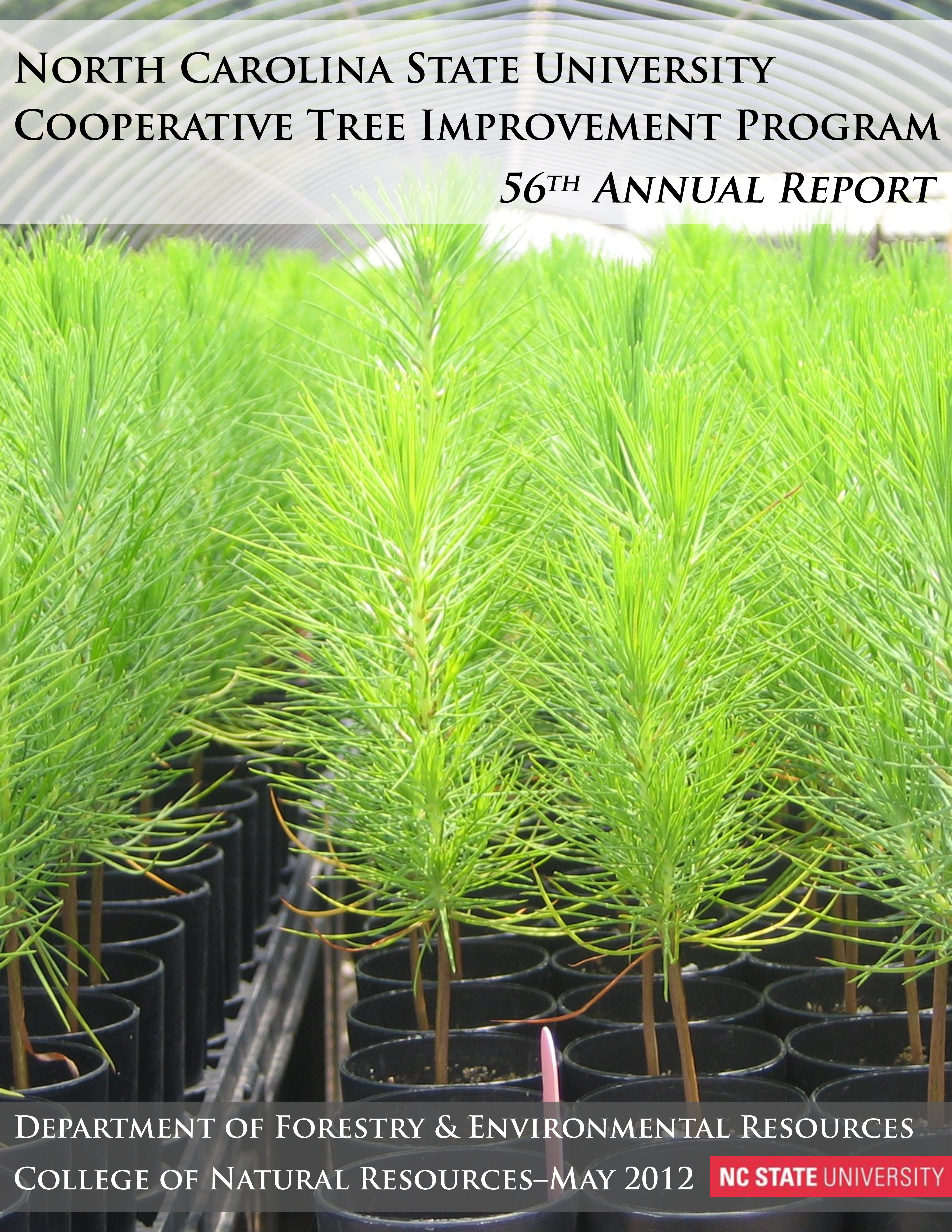
NORTH CAROLINA STATE UNIVERSITY COOPERATIVE TREE IMPROVEMENT PROGRAM

56TH ANNUAL REPORT



DEPARTMENT OF FORESTRY & ENVIRONMENTAL RESOURCES

COLLEGE OF NATURAL RESOURCES–MAY 2012

NC STATE UNIVERSITY

56th Annual Report

May 2012

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EXECUTIVE SUMMARY

Tree improvement has not only impacted the productivity and economic value of loblolly pine plantations for landowners in the South, but has also increased carbon sequestration significantly. Breeding and deployment of improved germplasm provides benefits to landowners and society not only today but for generations to come.

SELECTION, BREEDING AND TESTING

- 81% of the total PMX (pollen mix) breeding is complete. The Northern Sawtimber Elite Breeding (N-SEPop) testing program is underway; seedlings are growing at NCSU and clonal testing will commence in 2013.
- The testing of the Livingston Parish Plantation Selection program is complete and 10 trials were measured at age 6. Outstanding parents were identified for growth and quality traits with plans to include them in the 4th-cycle breeding populations.
- The average survival in the 2011 Longleaf Pine Genetic Tests across the 8 test sites was 68% and average survival by family was 0% to 92%. These family differences were under weak genetic control with $h^2_F=0.24$.
- USDA Plant Hardiness Zone has been incorporated into NCSU-CTIP ***PRS***™ for enhanced deployment decisions.

SEED PRODUCTION

Cooperative members collected 25 tons of loblolly pine seed in 2011, an increase of over 50% from 2010. Enough seed was harvested from 3rd-cycle orchards to plant over 400,000 acres next year with the most advanced open-pollinated families available.

RESEARCH

- Clonal varieties of loblolly pine were analyzed for variation of fermentable sugar yield from enzymatic hydrolysis. Large differences were found between treatments in the cost/gallon of ethanol required to achieve a 12% IRR.
- In a study to evaluate the effects of genetics and silviculture and their interactions on loblolly pine growth and wood properties, silviculture by genetics interaction effects were non-significant for all traits, and family rankings across treatment combinations were generally stable for growth, form, and wood property traits.
- Inbreeding effects on wood density and bending strength were not significant in a 23-year-old inbreeding study in eastern NC.
- Factor analytic methods were very effective in providing accurate predictions of genotypes and are a natural framework for modeling GxE interaction in genetic trials of loblolly pine.
- A large study to evaluate 20 of the Cooperative's fastest-growing open-pollinated families with bioenergy feedstock potential, was established in the Piedmont of NC. Genetic differences for biomass and sawtimber production will be evaluated in this long-term trial.
- Research in genomic selection addressed questions like: How many marker loci must be genotyped to allow estimates of breeding value to approach the accuracy obtained by using pedigree information alone? What structure of mating design and breeding population will be best suited to the application of genomic selection? What genotyping methods will be most cost-effective and reproducible for high-throughput genotyping?
- The USDA AFRI grants, Pine Integrated Network Education, Mitigation and Adaptation Project (PINEMAP) and the Southern Partnership for an Integrated Biomass Supply System (IBSS), are well underway with field and laboratory sampling commencing this last year.

ASSOCIATED ACTIVITIES, GRADUATE STUDENTS, COOPERATIVE STAFF, MEMBERSHIP

This year has been an active year for TIP and outreach activities. We hosted workshops, short courses, contact meetings, and started making appearances at various forestry association meetings. Jadie Andrews joined the staff as a research assistant and Fikret Isik started as Associate Director. There are 10 Full Members, 17 Contributing Members, and 2 Research Associate Members in the Cooperative.

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TREE IMPROVEMENT COOPERATIVE – IMPACT AND RELEVANCE

I like talking about **impact** and **relevance** when I talk about what the Cooperative Tree Improvement Program has meant to landowners, foresters, the economic wellbeing of the South, and the ecosystem services that improved pine plantations provide. It's relatively easy to quantify the increased productivity and economic value of our breeding programs, but we often overlook the ecological significance that has resulted. Below is the abstract of a paper that former PhD student Mike Aspinwall, John King, and I recently published in Forest Science - *Carbon Sequestration from 40 Years of Planting Genetically Improved Loblolly Pine across the Southeast United States*:

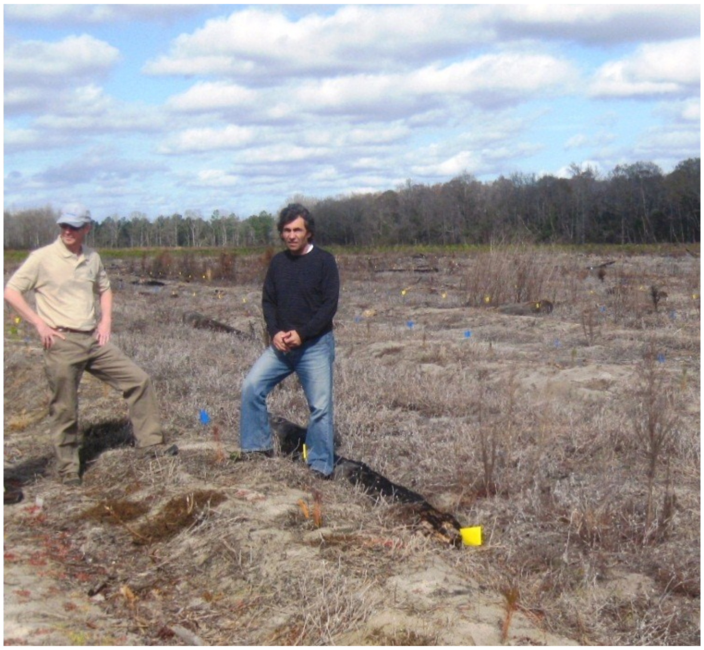
> Highly productive, widely deployed genetically improved loblolly pine (*Pinus taeda* L.) may play an important role in mitigating rising atmospheric CO_2 via carbon (C) sequestration. To understand the role of loblolly pine genetic improvement in future C sequestration strategies, we examined the historical (1968 – 2007) impact of operationally deploying improved families of loblolly pine on productivity and C sequestration across the southeast United States. Since 1977, nearly 100% of loblolly pine plantations in the southeast United States have been established with genetically improved loblolly pine. In recent years, more than 400,000 ha of genetically improved loblolly pine are planted annually. Between 1968 and 2007, we estimate that genetically improved loblolly pine plantations have produced a total of 25.6 billion m^3 of stemwood volume and have sequestered 9,865 Tg C in live and dead biomass. Our estimates also indicate that genetic improvement has resulted in an additional 3.7 billion m^3 (17% increase) and 1,100 Tg C (13%) of volume production and C sequestration, respectively, relative to volume production and C sequestration with no genetic improvement. We expect that loblolly pine plantation C sequestration will increase as more productive families and clones are deployed and as currently deployed genetic material continues to mature. Together, genetic improvement, intensive silvicultural, and longer rotations aimed at producing long-lived wood products will be important tools for maximizing C sequestration in loblolly pine plantations.
> (Published online April 12, 2012; http://dx.doi.org/10.5849/forsci.11-058)

Mike did a wonderful job of estimating what impact faster-growing plantations have had on carbon sequestration over the past four decades. Records from cooperators of seed harvests from 1st-, 2nd-, and 3rd-generation seed orchards and the number of mass-produced specific crosses and clonal varieties allowed us to estimate the operational gains across the landscape. In the early years, the impacts were modest with relatively few acres of improved plantations being established. As seed production increased, and virtually every acre of plantation was established with genetically improved seedlings, the impact has risen dramatically. Today, about 10% of loblolly pine plantations are established with specific crosses or clonal varieties. Based on seeds harvested from 3rd-cycle orchards last year (see Seed and Cone Yields, p. 10), over 400,000 acres can be planted next year with the most advanced open-pollinated families available. The impact that these more productive and valuable plantations will have on carbon sequestration, income to landowners, and other ecosystem services will be much greater than what is being harvested today.

Our faculty, staff, and students are also having significant impacts and relevance to the tree improvement and scientific communities. Our group with other scientists of the Conifer Translational Genomic Network (CTGN - http://dendrome.ucdavis.edu/ctgn/) was honored at a ceremony in Washington, DC on September 14, 2011 with the prestigious US Department of Agriculture Secretary's Honor Award in the category of Ensuring our national forests and private working lands are conserved, restored, and made more resilient to climate change, while enhancing our water resources. The CTGN team members included NCSU TIP scientists Ross Whetten, Fikret Isik, and even me. These awards are the most prestigious Departmental awards presented by the Secretary of Agriculture and recognize exceptional leadership, contributions, or public service by individuals or groups who support the mission/goals of the USDA.

Fikret and Ross were also recognized by the international tree improvement community for their contribution in application of genomic tools in forest tree breeding. The NCSUCTIP program organized an international week-long workshop on genetic data analysis in May 2011 at the Department of Forestry and Environmental Resources. The workshop included classical progeny test data analysis as well as analysis of large number of markers and genomic selections approaches. Ross and Fikret taught the workshop with help from Drs. Christian Maltecca (NCSU Animal Science) and Jim Holland (NCSU Crops Science). Ross and Fikret were also invited to teach genomic selection workshops in Brazil (July) and in France (November) in 2011 and were invited to teach a similar workshop in Europe in June 2012. Our group's scientists also authored two teaching modules on marker aided selection and genomic selection for the CTGN project extension efforts. The YouTube Videos of modules (14 and 15) can be watched at: http://www.extension.org/pages/60370/conifer-translational-genomics-network-online-modules. Our work in the emerging field of genomic selection and forest tree improvement will help maintain our position as leaders in the field of tree breeding.

I would be remiss if I did not emphasize all of the Tree Improvement staff for their incredible dedication to the Cooperative Tree Improvement Program and the hard work they put in every day to make this program as productive and valuable as it is. I want to thank Jadie Andrews (our newest employee), Tori Brooks, Saul Garcia, Jim Grissom, Fikret Isik, J.B. Jett (our veteran), Josh Steiger, and Ross Whetten. I say it all the time at meetings, but I want to put it in print – there is no more hard-working, dedicated group of tree breeders anywhere.



Ross Whetten and Fikret Isik not only contribute with their analytical and genomics expertise, but they also work with cooperators on breeding strategies, progeny testing (pictured above), and orchard management.

Special recognition needs to go to Fikret Isik whose contributions to the Cooperative have increased dramatically the last few years. Fikret is now Associate Director of the Cooperative in recognition of his increased responsibilities and critical work in the program. In addition to his teaching, research, and service activities in the Department, Fikret's role will be to support the Cooperative on a range of technical issues, including quantitative genetic analyses, developing breeding strategies for the genomic era, pedigree management, and genomic selection approaches.

Tree improvement does have **impact** and **relevance** in areas that often go unmentioned and unrecognized. Too often, we get caught up in our discussions of breeding strategies, genetic gain, seed orchard management, linear mixed models, genomic selection, and wood properties. Take every opportunity to emphasize to the public the importance of what each of us has done for today's landowners and society, but more importantly for what we are doing for our children, grandchildren, and generations to come.

Steve McKeand

May 2012

SELECTION, BREEDING, AND TESTING

Third-cycle Mainline Breeding Progress

Mainline breeding progress has made good progress since last year, with 81% of the total PMX (pollen mix) breeding complete. By region, the Coastal PMX breeding is the farthest along, with 85% of breeding completed. However, Piedmont and Northern breeding is moving along nicely with 78% and 80% of breeding finished, respectively.

With several members now finished or nearly finished with PMX breeding, more resources are being put into within subline full-Sib crossing. Moving forward, it is important that we quickly and efficiently make full-sib crosses, as they will generate seed responsible for within-family selections to be used in the next breeding cycle. As PMX breeding nears completion and full-sib breeding ramps up, the Cooperative is in a good position to finish third cycle breeding in the near future.

Table 1. Summary of 3rd Cycle PMX breeding progress through Spring 2012

Type of crosses	Breeding Region	# of Sublines	Total Parents	# Crosses producing Seed	#Crosses of Sufficient Seed	% Done
Polycross	Coastal	32	368*	329	313	85%
	Piedmont	31	316	250	247	78%
	Northern	13	180	164	144	80%
	Total	**76**	**864**	**743**	**704**	**81%**
PMX checks	Coastal	--	10	10	10	100%
	Piedmont	--	10	10	10	100%
	Northern	--	12**	12	12	100%
PMX CFs	Coastal	--	7	7	7	100%
	Piedmont	--	7	7	6	86%
	Northern	--	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 and late 2nd selections

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

Third-cycle Elite Breeding Progress

Atlantic Coastal Elite (ACE) Population

Breeding of elite material in the Cooperative has progressed at a rapid pace over the past several years. In fact, this coming winter we will begin collecting three year data from clonal trials established from our Atlantic Coastal Elite (ACE) Population.

Fikret Isik discusses details of the 2-year-old ACE test with the Westervelt tree improvement team – George Surritte, Tommy Conwell, and Glenn Free.

Sawtimber Elite Population

The Sawtimber Elite Population (SEPop) is an accumulation of the very best selections across all three breeding regions based upon new and improved BLUP breeding values. These populations are comprised of straight, high-volume, rust-resistant selections that should be great for sawtimber. The SEPop, like our mainline population, is divided into a Coastal, Northern, and Piedmont region. In each region, crosses were assigned based upon the breeding values for volume gain, stem straightness, forking, and rust incidence.

Overall, breeding progress for the SEPop has been exceptional. The effort put forth at the Arrowhead Breeding Center combined with the in-kind work done by Cooperative members has resulted in rapid progress to date. In fact, cone collection this past fall yielded enough seed in our Northern SEPop to allow for clonal testing to commence (see N-SEPop below). The flower crop was a bit low this spring, so not as much breeding was done as in past years. In the Coastal region, 29 crosses were made, totaling well over 500 flowers. For our Piedmont selections, 440 flowers were bred across 19 crosses.

Northern SEPop – After cone collection in Fall 2011, we had sufficient seed from a majority of the original 52 Northern SEPop crosses as well as from crosses in the Elite Northern Population (EN-1, EN-2, EN-3). There were space constraints at the NC State University Horticulture Field Laboratory, so the decision was made to begin the process of clonally replicating individual genotypes within each full-sib family using rooted cuttings from the top 23 crosses. To hasten this process by a full year, seed from the 23 top crosses as well as 7 common family checks were put into stratification before Christmas in 2011. In late January 2012, these seed were sown in our greenhouse and by mid-March (see picture to the right) we had young, healthy seedlings. Our plan is to transplant the seedlings into 3 gallon pots in mid May and begin managing the seedlings so that they will be ready for hedging in early August. Ideally, we will have roughly 40 hedges per family to produce clonal replicates, giving us great power when making within-family selections. At this pace, by next spring, the hedges will have sufficient shoots and the process of collecting and sticking cuttings can begin.

Northern Elite SEPop germinants being grown in the greenhouse in Raleigh, NC.

Piedmont Elite Diallel Population (PEDP)

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.

The Piedmont Elite Diallel Population (PEDP) was compiled from full-sib families produced from a disconnected diallel mating design of 23 parents from two elite piedmont diallels. We selected 40 crosses based on their index breeding values for growth and quality traits, but poor germination from two families left us with 38 crosses. With fusiform rust a pest of concern in the Piedmont region, and the positive results we saw with the Atlantic Coastal Elite (ACE) Population, pre-screening the PEDP for resistance was a logical first step. In spring 2011, full-sib seedlings (3,656) were challenged at the USDA Forest Service Resistance Screening Center (RSC) in Ashville, NC with a 5-zone bulked inocula at 50,000 sp/ml. In addition, six common family checklots were included in the screening trial. The checklots were represented twice, each family having samples screened and not screened for rust. This duplication will allow us to have a set of checks that can be used to measure productivity and quality traits, as well as gauge field rust since some of the checks will not have been pre-screened. Depending on the seeds available, each cross was represented by 10 to 120 seedlings. Disease incidence was observed at 3 and 5 months after inoculation.

A generalized linear mixed model was fit to the data to estimate variances and heritabilities. As we found with the ACE population, there was considerable genetic variation for fusiform rust disease in the population. Half-sib family heritability was 0.91 ± 0.015, broad-sense full-sib family heritability was lower (0.75 ± 0.056) mainly because of the smaller number of full-sib progeny for a given cross compared to half-sib families. There was a huge range in the predicted rust breeding values for parents, ranging from 0.15 to 0.91. Full-sib genetic values ranged from 0.14 to 0.84. We selected 18 crosses based on the resulting analysis and number of available seedlings that we could reclaim from the RSC.

Experimental trees and checklots combined, we potted 1,138 seedlings in Fall 2011. Transplanting survival was very high with only a handful of trees dying. We will stump these trees at the end of Spring 2012, and hedge at the end of Summer 2012; cuttings will be rooted in Spring 2013. If the ACE trials were any indicator, we hope to have the necessary rooted cuttings for the PEDP trials wrapped up in one series of harvesting cuttings. Plans are to establish field trials fall of 2013.

PEDP seedlings came back from the RSC in October, 2011 (Saul Garcia and Maria Wilkes, pictured below) and were transplanted into 3-gallon pots on March 20, 2012. Seedlings will be hedged in the summer of 2012 to produce cuttings in 2013 for clonal tests.

Above: Saul Garcia and Maria Wilkes discussing the PEDP seedling survivors from Resistance Screening Center and plans for repotting them into hedges.

Breeding came early this year for the Southeast US. The picture on the right shows flowers at stages 2 and 3 on February 17, 2012, about 3 weeks earlier than normal.

Third-Cycle Progeny Testing

Since the winter of 2004-2005, Cooperative members have installed 78 polymix (PMX) tests across all three breeding regions. In the fall/winter of 2011-2012, a total of 5 tests were established in the Coastal Plain region (CPMX5) by Cooperators (see Table 1). Over the last several years, Cooperators have excelled in establishing more of these progeny trials along with more of the full-sib family block plots.

In April 2012, seed was sent out to cooperators for the establishment of Coastal region PMX tests (series 6) in 2012-2013. As well, seed was sent for establishment of Piedmont PMX tests (series 4). A total of 3 Coastal tests and 2 Piedmont tests comprised of about 60 families were distributed for Cooperative members. This seed will be sown and seedlings will be established into field progeny tests.

Among other activities in the third-cycle progeny testing, in fall 2011 the second PMX series for Coastal region tests, and first Piedmont PMX test series, were measured and analyzed for Breeding Values at age 6 years (see earlier section). Also, four Checklot Comparison trials, designed to link second-cycle and third-cycle checklots, were measured at age 6 years.

Table 1. Third-cycle Polymix (PMX) progeny tests established through year 2011.

Region	Test Series	2004-2005	2005-2006	2006-2007	2007-2009	2009-2012	Total
Coastal	CPMX1	15					*15*
Coastal	CPMX2		5	5			*10*
Coastal	CPMX3			5	5		*10*
Coastal	CPMX4				8		*8*
Coastal	CPMX5					5	*5*
Piedmont	PPMX1		3	5			*8*
Piedmont	PPMX2				8		*8*
Piedmont	PPMX3					5	*5*
Northern	NPMX1				4		*4*
Northern	NPMX2					4	*4*
	Total	**15**	**8**	**15**	**25**	**14**	***77***

Progeny Test Evaluation of Plantation Selections of Livingston Parish Loblolly Pine

Based on early provenance studies in the 1950's and 60's, loblolly pine seed sources from Livingston Parish, Louisiana became renowned for characteristics of good resistance to fusiform rust as well as good growth across a wide geographic area in the southeastern US. Loblolly pine from Livingston Parish (L.P) and nearby Parishes and counties were widely planted during the 1970's on more than 300,000 acres from south Arkansas to north Florida and beyond. In the 1980's, Marvin Zoerb with Union Camp Corporation (now International Paper) recognized the value of the L.P. seed sources and started making selections from plantations that originated from wild-stand collections. In the 1990's, more selections from plantations of L.P. seed sources were made by other members of the Cooperative, and a total of 154 plantation selections were available for testing.

Initial plans were to use a pollen-mix (PMX) breeding and testing scheme to determine which, if any, of the selections from the Livingston Parish plantations were worthy of including in the Cooperative's Coastal breeding population. A mix of pollens from 12 average (based on height breeding values) Atlantic Coastal Plain parents was initially used to breed the Union Camp selections. The Cooperative's standard 3rd cycle Coastal pollen mix was used to breed all remaining selections. The two pollen mixes were very similar for growth breeding values.

A total of 10 PMX tests were established, 4 by Union Camp (Series 492) and 6 by other Cooperative members (Series 550). PMX families plus checks (CC3, CC4, CC5, SOM4, 7056, LPCC) were in each test Series 492. Only the 7-56 check was included in Series 550. The experimental design is randomized complete blocks with 20 blocks per location and single-tree-plots. Each check is represented by 1 seedling in each rep. In Series 492, 23 PMX families were tested whereas in Series 550, a separate set of 85 PMX families were tested.

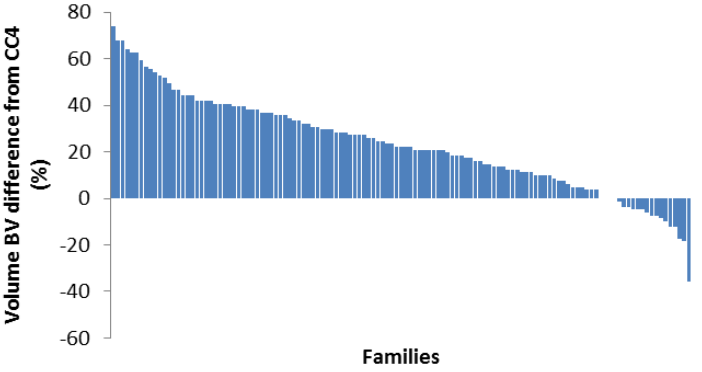


Figure 1. There are outstanding families in L.P. series. The top family had 74% greater volume BV and 36% better straightness BV over the CCK checklot.

All trials were measured at age 6 years and summarized and as expected, there were some outstanding parents identified for growth traits (Figure 1) as well as for other traits (not shown). Heritability estimates for all traits were typical of other test series (Table 2).

Our intention is to select parents with acceptable growth and quality attributes and include these into the 4th-cycle breeding populations. These L.P. trees are not related to other individuals in the mainline or elite populations, so they will increase the effective population size of the breeding population as well as adding to the genetic quality. This spring, 9 outstanding selections were identified (Figure 2) for topgrafting at the Cooperative's Arrowhead Breeding Center in Georgia.

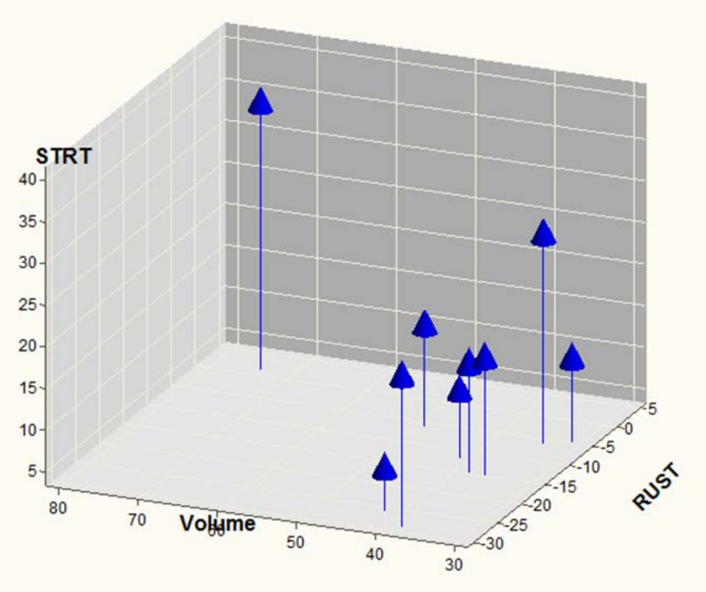


Figure 2. Three dimensional plot of selections topgrafted at the Arrowhead Breeding Center. The top selection in the upper left-hand corner is outstanding for volume and straightness but about the same as the checklot for fusiform rust disease incidence. The other 8 selections combine good growth and form traits.

Series	Height	Volume	Strt	Rust	ForkRam
492	0.57 ±0.147	0.31 ±0.091	0.41 ±0.111	0.27 ±0.019	0.04 ±0.002
550	0.25 ±0.043	0.17 ±0.031	0.12 ±0.026	0.39 ±0.003	0.03 ±0.001

Table 2. Individual-tree heritability estimates ± standard error for two test series

The Livingston Parish plantation selection effort was a large effort by many Cooperative members and took several years to complete the initial testing. With the breeding values in hand, we are poised to use this valuable population to enhance both the short - and long-term breeding populations for the Coastal Plain.

Longleaf Genetic Trials – Update and Plans

Background

In early 2010, the Cooperative and the US Forest Service began collaboration on a longleaf pine genetic testing effort that we hope will be a valuable contribution to the restoration of the species. The trials will serve as platforms to study the effects of climate change and adaptability while also allowing data to be collected on various growth traits and disease incidence, which will help us better understand the genetic variation in the species. In the fall/winter of 2010-2011, 10 long-term longleaf pine field trials were established across the native range of the species. In all, 133 seedlots are being tested. Most seed were collected from selections in seed orchards, with the exception of 15 seedlots collected from natural stands.

Update

Overall, first year survival of the 10 longleaf tests was marginal. Two tests were completely lost due to the harsh winter and extreme drought of 2011. However, the other 8 tests faired reasonably well, considering the drought conditions across most of the southeast US. According to the US Drought Monitor, the summer months of 2011 (May 1 – July 31) ranked 8th driest on record (http://droughtmonitor.unl.edu/), making 2011 a difficult year for longleaf establishment.

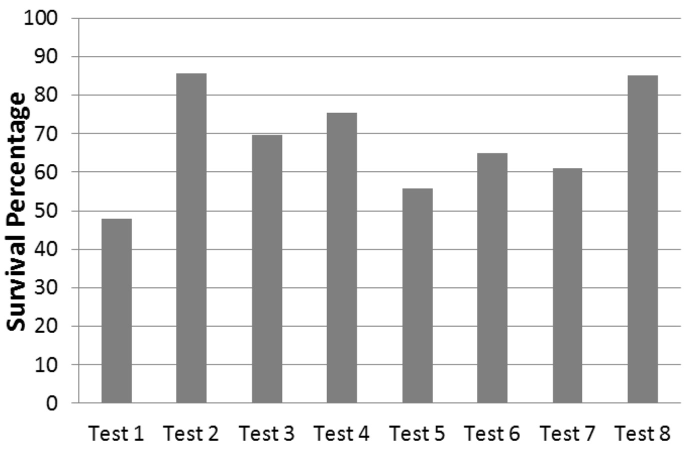
The average survival across the 8 test sites was 68% and ranged from 48% to 86% (Figure 1). As expected from the range in test survival means, test location had a statistically significant effect ($p<.0001$) on first-year survival. Furthermore, average survival by family ranged dramatically, from 0% to 92%, and these family differences were under weak genetic control with a family-mean heritability of 0.24 (SE=.18).

A Weyerheauser longleaf progeny trial after its first growing season in Washington Parish, LA.

Plans

Height at age 2 or 3 years will be measured to assess emergence from the grass stage. Depending on the growth rate, height, diameter, disease incidence, and stem form will be measured at age 8 to 10 years. Additionally, sites will be monitored by USFS, where research on climate change, adaptability, and carbon sequestration will be done.

Figure 1. First-year survival for each test site.



Loblolly Pine Performance Rating System (PRS™) Updates

Many landowners are often not knowledgeable about the genetic quality of the seedlings they purchase. If landowners were better informed of the benefits of using specific families for forest regeneration, then they can determine when it is appropriate to invest in the higher priced and higher genetic quality seedlings to recognize the increase in the net present value of their plantation investment. Because of the need for specific genetic info, the NCSU Cooperative Tree Improvement Program (NCSUCTIP) developed the Loblolly Pine Performance Rating System (PRS™) as a service to landowners, nursery managers, the tree improvement community, and loblolly pine breeders. The PRS™ expresses the genetic potential of a family for stem volume production, resistance to fusiform rust disease, stem straightness, and forking. Members are using the PRS™ to relate information to internal units within organizations as well as with customers.

We are continually improving the quality of information in the PRS™ database. In June 2011, an updated Coastal Plain region PRS™ database was distributed to Cooperative members. This version fully incorporated the 2011 "Big BLUP" analysis results (see 52nd and 53rd Annual Reports for descriptions of the "Big BLUP" project). These tools have been well received and widely used. The flexible BLUP protocol can seamlessly integrate new phenotypic data; for instance the new third-cycle PMX parental info was added in 2011.

2011 Enhancements to the PRS™ database: Deployment to a specified county location using minimum winter temperatures (Plant Hardiness Zones)

In PRS™ 2011 v3, we use Minimum Winter Temperature (e.g. USDA Plant Hardiness Zones - PHZ) to guide deployment decisions. In general, families from warmer origins will grow faster than seedlings from the local seed source, but there is a risk of maladaptation if the seedlings are moved from too warm of a region (for details, see Schmidtling 2001, ***Southern Pine Seed Sources***, available at: http://www.srs.fs.usda.gov/pubs/gtr/gtr_srs044.pdf).

Genetic Origin of a family (selected parent): The average min. winter temperature (*MinTemp*) for the geographic origin is derived in one of two ways:

1) First-generation or plantation selections (unknown parentage) – *MinTemp* of the county where the original selected tree (ortet) was located.
2) Advanced-generation selection (known parentage) – The average of the *MinTemp* of the counties where its parental selections were located.

For full-sib families, *MinTemp* for both the female parent AND the male (pollen) parent are averaged to estimate the *MinTemp* for the cross. Likewise, for open-pollinated (OP) progeny, *MinTemp* factors into both the female parent AND the male pollen cloud parent for filtering. We make the same assumptions for the cold-hardiness of the pollen cloud that we make for the breeding values of the pollen cloud. We take the average *MinTemp* of all the selections in the orchard and the estimate of *MinTemp* of pollen coming in from outside the orchard to estimate the *MinTemp* of the pollen cloud; we then average this with the female parent's *MinTemp* to estimate the *MinTemp* of the OP family.

Users of the new PRS™ are asked to specify a *MinTemp* value difference that they are willing to accept for deployment. Our standard recommendation is to deploy families that have a *MinTemp* value difference of 5º F (e.g. if the *MinTemp* for the county where the trees will be planted is 15º F, and the *MinTemp* of the family is no more than 20º F, then it is an acceptable family for that county). Users are free to accept any *MinTemp* value they want, but they must understand the risk versus benefit of planting families from different geographic origin.

As we reach out to more landowners and forestry consultants through workshops, PRS™ is always high on our list of items to promote. Interested parties can now visit the TIP website at, www.treeimprovement.org/prs, to familiarize themselves with how to interpret PRS™ for their regeneration decisions.

SEED AND CONE YIELDS

Cooperative members collected 25 tons of loblolly pine seed in 2011 (Table 1). Compared to last year, overall seed yields were up dramatically, an increase of over 50%! Additionally, average seed yield per bushel increased significantly, contributing to the higher seed harvest.

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 10 years, cooperators have produced sufficient quantities of improved seed and have been increasingly selective with collecting from only the best parents in the orchards.

From 1968 to 2011, nearly 2.4 million pounds of improved seed have been produced by Cooperative members. At 12,000 seedlings per pound, this is enough seed to grow over about 29 billion improved seedlings. We look forward to greater production from advanced generation seed orchards in the near future!

Provenance	Bushels Of Cones		Pounds Of Seed		Pounds per Bushel	
	2011	2010	2011	2010	2011	2010
Coastal 1.0	0	0	0	0	N/A	N/A
Coastal 2.0	12352	8103	18131	11387	1.467843	1.405282
Coastal 3.0	10333	3363	14572	3857	1.410323	1.146893
Piedmont 1.0	0	0	0	0	N/A	N/A
Piedmont 2.0	9395	7026	11113	8992	1.182839	1.279818
Piedmont 3.0	5277	1202	5040	1283	0.954987	1.067388
Northern 2.0	1487	1706	2190	1037	1.472764	0.607855
Northern 3.0	441	262	332	224	0.752834	0.854962
Totals	***39285***	***21662***	***51378***	***26780***	***1.39***	***1.06***

Table 1. Comparison of 2011 seed and cone yields with previous year.

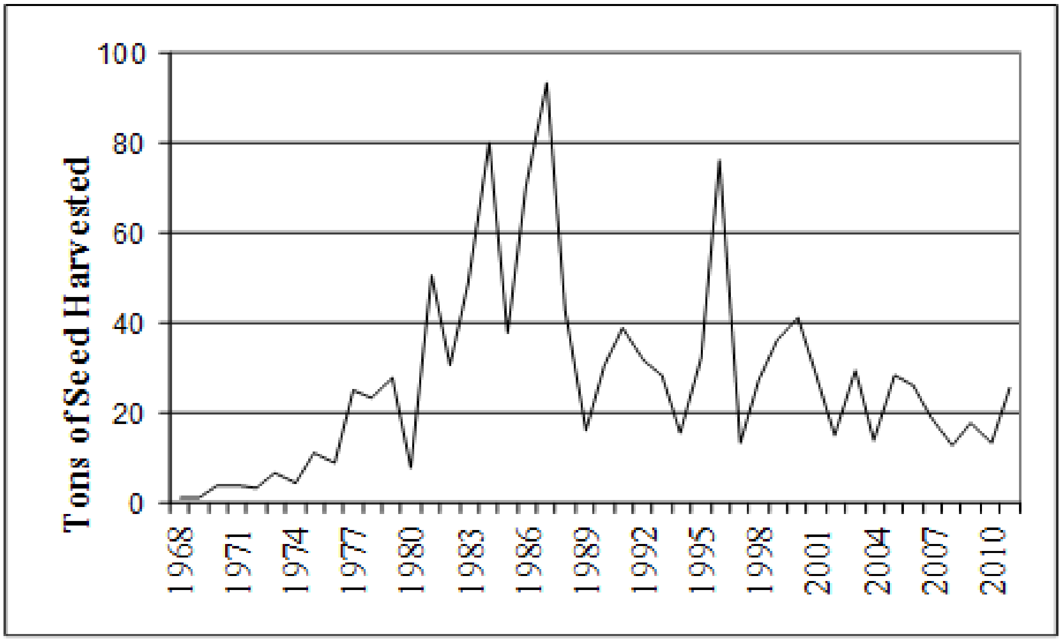


Figure 1. Annual seed yields from 1968 to 2011.

RESEARCH

Variation in Sugar Yield from Enzymatic Hydrolysis of Loblolly Pine[1]

Clonal varieties of loblolly pine were tested to characterize variation in yield of fermentable sugars from enzymatic hydrolysis. Clonal varieties made available by Plum Creek and CellFor were divided into groups using a cluster analysis based on near-infrared (NIR) spectra of ground wood samples from multiple individual trees, or ramets, of each of the clonal genotypes. NIR spectra reflect chemical and physical wood properties, so the clustering should have produced groups of clones that are similar for some combination of these traits. If a cluster can be identified that is superior for sugar yield, this may provide a quick way of identifying additional superior genotypes for sugar production. The cluster analysis was performed on 178 clones, and a subset of these clones was selected for enzymatic hydrolysis.

Three different pretreatments were tested: dilute acid, alkaline, and alkaline with mechanical beating. The purpose of pretreatment is to release the cellulose from the lignin, thereby making the cellulose available for enzymatic hydrolysis. A moderate level of enzyme was used for the dilute acid pretreatment, and three levels of enzyme (low, moderate, and high) were used for the alkaline and alkaline with mechanical beating pretreatments, for a total of seven treatments. Each treatment was applied to a mixture of wood chips representing each clone. This mixture was composed of three ramets of a given clone. Mean sugar yields were calculated for each treatment (Table 1). The alkaline pretreatments with mechanical beating produced more sugars than the dilute acid or alkaline pretreatments. Additionally, increasing enzyme levels also produced greater yields of sugars.

Table 1. Mean sugar yields by treatment. Different letters indicate significant differences between treatments.

Treatment	Mean Sugar Produced (mg sugar/g wood)	Grouping
Dilute Acid, Low Enzyme	210 (st. dev. 19.9)	A
Alkaline, Low Enzyme	228 (52.8)	A
Alkaline, Moderate Enzyme	332 (62.6)	B
Alkaline, High Enzyme	414 (59.9)	B
Alkaline with beating, Low Enzyme	341 (60.6)	C
Alkaline with beating, Moderate Enzyme	444 (65.5)	D
Alkaline with beating, High Enzyme	521 (34.9)	E

Table 2. Mean sugar yields by cluster (groups of similar clones) for the treatment with alkaline plus mechanical beating pretreatment and a high level of enzyme. Different letters indicate significant differences between clusters.

Cluster	Mean (mg sugar/g wood)	Grouping
3	559	A
4	517	B
2	513	B
6	511	B
1	503	B
5	496	B

Mean separation tests indicate significant pairwise differences between clusters within several of the treatments. However, the alkaline plus mechanical beating pretreatment with a high level of enzyme is the only treatment for which one cluster is significantly different from all others (Table 2).

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[1] *This is a summary of David Barker's PhD research. David hopes to complete his degree by December 2012.*

Financial analysis was performed on the sugar yield data from the experiment using process models for a loblolly biorefinery processing 500,000 bone dry tons of biomass per year. These biorefinery process models were developed by colleagues in the Department of Forest Biomaterials at NCSU under a grant from the Biofuels Center of NC. The goal of the analysis was to estimate the effect of conversion method, cluster membership, clone, enzyme cost, and biomass cost on the economic feasibility of an industrial-scale biorefinery using loblolly pine as a feedstock. All scenarios modeled use a 12% internal rate of return (IRR). This IRR is higher than is often used in economic analyses of timberland investments, but it is reflective of the higher returns generally expected by investors in pulp mill and ethanol production operations.

Above: David Barker taking cores from clonal varieties.

Below: A sample core and resulting grounds for NIR.

Large differences were found between treatments in the cost/gallon of ethanol required to achieve a 12% internal rate of return (IRR) (Table 3). The dilute acid treatment was the worst, and this was due to lower sugar yields and higher chemical costs than the alkaline treatments. The alkaline treatments have a lower ethanol cost/gallon, but there is not a clear positive relationship between ethanol price and mean sugar yields (Table 1), indicating the presence of other important factors that affect ethanol cost. The two lowest ethanol prices were obtained from use of low to moderate enzyme doses and mechanical beating. Current high enzyme costs offset the increased sugar yields gained from using the highest level of enzyme. The increased sugar yields from Cluster 3 clones reduce the cost of ethanol by varying degrees.

Table 3. Cost/gallon of ethanol required to achieve a 12% IRR, by treatment.

Treatment	Cost/gal (average)	Cost/gal (Cluster 3)
Dilute acid, 20 FPU	$8.83	$8.81
Alkaline, 10 FPU	$6.11	$5.32
Alkaline, 20 FPU	$5.40	$4.99
Alkaline, 40 FPU	$6.52	$6.26
Alkaline w/ beating, 10 FPU	$4.83	$4.25
Alkaline w/ beating, 20 FPU	$4.81	$4.45
Alkaline w/ beating, 40 FPU	$6.18	$6.01

These results indicate that potential does exist for selecting clones based on biofuel potential. The statistical significance of NIR cluster for several of the treatments is encouraging, as it indicates that it may be possible to create a rapid method for screening clones for biofuel potential. This method would be based on the NIR spectra of the clones, which are less costly to obtain than results from a wet chemistry analysis of sugar yields. However, while these results indicate some impact from genetic variation, we are collecting further data to characterize the magnitude of this contribution.

Genetic and Silviculture Effects on Loblolly Pine Growth, Form, and Wood Properties

Dr. Daniel Gräns completed his PhD this spring and is back in Sweden teaching at The School for Forest Management, Swedish University of Agricultural Sciences at Skinnskatteberg. Daniel's dissertation is entitled "Effects of Genetics and Silviculture and Their Interactions on Loblolly Pine and Norway Spruce Growth and Wood Properties". Below is a summary of his work with loblolly pine.

The effects of imposed silvicultural treatments and genetics were investigated in a 16-year-old loblolly pine trial established by International Paper Co. at their Southlands Forest near Bainbridge Georgia. The trial was designed with different fertilizer and herbicide treatment combinations applied to 25 open-pollinated first- and second-generation families. Focus in the study was directed towards analyzing growth, form, and wood property traits having a large impact on volume production and the quality of solid wood products. In the combined fertilization and herbicide treatment plots, the mean for individual-tree stem volume was 185.2 dm^3 (6.54 ft^3), while in the other extreme, the control plots, mean stem volume was 91.2 dm^3 (3.22 ft^3). The variation in traits such as height, sweep, branch angle, percentage trees with forking defects, and fusiform rust incidence was also studied, and there were large, significant differences among treatment combinations for height, volume, sweep, and forking defect (Table 1).

Family effects were significant for all traits when pooled across all treatments. Individual-tree heritability values were 0.26 for height, 0.33 for volume, 0.10 for sweep, 0.39 for branch angle, 0.29 for forking defect, and 0.23 for rust incidence. Pooled family mean heritability values were 0.85 for height, 0.86 for volume, 0.60 for sweep, 0.90 for branch angle, 0.87 for forking defect, and 0.84 for rust. Genetic and phenotypic correlations between growth and form traits were generally rather low and non-significant except for a few combinations of traits.

In addition to growth and form traits, variation in wood density and acoustic velocity (a surrogate trait for wood stiffness as measured with the TreeSonic) was assessed. Squared acoustic velocity ranged from 14.5 km^2/s^2 in the fertilization only plots to 16.4 km^2/s^2 in the herbicides only plots, indicating that trees in the herbicide plots had stiffer wood compared to trees in other treatments (Table 2). Average core density was higher in the control plots (465 kg/m^3) and was significantly lower (450 kg/m^3) in the combined fertilization and herbicide plots. Ring 2-6 core section density (all juvenile wood) varied from 445 kg/m^3 in the control plot to 435 kg/m^3 in the combined fertilization and herbicide plots, and ring 7-16 core section density varied from 590 kg/m^3 in the herbicide only plots to 538 kg/m^3 in the fertilization only plots.

Table 1. Treatment combination means for growth, sweep, branch angle, forking defect and rust incidence. Different letters following treatment combination means for a given trait indicate significant differences between treatment combination means at the $p \leq 0.05$ level.

Treatment combination	Height (m)	Volume (dm^3)	Sweep (cm)	Branch angle (deg.)	Forking def.* (%)	Rust (%)
Control	13.9 **a**	91.2 **a**	1.9 **a**	54.6 **a**	18.6 **a**	28.5 **a**
Herbicides	16.0 **b**	129.6 **b**	2.3 **b**	55.7 **ab**	19.5 **a**	25.1 **a**
Fertilization	16.4 **c**	147.9 **c**	2.0 **a**	55.8 **b**	24.0 **a**	25.8 **a**
Fert.+Herb.	17.5 **d**	185.2 **d**	2.4 **b**	55.3 **ab**	34.2 **b**	24.7 **a**

*Forking defect is a combination of forking and ramicorn branching.

Table 2. Treatment combination means for wood quality traits. Different letters in a column indicate significant differences between treatment combination means at the $p \leq 0.05$ level.

Treatment combination	Velocity^2 (km^2/s^2)	Whole core dens. (kg/m^3)	Ring 2-6 dens. (kg/m^3)	Ring 7-16 dens. (kg/m^3)
Control	14.7 **c**	464.6 **a**	445.2 **a**	570.1 **b**
Herbicides	16.4 **a**	459.8 **a**	442.3 **ab**	589.5 **a**
Fertilization	14.5 **c**	453.8 **b**	437.4 **bc**	537.9 **d**
Fert.+ Herb.	15.7 **b**	450.0 **b**	435.4 **c**	552.2 **c**

Genetic effects were significant for all wood property traits, and there was a wide range in family means for each trait. When pooled across treatments, individual-tree heritability values were 0.41 for squared velocity, 0.32 for whole core weighted density, 0.28, for ring 2-6 core section density, and 0.78 for ring 7-16 core section density. Pooled family mean heritability values were 0.90 for squared velocity, 0.88 for whole core weighted density, 0.86 for ring 2-6 core section density, and 0.95 for ring 7-16 core section density. Genetic and phenotypic correlations were significant between several combinations of wood quality traits. In contrast, correlations of wood quality traits with growth and form traits were generally low and non-significant.

Silviculture by genetics interaction effects were non-significant for all traits, and family rankings across treatment combinations were generally stable for growth, form, and wood property traits (i.e. families that were high ranking in one treatment were generally high ranking for all treatments; see example in Figure 1). For improved open-pollinated Coastal loblolly pine families, the risk of losing value in terms of growth, stem form, and wood quality due to suboptimal matching between genetic material and silvicultural prescriptions is generally low.

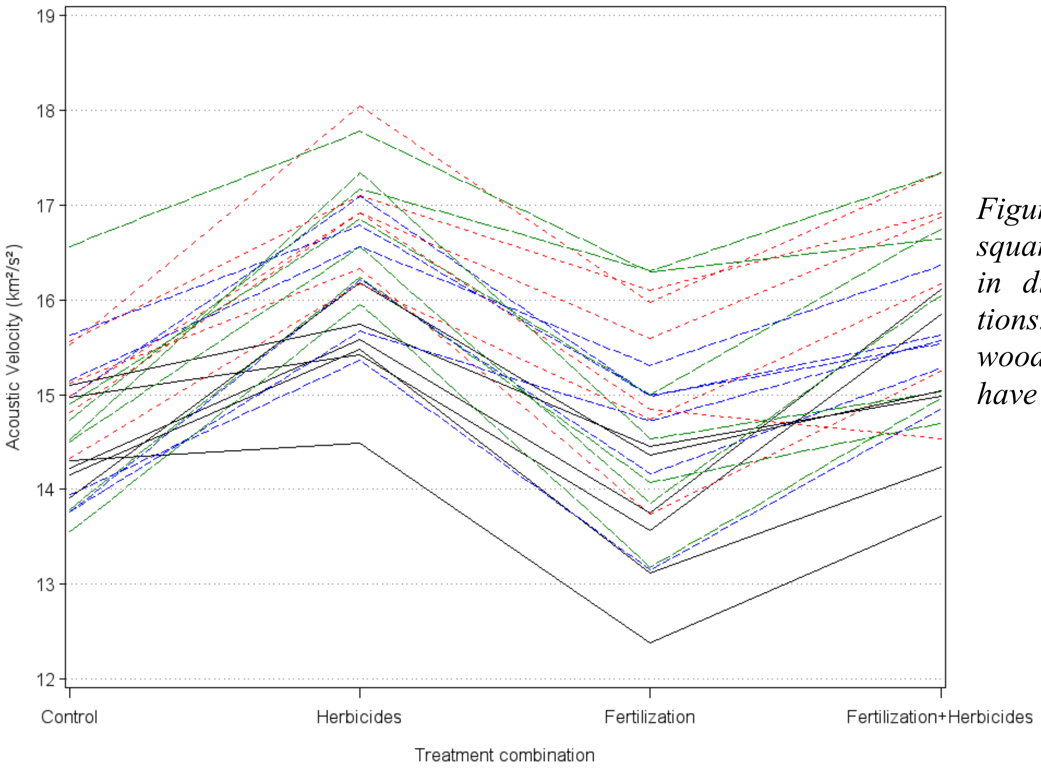

Figure 1. Family means for squared acoustic velocity (km^2/s^2) in different treatment combinations. Overall, families with stiff wood in one treatment tended to have stiff wood in all treatments.

Effect of Inbreeding in 2nd Generation Families of Loblolly Pine[1]

As the Cooperative develops its future breeding strategies with loblolly pine, it is important for breeders to understand the impact that inbreeding can have on growth and quality traits. Inbreeding can increase rapidly in intensively selected breeding populations and is generally detrimental in outcrossing species such as loblolly pine. Managing inbreeding and the deleterious effects on metric traits is a primary objective in forest tree breeding programs. The goal of this research is to investigate the magnitude of inbreeding depression on growth and wood quality traits in genetically improved families of loblolly pine.

Effects of inbreeding were quantified for 10 Coastal and 10 Piedmont loblolly pine families. Each of 10 selected 2nd-generation parents was selfed and bred to other related and non-related selections to provide a gradient of inbreeding coefficients (F) of 0, 0.125, 0.25, and 0.5. Progeny from each cross were planted in field trials in 1988, and measurements were collected at age 9. There was generally a linear decrease in metric traits with increase in inbreeding coefficient and a significant female by inbreeding coefficient interaction. A summary of the age 9 results can be found in the Cooperative's 42nd Annual Report from 1998.

In the years following the age 9 measurements, test sites were lost or abandoned, and only Weyerhaeuser's test site in Beaufort County, NC remains intact allowing for additional growth and wood quality measurements. In winter 2012 at age 23 years, height and diameter at breast height (DBH) measurements were collected, and individual tree volumes were calculated using a standard volume equation. Wood strength data were collected using a TreeSonic (http://www.fakopp.com/site/treesonic) that measures the time of travel for a stress wave sent between two transducers that are inserted into a tree, and correlates with bending strength or modulus of elasticity. Specific gravity was measured on 12mm cores taken at breast height using the volumetric method.

As expected at age 23, height decreased as inbreeding coefficient increased. Volume decreased with increased inbreeding, except for the selfs (Table 1). Survival can explain the better individual-tree volume growth of the selfs. Survival decreased as level of inbreeding increased, and survival was dramatically lower for the selfed individuals (40%). The greater growing space in the self plots resulted in trees with higher volume.

Above: Graham Ford, David Barker & Tori Brooks taking measurements on Inbreeding Study in NC.

The trends of inbreeding depression in the growth traits at age 23 are consistent with the results at age 9 and for similar research in other pines and conifers. Growth traits are tied to overall fitness and reproductive capability, and population genetic models predict that traits which influence fitness will more likely exhibit inbreeding depression. This is due in part to the presence of deleterious, recessive alleles that increase in frequency as inbreeding occurs.

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[1] This is a summary of Graham Ford's MS research project. Graham plans to defend his Masters thesis in the coming months.

For wood quality traits, there was no inbreeding effect; outcrossed trees had the same wood specific gravity or wood strength as inbred trees (Table 1). It is possible that wood quality traits have fewer lethal or deleterious recessive loci, accounting for the absence of a significant change in wood strength or density with increased levels of inbreeding. Wu, Matheson, and Abarquez (2002, Annals of Forest Science 59: 557-562) conducted similar research in radiata pine. These researchers suggest that wood density displays little inbreeding depression because it is not as integral to life history as growth traits.

More analyses are underway to investigate family differences in inbreeding effect and how breeders can better manage inbreeding in advanced generations.

Table 1. Age 23 Growth and Wood Quality Trait Means at the Different Inbreeding Levels

Mating	Height (ft.)	Volume (cu. ft.)	Survival (%)	Specific Gravity	Time of Flight (microseconds)
Outcross (F=0)	74.6	14.52	62.9	0.454	240.5
Half-Sib (F=0.125)	73.7	13.98	62.9	0.458	247.5
Full-Sib (F=0.25)	70.4	11.98	52.5	0.457	246.8
Self (F=0.5)	71.4	15.16	39.8	0.452	247.2

Genetic Analysis of Diallel Progeny Test Data in *Pinus taeda* Using Factor Analytic Linear Mixed Models[1]

In plant breeding programs, analysis of data from multi environmental trials within a mixed model framework is common practice to evaluate the response of genotypes across different environments (t) and to understand the extent of genotype by environment (GxE) interaction. An unstructured covariance matrix with $t(t + 1)/2$ parameters is commonly used to account for heterogeneity in variances and correlations between environments. However, this structure requires a large number of parameters to be estimated, and convergence of models becomes difficult as the number of environments increases. The factor analytic (FA) variance structure is a parsimonious form of the unstructured variance structure (US) and is used to approximate the unstructured form of the genetic variance–covariance matrix in the model. If k factors are fit, then $t(k+1)- k(k-1)/2$ parameters are estimated, resulting in a large reduction in the number of parameters to be estimated.

In this study, the efficiency of factor analytic covariance structures was investigated using multi-environment loblolly pine progeny test data from Plum Creek Timber Company, Inc. (142 crosses from 19 parents) across 10 sites. We demonstrated that among all models tested, the FA model was the model of best fit. FA models were robust with high predictive accuracy and provided more accurate estimates of overall variety effects. There was significant heterogeneity among trials for estimates of GCA and SCA effects, the ratio of SCA to GCA genetic variance, and individual narrow-sense heritabilities. On average, all 10 environments had an additive genetic correlation of 0.83 and a non-additive genetic correlation of 0.91, suggesting that GxE should not be of concern for this population in the environments where genotypes were tested.

Since factor analytic models capture both variance and the covariance at the genetic level better than simpler models, they provide more accurate predictions of genotypes and are a natural framework for modeling GxE interaction. FA models proved to be superior to account for heterogeneity in these loblolly pine progeny test data, and tree breeders should be using them for multi-environment trials in forest tree breeding programs.

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[1] *This is a summary of part of Funda Öğüt's PhD research. Funda plans to defend her dissertation this summer and will return to Turkey and join the faculty of Artvin Çoruh University.*

NC Biofuels Study Update

Grown across millions of acres and found on a wide range of sites in the Southeast, loblolly pine is a rapid-growing tree that does not compete with America's food production and may be an ideal feedstock for the developing biofuels industry. Substantial genetic differences exist that will influence biomass/biofuel production, and by planting genetically superior trees with desirable biomass/biofuel traits, it may be possible to dramatically increase the amount of biomass produced at any given site.

At a study site in Butner, NC in the northeastern Piedmont, we are testing 20 of the Cooperative's fastest-growing open-pollinated families, each with varying potential for bioenergy feedstock. Ten of the top volume families adapted to the Piedmont region as well as 10 of the top volume families from the Coastal region were included in the study. The Coastal families are faster growing than the Piedmont families, but they will be less adapted to this Piedmont site. By incorporating the high-risk Coastal material, we will be able to evaluate the risk/reward of planting material that is not adapted to the region.

Tree spacing is at a high density, 6'x7' or 1037 trees per acre. Additionally, we are incorporating a thinning and no-thinning treatment. The no-thin plots can be harvested at age 8-10 years to assess genetic differences for biomass/biofuel production, while the thin plots will be thinned to 12'x14' spacing (259 tpa) to allow the stand to develop into sawtimber. We believe these different thinning regimes will be valuable to demonstrate to forest landowners that planting loblolly pine as a dedicated energy crop will provide options both for early harvest for biomass and/or longer rotations for sawtimber production.

Progress

Seedlings from the 20 families were grown at NCSU during the summer of 2011. The trees were planted by our students and staff this January and February. A balanced fertilizer was applied to each seedling to enhance growth and uniformity. The study is located on old field sites, so there is significant herbaceous competition. Glyphosate was applied in later summer of 2011 to eliminate some competition, and a banded herbicide treatment (Sulfometuron methyl and Imazapyr) was applied over the top of the trees this spring.

Graduate Student Graham Ford planting a seedling at Butner, NC field site.

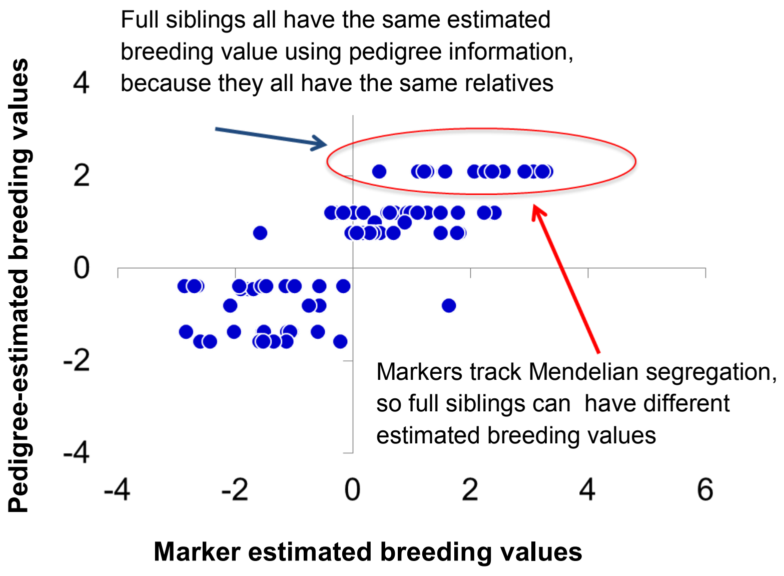
The field site will be managed by the NCSU Cooperative Tree Improvement program for 3 years. In addition, growth and survival will be measured after the first and second growing seasons. After 3 years, the Biofuels Center will be responsible for maintenance and management. However, if funding is available, the Tree Improvement Cooperative will continue to have involvement in the study and support more detailed measurements. In all, this project will serve as a platform to evaluate different pine varieties ranging in biofuels/biomass potential under different cropping systems that can provide landowners with an economic incentive to plant loblolly pine as a dedicated energy crop.

Genomic selection and cost-effective genotyping

There has been considerable discussion of the potential applications of genomic selection (GS) in forest tree breeding, based on advances made in both genotyping technology and statistical methods. This approach has been applied in several livestock breeding programs, most successfully in dairy cattle. The central concept of GS is to use large numbers of markers, ranging from 30,000 to over 700,000 marker loci, to track the inheritance of specific regions of chromosomes and associate genetic variation with phenotypic variation. This concept can be applied using BLUP methods already used by tree breeders, but some key questions still need to be answered. How many marker loci must be genotyped to allow estimates of breeding value to approach the accuracy obtained by using pedigree information alone? What structure of mating design and breeding population will be best suited to the application of genomic selection? What genotyping methods will be most cost-effective and reproducible for high-throughput genotyping?

We are actively working to find answers to these and other questions, and remain optimistic that the pace of progress in research will bring genomic selection into application in tree breeding programs. One area in which progress has been made in the past year is that of high-throughput genotyping. A publication by Elshire et al (PLoS One 6(5): e19379, 2011) reported methods for cost-effective genotyping by sequencing in maize, and a recent publication by Poland et al (PLoS One 7(2):e32253, 2012) modified that method to work in hexaploid wheat, which has a genome almost as large as pine.

We conducted an experimental test of the Elshire et al. genotyping-by-sequencing (GBS) protocol with DNA samples from three different conifer species, using several sample preparation procedures, and obtained 144 million sequencing reads from 93 samples, at a cost of about $3000 in reagents and laboratory costs (not including labor). Analysis of this dataset using software tools developed by the Institute for Genomic Diversity at Cornell University showed that about 136.5 million of the 144 million sequencing reads contained one of the 93 identifying “barcode” sequences used to identify the specific samples in the pooled sample that was sequenced. Depending on the threshold of missing data allowed, the software detects anywhere from 10,000 to over 220,000 putative genetic marker loci, but marker detection reproducibility across different individuals is low. Analysis of this trial dataset is continuing, but a second experiment is underway to test the modified protocol reported by Poland et al, and updates to the analytical software in the next few weeks will mean that both datasets can be analyzed in parallel with more powerful tools.



Breeding values estimated from marker data (horizontal axis) versus those estimated based on pedigree information of loblolly pine clones, in the absence of phenotypic data. Without phenotypic data, classical pedigree-based analysis cannot predict differences in expected breeding value among clones within a family (vertical axis). This is not the case when genetic markers are used to estimate breeding values (Zapata-Valenzuela thesis 2011).

Update on the Pine Integrated Network Education, Mitigation and Adaptation Project (PINEMAP)

PINEMAP is a multi-disciplinary, multi-institutional award from USDA focused on increasing the resilience of southern pine plantations to climate change, and on increasing the amount of carbon stored by pine plantations, through improvements in management strategies and planting stock. Eight university cooperative research programs, including the three breeding cooperatives plus silviculture, management, modeling, and economic analysis cooperatives, are involved, along with university researchers at institutions across the southeastern US. The project is organized into six Aim Groups which are focused on meeting different aspects of the overall project objectives, with significant effort devoted to integration results across Aim Groups to synthesize the various research and outreach activities into a coherent whole. Key areas of progress in the first year include establishment of four field sites for study of pine plantation growth responses to changes in water availability under differing levels of nutrient availability; collection of data from existing databases on relative performance of pine genetic entries under differing climate conditions; establishment of a monitoring network to collect data on water, nitrogen, and carbon cycling in pine plantations; establishment of a modeling dataset of climate and growth data for use in future modeling work; and development of educational and outreach materials for use in K-12 and university education as well as outreach to owners and managers of southern pine forestlands.

NCSU Tree Improvement Program hired Alfredo Farjat as a PhD student in 2011 to work on the progeny test data to answer some of the questions related to Aim 3. Alfredo is working on a geo-spatial statistical model to estimate the effect of climatic variables such as annual average minimum temperatures on loblolly pine performance throughout the southern US. Alfredo is using the Plantation Selection Seed Source Study (PSSSS) that is replicated on 22 locations across the southern United States (Figure 1). In the map, annual average minimum temperatures are estimated using a Kriging approach with data from weather stations throughout the southeastern US from 1980 to 2010. Such models can be used to estimate the probability of different climatic scenarios and can guide future decisions. One of the questions Alfredo will be studying is the interactions between pine families and climatic variables to examine the norm of reaction of families and draw guidelines for future deployment of loblolly pine varieties under different average minimum temperatures scenarios.

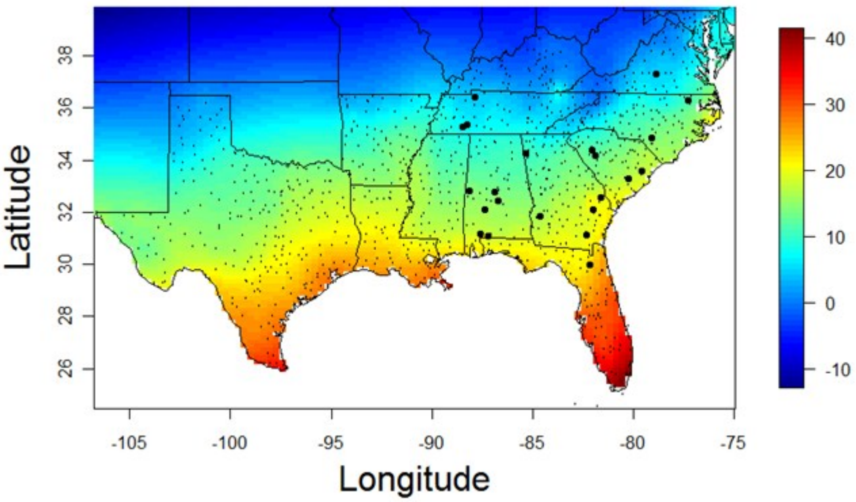


Figure 1. Estimated annual average minimum temperature throughout the southern United States using data from weather stations from 1994 to 2005. The small black points represent the location of the weather stations. The larger black points represent the location of the planting sites of the PSSSS study.

A full report for the first year of the project can be downloaded at: http://www.pinemap.org/reports/annual-reports/Year%201%20Annual%20Report.pdf.

Update on the Southern Partnership for an Integrated Biomass Supply System (IBSS)

This project is another multi-disciplinary, multi-institutional award from USDA, focused on fostering development of industrial production of liquid transportation fuels from biomass in the southeastern US. The award was announced in August of 2011, and research efforts are just getting underway. David Barker, whose research to date has been supported by a grant from the Biofuels Center of North Carolina and funds from the College of Natural Resources, is working to extend the research findings of his previous work. That work discovered genetic differences among pines in the efficiency with which pine chips pre-treated to reduce lignin content can be converted to fermentable sugars by enzymatic hydrolysis. David has collected 300 additional wood cores from a set of genetic materials provided by collaboration with Plum Creek and CellFor, and is now preparing those samples for near-infrared spectroscopy analysis to test the reproducibility of the analytical results obtained in his previous experiments. Genetic differences in pine wood chemical and physical properties have been previously demonstrated, but the novel aspect of this research is to test the effects of those differences on the efficiency of biomass conversion to biofuel. More information about the IBSS project is available on the website at http://www.se-ibss.org/.

Grants received

The summer of 2010 was a busy time for soliciting grant funds for southern pine research, and it kept the TIP staff busy from May to December of that year writing proposals. We reported last year that we were successful on three proposals and awaiting final word on the fourth. We are now happy to report that in August of 2011 we got the funding for the fourth project and the list below is a show of all our successes:

Continuing Grants

Fikret Isik is a co-principal investigator with Vincent Chiang and Ron Sederoff on a NSF Plant Genome grant, Regulation and Modeling of Lignin Biosynthesis. TIP portion: $43,548. 09/09 to 08/13.

Ross Whetten, Steve McKeand, Fikret Isik. Subcontract with the University of Florida, USDA Coordinated Agricultural Project (CAP) “Integrating research, education and extension for enhancing southern pine climate change mitigation and adaptation”. TIP portion: $867,665. 3/11 to 2/16.

Steve McKeand, Bronson Bullock, Ross Whetten, and Fikret Isik. Biofuels Center of North Carolina. “Loblolly Pine Biomass Genetics/Cropping Study”. $148,419. 7/11 to 9/14.

New Grants

Ross Whetten, Steve McKeand, and Fikret Isik. USDA via subcontract with the University of Tennessee. Integrated Biomass Supply Systems (IBSS). TIP portion: $652,369. 8/11-7/16.

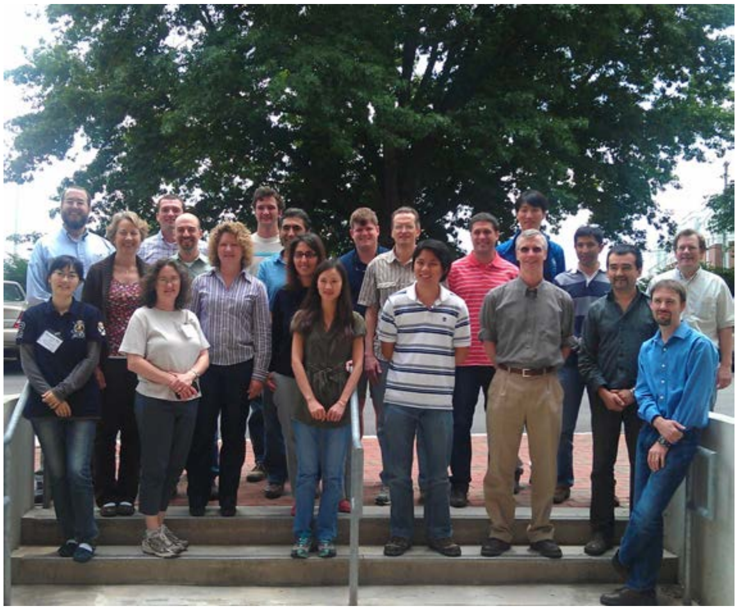
Fikret Isik is a co-principal investigator with Vincent Chiang on a U.S. Department of Energy grant, Modeling of Cellulose, Hemicellulose and Lignin-Carbohydrate Complex Formation and Regulation to Understand Plant Cell Wall Structure. TIP Portion: $20,223. 9/11-8/14.

ASSOCIATED ACTIVITIES

Meetings, Workshops, and Short Courses

2011-12 has been an active year for TIP and outreach activities. We hosted workshops, short courses, contact meetings and started making appearances at various forestry association meetings. The idea is to spread the word of the value of Tree Improvement activities and the *PRS*™ rating system. Landowners are asking questions and are excited to know they have so many deployment options for their regeneration needs. Look for our booth at upcoming meetings – we come fully stocked with peppermint patties which seems to bring folks in like moths to a flame!

NCSU Tree Improvement Program organized The Genetic Data Analysis Workshop on May 16-20, 2011. The workshop was taught by the Tree Improvement Program scientists Drs. Fikret Isik and Ross Whetten; also Dr. James Holland (NCSU Crop Science) and Dr. Christian Maltecca (NCSU Animal Genetics). The event attracted attendees from various countries who wanted to learn more about analyzing phenotypic and genomic data for plant and animal breeding. Fikret and Ross were also invited to teach a one-day workshop on genome-wide selection methods in forest trees during the IUFRO Tree Biotechnology Conference in Bahia, Brazil on June 26, 1011. The workshops were such successes that Fikret and Ross were invited to teach a short workshop in Bordeaux, France in November 2011 and are invited to teach a week-long workshop on genetic data analysis in Prague, Czech Republic on June 11-15, 2012. We are glad that Fikret and Ross are on our team and proud they are getting recognition for their contribution to the genomic and phenotypic data analysis for breeding.



Pictured Above: Instructors and attendees of The Genetic Data Analysis Workshop held in Raleigh, NC on May 16-20, 2011. The week long workshop attracted both industry and academia attendees from all over the US (including Hawaii), Chile, and Korea.

After a long 4-year break, we blew off the dust and brought back the Tree Improvement Short Course. The course was held in Charleston, SC with ArborGen, Inc. hosting the field tours. We restructured the class and updated the manual to incorporate the needs of not only the on-the-ground tree breeders but the landowners and managers. It was a great course and field tour for the TIP newbies and a good refresher for the regulars. TIP members can find the new and improved Tree Improvement Manual under the members login at www.TreeImprovement.org.

Pictured left: TIP Short course field tour hosted by ArborGen, LLC.

The annual Contact meeting was hosted by NCSU Tree Improvement and the Georgia Forestry Commission on November 9-10, 2011 in Macon, Georgia. Attendees took a field tour of the Arrowhead Breeding Center in Cochran, GA and got an overview of the collaboration that GFC and NCSU have had in building a centralized breeding center model. Despite the threat of rain; we had perfect weather for the fish fry and celebrated the VDF's Ones Bitoki becoming a US citizen and Richard Bryant's retirement from ArborGen...leaving Early McCall of Rayonier as "the last man standing"! As always, one can expect a little adventure when attending a Tree Improvement Contact Meeting, and this year certainly did not disappoint. Bus troubles in the middle of nowhere and in the pitch black – you got it! Luckily Tori hired a local company so the group was saved after only 30 minutes of being stranded. Stay tuned for next year's adventure; it is certainly not for the faint of heart!

Richard Bryant receiving his TIP uniform to sport during his retirement. Congratulations Richard!

This year the NCSU Department of Forestry and Environmental Resources hosted the Stihl® Timbersports® Competition on March 15-17, 2012, and in conjunction with the competition they had an Arbor Day celebration at the NC State Farmers Market in Raleigh on the 16th of March. We gave away pine seedlings and educated the young and old about Tree Improvement and ***PRS***™. Our game, again, attracted the masses including Smokey the Bear...who by the way needs to brush up on his ***PRS***™ knowledge and work on his bucket toss!

Above: Saul helping a young girl play the PRS game.

Right: Jadie and Smokey the Bear at the TIP booth.

Staff

The staff grew this year with the addition of Jadie Andrews. Jadie has been working as an undergraduate with the program for over 3 years, and when the opportunity to work on the PINEMAP and IBSS projects became available he was a perfect fit. We had another change in staffing with the promotion of Fikret Isik here at the university and his acceptance of the Associate Director title for the Cooperative. He, along with Ross Whetten, have elevated the program with their innovative approaches to genetic analysis and using these tools in developing future breeding and testing strategies.

TIP Staff: (Left to right)

Back row: Josh Steiger, J.B. Jett, Jim Grissom, Saul Garcia, and Fikret Isik

Front Row: Jadie Andrews, Steve McKeand, Tori Brooks, and Ross Whetten

Visitors

Isabel Deliberali joined the group in January 2012 as an undergraduate intern from the University of Sao Paulo, Brazil. The majority of her 6 month internship has been working on the Improvement of Cold Tolerant Eucalyptus Species (ICES) but she has been able to get in some Tree Improvement along the way! She has been sitting in on the FOR 411 class taught by Steve and Ross and she rolled up her sleeves and did a little tree planting for the Biofuels Center project in Butner, NC. She has been a great addition to the group and we hope she has found her internship equally fulfilling.

We also had two visitors from the Forest Tree Breeding Center, Forestry and Forest Products Institute in Japan. Drs. Masahiro Miura and Yuichiro Hiraoka visited with the staff and gave a seminar on Tree Improvement in Japan. They then wisely flew down to the Arrowhead Breeding Center in Cochran, GA to get a snapshot of tree breeding efforts in the Southeast for loblolly pine before meeting with the staff at University of Florida. It is always interesting to see the similarities between the programs but also the stark differences like terrain and what traits they value and breed for. It was nice for the staff and students to interact with tree breeders from another part of the world.

Isabel Deliberali can usually be found tending to a sea of eucalypt seedlings at the Horticultural Field Lab at NCSU.

Teaching

Teaching both graduate and undergraduate courses is one of our top priorities here at NC State 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). Ross also teaches FOR 350, Professional Development III: Ethical Dilemmas in Natural Resource Management, and BIT 815, Deep Sequencing Data Analysis. From the course description (http://www4.ncsu.edu/~rosswhet/BIT815.html), the material looks plenty challenging for most of us. Fikret Isik's and John Frampton's class, NR554 - Introduction to Data Analysis, has become very popular in the College and has become a "must take" course for many students.

TIP Grad Students: (L to R)

David Barker, Bo Dixon, Graham Ford, Alfredo Farjat, and Greg Albert (the new guy!)

Missing: Funda Öğüt

Graduate Students

This past year was another very successful year for graduating Tree Improvement students which include the following: Daniel Gräns, Jaime Zapata, and Aaron Chamblee. Daniel is continuing his teaching appointment at The School for Forest Management, Swedish University of Agricultural Sciences at Skinnskatteberg. Jaime has returned to Chile and continuing his career at Arauco-BIOFOREST in the Bioinformatics Unit. Aaron is working for the forestry consultant group based in Raleigh, NC. We are proud of all these folks and their outstanding accomplishments.

Current Students

David Barker, PhD Candidate– Genetic differences in loblolly pine for conversion efficiency for cellulosic ethanol.

Bo Dixon, MS - Economic effects of tree improvement.

Alfredo Farjat, PhD - The effect of climatic variables on pine growth.

Graham Ford, MS - Inbreeding effects in loblolly pine.

Funda Öğüt, PhD Candidate – Molecular aided selection and breeding in loblolly pine.

Josh Steiger, MS – Impacts of different levels of genetic homogeneity in loblolly pine in a genetics x spacing x thinning trial in Eastern, NC.

Membership in the NCSU Cooperative Tree Improvement Program

This year has proved to be a busy year for membership with F&W Forestry Services, The Campbell Group, White City Nursery coming on board as Contributing members. We also saw the re-emergence of the Research Associate Member with Biofuels Center of NC moving to that level and the USDA Forest Service joining in January 2012. Welcome to all!

On the negative side, in December 2011, CellFor filed for creditor protection under the Companies' Creditors Arrangement Act ("CCAA"), a Canadian court process that is analogous to the Chapter 11 process in the United States. At the time of this writing, we do not know what will happen with the company, but we lost CellFor as a member. CellFor was instrumental in developing operational-scale clonal forestry for loblolly pine in the southern US. The biggest obstacle they faced was the miserable economy the last few years. Once production reached significant levels, the "Great Recession" hit, and they were unable to overcome these economic hurdles.

We wish the best to our friends at CellFor, Bob Weir, Nick Muir, Andy Benowicz, Jason Watson, Greg Hay, John Pait, and others. It's always tough to watch your friends go through such difficult times.

Full Members

ArborGen, Inc.

Georgia Forestry Commission

Hancock Timber Resources Group

North Carolina Division of Forest Resources

Plum Creek Timber Company

Rayonier, Inc.

Tennessee Department of Agriculture

The Westervelt Company

Virginia Department of Forestry

Weyerhaeuser Company

Research Associate Members

Biofuels Center of North Carolina

Contributing Members

American Forest Management, Inc.

Dougherty & Dougherty Forestry Services, Inc.

F&W 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

The Campbell Group

Timberland Investment Resources , LLC

White City Nursery

Z.V. Pate, Inc.

Publications of Special Interest To Members (2009-2012)

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://treeimprovement.org/public/publications/publications

2012

Aspinwall, M.J., S.E. McKeand, and J.S. King. 2012. Carbon sequestration from 40 years of planting genetically improved loblolly pine across the southeast United States For. Sci. (published online April 12, 2012; http://dx.doi.org/10.5849/forsci.11-058.).

Cumbie, W.P., F. Isik, and S.E. McKeand. 2012. Genetic improvement of sawtimber potential in loblolly pine. For. Sci. In press.

Espinoza, J.A., H.L. Allen, S.E. McKeand, and P.M. Dougherty. 2012. Stem sinuosity in loblolly pine with nitrogen and calcium additions. For. Ecol. and Manag. 265: 55–61.

Isik, F., H.V. Amerson, R.W. Whetten, S.A. Garcia, and S.E. McKeand. 2012. Interactions of Fr genes and mixed-pathogen inocula in the loblolly pine-fusiform rust pathosystem. Tree Genetics & Genomes. 8(1): 15-25.

McKeand, S. and R. Pohl. 2012. The Arrowhead Tree Breeding Center, $483 million present value to the citizens of Georgia and the South. Georgia Forestry Today. 8(1): 24-29.

Zapata-Valenzuela, J., F. Isik, C. Maltecca, J. Wegrzyn, D. Neale, S. McKeand, and R. Whetten. 2012. SNP markers trace familial linkages in a cloned population of *Pinus taeda* – prospects for genomic selection. Tree Genetics and Genomes (in press).

2011

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

Aspinwall, M.J., J.S. King, J-C. Domec, S.E. McKeand and F. Isik. 2011. Genetic effects on transpiration, canopy conductance, stomatal sensitivity to vapor pressure deficit, and cavitation resistance in loblolly pine. Ecohydrology. Published online in Wiley Online Library (wileyonlinelibrary.com) DOI: 10.1002/eco.197.

Aspinwall, M.J., J.S. King, S.E. McKeand, and B.P. Bullock. 2011. Genetic effects on stand-level uniformity, and above - and belowground biomass production in juvenile loblolly pine. Forest Ecology and Management. 262: 609–619.

Aspinwall, M.J., J.S. King, S.E. McKeand, and J-C. Domec. 2011. Leaf-level gas-exchange uniformity and photosynthetic capacity among loblolly pine (*Pinus taeda* L.) genotypes of contrasting inherent genetic variation. Tree Physiology. 31: 78-91.

Cumbie, W.P., F. Isik, B. Li, and B. Goldfarb. 2011. Effects of propagule type on genetic parameters of wood density and growth in a loblolly pine progeny test at ages 10 and 11 years. Tree Genetics & Genomes, 7:1147–1158, DOI 10.1007/s11295-011-0402-6.

Cumbie, W.P, A.J. Eckert, J.L. Wegrzyn, R.W. Whetten, D.B. Neale, and B.Goldfarb. 2011. Association genetics of carbon isotope discrimination, height, and foliar nitrogen in a natural population of *Pinus taeda* L. Heredity advance online publication 19 January 2011; doi: 10.1038/hdy.2010.168.

El-Kassaby, Y.A., S. Mansfield, F. Isik, and M. Stoehr. 2011. In situ wood quality assessment in Douglas-fir. Tree Genetics & Genomes, 7:553-561. DOI: 10.1007 /s11295 - 010-0355-1

Grissom, J.E., J.H. Roberds, C.D. Nelson, F.T. Isik, S.E. McKeand, and R. Rousseau. 2011. Genetic variation in a longleaf pine population: a long-term field study of a 13-parent diallel. P. 51-53. In: Proc. 31th Southern Forest Tree Improvement Conf., Biloxi, MS. http://www.sftic.org.

Isik, F., C.R. Mora and L.R. Schimleck. 2011. Genetic variation in *Pinus taeda* wood properties predicted using non-destructive techniques. Annals of Forest Science 68:283–293, DOI 10.1007/s13595-011-0035-9

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Sherrill, J.R., B.P. Bullock, T.J. Mullin, S.E. McKeand, and R.P. Purnell. 2011. Total and merchantable stem volume equations for mid-rotation loblolly pine (Pinus taeda L.). South. J. App. For. 35:105-108.

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Aspinwall, M.J., B. Li, S.E. McKeand, F. Isik, and M.L. Gumpertz. 2010. Prediction of whole-stem α-cellulose yield, lignin content, and wood density in juvenile and mature loblolly pine. South. J. Appl. For. 34:84-90.

Dougherty, D., R. Bryant, H. Burkhart, P. Dougherty, S. Jones, S. McKeand. 2010. Valuing tomorrow’s loblolly pine plantations today. Forest Landowner. Jan./Feb. 2010: 19-21.

Eckard, J.T., F. Isik, B. Bullock, B. Li, and M. Gumpertz. 2010. Selection efficiency for solid wood traits in *Pinus taeda* using time-of-flight acoustic and micro-drill resistance methods. For. Sci. 56(3), 233-241.

McKeand, S., P. Cumbie, and B. Abt. 2010. Investment in genetically improved loblolly pine – landowner benefits today and for generations to come. Forest Landowner. Jan./Feb. 2010: 27-29.

Nelson, C.D., T. L. Kubisiak, and H. V. Amerson. 2010. Unravelling and managing fusiform rust disease: a model approach for coevolved forest tree pathosystems. For. Path. 40: 64–72.

Whetten, R.W. and R. Kellison. 2010. Research gap analysis for application of biotechnology to sustaining US forests. J. Forestry 108(4):193-201.

Xiong, S.J., F. Isik, S.E. McKeand and R.W. Whetten. 2010. Genetic variation of stem forking in loblolly pine. For. Sci. 56: 429-436.

2009

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Barker, D.K., S.E. McKeand, F. Isik, and R.W. Whetten. 2009.Analysis of biomass production in young loblolly pine. P. 66-68. In: Proc. 30th South. For. Tree Impr. Conf., Blacksburg, VA.

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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.

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TREE IMPROVEMENT
PROGRAM NC STATE UNIVERSITY

The Cooperative has made a major push to evaluate loblolly pine families for their potential for bioenergy and biofuels value. With funding from the Biofuels Center of North Carolina, we planted a large field trial this year near Butner, NC to evaluate both Coastal and Piedmont families with varying degrees of biofuels and sawtimber attributes. Can landowners benefit from the new, emerging biomass markets and also realize long-term financial rewards from harvesting high-value sawtimber after thinning for biomass?