genetic Variation in Water relations, Physiology, Biochemistry, and Biomass Partitioning in Loblolly Pine .....	9
Plantation Selection Seed Source Study: A look into the base selection materials .....	11
Conifer Translational Genomics Network Progress .....	13
Association Genetics to Improve Sawtimber Quality in Loblolly Pine .....	14
Molecular Marker Fingerprinting to Establish Parentage and Clone Identity .....	16
Grants Received .....	17
<b>ASSOCIATED ACTIVITIES</b> .....	<b>18</b>
Teaching .....	18
Welcome to TreeImprovement.org .....	18
Meetings, Workshops, and Short Courses .....	19
Visiting Scientist .....	19
<b>COOPERATIVE STAFF</b> .....	<b>20</b>
<b>GRADUATE STUDENTS</b> .....	<b>20</b>
<b>MEMBERSHIP</b> .....	<b>22</b>
<b>PUBLICATIONS OF SPECIAL INTEREST TO MEMBERS</b> .....	<b>23</b>



## TREE IMPROVEMENT COOPERATIVE HAPPENINGS

Survival mode – that pretty well describes the situation that many of us find ourselves in these days. It's discouraging when you know how valuable tree improvement is to the long-term sustainability and economic development of the region, and budget constraints limit your ability to do what you know would bring tremendous financial returns. I think most of you know that I am the “eternal optimist” when it comes to southern forestry and the benefits of tree improvement, but even my optimism has been challenged these past several months. Fortunately, when I see the progress that our Cooperative has made in the breeding program, and the deployment of incredibly valuable new genotypes to landowners, I realize that we are still able to do great things despite the dismal economy.

A very important sign of the Cooperative moving forward is the progress made on our various elite breeding populations. The spring of 2010 marked the third 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. 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 field testing phase of the Atlantic Coastal Elite (ACE) Population is now underway. Clonally replicated trials of these outstanding crosses were planted in 6 trials from North Carolina to Alabama. Three more will be planted this fall. The effort is also indicative of the push to get as much “bang for the buck” out of our breeding program even when times are tough.

The increase in Cooperative membership is also an indication that companies and state agencies continue to view tree improvement as a critical component of their forestry programs and recognize the value of using the best genetics and supporting the development of the genetic resource. Since starting the Contributing Membership option in 2008, 14 businesses have joined the program (see membership list on p. 21). This year, we have added 4 new members: American Forest Management, Biofuels Center of North Carolina, Milliken Forestry Company, and Pacolet Milliken Enterprises.

Unfortunately, the tough economic times have resulted in a significant loss to the program. After 40 years as a member of the Tree Improvement Cooperative, Smurfit-Stone Container Corporation filed for bankruptcy in 2009, and that was the beginning of the end for the tree improvement and nursery program. Container Corporation of America joined the Cooperative in 1969 and operated out of Callahan, FL and Brewton, AL. In 1986, Jefferson Smurfit bought the company, and in 1998 the name was changed to the present S-SCC. We wish the best to our friends Tyler Eckard and Doug Shelbourne.

Survival mode – yes, we will survive, and we will flourish due to the support from our members, the Department of Forestry and Environmental Resources and the College of Natural Resources at NC State, our granting agencies, and the multitude of landowners who benefit from the increased productivity and value they receive from our breeding programs.

*Steve McKeand  
May 2010*

## SELECTION, BREEDING, AND TESTING

### THIRD-CYCLE MAINLINE BREEDING PROGRESS

Mainline breeding has progressed since last year, with 70% of the total PMX (pollen mix) breeding complete. Of the remaining selections to breed, approximately 45% have 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 polycross mating for among family selection and full-sib mating for within-family selection. The numbers of completed PMX crosses are summarized in Table 1. The Coastal region has made the most progress with 82% of PMX breeding complete as of Fall 2009.

Full-Sib breeding progress has continued but has slowed due to the recent changes that placed priority on completing PMX crosses. The decision was made to emphasize PMX breeding to ensure proper ranking of parents within each subline which is determined by measuring the progeny of the selections. These full-sib crosses will generate seeds for within-family selections for the next breeding cycle.

Three third-cycle check seed lots have been created for each of the three regions by using the same 20-parent pollen mix applied to 10 females of the 20 selections. Checklot breeding has been completed in all three regions (Table 1). Thanks to the breeding efforts by full members.

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 20-parent pollen mix. These families are included in all tests to contribute to estimates of variance components and to provide better family comparisons among test series. Nearly all breeding is complete on the common family checks, allowing for more effort to be put elsewhere.



*The 2010 flower crop was excellent throughout the South and good breeding progress was made.*

Table 1. Summary of 3<sup>rd</sup> Cycle PMX breeding progress through Spring 2010

Type of crosses	Breeding Region	# Cooperators	# of Sublines	Total Parents	# Crosses producing Seed	#Crosses of Sufficient Seed	% Done
Polycross	Coastal	8	32	370*	329	304	82%
	Piedmont	8	31	308	207	167	54%
	Northern	6	13	182	164	130	71%
<b>Polycross</b>	<b>Total</b>	<b>22</b>	<b>76</b>	<b>860</b>	<b>700</b>	<b>601</b>	<b>70%</b>
PMX checks	Coastal	8	--	10	10	10	100%
	Piedmont	8	--	10	10	10	100%
	Northern	6	--	12**	12	12	100%
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 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

\* Number includes Livingston Parish selection

\*\*There are two alternative PMX checks, but only 10 are to be used in checklot mix

**THIRD-CYCLE PROGENY TESTING**

Polycross tests: Since fall of 2009, eight more PMX tests have been established by cooperative members (Table 2). In the Northern region, four tests were established to complete the second (NPMX-2) of three testing series. In the Piedmont region, testing of selections in series 3 began. To date, four of the six tests that make up the PPMX-3 series have been established. The remaining two tests are being grown and will be planted this fall.

Plans are now being made to establish test series number five of six in the Coastal region. We will most likely have enough seed in inventory by this coming fall to have all eight tests sent out for fall 2011 test establishments. In the coming months, we will need to coordinate with members to grow the seedlings, as well as establish field tests.

To date, a total of 71 PMX progeny tests have been established in the third-cycle. As a result, there are only one Northern, two Coastal, and two Piedmont test series remaining. Our hope is to complete breeding and test establishment of all third cycle selections within the next two to three years.

*Table 2. Third-cycle progeny tests established through spring 2010. A total of 71 tests have been planted.*

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					4	4
Northern	NPMX1				4		4
Northern	NPMX2					4	4

Full-Sib controlled cross tests: Full-Sib controlled-cross tests are very important since they are the source of 4<sup>th</sup>-cycle selections. By ranking the parents through testing in the PMX tests, the full-sib crosses are designed to optimize the probability of creating superior trees.

Recently, the decision was made to put breeding and testing priority on PMX assignments. Consequently, the pace of full-sib breeding has been slowed. However, over the last several years, cooperators have been able to establish numerous full-sib block (FSB) progeny trials (Table 3). Each member is encouraged not to delay the installation of these tests. Once adequate seed is collected from a few controlled crosses, FSB plots can be installed.

*Table 3. Third-cycle Full-Sib Block (FSB) progeny tests established through spring 2010.*

Region	Test Series	2005-2006	2006-2007	2007-2008	2008-2009	2009-2010	# Families
Coastal	07-CFSB1	2					19
Coastal	11-CFSB1	2					11
Coastal	11-CFSB2		2				28
Coastal	26-CFSB1			2			16
Coastal	22-CFSB1			2			23
Coastal	22-CFSB2				2		31
Northern	16-NFSB1					2	36
Northern	20-NFSB2					2	36

### ***THIRD-CYCLE ELITE BREEDING***

In 2007, breeding of the Elite Coastal diallels was completed, allowing the Cooperative to move forward with rust screening and clonal testing in the Atlantic Coastal Elite test series (see ACE, p. 5). In the coming years, the importance of this material will prove to be immense for the Cooperative and regeneration efforts throughout the southeastern US.

In 2008, the cooperative established the Sawtimber Elite Population (SEPop). As a result of this population, the statuses of the Northern Elite (EN) and Piedmont Elite (EP) populations have slightly changed. In the Northern region, we will include any completed EN crosses into the northern region SEPop trials. But, we will not plan to complete all EN crosses from the original third-cycle plan. The Piedmont region will be handled in a similar manner; we will complete EP-1 and EP-3, but not EP-2. Based on the changes previously mentioned, 60% of the Piedmont Elite breeding has been completed.

#### Sawtimber Elite Population

The Sawtimber Elite Population is an accumulation of the very best selections across all three breeding regions based upon the new and improved BLUP breeding values. This population is comprised of straight, rust-resistant, high-volume selections that should produce high-value sawtimber trees. The SEPop, like our mainline population, is divided into a Coastal, Northern, and Piedmont regions. The Coastal region has been assigned 75 crosses, while the Piedmont and Northern have less with 36 and 52 assigned crosses. Crosses were assigned based upon complementing breeding values for volume gain, stem straightness, forking, and rust incidence for the parents in each region.

In the spring of 2010, good progress was made on the Sawtimber Elite Population at the Arrowhead Breeding Center (ABC), located at Georgia Forestry Commission's Arrowhead Seed Orchard. In total, nearly 250 bags were pollinated, representing 102 of the 163 crosses. In addition, approximately 300 grafts were made to bulk up existing selections as well as to add new selections to the population.

This year's SEPop breeding was greatly accelerated by the pollen collection efforts of cooperators, allowing crosses to be made without waiting for pollen production at Arrowhead. Thanks to cooperators for their efforts in providing scion and pollen for this elite population.

*The flower crop at the Arrowhead Breeding Center was excellent this spring. For the SEPop breeding, we averaged almost 10 female strobili per pollination bag. Patrick Cumbie and Josh Steiger (pictured in the aerial lift) spent much of February and March working at the ABC.*

*Once again, without the contributions of the Georgia Forestry Commission, especially Russ Pohl and Terry Butts, our effort with SEPop would not be possible.*





## ***THE ATLANTIC COASTAL ELITE POPULATION (ACE): Progress & Plans***

Over the past several years, tremendous Cooperative time, effort, and resources have been put towards our Atlantic Coastal Elite (ACE) Population. Consisting of 55 full-sib crosses, the breeding effort that went into these elite selections required a substantial amount of effort from our members. After several years of breeding, enough seed was produced by the fall of 2007 to begin the process of testing the progeny of these elite crosses. The first step in testing the ACE material was inoculating seedlings with a very high spore load of fusiform rust. After inoculation, only the seedlings that were free of rust galls were kept for further testing, thus increasing the probability that the progeny from these selections are resistant to rust. By deploying rust resistant trees, the economic and ecological value of the material is further increased.

Furthermore, to maximize the genetic gains of the population, clonal testing of the progeny is being used to increase the within-family heritabilities. The increased within-family selection power gives greater likelihood that the forward selections can be confidently grafted directly into orchards for seed production.



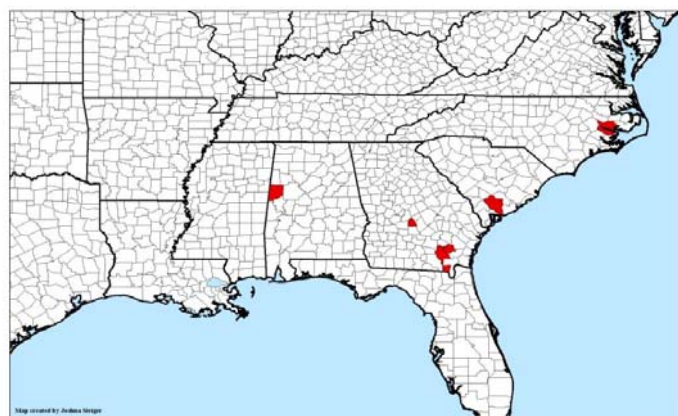
*About 20,000 rooted cuttings were propagated at NCSU in 2009. These trees went to the field in 2010. This is the Plum Creek test site in Wayne County, GA.*

In summer 2009, we finished clonally propagating the ACE population, and approximately 20,000 trees representing 2,442 different genotypes were produced. This is enough trees to test nearly every genotype across nine test sites, ensuring that the top performing clones will be selected. In addition, seedling checks were included in each test to assess the resistance to fusiform rust in the experimental population and to make this population directly comparable to other Cooperative breeding populations.

This past winter, the Cooperative established six of the nine clonal tests across four different states. By planting across such a broad geographic region (see map below), we minimize the risk of mortality due to climatic events and increase the exposure to various strains of the rust pathogen. The experimental design is an Alpha Cyclic Incomplete Block Design with row-column configuration, an efficient design to account for within-site heterogeneity when a large numbers of entries (~2400 clones) are being tested.

By this coming winter, the remaining three ACE field tests will be established, and survival assessments of the six tests will be made. Measurement schedules have not been finalized, but we plan to measure each test after the 3<sup>rd</sup> and 6<sup>th</sup> growing seasons. In addition to the standard measurements (heights and diameter, fusiform rust incidence, straightness, and forking), we will likely assess other stem and wood quality traits.

County Locations of Atlantic Coastal Elite Test Sites





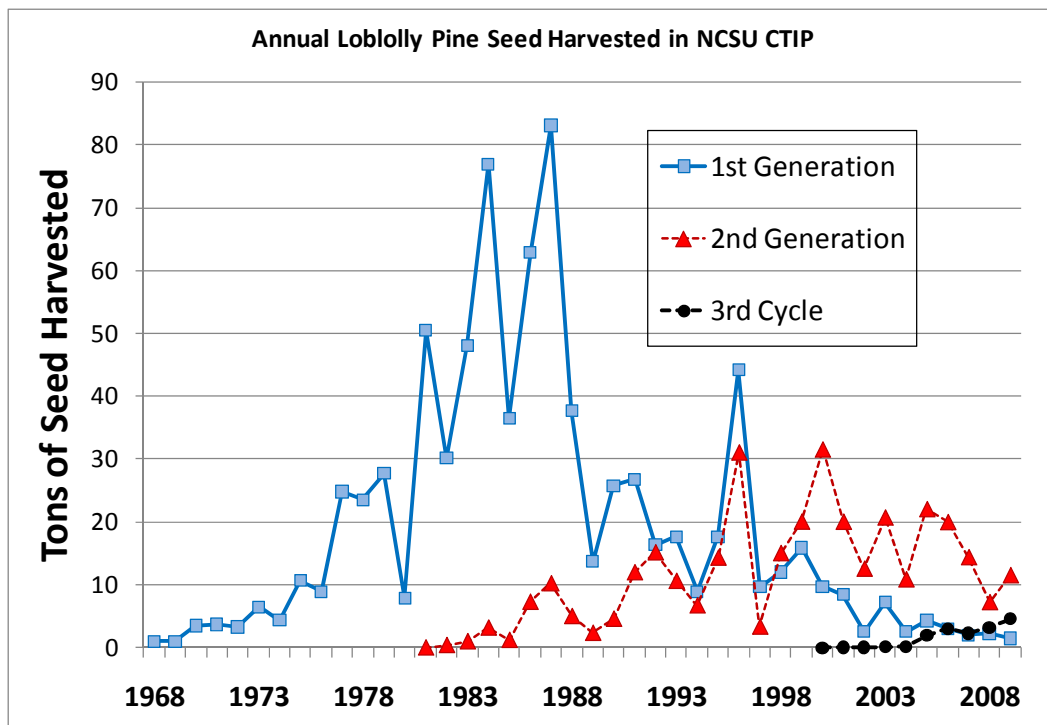
## CONE AND SEED YIELDS

Cooperative members collected 17.6 tons of loblolly pine seed in 2009 (Table 1). The 2009 harvest is 40% greater than the 2008 harvest but lower than the 10-year average of 24 tons per year. Third-cycle orchard harvests continue to increase and accounted for 26% of the seed harvested in 2009. Second-generation orchards made up 66% of the 2009 harvest while first-generation orchards decreased from 12% in 2008 to 8% of the harvest in 2009. Coastal orchard seed made up 73% of the total harvest followed by Piedmont (26%) and Northern (1%).

*Table 1. Comparison of 2009 seed and cone yields with previous year.*

Provenance	Bushels Of Cones		Pounds Of Seed		Pounds per Bushel	
	2009	2008	2009	2008	2009	2008
Coastal 1.0	526	3117	821	4353	1.56	1.40
Coastal 2.0	12042	7281	17938	11314	1.49	1.55
Coastal 3.0	5327	3421	6856	4419	1.29	1.29
Piedmont 1.0	1622	16	2121	27	1.31	1.69
Piedmont 2.0	3440	2043	4599	2786	1.34	1.36
Piedmont 3.0	1913	1584	2301	1957	1.20	1.24
Northern 2.0	525	253	494	340	0.94	1.34
Totals	25395	17715	35130	25196	1.38	1.42

Annual seed yields have varied over the years (Figure 1) due to regeneration needs, changes in membership within the Cooperative, environmental factors, and growth and technology within the industry. For the last 10 years, cooperators have produced sufficient quantities of improved seed, and therefore have been making selective harvests that average 24 tons of seed per year. The decrease in harvest from first-generation orchards, and the increase from the third-cycle orchards is shown in Figure 1.



*Figure 1. Annual seed yields from 1968 to 2008.*

From 1968 to 2009, over 2.3 million pounds of improved seed have been produced by Cooperative members (Figure 2). At 12,000 seedlings per pound, this is enough seed to grow over 27 billion improved seedlings. We look forward to greater production from advanced generation seed orchards in the near future!

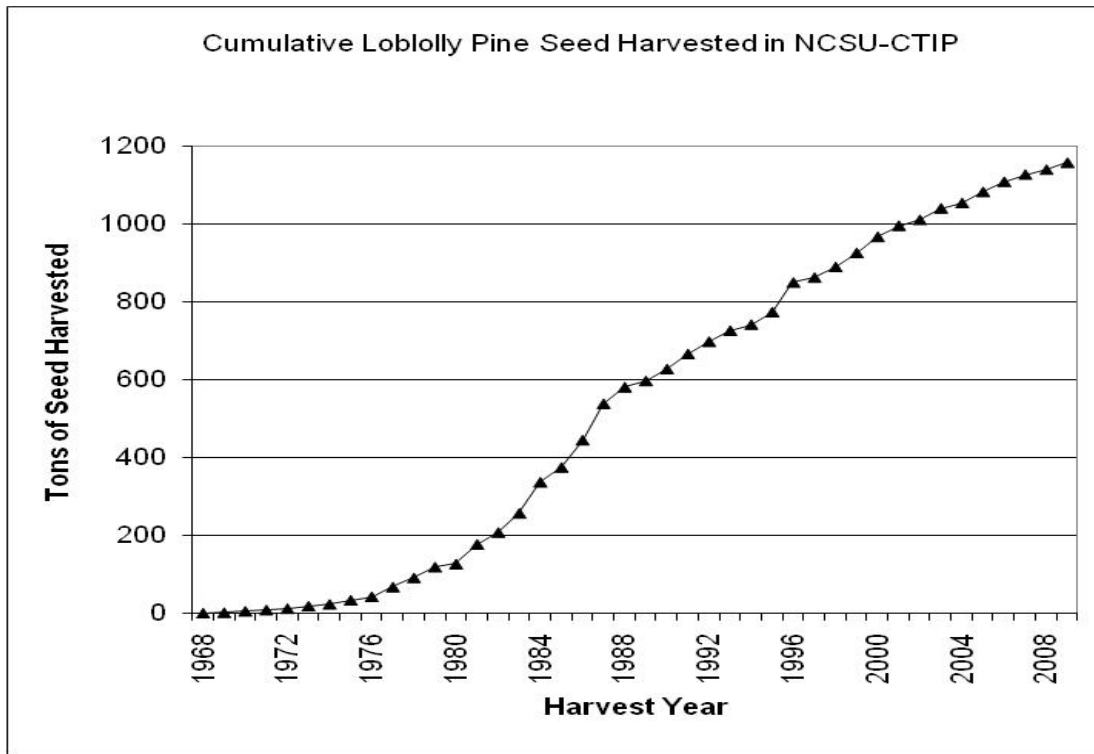


Figure 2. Cumulative seed yields for the Cooperative Tree Improvement Program starting from recorded amounts in 1968. Approximately 1,157 tons of improved seed harvested over the past 41 years.



Young 3<sup>rd</sup>-cycle seed orchards such as ArborGen's Piedmont loblolly orchard in Selma, AL are producing more of the seeds harvested by Cooperative members each year. Third-cycle orchard harvests accounted for 26% of the seed harvested in 2009.

## RESEARCH

### Genetic Analysis of Forking Defects in Loblolly Pine<sup>1</sup>

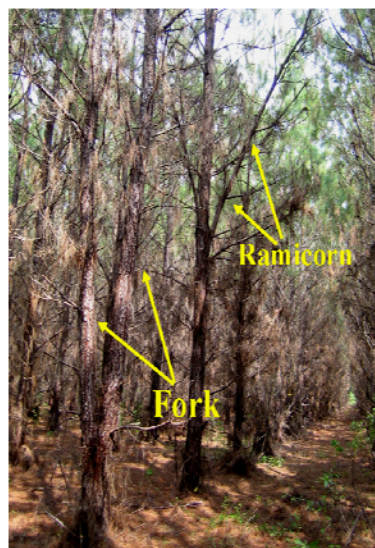
Forking defects, including stem forking and ramicorn branching, are serious stem-quality problems in loblolly pine. The presence of forked stems and ramicorn branches greatly reduces wood quality and quantity and the merchantability of the wood. Assessing forking traits may enable breeders to successfully breed and deploy non-forked genotypes in breeding populations. The objectives of this study were to assess the genetic basis of forking in loblolly pine by assessing trees in a large number of diallel tests (268 test series, ~ 1000 tests in the 2<sup>nd</sup>-generation testing program) and in a clonal test of MeadWestvaco in South Carolina.

In the diallel tests, the percentage of forking averaged 18%, and ranged from 4% to 80% across different test series. The individual-tree heritability was low (0.06), but family-mean heritabilities were moderately high (half-sib=0.76, narrow-sense full-sib=0.59, broad-sense full-sib =0.71), indicating that forking is partially under genetic control at the family level. By using half-sib family selection with selection differential of 20%, the genetic gain could be achieved with 12% to 23% reduction of forking across different regions. A weak unfavorable genetic correlation (0.18) was found between forking and height, suggesting that selection for growth alone will negatively impact forking in loblolly pine. A favorable genetic correlation (0.33) was found between forking and straightness.

In the clonal test, stem forking and ramicorn branching were serious problems in some clones. Forking averaged 17% with clone means ranging from 0% to 73%, and ramicorn branching averaged 24%, with clone means ranging from 3% to 50%. The estimated clone-mean repeatabilities were 0.86 and 0.67 for fork and ramicorn, respectively. Unfavorable genetic correlations between growth traits and forking suggest that selection for either

trait alone will negatively affect the genetic response for the other. A moderate positive genetic correlation between stem forking and ramicorn branching indicates that both traits may be partially controlled by the same genes and could be improved simultaneously.

Co-segregation analysis of markers and QTL for forking was conducted using the clonally replicated progenies of the outbred full-sib family from the clonal test. The linkage map was constructed with three types of markers containing various segregation patterns (F2, BC1 and BC2). A linkage map was achieved with 18 linkage groups defined by 409 SNP markers. A single-marker analysis of 1257 loci identified 11 and 9 markers that were significantly associated with stem forking and ramicorn branching, respectively. Those SNP markers explained 7% to 10% of the total phenotypic variations for forking defects. By using the interval mapping method, two QTL were identified for forking, and three QTL were identified for ramicorn branching. Two QTL were detected for both traits using multiple traits analysis, suggesting there are pleiotropic effects on both traits. The results from this study further confirmed that there is genetic control for forking defects, and genetic gain can be achieved through family and clonal selection. With the identified QTL influencing forking defects, marker-assisted selection can be considered in decreasing forking defect in the breeding population of loblolly pine.



*The presence of forked stems and ramicorn braches greatly reduces the economic value of the wood. By using family selection with a selection differential of 20%, forking could be reduced 12% to 23% across the different regions of the Cooperative.*

<sup>1</sup> Jin (Sherry) Xiong successfully defended her PhD dissertation in May, 2010. This is a summary of her work.



## Genetic Variation in Water relations, Physiology, Biochemistry, and Biomass Partitioning in Loblolly Pine<sup>2</sup>

Genetic differences in growth rate and uniformity may influence stand-level resource capture and productivity. Furthermore, genetic differences in physiological process rates, allocation to secondary biochemistry, and biomass partitioning could have major implications for ecosystem sustainability, carbon sequestration, and biogeochemical cycling. We hypothesized that genetically homogeneous genotypes (i.e. clones) would show more uniformity in height and diameter growth in a plantation setting relative more genetically diverse full-sib and half-sib family genotypes. We also examined genetic effects on physiological process rates, carbon allocation to secondary biochemistry, and above- and below-ground biomass partitioning in relation to genotype productivity. To determine genetic effects on physiology, biochemistry and biomass production, we grew nine genotypes in a plantation setting for three years and quantified differences in growth, gas-exchange rates, stem hydraulic traits, and foliar secondary biochemistry.

Growth, physiology, foliar biochemistry (total phenolics, condensed tannins, soluble starch and sugar) and biomass partitioning/production were measured on nine different genotypes planted as a complement to the Hofmann Forest Genetics × Spacing × Thinning (GST) study located in Onslow County, NC. This study was planted in 2006 and was composed of twenty replications of three genotypes from within each of three genetic groups (clones, open-pollinated families, and full-sib families) and was measured during the first 3 growing seasons. Additionally, we measured growth and foliar biochemistry in a 9-year-old clonal trial replicated across sites in GA and SC. Eight clones representing a range of potential productivity were selected for sampling to determine the relationship between growth and C allocation to secondary biochemistry.

In our results, one clone showed significantly higher uniformity in height and diameter growth relative to one half-sib family, however, less genetically diverse half-sib and full-sib families generally showed levels of uniformity comparable to that of clones. Although we found significant differences in percent stem wood biomass, fine root partitioning and stem internode length among genotypes, these differences were not consistently related to genotype differences in productivity. The main determinant of productivity differences among genotypes was the rate of mean height and ground-line diameter increase between May and August.

We found no genotype differences in any leaf-level gas exchange trait except intrinsic water-use efficiency (WUEi). Clones, full-sibs, and half-sibs showed no significant differences in physiological uniformity. Tree volume was significantly and positively correlated with net photosynthetic rate and WUEi, but the relationship varied by season. Individual tree volume growth and genotype mean volume were positively correlated with needle dark respiration.

In our water relations study, more productive genotypes tended to have higher rates of transpiration and canopy conductance. There was no consistent trend in uniformity within genotypes with varying amounts of inherent genetic diversity. We also found no differences in stem hydraulic properties or cavitation resistance among genotypes. Genotype mean spring-time canopy conductance and stomatal sensitivity were negatively correlated with genotype maximum hydraulic conductivity, and the full embolism point which suggests that genotypes with more rapid water use may be less sensitive to increases in atmospheric demand.

In the Hofmann Forest study, we found moderate differences in total phenolics among genotypes, and a weak negative association between volume and concentrations of total phenolics. In the clonal study, there was a significant site × clone effect for total phenolics. In the lowest productivity clones, total phenolics concentrations increased by 21% to

---

<sup>2</sup> Mike Aspinwall successfully defended his PhD dissertation in May, 2010. This is a summary of his work.

23% at the less productive GA site relative to the more productive SC site. More productive clones showed little variation in mean total phenolics concentration among sites, which suggests that superior clones exhibited less plasticity in production of secondary defense compounds.

Overall, our results provided little support of the hypothesis that more genetically homogeneous individuals would show greater uniformity in a plantation setting, at least not in the first three years. Also, differences in biomass partitioning may be partially related to genetic differences in productivity, but actual height and ground-line diameter growth rate over time was the best indicator of productivity. Uniformity in leaf-level physiological process rates were not consistently related to the amount of genetic variation within a given genotype, and WUEi, net photosynthetic rate, and dark respiration were the leaf-level physiological parameters that were most consistently related to individual tree and genotype productivity. In conclusion, the results of our study imply that silvicultural prescriptions should be appropriately matched to individual clone performance so that growth and uniformity can be maximized. Further investigation of genotype differences in stand-level uniformity, water-use, carbon fixation and carbon allocation may provide additional insight into genetic differences in productivity, carbon sequestration, and resource capture.



*Sometimes graduate students spend way too much time in the field harvesting trees and digging roots and start doing “unusual” things to entertain themselves.*

*Mike Aspinwall successfully defended his PhD dissertation in May 2010 and he headed to the University of Texas in Austin for a post-doc position in ecophysiology and genetics.*

## Plantation Selection Seed Source Study: A look into the base selection materials<sup>3</sup>

Studies of natural loblolly pine populations, like the Southwide Pine Seed Source Study, do a good job of defining characteristics of local pine populations in relation to genotype by environment effects (GxE) across many regions. These studies have been invaluable in defining seed source characteristics that could be used for making selections for growth, adaptability, and fusiform rust resistance. Geographic variation of traits in genetically improved pines with relation to GxE have also been described in previous studies, such as the Good General Combiner trials and many short range seed source or provenance studies. Most studies, however, do not carry a balanced set of genetic material from different regions, lack wide spread test sites, or lack large enough samples of families from different regions that could have the same potential as the seed source studies for natural pine populations.

The Plantation Selection Seed Source Study (PSSSS) contrasts these studies in that it is the first seed source study with loblolly pine to use genetically improved material, first generation plantation selections, with a widespread, balanced design. First-generation plantation selections were used for the PSSSS trials because they were supposed to make up ~85% of the future generations and were more intensively selected for growth, form, and fusiform rust resistance traits than other first-generation material. Another important aspect of these selections is that some were made from plantations containing non-local seed sources. Will the seed source variation using these plantation selections be comparable to previous seed source studies of natural populations? Some of the objectives of this study were to establish a potential baseline for the stability, gains, variation, and genetic correlation of growth and rust resistance traits across sites and populations.

Plantation selections were chosen from seven regions in the southeastern United States (Figure 1). In each region, 20 female parents were randomly selected from plantation selections and were then mated with a single pollen mix of 40 different pollen parents from the same region.

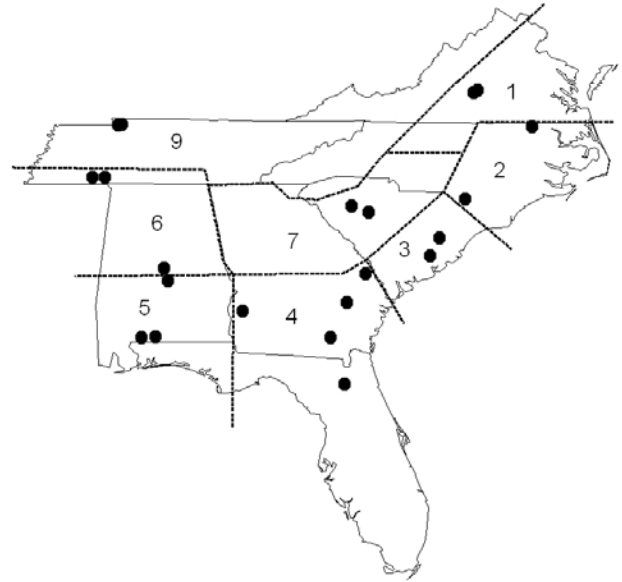


Figure 1. Plantation selection regions (1-7, 9) are indicated by dashed lines Test locations for the PSSSS trials analyzed are indicated by the black dots.

Each test, except in the Piedmont of Virginia and northern Tennessee, contains 140 families with 20 families per source from the seven regions. Each test had single-tree-plots replicated 24 times. Each test also included 4 checklot seedlings per rep. Most tests were planted on a 9'x9' or 8'x10' spacing, and most were planted between 1994 and 1997. At year 8, height, diameter at breast height, straightness, rust incidence, and survival were measured with the exception of tests 17 and 23 which were measured only at year 4.

The overall mean survival for all the tests was 87% with a range of survival on sites 56-96% (Table 1). The single site results showed some drastic differences in several traits; percent galled means ranged from 0 to 84%, and volume varied from 9.4 to 29.8 ft<sup>3</sup> when not including the 4 year data (Table 1). The commercial check lots means for most traits

<sup>3</sup> The PSSSS is the basis for Aaron Chamblee's MS research.

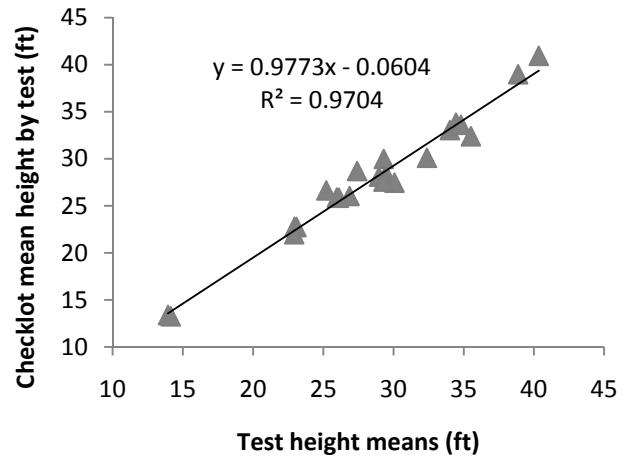


matched very well with site means, such as height where they had a nearly 1:1 relationship with test means (Figure 2).

Genetic analyses are underway, and will be the basis for Aaron's MS thesis.



*This one-year-old PSSSS trial planted in 1994 in Berkeley County, SC is part of Aaron Chamblee's data set for his MS thesis.*



*Figure 2. Test means vs. checklot means by site for height show that the checklots are very predictable.*

*Table 1. Test site locations and trait means*

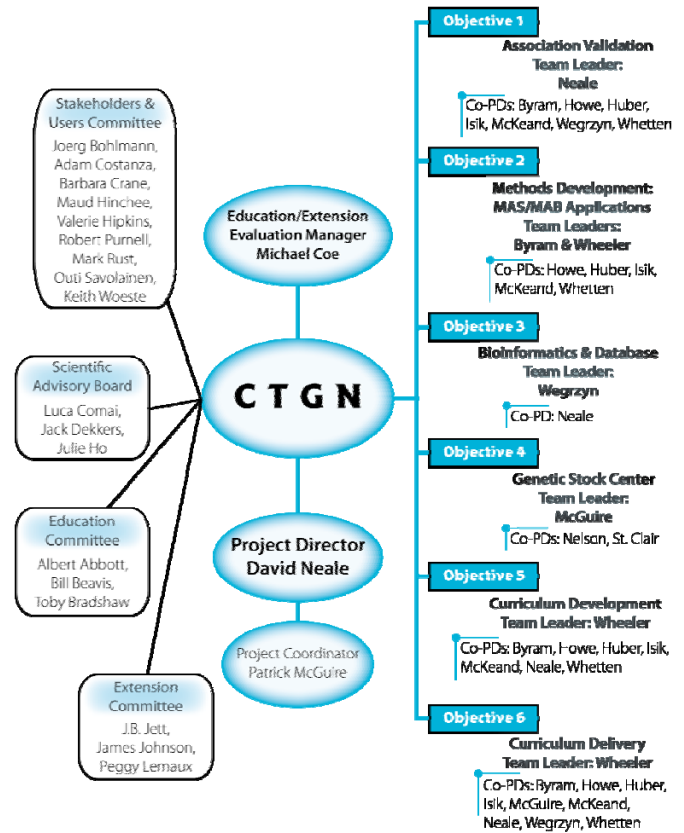
Test	State	County	Survival	Rust Incidence	Height (ft)	DBH (in)	Volume (ft <sup>3</sup> )
1	VA	APPOMATTOX	0.93	0.00	26.9	5.5	16.3
2	VA	APPOMATTOX	0.91	0.01	29.5	5.8	19.9
3	NC	NORTHAMPTON	0.63	0.84	26.0	6.0	20.1
4	NC	ROBESON	0.85	0.49	34.0	6.4	28.3
5	SC	WILLIAMSBURG	0.93	0.45	25.2	4.8	12.8
7	SC	BERKELEY	0.82	0.72	38.9	6.8	36.5
8	GA	SCREVEN	0.92	0.55	29.3	5.7	19.7
9	GA	TATTNALL	0.93	0.51	40.3	6.4	34.1
10	GA	RANDOLPH	0.90	0.61	23.0	4.5	9.9
11	FL	BRADFORD	0.92	0.62	27.4	4.4	11.9
12	GA	WARE	0.94	0.23	26.1	4.1	9.4
13	AL	ESCAMBIA	0.89	0.09	34.5	5.4	21.4
15	AL	ESCAMBIA	0.96	0.31	34.8	6.5	29.8
16	AL	AUTAUGA	0.56	0.69	29.0	6.1	21.6
17	AL	SUMTER	0.86	0.25	13.9	3.2	3.2
18	TN	MCNAIRY	0.93	0.05	32.4	6.5	28.2
19	AL	CHILTON	0.96	0.25	35.5	6.0	27.0
20	TN	HARDIN	0.77	0.01	29.5	6.3	24.0
21	SC	NEWBERRY	0.85	0.69	30.1	6.5	26.5
22	SC	LAURENS	0.92	0.47	29.3	5.6	18.5
23	GA	FLOYD	0.95	0.20	14.1	2.9	2.4
24	TN	STEWART	0.82	0.00	23.1	5.4	14.0
25	TN	STEWART	0.85	0.00	22.9	5.4	13.7

## Conifer Translational Genomics Network Progress

The CTGN is a Coordinated Agricultural Project supported by the Agriculture and Food Research Institute (AFRI) of the US Department of Agriculture, and represents a cooperative effort of six universities (UC-Davis, NC State, Univ. of Georgia, Univ. of Florida, Texas A&M, and Oregon State) and the US Forest Service. The objectives include testing thousands of single-nucleotide polymorphism (SNP) genetic markers for association with phenotypes of trees in the breeding programs, and developing strategies for applying the marker-phenotype associations to improve the rate or efficiency of the breeding programs.

After two and a half years of supported research, the CTGN project has yielded genotypes for about 4800 useful SNP loci in a set of about 1500 trees from the Cooperative breeding population, and we expect to receive some additional genotype data to bring the total to almost 1700 trees. Multiple independent subsets of the genotyped trees are being used in independent association tests, to allow comparison of the results obtained from one analysis with those from an independent analysis.

To date, these SNP genotypes have been used in three different types of populations – unrelated selections sampled from the breeding population, using breeding values estimated from progeny test data; a set of full-sib and half-sib families in a structured mating design, using genetic values estimated from clonally-replicated field plantings, and a large single full-sib family, using genetic values estimated from clonally-replicated field plantings. Each of these independent sets of trees contains 180 to 220 individuals, which is a relatively small number for traditional association genetics studies. However, the availability of three separate populations will allow comparison of results among the separate analyses to further test hypotheses of marker-trait associations, and they will provide unbiased estimates of the proportion of genetic variation accounted for by each genetic marker.



CTGN Management Plan from:

[http://dendrome.ucdavis.edu/ctgn/files/ctgn\\_mgt\\_plan.png](http://dendrome.ucdavis.edu/ctgn/files/ctgn_mgt_plan.png)

It is important to note that the use of a single large full-sib family to test for relationships among phenotypes and genetic markers constitutes a linkage analysis experiment rather than an association genetics experiment. In linkage analysis, a much larger segment of each chromosome is expected to show a correlation between genotype and phenotype, and so the number of SNP markers associated with phenotypes in this family is expected to be much larger than the number of markers associated with phenotypes in the population of unrelated individuals, or in the set of multiple families from a structured mating design. The appropriate set of comparisons, then, is to begin with the results from the full-sib family, and test each of those SNPs for presence among the SNPs identified in the other two populations, so that initial discovery uses the population with the maximum sensitivity, and validation uses additional populations with a reduced probability of false positive associations.

The phenotypes for which breeding values are available in the cooperative database are height, volume, straightness, forking, and rust disease incidence. A total of 1257 SNPs were tested for linkage to phenotypes in the full-sib family experiment, using a false discovery rate of 5% as the threshold of significance. Of the 1257 SNPs tested, 80 were linked with the height phenotype,

234 were linked with volume, and 162 were linked with forking defects. Additional testing of these SNPs for association with these phenotypes in the other populations is now underway, in order to further narrow the field of candidate SNPs to those that are reproducibly associated with these important phenotypes in multiple independent samples from the breeding population.

## Association Genetics to Improve Sawtimber Quality in Loblolly Pine

The development of genomic resources in loblolly pine offers the potential to improve economically important traits through the discovery of DNA sequence variation that is associated with phenotypic trait variation. Recent studies have found associations between SNPs (Single Nucleotide Polymorphisms) and variation in traits such as pitch canker infection, wood density, foliar nitrogen content, and water use efficiency. New approaches and methods are being developed that focus on the utilization of many markers for the selection and breeding of economically important plant and animal species. In this study, we use an association genetics approach to test for SNPs that explain variation in sawtimber quality related traits.

As part of the Conifer Translational Genomics Network project (USDA-CAP), over 1500 Cooperative selections are genotyped for 4800 SNPs. For this study we analyzed 200 unrelated first- and second-generation selections from Coastal and Piedmont provenances, using parental breeding values for volume, rust incidence, straightness, and forking from the recent “BIG BLUP” analysis as phenotypes. Additionally we tested for associations with the sawtimber index score for each selection (based on the Lower Gulf Elite sawtimber analysis – see p.11-13 in the 2009 Annual Report). In this case we only have genotypes for the tested selection, and thus we are associating SNPs with variation among breeding values.

Association testing revealed 278 SNPs associated with the sawtimber index and slightly fewer SNPs for individual trait breeding values prior to multiple test correction (Table 1). The number of shared SNPs appears to follow the strength of correlations

among traits. The correlation of the trait-trait correlation and the number of SNPs in common was 0.91. For example, height and volume are most highly correlated with the sawtimber index value and shared 121 and 165 SNPs in common, respectively. This suggests that using markers to improve a composite trait, such as an index value, could result in a correlated response in the individual traits.

*Table 1. Significant SNPs ( $p < 0.05$ ) for each trait (diagonal), SNPs shared in common by two traits (above), and correlations among traits (below). Saw = sawtimber index; Vol = Volume; Ht = Height; STRT = Straightness*

	<b>Saw</b>	<b>Vol</b>	<b>Ht</b>	<b>STRT</b>	<b>Rust</b>	<b>Fork</b>
<b>Saw</b>	<b>312</b>	165	121	29	94	39
<b>Vol</b>	0.85	<b>269</b>	151	20	34	15
<b>Ht</b>	0.73	0.85	<b>296</b>	15	35	19
<b>STRT</b>	0.29	-0.1	0.01	<b>258</b>	21	22
<b>Rust</b>	-0.58	-0.21	-0.14	-0.17	<b>282</b>	21
<b>Fork</b>	-0.36	-0.06	0.01	-0.27	0.26	<b>275</b>

After correction for multiple testing, there were 14 significant SNP-phenotype associations for sawtimber index, volume, and straightness, but no significant SNPs survived multiple test correction for height, rust, or forking. Minor allele frequencies for significantly associated SNPs ranged from 0.03 to 0.43, and each SNP explained 9 to 11% ( $r^2$ ) of the variation in the linear model. Five SNPs were located in sequences similar to known genes or sequences. SNP 0\_2222\_02\_74 was similar to a GRAS family transcription factor, SNP



0\_12452\_03\_87 was similar to a CDPK adapter protein in Arabidopsis, SNP 0\_15969\_01\_107 was similar to the at5g35570 k2k18\_1 sequence in Arabidopsis, SNP 0\_9918\_01\_641 was similar to a putative lysophosphatidic acid acyltransferase, and SNP 0\_14875\_01\_63 was similar to a calmodulin-binding family protein (Table2). The remaining SNPs were located in previously undescribed sequences.

Future work will incorporate a greater number of SNPs to estimate the effect each SNP has on economically important traits to create a marker-based index for the prediction of progeny performance, and use of an independent population of trees to obtain unbiased estimates of the magnitude of SNP effects on phenotypes. In

previously-reported association analyses in forest trees, individuals were genotyped and individual tree phenotypes were used in the marker analysis. In this case, we are combining BLUP-based breeding values from the Cooperative breeding program, which have much higher heritabilities than individual-tree phenotypes, and the genotypes of the parents in the breeding program. Ultimately we seek to find SNPs that predict sawtimber quality traits in progeny of these parents, to guide within-family selection. As genomic data become more available, we anticipate the implementation of genomic selection in which the effects of many markers are used to predict the phenotype of an individual. This will likely reduce generation time in future cycles of improvement of loblolly pine.

*Table 2. Significant marker-trait associations after multiple testing correction for sawtimber index, volume, and straightness. Height, forking and rust incidence had no significant associations after multiple test correction. MAF is the minor allele frequency; qvalue is an adjustment for multiple test corrections.*

Trait	SNP Locus	Annotation	SNP	MAF	$r^2$	p-value	q-value
Saw	0_13919_02_271	NA	[A/G]	0.04	0.11	2.6E-06	0.01
Saw	0_16546_01_530	NA	[G/A]	0.11	0.10	8.5E-05	0.10
Saw	0_2222_02_74	GRAS family transcription factor	[A/T]	0.26	0.09	9.3E-05	0.10
Saw	0_8448_02_252	NA	[G/A]	0.18	0.09	1.0E-04	0.10
Volume	0_8448_02_252	NA	[G/A]	0.18	0.13	2.1E-06	0.01
Volume	0_13919_02_271	NA	[A/G]	0.04	0.10	4.3E-06	0.01
Volume	0_16729_01_535	NA	[A/G]	0.12	0.09	7.5E-05	0.07
Volume	0_16710_02_265	NA	[G/A]	0.43	0.10	8.5E-05	0.07
Volume	0_12452_03_87	af384822_1 cdpk adapter protein 1	[G/A]	0.03	0.08	9.5E-05	0.07
Straightness	0_15969_01_107	at5g35570 k2k18_1	[C/G]	0.05	0.11	8.7E-06	0.04
Straightness	0_9918_01_641	ac068923_13 lysophosphatidic acid	[A/G]	0.07	0.10	3.9E-05	0.06
Straightness	0_14875_01_63	calmodulin- binding family protein	[A/G]	0.04	0.10	5.3E-05	0.06
Straightness	CL2166Contig1_01_105	NA	[C/A]	0.12	0.10	5.6E-05	0.06

## Molecular Marker Fingerprinting to Establish Parentage and Clone Identity

A question arose in the fall of 2009 regarding the identity of several ramets labeled as identical copies of a Piedmont selection, hereafter called “X”. A Cooperative orchard manager noticed that some ramets of selection X produced egg-shaped cones (“ovoid-cone” phenotype – left in picture), while some others produced cones that were longer, narrower, and curved (“curved-cone” phenotype – right in picture).



Foliage samples were taken of the ramets in question, and microsatellite markers were used to test for genetic differences between the different phenotypic classes. The marker results indicated that the “ovoid-cone” and “curved-cone” phenotypic classes also differed in the alleles at microsatellite loci, suggesting that at least one of these ramets is mis-labeled, and is not truly selection X.

In order to test for similarity between the two classes of ramets in the cooperator's orchard and the original ramets of selection X, foliage was sampled from a clone bank where several original ramets of selection X are archived. Microsatellite marker analysis of DNA extracted from the foliage samples showed that one differed from the others in the microsatellite alleles present, and the two different genotypes detected in the foliage samples agreed with the two genotypes detected in the cooperator's orchard. This result suggested that the presence of

two different genotypes labeled as selection X dated back to the original collection of ramets used as parents in the progeny testing of the selection.

The next objective was to determine which of the two genotypes was used as the parent for the progeny testing, because the genotype that was actually the parent of the measured progeny is the one that earned the rating listed for the selection in the cooperative database. Fortunately, two sites where progeny tests including this selection were planted are still accessible, and the progeny tests are still intact. Foliage samples were collected from 94 progeny at two sites, or about one-third of the progeny descended from this selection planted in those two sites. In addition, foliage samples were also taken from all surviving ramets labeled selection X in the clone bank, along with samples of multiple ramets of other selections grafted and bred at the same time. The other selections that were grafted and bred at the same time were considered the most likely contaminants, and the genotype of one of those other selections was considered likely to match the genotype of the trees mis-labeled as selection X.

DNA was isolated from 118 foliage samples and the isolated DNAs were genotyped with a set of 53 single-nucleotide polymorphism (SNP) markers. The genotyping was quite successful; only one of the 118 samples failed to yield genotypes for at least 95% of the SNP loci assayed. The sample that failed was one of the progeny samples, and that individual was excluded from further analysis.

The genotype data were analyzed using a computer program called Cervus (v 3), available from <http://www.fieldgenetics.com>. The genotypes of all ramets labeled as selection X in the clone bank were identical – no difference was detected using the SNP markers, in contrast to the result reported by the cooperator based on microsatellite marker genotyping. All ramets of other selections in the clone bank were also identical within selection. This result provides no evidence of any problem with mis-labeling or confusion of genotypes within the clone bank. A representative genotype for each selection tested within the progeny test series was used as a candidate parent for analysis with the

Cervus program, along with the 93 high-quality progeny samples. No prior information regarding the candidate parents of each progeny sample, based on progeny test records, was provided to the computer program; the program was provided only with 93 progeny genotypes and the putative parent genotypes, and asked to assign parents to each offspring in the progeny test. The software did this successfully, and all progeny listed in the progeny test records as descended from selection X were identified by the software as offspring of selection X, for 100% agreement between the progeny test records and the molecular marker genotypes.

The question remains unanswered as to why the SNP genotyping results disagree with the microsatellite results for the ramets of selection X within the clone bank of original breeding stock. Additional experiments will be required to determine if this is truly a disagreement between the different types of molecular markers, or if it is a result of incorrect sampling in the clone bank by one of the two groups that independently sampled the surviving original ramets.

## Grants Received

Competitive grants continue to be important to our program. They provide the funding for graduate students and faculty time for research and for fueling the long-term viability and innovation that is critical for tree improvement and forest genetics in the years to come. Listed below are current grants:

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.

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. 09/09 to 08/13.



*Topgrafting continued this year at the Arrowhead Breeding Center. Approximately 300 grafts were made to bulk up existing selections as well as to add new selections to the Sawtimber Elite Population.*

*Both Josh Steiger (left) and Ross Whetten (right) have become “grafting masters” the last couple of year.*

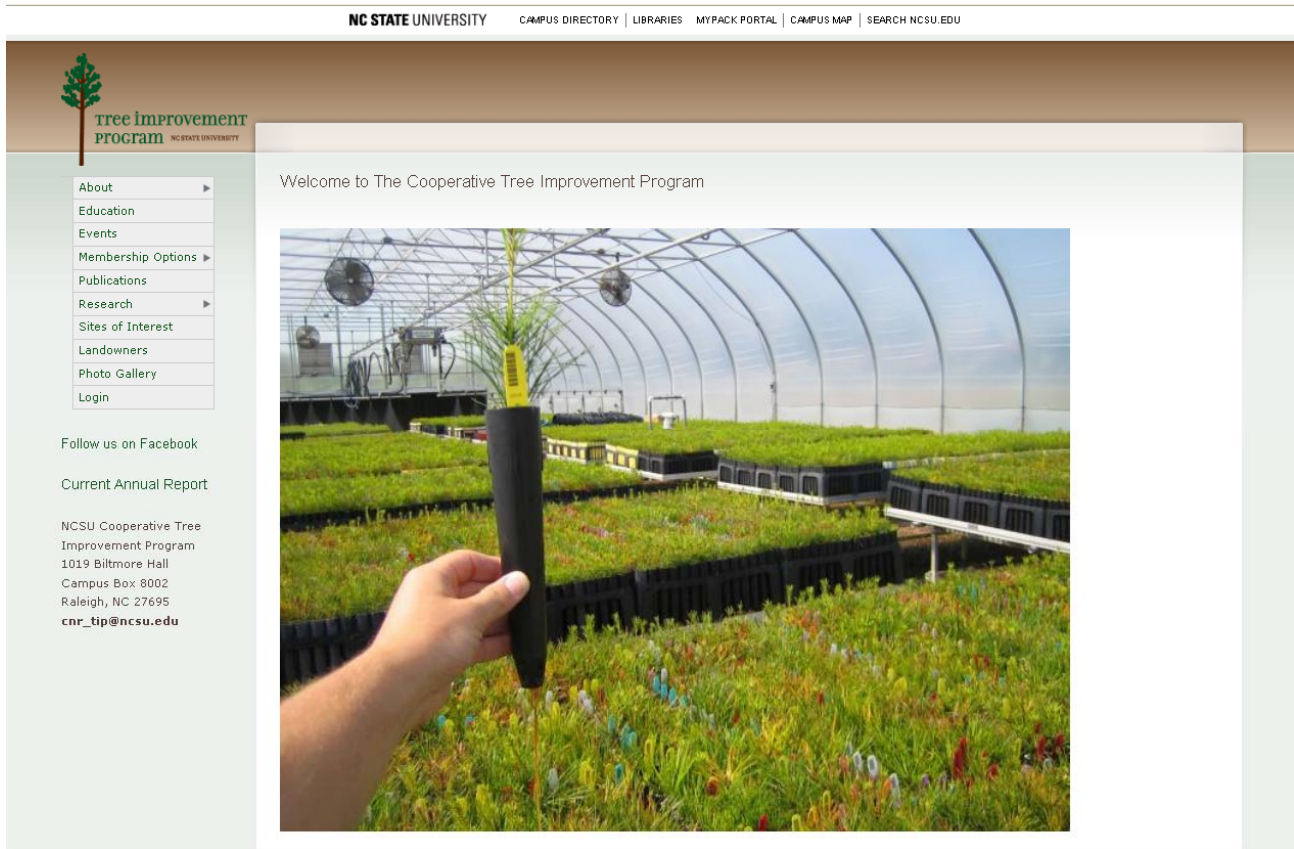


## ASSOCIATED ACTIVITIES

### Teaching

Teaching both graduate and undergraduate courses is at the center of a University. 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 – formerly FOR 611). Fikret Isik teaches in the Advanced Analytics program (AA591a, Statistical Foundation for Analytics and AA591b, Logistic Regression Models) as well as two courses in the College of Natural Resources (FOR728, Quantitative Forest Genetics and NR554, Introduction to Data Analysis). Ross also teaches FOR 610, Forest Genetics Seminar and FOR 350, Professional Development III: Ethical Dilemmas in Natural Resource Management

### Welcome to TreeImprovement.org



As mentioned at last year's annual meeting, we have overhauled our website once again. Along with the new website, we took on a new web address, <http://www.treeimprovement.org>, that we think makes it easier to remember how to find us.

Some may remember that our previous website was launched about 3 years ago, so why the change? The simple answer is that we rapidly outgrew our previous design. With the previous site we were locked into a rigid design layout, limited in physical space, and all changes had to be routed through one person. Our new site layout is much more flexible in both terms of growth and management of content. We have essentially an unlimited amount of space in which to grow, flexibility in the types of content made available, and the entire staff can now help manage areas of the website.



The new site has already brought us a new level of visibility on the web. The previous site was not search engine friendly, so when using any of the major search engines (Google, Yahoo, etc) to search “tree improvement” or even “NCSU tree improvement”, we would be no higher than five or six, and sometimes as low as fifteen. With the new site, and in less than six-months, we consistently rank in the top five results using the same criteria. We hope this improvement in search standings will help us better reach and inform people interested in learning about what the Cooperative does.

Over the coming months, we hope to continue to fill the site with information that will be useful to our cooperators, such as more technical information and training tools. For the general public and those people coming to learn about the tree improvement process and our Cooperative in general, we hope to communicate the aim and objective of the Cooperative, and that this tool aids in outreach and recruitment.

## Meetings, Workshops, and Short Courses

In December 2009, we had a workshop and the annual Contact meeting in Tuscaloosa, AL. The one day workshop titled, “The Value of Forest Genetics for Progressive Landowners” brought in almost 80 attendees to hear about genetics and tree improvement and how they can use these tools to increase stand value. The workshop was open to TIP members, but the bulk of the group consisted of consultants and private landowners in MS, AL, and GA. A combined field tour hosted by Westervelt had close to 100 folks (see group picture below) out in the field, and we got a wonderful mill tour, learned about more carbon credits, and got a look into a high production container nursery and seed orchard. Minus the bus getting stuck in the field right before happy hour, the trip was fantastic, and at the end of the day we celebrated with tasty meal of BBQ and Brisket. Thanks to Tommy Conwell, Glenn Free, George Surritte, and all the Westervelt team for being great hosts.



## Visiting Scientist

Andres Hernandez joined us in December 2009 from Universidad de Concepción, Chile. He is a PhD candidate studying under Dr. Rafael Rubilar, professor and co-director of the Forest Nutrition Cooperative and was supported by UDC, the National Commission for Scientific and Technological Research (CONICYT) and Project Mecesusup. Andres’ research is looking at rooted cuttings and laceration techniques in *Pinus radiata* and examining resulting root growth and morphology and nutritional effects for each corresponding technique. He worked with staff from Forest Nutrition Cooperative to determine nutritional values and sat in on Steve and Ross’s Forest Genetics class. We enjoyed having him here, and we wish him the best in completing his PhD program.



## Tree Improvement Cooperative Staff

For most of the year the Cooperative staff remained stable, but this July we will be saying goodbye to Patrick Cumbie. He will be finishing his PhD program and will be joining ArborGen as a Senior Scientist. His innovative marks have been left on several Cooperative projects like his work on evaluating our elite populations for Sawtimber values and work on Atlantic Coastal Elite populations, breeding center, work equivalents, Lower Gulf Elite analysis, and marker association research in evaluating sawtimber and drought tolerance. His contributions to our group will be sorely missed, but we wish him luck and look forward to his contributions as a NCSUCTIP member.

I know I say this every year, but there is no more dedicated group of hard-working individuals anywhere. These are the folks that make this Cooperative program the success that it is.



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

**Graduate Students** – Even with a tough economy, we are dedicated to supporting forest research and training individuals who will continue to contribute to forestry and tree improvement. Below are the graduate students and their corresponding forest research.

**Mike Aspinwall, PhD Candidate** - Evaluating genetic and physiology effects in a Growth-Genetics-Spacing trial. Mike successfully defended his dissertation May 4, 2010 and is heading to the University of Texas for a post-doc.

**David Barker, PhD** - Evaluating genetic differences in loblolly pine in conversion efficiency for cellulosic ethanol.

**Aaron Chamblee, MS** - Evaluating geographic variation in resistance of loblolly pine to fusiform rust disease using the Plantation Selection Seed Source Study as population.

**Patrick Cumbie, PhD Candidate** – Identification of molecular markers associated with phenotypic variation in growth, water use efficiency, and stem quality traits in loblolly pine.

**Daniel Gräns, PhD Candidate** - Evaluating solid wood properties in loblolly pine and Norway spruce.

**George Khan- MS** - Development of species specific SNP markers to distinguish different populations and species of pines.

**Funda Ögüt, PhD** – Genomic selection approaches in breeding loblolly pine.

**Ben Smith, PhD Candidate** - Incorporating realized gain from genetic improvement into loblolly pine growth and yield models.

**Sherry Xiong, PhD Candidate** - Genetic factors in forking in loblolly pine. Sherry successfully defended her dissertation May 4, 2010.

**Jaime Zapata, PhD** - Develop breeding strategies that incorporate molecular markers into the selection process for loblolly pine breeding.

---

### **William J. (Bill) Lowe – 1946-2010**

*Our Cooperative and tree improvement programs across the South lost a real friend with the passing of William J. (Bill) Lowe on March 31, 2010. For those that did not know Bill, he was Geneticist and Director of the Western Gulf Forest Tree Improvement Program from 1975 to 2001. Few knew southern tree improvement as well as Bill, and few did a better job of conveying this knowledge to foresters and tree breeders as is shown in the picture (Bill is in the white cap). Bill was a loyal friend and always a staunch partner with our program and others in the southern US. He will be missed.*





## **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.

Unfortunately, after 40 years as a member of the Cooperative, Smurfit-Stone Container Corporation filed for bankruptcy in 2009, and that was the beginning of the end for the tree improvement and nursery program. We wish the best to our friends Tyler Eckard and Doug Shelbourne.

New Contributing Members in FY2010 are American Forest Management, Biofuels Center of North Carolina, Milliken Forestry Company, and Pacolet Milliken Enterprises.

### **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**

**American Forest Management**

**Biofuels Center of North Carolina**

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

**International Forest Company**

**Jordan Lumber & Supply Company**

**Milliken Forestry Company**

**Molpus Timberlands Management, LLC**

**North Carolina Natural Resource Foundation**

**Pacolet Milliken Enterprises**

**ProFor**

**Resource Management Service, LLC**

**Scotch Land Management, LLC**

**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://www.treeimprovement.org/publications>

### 2010

- Aspinwall, M.J., B. Li, S.E. McKeand, F. Isik, and M.L. Gumpertz. 2010. Prediction of whole-stem  $\alpha$ -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.
- 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.
- 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., Skrøppa, T., Stoehr, M., and Yanchuk, A.D. 2010. Economic importance, breeding objectives and achievements. Chapter 2 In: *Genomics of Conifers*. Edited by: C. Plomion and J. Bousquet. Volume in *Genomics of Industrial Crops*, Series editor: C. Kole. Science Publishers, Inc., New Hampshire; Edenbridge Ltd., UK. (in press).
- 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* (in press).
- Xiong, S.J., F. Isik, S.E. McKeand and R.W. Whetten. 2010. Genetic variation of stem forking in loblolly pine. *For. Sci.* (in press).

### 2009

- Bains, B., F. Isik, W. Strong, B. Jaquish, J.A. McLean, Y.A. El-Kassaby. 2009. Genetic susceptibility of spruce to gall forming adelgids (Hemiptera: Adelgidae). *Can. J. Forest Research*.39: 2536-2541.
- Cumbie, P.W., R. Whetten, B. Goldfarb, J.L. Wegrzyn, A.J. Eckert, and D.B. Neale. 2009. Association genetics of water relations and early growth in loblolly pine. In: *Proc. 30th South. For. Tree Impr. Conf.*, Blacksburg, VA. (in press).
- Frampton, L.J, F. Isik, M. Benson, and AM Braham. 2009. Variation in resistance to *Phytophthora* root rot within Turkish and Trojan Fir. The IUFRO 9th International Christmas Tree Research and Extension Conference. 14-18 September 2009, Corvallis, OR. USA.
- Gräns, D., B. Hannrup, F. Isik, S-O Lundqvist, and S. McKeand. 2009. Genetic variation and relationships to growth traits for microfibril angle, wood density, and modulus of elasticity in a Norway spruce (*Picea abies*) clonal trial in southern Sweden. *Scandinavian J. For. Res.* 24(6): 494-503.
- Isik, F., H. Amerson, R. Whetten, S. Garcia, S. McKeand. 2009. Fusiform rust Fr genes by bulked inocula interactions in loblolly pine. In: *Proc. 30th South. For. Tree Impr. Conf.*, Blacksburg, VA. (in press).
- McKeand, S.E. 2009. Genetically improved loblolly pine – have we reached our limit? P. 3, 5 In: *Treeline*, Newsletter of the North Carolina Forestry Association. Jan/Feb 2009.
- McKeand, S., Steiger, J, and Crane, B. 2009. Progeny testing of longleaf pine seed orchards – plans for 2009. *The Longleaf Leader*, Newsletter of The Longleaf Alliance. 2(3): 8-9.
- McKeand, S., R. Whetten, P. Cumbie, J. Steiger, F. Isik, T. Batista-Brooks, S. Garcia, and J.B. Jett. 2009. What are the consequences of reduced effort in tree improvement? In: *Proc. 30th South. For. Tree Impr. Conf.*, Blacksburg, VA. (in press).

- Mora, C.R., L.R. Schimleck, F. Isik, J.M. Mahon, Jr., A. Clark III, and R.F. Daniels. 2009. Relationships between acoustic variables and different measures of stiffness in standing *Pinus taeda* trees. *Can. J. For. Res* 39:1421-1492.
- 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. 2009. Evolution of genome size and complexity in *Pinus*. *PLoS ONE* 4(2): e4332. doi:10.1371/journal.pone.0004332
- Morse A.M., R.W. Whetten, C. Dubos, M.M. Campbell. 2009 Post-translational modification of a R2R3-MYB transcription factor during xylem development. *New Phytologist* 183: 1001-1013
- Whetten R.W., S. Valenzuela, L.J. Frampton. 2009. Polymerase chain reaction preparation of template for massively parallel pyrosequencing. *J. Biomol. Tech.* 20:128 – 134

## 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: 1676-1689.
- 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

- Cumbie, W.P., S.E. McKeand, J. Espinoza, F. Isik, and J.E. Grissom. 2007. Improvements in stem form and growth of elite genotypes in loblolly pine. P. 108-111. In: *Proc. 29th South. For. Tree Impr. Conf., Galveston, TX.* Galveston, TX.
- McKeand, S.E., B.J. Zobel, T.D. Byram, and D.A. Huber. 2007. Southern pine tree improvement – a living success story. P. 3-6, In: *Proc. 28th South. For. Tree Impr. Conf., Galveston, TX.*



December Sunset at Westervelt's 3<sup>rd</sup>-Cycle  
Loblolly Pine Seed Orchard