

Tree improvement Program

NC STATE UNIVERSITY

50th Annual Report

Department of Forestry and Environmental Resources
College of Natural Resources
North Carolina State University

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NC STATE UNIVERSITY

EXECUTIVE SUMMARY

50 Years of Tree Improvement at NC State

Because of the foresight of our leaders in industry and government since 1956, members of the Cooperative Tree Improvement Program have been major players in increasing the productivity of southern forests over the past 50 years. Over 1000 tons of genetically improved loblolly pine seed have been produced, and forest landowners in the South are responsible for over $\frac{3}{4}$ of the nation's annual tree planting. The financial benefits from our tree improvement efforts are enormous to these landowners.

At NC State, the Cooperative has been a major player in the educational, research, and extension mission of the Department of Forestry and Environmental Resources and the College of Natural Resources. Over 200 graduate students have studied in our program, and the 800+ publications arising from their research and the research of scientists and staff is testimony to the intellectual leadership over the years.

SELECTION, BREEDING, AND TESTING

The first of the full-sib block-plot tests for within family selection were established this year. Progress continues on the pollen mix (PMX) breeding and test establishment. The first of the Piedmont PMX tests went to the field, and the second Coastal PMX test series got started. All the remaining checklot comparison tests should be planted by the end of this year. More effort has been focused on breeding in the third-cycle elite populations, and tests should be in the ground in the next 3 years.

PROGRESS REPORTS FOR RESEARCH

Efforts to understand the fusiform rust – loblolly pine pathosystem have accelerated with the completion of the IPM Study and the initiation of the Fr Gene Discovery Project. The discovery of more major genes for rust resistance in these elite pedigrees will enable us to more successfully breed and deploy rust-resistant material.

At the Hofmann Forest, a 65-acre study was established to research the impacts of different levels of genetic homogeneity on growth and yield and ecophysiology of loblolly pine stands. This will be a long-term field laboratory for biometricians, physiologists, and geneticists to better understand the stand dynamics and ecophysiology of our genetic material.

Funding from the National Science Foundation allowed NC State scientists and students to collaborate with other scientists around the country in the Allele Discovery of Economic Pine Traits 2 project (ADEPT 2). This national research program has the overall purpose to understand the molecular genetics of complex traits and the relationship between natural genetic and phenotypic variation in forest trees.

There were different CAD wild type alleles in parents that were assessed in diallel tests for growth and wood quality traits, but these differences could not explain the specific combining (e.g. full-sib) effect that was seen.

Family and clonal differences in microfibril angle (MFA) were large and suggest that selection for lower MFA would be effective in increasing lumber strength, stiffness, and stability.

SEED PRODUCTION

Cone and seed production was substantially higher in 2005 compared to 2004. The 56,752 pounds of loblolly pine seed contributed to the total of 1,082 tons of seed produced by Cooperative members since 1968.

ASSOCIATED ACTIVITIES

Cooperative personnel have been involved with many activities including advisement on planting specific genotypes on the Hofmann Forest. The Cooperative hosted the 28th Southern Forest Tree Improvement Conference in Raleigh in June 2005. We have participated in numerous workshops and have had several visiting scientists to our Raleigh office.

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50 Years of Tree Improvement at NC State

“*So What?*” To really understand the impact of a research project, a student thesis, a day’s work, or 50 years of tree improvement, I always like to ask the question, *So What?* What better time to reflect on the impacts that our Cooperative Tree Improvement Program has had on forest productivity, forest landowners, the forest products industry, members of the Cooperative, NC State University, and the individuals whose lives have been touched by this program? In 1956, the *N.C. State – Industry Cooperative Forest Tree Improvement Program* was formed. Plantation forestry with southern pines was still a fairly new venture, and industry leaders knew that planting the best genetic material possible would result in increased productivity and forest value. Taking the lessons from plant and animal breeders, the Cooperative launched an aggressive selection, breeding, and testing program that continues today.

To prepare for writing this year’s annual report, I read 49 years of Annual Reports of the Tree Improvement Cooperative. I thought I knew most of what had occurred, but I had numerous surprises and revelations. Some of the impressions and reactions to the years of work are:

- Cooperative members, leaders, staff, and students have had incredible foresight to lead forestry, land management, and tree improvement in the direction they have gone. With the best silviculture and the best genetics, mean annual increments of 10 tons of wood per acre per year are now possible with plantations of loblolly pine in the southern US.
- The increase in forest productivity coming from producing over 1,000 tons (that’s over 2 million pounds) of genetically improved seed over the years. This is enough seed to plant **over 30 billion seedlings**.
- Members of our Cooperative and the other two southern tree improvement cooperatives are currently responsible for planting over 1 billion southern pine seedlings each year, over 75% of the annual tree planting in the US.
- Depending on site index, intensity of management, stumpage prices, and internal rates of return, landowners can easily realize between \$50 per acre (0.4 hectares) to over \$300 per acre of net present value by planting outstanding families that increase site index (SI₂₅) by five feet (1.52 m).
- The educational mission of NC State has been enhanced with over 200 graduate students educated and numerous undergraduates taught. Funding for student research has always been a Cooperative priority. These students have become leaders in industries, universities, and governments around the world. What a network of friends and colleagues we have!
- The 800+ publications representing the intellectual leadership and innovation that has come from our faculty, staff, and students signifies the impact the research program has had on the scientific community.
- Millions of dollars of grants and contracts that built upon the Cooperative’s infrastructure and support from cooperators have contributed immensely to the research mission of the University.
- The list of short courses, workshops, talks and presentations to numerous groups is most impressive. Outreach to Cooperative members, landowners, foresters, and the public has been a priority of the program from the beginning. We strive to make the work understandable and useful to all users.

What an impressive list of accomplishments to answer the question, “*So What?*”

1950's - The Beginning

Dr. Bruce Zobel was recruited from the Texas Forest Service in January 1957 to be the director of the Cooperative. Bruce's leadership in the early years was instrumental to the success of the program. Bob McElwee actually preceded Bruce by several months and was the first scientist with the Cooperative and was responsible for much of its early success.

From the first annual report in June 1957, "Activities in the forest tree improvement program can be arbitrarily divided into three parts; (1) help, advice, and consultation with the supporting industries on management problems relating to tree improvement, (2) basic research on problems common to the supporting industries, (3) working with and guiding graduate students in the forest genetics field." This still describes the main operation today.

Tree selection was the first big push in the Cooperative. Without a genetic base, little progress could be made. The majority of staff time was spent with cooperators looking for superior trees throughout the Southeast.



Bob McElwee and Bruce Zobel were instrumental in getting the Cooperative off to a good start in the late 1950's.

Bruce Zobel realized that getting trees selected and established into seed orchards would take many years, so to maintain the support of members, research on wood properties was an early focus of the program. The value of wood properties to the industry was clear, but little was known about the basics – tree to tree variation, the differences between juvenile and mature wood, and other wood properties questions were all emphasized.

One interesting quote from the 1957 Annual Report is "These figures are rather important, showing that in the shorter rotations, a higher proportion of very low specific gravity wood (juvenile wood) is produced." This sounds like something we recently wrote in a *Journal of Forestry* article about the economics of tree improvement.

1960's - Things Get Going

Once the Cooperative was functioning, the focus was on making selections and establishing orchards to get genetic gain to the woods. There were so many questions to understand – what traits are most important in the selection program? How can we improve grafting success for orchard establishment (the goal was to have 25% grafting success in early seed orchards!)? When will orchards become productive? How long will they produce seed? How should orchards be managed to enhance seed production? How should we breed and test the hundreds of selections? Despite the unknowns, tremendous strides were taken.

Orchards started producing significant quantities of seed in the late 1960's and tree improvement started having real impacts on forest productivity about 10 years after the Cooperative started. Some operational measures of success during this very busy decade were:

- 2450 acres of seed orchards (1769 acres of loblolly)
- Quantities of seeds harvested to grow 40 million seedlings
- 5595 control-cross families planted in progeny tests

Early research focused on wood quality looking at genetics and silvicultural control of wood quality. Companies collaborated to do mill studies worth hundreds of thousands of dollars. The Cooperative received funding from many sources to conduct research: “A larger share of the operating funds however, is coming from non-industrial sources... The NSF grant, Experiment Station Funds, and fellowships from various sources such as NSF and HEW have helped the program to expand both in size and scope of operation.” This could have been written in 2006.

The Heritability Study in Bainbridge, GA exemplifies the type of research that has been conducted over the years. Conceived in the late 1950’s, the Heritability Study was a collaborative effort between International Paper Company and the Tree Improvement Cooperative. Research support came from many sources including a \$75,000 NSF grant. Dozens of graduate students and scientists worked on the study for more than 25 years.

Graduate education was a priority from the beginning. During the 1960’s there were typically 15-20 graduate students working on research projects of direct interest to the Cooperative members. Funding came from members’ dues, College funds, research grants, and international governments and agencies funding international students.

The forest genetics “team” at NC State was starting to grow. The Coop staff grew to include Bob Kellison, Jim Roberds, J.B. Jett, Jerry Sprague, and Alice Hatcher. Associated faculty included Ellis Cowling, Chuck Davey, Jack Duffield, Gene Namkoong, Tom Perry, and Bud Saylor.

In the mid 1960’s there was a big push to increase forest productivity – mill expansions, labor shortages, conversion of timberland to farmland, large construction projects, roads, suburban sprawl, emphasis on recreation were all mentioned as major influences on forest management for timber production. “We must produce more on less land.” Once again, prophetic words were spoken.



Bowater’s Carters Nursery and Seed Orchard complex near Chatsworth, GA.

1970’s - Genetic Gain to the Forest

The 1970’s was a period when seed orchards started producing huge amounts of seed, genetic tests were yielding valuable information about the “winners and losers” in orchards, and forestland was being significantly impacted by tree improvement. The Cooperative made a conscious decision to “...concentrate activities on the tree breeding and applied silvicultural aspect and, secondly, to carry on as much supporting research as funding and staff time will permit.” Cutbacks of funding and fewer grants pushed the program in this direction.

The 1970’s could be considered the “Golden Age” for seed orchards in the South. So much was learned about how to manage orchards to get early and heavy seed production that gains going to the forest far exceeded expectations. The knowledge gained from fertilization, irrigation, subsoiling, insect control, and turf management studies is still relevant and valuable to orchard managers today.

This was a critical decade for expansion of the genetic base of the Cooperative. A huge effort (estimated cost was over \$1,300,000) by everyone in the program resulted in more than 3000 new Plantation Selections. Today, approximately 80% of the selections in our 3rd-Cycle Seed Orchards are established with these parents. The projected gains in forest productivity are incredible, due in a large part to the investment made over the last 30 years to select, breed, and test this population. The Plantation Selection program is an excellent reminder that the work we do today in tree improvement will have impacts in perpetuity.

The first operational family-block plantings were established by Weyerhaeuser Company in 1974-75. This innovative practice from 30 years ago has had as much impact on forest productivity in the South as any other genetics tool we have employed. The recognition that the best families planted on the best sites will yield the greatest operational gains has become fundamental to our silvicultural systems today.

All good things come to an end and other good things begin. Dr. Bruce Zobel retired as Director of the Cooperative in 1979 and Dr. Bob Weir assumed the leadership. As the first generation of breeding came to an end, Bruce decided to step down and “take it easy” – yeah right!

1980's - Research to Enhance Productivity

The 2nd-generation program really took off in the 1980's. Under the direction of the Cooperative's staff of scientists that included Bob Weir, J.B. Jett, John Talbert, Steve McKeand, and Dr. Floyd Bridgwater with the USDA Forest Service, the program's second round of breeding, testing and selection was even more intensive and innovative than the first round. Accelerated breeding methods to speed up the testing cycle were researched and put into practice. Disconnected half-diallels were used in the breeding and testing program, and all cooperators focused on doing the best testing possible on uniform sites. We are still benefiting from the high-quality of data today.

The research efforts of the Cooperative were significantly increased during this period:

- Seed orchard management studies to evaluate supplemental mass pollination, fertilization, irrigation, subsoiling and turf management helped to increase the quality and quantity of seed produced.
- Selection methods were refined to optimize the financial benefits of choosing the best genotypes at the appropriate age.
- Genetic variation trials were evaluated and new ones established to guide deployment decisions within and across geographic regions.
- The first use of molecular genetics in the program actually started in the late 1970's when Tom Adams from Oregon State and his students used isozymes to evaluate selfing and pollen contamination in seed orchards. Error rates in control crosses were also estimated using markers. The Cooperative established an isozyme laboratory in 1981.
- Computer modeling efforts to help direct future breeding strategies began.



Tree grading of second-generation selections by Cooperative staff (Jerry Sprague, John Talbert, and J.B. Jett pictured) was a huge effort in the 1970's.

During the 1980's the forest product industry started taking a hard look at forest land ownership. Little did we realize how much ownership patterns would change over the next 20 years.

1990's - Continued Improvement and New Strategies

I suppose that one measure of success is when one is taken for granted. Tree improvement had become such an integral component of silviculture and forest management that sometimes we got overlooked. Fortunately, in the mid 1990's there was another big push to increase forest productivity and tree improvement helped play a major role in the revitalization of the industry. Genetic gains from rouged 2nd-generation orchards were very large, and the use of family block plantings to realize the greatest gains had become common practice.

The 3rd-cycle selection effort was in full swing. It is ironic that the first selection in this cycle made in 1990 was due to Hurricane Hugo's damage. Selection 113001 and others were made a year early due to the storm's devastation of tests. The diallel breeding was completed and final tests were established in 1996.

Two long-time faculty members left the Cooperative during this time. After 26 years, J.B. Jett left to become Associate Dean for Research and Extension in the College, and Floyd Bridgwater left to move to Texas A&M with the USDA Forest Service. Their contributions to the Cooperative and forest genetics in general were immeasurable. Dr. Bailian Li joined the staff in 1995 after J.B. Jett left.



Breeding the 2nd-generation and plantation selections progressed rapidly through the 1990's. Progeny from almost 4000 parent trees were evaluated in diallel tests by cooperators.

A major benefit to Cooperators was the development of a relational database. Alice Hatcher's efforts resulted in a relatively easy way to bring the morass of data and summaries together so that tree breeders and orchard managers could use them. The database has evolved over the years, but these initial efforts were critical for the program. The first BLUP breeding values were estimated from our diallel tests in 1997. Bailian Li was instrumental in developing analytical procedures that could handle the complex data and provide useful BV's for the program.

The research efforts remained strong throughout this period. Efforts with computer modeling and simulations, silviculture-genetics studies, wood quality studies, the first QTL trials, elite populations, and the Rooted Cutting Program all indicated that the intellectual well being of the program was very healthy.

A milestone occurred in 1995 when the IPCo Heritability Study in Bainbridge, GA was cut down. The science that emanated from that trial, the graduate theses that resulted from the data, and the camaraderie among students and staff during measurement trips were all significant components of the Cooperative's history. It was tough to see an old friend go.

Another prophetic message from the 1999 Annual Report suggesting future turmoil "...we are being challenged by the most disruptive changes in membership in the 43-year history of the Cooperative. Mergers among industrial members..."

2000's - Unprecedented Change in Forestland Ownership

For the first 5+ years of the new millennium, one word resonates in tree improvement, forest management, and land ownership - *change*. In 2000 Bob Weir, Alice Hatcher, and Jerry Sprague retired after 30 years of dedicated service to the Cooperative, and Dr. Tim Mullin took over as Director. At NC State we've had a new Department Head, 3 Deans, 4 Provosts, a new Chancellor, and new System President. In 2005, Tim Mullin left the program, and Bailian Li and Steve McKeand were named co-directors. A year later, Bailian has taken a leave to be Interim Vice Provost for International Affairs.

If that's not enough change, consider the divestitures, mergers, and acquisitions by forest products companies resulting in the loss of Bowater, Champion International, and Union Camp as original Cooperative members. Because of budget cuts and other woes the states of South Carolina and Alabama ceased efforts on their tree improvement programs. Timber Investment Management Organizations (TIMOs), Real Estate Investment Trusts (REITs), and Biotechnology companies now make up 1/3 of our membership. International Paper Company has just announced the sale of its forestlands. At the time of this writing, Resource Management Service, LLC, Forest Investment Associates and other investors, and an investor group led by TimberStar will be acquiring the bulk of IPCo's southern U.S. forest land base.

Through all of this, the support and effort by our members and dedicated staff have not faltered. The third cycle of breeding and testing is well underway, and the gains being deployed and the financial benefits to landowners from using the best genetic material have never been greater. We are learning more about the genetic control of important wood properties such as microfibril angle, cellulose and lignin content, and solid wood properties and pulping characteristics. With our molecular genetics colleagues we better understand the value of a single gene, *cad-n1*, and its effect on wood properties and enhanced growth. The loblolly pine – fusiform rust pathosystem is not nearly as mysterious due to Henry Amerson's research in understanding major gene resistance. The decades-long goal to get full-sib families and selected clones (varieties) deployed operationally in substantial numbers has become a reality for many of our members.

We should be proud of our 50 years of accomplishments with an eye towards ...



Cooperators are using the latest technologies like somatic embryogenesis to produce propagules of elite varieties (right) and millions of full-sib seedlings (above) for reforestation.

The Future

Despite the upheaval in landownership, Cooperative membership changes, floods, fires, storms, and pestilence, the Cooperative Tree Improvement Program remains strong and its members remain committed to our mission:

To Economically Increase Forest Productivity Through Genetic Manipulation of Forest Tree Populations

Good times and bad will come, but optimism prevails as we start the next 50 years of genetic resource development to help produce a reliable, ecologically sustainable, and economically affordable supply of wood.



*Steve McKeand
May 2006*

SELECTION, BREEDING, AND TESTING

3rd-Cycle Mainline Breeding Progress

The third-cycle breeding program is well underway, with breeding of the sublimes accelerating in the last few years. In a few cases, breeding responsibilities have been transferred due to members leaving the Cooperative (namely, South Carolina For. Com. and Alabama For. Com.). Of the total 77 breeding sublimes, 11 sublimes were re-assigned to current Cooperators. Sublime selections have been grafted into breeding orchards or top-grafted on to big trees for breeding by each Cooperator.

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 crosses are summarized in Table 1. Crossing was particularly active in the Coastal and Piedmont regions, with the Northern region showing some progress.

Cooperative members within each region have grafted all parents selected for generating the pollen mix (PMX) for polycross mating. Three third-cycle check seed lots are being created for each of the three regions by using the same 20-parent PMX applied to 10 of the selections as females. Checklot crossing has progressed well in the Coastal and Piedmont regions (Table 1). Sufficient quantity of checklot seeds has been produced for all three regions (Coastal, Piedmont, and Northern) for establishing the PMX tests in 2006. More seeds will be needed to support more test series that will be established in the future.

In addition to the third-cycle checklots, several well-characterized families (common family checks) from the first two cycles of breeding are being crossed with the pollen mix. These families will be included in all tests to contribute to estimates of variance components and provide better family comparisons. Seeds from some common family checks have been collected for each breeding region (Table 1).

Full-sib controlled crossing has also commenced in the last few years, and full-sib seeds have been produced (Table 1). Seed production from full-sib cross mating is expected to rise in the coming years as the third-cycle selections produce more flowers. These full-sib crosses will generate seeds for within-family selection for the next breeding cycle.



When young trees flower heavily like this selection in the Virginia Department of Forestry breeding orchard at New Kent, rapid progress in the 3rd-cycle program can be made.

Table 1. Summary of third-cycle breeding progress as of Fall 2005

Type of crosses	Breeding Region	# Cooperators	# Sublines	Total # Parents	# Crosses producing Seed	# Crosses of Sufficient Seed	% Done Crosses
Polycross	Coastal	9	32	384	201	146	38.02%
	Piedmont	8	31	364	105	67	18.4%
	Northern	6	14	177	46	26	14.7 %
Polycross	Total	15	77	925	352	239	25.8 %
PMX checks	Coastal	9	--	10	10	10	100 %
	Piedmont	8	--	10	10	9	90 %
	Northern	6	--	10	10	5	50 %
PMX CFs	Coastal	9	--	7	7	6	85.7 %
	Piedmont	8	--	7	5	5	71.4 %
	Northern	6	--	7	3	2	28.6 %
Full-Sib	Coastal	9	32	235	97	75	31.9 %
	Piedmont	8	31	186	20	16	8.6 %
	Northern	5	13	81	17	5	6.17 %
Full-Sib	Total	15	76	502	134	96	19.1 %

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

PMX checks: Polycrossed checklot mixes

PMX CFs: Polycrossed Common Family checks

3rd-Cycle Progeny Testing

Polycross tests

In the fall/winter of 2004-05, the first series of 15 third-cycle polycross (PMX) tests were established for the Coastal region. Nine members of the Cooperative contributed to this ground-breaking effort. In 2006, more third-cycle PMX tests were established in both the Coastal and the Piedmont regions. In both regions, 5 tests of new PMX test series were grown and established in winter 2005-2006 (Table 2).

With the seeds harvested in the fall of 2005, we will sow more PMX tests of these same series this spring for field planting in 2007. Seeds have been distributed to members for establishing 10 tests to complete the Coastal Series C-PMX2 and 5 tests to begin the Coastal Series C-PMX3. P-PMX1 test series will also be completed in 2007 with 5 tests being established in the Piedmont region. For the Coastal test series, the 15 tests will be distributed across the region from coastal South Carolina to the Florida panhandle. Similarly for the Piedmont test series, the 5 tests will be distributed to Cooperators across the Piedmont region from eastern Tennessee south to Alabama.

Full-Sib controlled cross tests

In 2006, the first few progeny tests of Full-Sib (FS) crosses were established by Cooperators. MeadWestvaco and International Paper Company each planted 2 field locations of Coastal FS selection plots. This testing effort begins the generation of a diverse, valuable population for within-family selections into the future.

Checklot comparison tests

The purpose of the Checklot Comparison (CComp) tests is to link second-cycle checklots (CC) from eight Test Areas with the new third-cycle checklots from each zone for gain estimation. In 2005, a set of CComp tests were established (Table 2). Seeds were distributed to members for establishing 5 tests in winter 2005-2006. These tests represent the second-cycle Test Areas 3, 4, 5, 6, and 7. In each test, checklots were planted in block plots, with 4 blocks per test. Five suitable CC's and the third-cycle checklots were planted in each test. In 2007, one additional test will be established to represent the second-cycle Test Area 2 (eastern NC).

Table 2. Third-cycle progeny tests established in Winter 2005-2006. The first 5 of the progeny tests in each series have now been established.

Region	Test Series	Number Cooperators	Number Tests	Location by States
Coastal	CPMX2	4	5	NC, SC, FL
Piedmont	PPMX1	5	5	SC, GA, AL, TN
Coastal	CComp	3	3	SC, GA, FL
Piedmont	CComp	2	2	GA, AL



One of the first Coastal PMX tests established by Joshua Management, LLC had only one tree die in the first year. We've been working with Sam Campbell to try to get him to do better next year!

3rd-Cycle Elite Breeding Progress

The expected gains from the Elite Breeding programs make it clear that getting this breeding underway is very important, and the cooperators have made great strides in accomplishing this task. With the Elite Breeding from 2005, the Coastal region is 44% complete with 86% of assigned crosses being made; Piedmont is 2% complete with 10% assigned crosses made; and Northern is 2% complete with 20% assigned crosses made. All indications are that great emphasis was made in the elite breeding front this spring, and we look forward to reporting much higher yields in Spring 2007.

IPCo Backup Grafting

In 2005, International Paper Company (IPCo) announced that a large amount of their land base would be sold, including breeding orchards and nurseries. Most of IPCo's breeding selections are well represented by other Cooperative members, but many selections were at risk of being lost given the uncertain future ownership. Several selections that are held only by IPCo are important to the Cooperative's breeding programs. It was unclear as to how the land sales would unfold, but it was essential that the Cooperative acted to prevent losing access to many selections.

Every Cooperator was willing to graft selected germplasm into their orchards or on remaining rootstock to ensure that these selections would be maintained by the Cooperative. In order to secure the highest priority selections, we ranked the IPCo trees based on breeding values for growth, form, rust resistance, and included those selections that met certain criteria. Finally, of those selections that met the criteria, we determined whether other members in the Cooperative held any ramets of each selection. The high-priority selections were then collected by International Paper and scions were sent throughout the Southeast to members of the Cooperative to graft into their clone banks or breeding orchards.

International Paper has been a major player in our Cooperative and was a founding member of the program. Preserving the hard work IPCo has accomplished over the decades was quite an undertaking. We want to thank the International Paper Company crew and all the Cooperators for their hard work in getting these selections established.



*Archives and clone banks are crucial for maintaining the Cooperative's genetic resources.
(Picture at the Tennessee Division of Forestry complex at Delano, TN)*

RESEARCH

Genotype by Pathogen Interactions in the Fusiform Rust – Loblolly Pine Pathosystem¹

Stability of fusiform rust resistance in selected loblolly pine families and pathogen virulence variation among diverse inocula sources were investigated in the Integrated Pest Management (IPM) project funded by the USDA. The experiments were carried out in a series of two greenhouse-screening trials. The specific objectives of the study were to (i) assess rust resistance stability and predictability in high-productivity loblolly pine families and (ii) identify families that are likely sources for future resistance gene (*Fr*) discovery and targets for genetic marker investigations and (iii) document virulence variation in the pathogen population across diverse inoculum sources.

High-productivity loblolly pine parent trees with a wide range of rust resistance were identified from the Cooperative breeding populations in the Atlantic and Gulf Coastal Plains. The parent trees were field-tested in the second-cycle diallel tests. Open-pollinated progeny from these selections and the susceptible checklot were rust-challenged at the USDA Forest Service Resistance Screening Center (RSC) in Asheville, NC, using standard RSC procedures. For each family, a target of 60 progeny (seedlings) were challenged with 10 different inocula (30-gall mixes from 10 different trees) from geographically distinct regions of the South. The experiment (inoculation of OP families) was repeated for each of 10 inocula. In total, 22,649 seedlings were screened. To estimate the probability of disease for each family, a generalized linear mixed model was fitted to binary data (diseased=1, not diseased=0).

Inocula were significantly different for percentage of disease occurrence. The mean gall forming of each inoculum varied from 35% to 57%. The most virulent inoculum had 12% greater incidence of

disease over the least disease causing inoculum. The means are averages across all families, including putative resistant and susceptible families.

Differences in family means were large, ranging from 12% (Family 23) to 77% (Family 3), resulting in a high family mean heritability of 0.98. A histogram of family disease means showed a bimodal distribution (two peaks) rather than a univariate normal distribution (Figure 1) also suggesting strong genetic effects and small environmental effects in family responses to pathogen attacks. Putative susceptible families (tested in field trials) were all diseased (susceptible) under controlled inoculation. A majority of putative resistant families were indeed resistant in greenhouse experiments (Figure 1). These families had an average of 12% to 40% disease. However, a group of putative resistant families were not resistant with more than 52% averages.

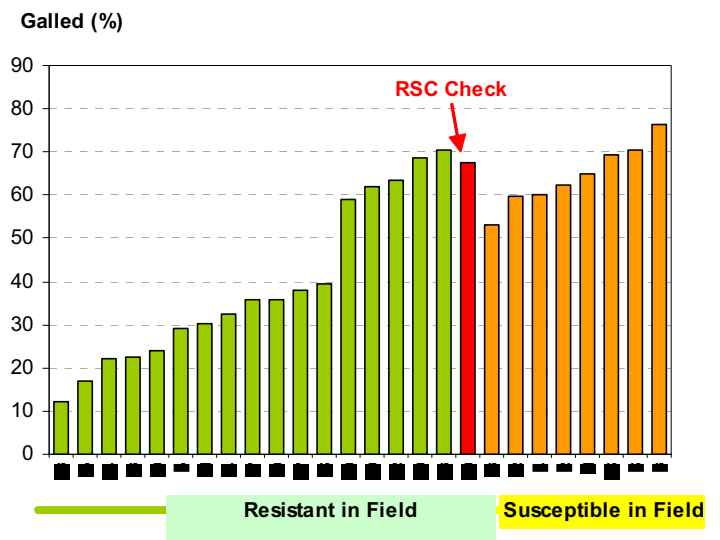


Figure 1. Family disease means across inocula after greenhouse inoculation. The red bar is the susceptible check used in screening trials. The orange bars are putative susceptible and the green bars are putative resistant families from diallel tests. A group of putative resistant families in the middle developed high average disease and were as susceptible as putative susceptible families.

¹ Most of this research was conducted by our collaborators, Henry Amerson and Saul Garcia, with the Fusiform Rust Program.

Genotype by inoculum interaction

The relative responses of families to the different inocula were not always stable but varied from one inoculum source to another for a few families. There was significant Inoculum x FAMILY interaction in the analysis of variance, but resistant families were no more interactive than susceptible families. One family (19) was particularly interactive to two inocula (Figure 2).

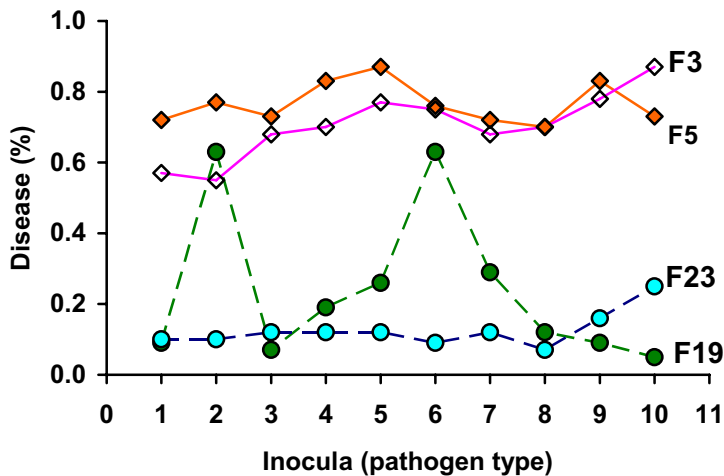


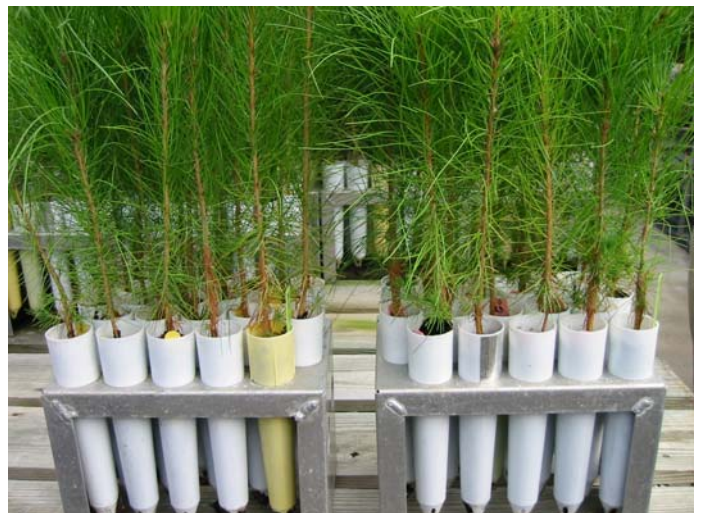
Figure 2. Response of two putative susceptible (F3 and F5) and two putative resistant (F23 and F19) families to different inocula. One resistant family was very susceptible when challenged with the pathogen genotypes 2 and 6.

Summary

- Inocula effects were significantly different in gall formation. The average disease percentage for each inoculum (across all families) ranged from 35% to 57%, suggesting that genotypes have been exposed to the pathogens adequately.
- We also found significant differences among families in response to inocula. Family disease means (%) showed a bimodal distribution (two peaks) rather than a univariate normal distribution suggesting possible resistance conferring genes in the putative resistant families.
- Some putative resistant families did not show resistance in greenhouse experiments when challenged with different inocula. However, all the putative susceptible families developed galls in the greenhouse experiments.
- Family by inoculum interaction was significant. But resistant families were no more interactive than susceptible families.
- The research is still ongoing. The results may help us to detect more resistant genes, particularly in resistant families. Understanding response of genotypes to different pathogens will help us to develop better deployment strategies. When the study is completed we will have broader knowledge of the level of variation among and within pathogen populations in the Southern United States (see next section).



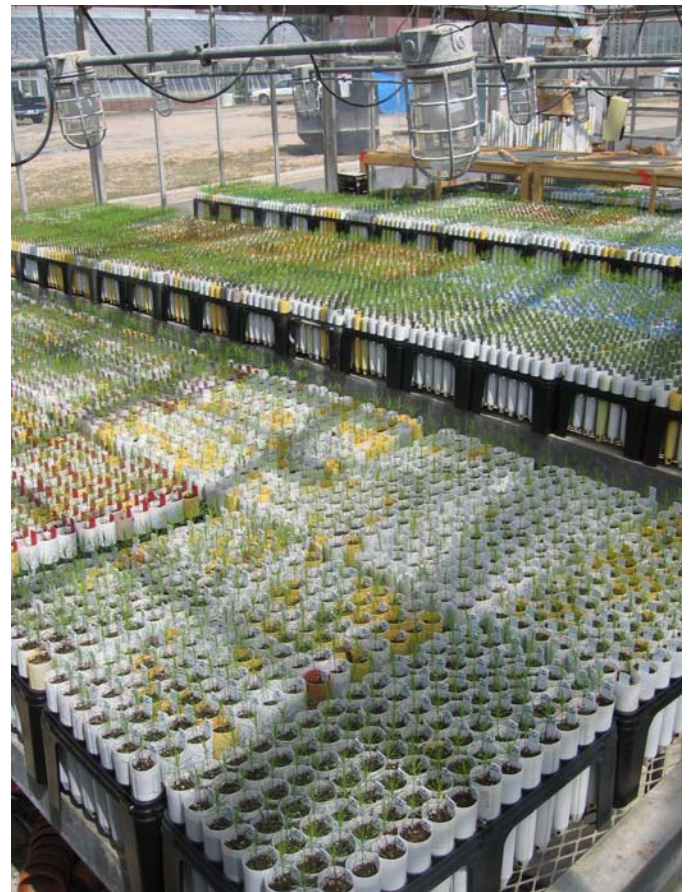
Seedlings were screened at the USDA Forest Service RSC to compare putatively resistant and susceptible families that were evaluated in the Cooperative's diallel tests.



Fr Gene Discovery

One objective of the Integrated Pest Management project (IPM project described on the previous pages) was to identify selections that potentially possess single effective resistance genes against fusiform rust. Progeny from 25 selections that were identified as resistant, based on our diallel field-test data, were used in the study. These seedlings were challenged with ten geographically different 30-gall inoculum mixes of fusiform rust to test the effectiveness and stability of the rust resistance. By analyzing the relationship of gall formation within each combination of family by 30-gall inoculum mix, we found 11 of the 25 selections to be candidates for major *Fr* gene discovery work.

In 2006 we will be challenging 100 progeny from each of the selections against several fusiform rust single genotype lines (SGL), using the USDA Forest Service Resistance Screening Center in Asheville, NC to conduct the inoculations. The fusiform rust SGLs reduce the genetic variation of the pathogen with regards to virulence, giving us a better chance to identify single resistance gene patterns from the host. At the time of transplanting, we harvested and stored the megagametophytes from each individual seedling for potential future molecular marker work. If and when single resistance gene patterns emerge from the phenotypic assessments, then genetic marker work will begin in an attempt to marker-tag the chromosome region(s) of the resistance gene(s). DNA from the stored megagametophytes will be used in the analysis because if *Fr* genes are present, only the maternal contribution would be tractable and useful. By using molecular markers to tag the *Fr* gene(s), we have a means to track the gene(s) in past pedigrees and use in future breeding strategies.



Saul Garcia (above right), Henry Amerson, and crew planted seeds, plucked megagametophytes, and cultivated over 11,000 seedlings for the Fr Gene Discovery Project that started this spring. These seedlings are now in Asheville, NC awaiting inoculation at the USDA Forest Service Resistance Screening Center.

Hofmann Forest Genetics x Spacing x Thinning (GST) Study¹

In January 2006, we initiated a 65-acre study on the Hofmann Forest located near Jacksonville, NC to research the impacts of different levels of genetic homogeneity on growth and yield and ecophysiology of loblolly pine stands. Impacts on both an individual-tree and stand-level basis will be studied for 10 different seedlots ranging from the clonal level to a seed orchard mix at two spacings and two thinning regimes. This study establishes a unique set of growth and yield block plots that will be used to study allometric trends and physiological traits in loblolly pine.

There is a lack of information on the effects of genetic homogeneity on individual tree and stand-level traits for loblolly pine. With the large increases in planting of specific genetic source, ranging from the open-pollinated (OP) to the clonal level, research is needed on how trees will competitively respond when all of their neighbors have the same (or similar) genetic background. The GST was installed to investigate the effects of genetic homogeneity on stand performance, while incorporating different spacing and thinning treatments.

Understanding carbon (C) allocation in loblolly pine is particularly important because of the intensive breeding and selection programs aimed at enhancing growth and product value. Selection for enhanced growth is almost certainly affecting stand-level secondary biochemistry, with implications for plant resistance to pests and ecosystem-level processes such as nutrient cycling and soil C formation.

One concern is that narrowing the genetic base through selective breeding for productivity may increase the risk of catastrophic pest outbreaks (facilitated by decreased constitutive phenolics) or create site limitations to growth. To date, this does not yet appear to be a problem, probably due to retention of adequate genetic diversity in the improved populations. However, the deployment of genetically identical populations (clones) in large blocks across the landscape gives this concern renewed urgency.

A total of ten genetic entries were chosen to represent the range of genetic material currently being operationally planted in the Southeast. The genetic entries include three clones, three full-sib families, three half-sib (OP) families, and one seed orchard mix. The information gleaned from the GST study will help address a wide range of research and operational questions including:

Being able to characterize the growth patterns in loblolly pine stands is crucial to proper land management and enables forest managers to plan for future harvests, mid-rotation treatments, and regeneration efforts. Forest growth and yield modelers need to be able to account for competition effects in prediction models to accurately reflect stand conditions. Understanding allometric trends in loblolly pine for different levels of genetic homogeneity, spacing, and thinning was a major motivation for the GST study. Specifically, researchers at NCSU and hopefully other institutions will use the GST study to address the following biometric and ecophysiology research issues:



Dr. Bronson Bullock was the brains behind much of the Hofmann GST, but at times, many of us were very concerned about following his lead.

¹ Drs. Bronson Bullock and John King are the scientific leaders on the GST. This trial would not be possible without their collaboration, intellectual contributions, and backbreaking work at the Hofmann.

Biometrics:

Diameter Distributions: Stand level diameter distributions will be derived across all genetic entries for comparing differences between the various levels of genetic homogeneity, spacing, and thinning effects. Diameter distributions can also be compared on a temporal scale, looking at how the distribution changes over time for a particular combination of genetic homogeneity, spacing, and thinning regime.

Spatial Dependency: Information on how stems in a stand compete with each other will aid in describing stand dynamics. High inter-tree competition for resources can be accounted for in spatial models and will increase the precision of stand-level growth projections. Spatial dependencies may differ between stands with a mix of genetic backgrounds and stands with a high level of genetic homogeneity. The neighbors of an individual tree influence the growth and survival of the subject tree; having neighbors that are genetically similar (or identical) to the subject tree will influence the spatial dependency between stems in a stand. The spatial dependency between stems in a stand can also be tracked over time to ascertain changes with growth and mortality.

Individual Stem Taper and Volume: Models to predict individual stem taper and volume across the treatments will be developed to compare differences in stem characteristics. Knowledge of how stems differ by treatment will aid in the development of more precise individual stem models.

Stand-level Volume Estimates: Data collected on a stand-level across the treatments in the GST study will be used to model stand-level volume. Comparisons by spacing, thinning, and genetic entry will be made in terms of stand volume. How the stand-level volume changes over time and across treatments will be assessed.

Tree Physiology:

In addition to the block plots, seedlings from all the seedlots were planted in greenhouse and single-tree plot trials and will be complementary and will provide opportunities for scaling of growth and physiological processes from small seedlings to stands. In the greenhouse study, detailed measurements will be collected frequently on photosynthesis, dark respiration, leaf area, above- and below-ground growth, biomass partitioning, fine root production and turnover, and seasonal concentrations of foliar carbon-based secondary components (CBSC). The greenhouse experiment will be terminated with a complete above- and below-ground harvest to look at detailed patterns of biomass accumulation and partitioning. These detailed studies will allow us to quantify genetic effects on whole-plant C allocation under conditions of low and high resource availability.



Undergrads, graduate students, staff, faculty, husbands and wives all helped plant the 65-acre Hofmann GST.

The single-tree plots at the Hofmann (adjacent to the block plot GST) will be measured for individual tree performance in a field setting. Above-ground growth, photosynthesis, nutrient uptake rates, and seasonal foliar CBSC will be assessed on a less frequent (monthly), but longer-term (years) basis. Of the 20 replicate trees per

genetic entry, 2 will be harvested at age 1, 2 and 3 years (6 total) to examine above- and below-ground biomass accumulation and partitioning, and to develop allometric biomass/volume equations. The harvested trees will be important for testing the hypothesis that increased productivity with genetic improvement is driven by increased resource use efficiency and greater growth efficiency.

The block-plots will be used to scale results of the greenhouse and single-tree-plot studies to the stand level. Annual assessments of stand-level growth (e.g. all measurement trees in the block plots) will be used to determine if differences in growth observed at the single-tree level are maintained when the trees are grown in genetically uniform stands. This growth analysis will rely on simple metrics such as height and diameter, to which the allometric models from the single-tree plots can be applied to arrive at volume or biomass production. In addition, the block plots will be used to examine ecosystem level properties, including: accumulation, nutrient content, and turnover of the forest floor; soil respiration (an index of belowground C allocation/cycling; incidence of fusiform rust, tipmoth, or other pathogens; soil N dynamics (content, mineralization, immobilization). These stand level assessments will allow us to determine the consequence of genetic improvement on important ecosystem properties such as C sequestration and nutrient cycling.

Experimental Design:

Three blocks (replications) of the study were established at the Hofmann Forest in January, 2006. Each replication consisted of two initial spacings, a 5 × 20 ft (436 TPA) and a 10 × 20 ft (218 TPA), with a thinning component for each initial spacing (thin and no-thin), with ten different genetic entries planted at each spacing. There are four levels of genetic control incorporated into the design:

CellFor Clones: C1, C2, C3 are excellent growing clones with good to excellent stem form and rust resistance that have been extensively tested throughout the South. The CellFor clones were grown as bareroot seedlings by Plum Creek Timber Company in their Jesup, GA nursery.

Full-sib Families (Mass Control Pollinated): (MCP1, MCP2, MCP3). The full-sib families were obtained from MeadWestvaco and were grown as bareroot seedlings in their nursery near Ravenel, SC

Half-sib Families (Open-pollinated): OP1, OP2, OP3 have excellent growth, form, and rust resistance were also obtained from MeadWestvaco.

Seed Orchard Mix: Seed for the Seed Orchard Mix came from International Paper Company's 2nd-generation coastal loblolly seed orchard near Bellville, GA, and seedlings were grown in their nursery near Selma, AL.

The design is a Split-Plot with the four combinations of spacing and thinning being the whole-plots and the genetic entry being the subplots. Each genetic entry was randomly assigned within each whole-plot. The measurement trees span 4-6 beds spaced 20 ft apart and run for 9 to 20 trees in each bed. Buffers were installed to reduce any adjacency effects. The plot size varies based on the treatment combination of spacing and thinning. The total area including all buffers is 63.9 acres.



Dr. John King surveys the results of several weeks of planting the Hofmann Forest GST. The GST alone is 65 acres in size. Almost 100 acres of genetics trials and demos have been planted on the Hofmann the last 2 years.

ADEPT2 - Association Genetics of Loblolly Pine

Understanding the relationship between phenotypic and genotypic variation is of key importance to tree breeders. In September 2005, funding from the National Science Foundation began for the Allele Discovery of Economic Pine Traits 2 project (ADEPT 2) under the direction of David Neale and Chuck Langley (UC Davis) and with collaboration with scientists at the University of Florida and Texas A & M University. The overall purpose of this project is to understand the genetics of complex traits and the relationship between natural genetic and phenotypic variation in forest trees. More specifically, the project has four main objectives: 1) determine 5000 candidate genes 2) discover sequence polymorphisms (hopefully, alleles) for the candidate genes 3) estimate the extent of linkage disequilibrium for a subset of candidate genes and 4) detect and verify associations between SNPs (single nucleotide polymorphisms) in 1000 candidate genes for wood property phenotypes, disease resistance phenotypes, gene expression phenotypes and drought tolerance phenotypes in multiple populations of loblolly pine. The focus at NCSU will be to develop and maintain a population of clones (rooted cuttings) for the project, and to detect genotype-phenotype associations in traits related to water relations and drought tolerance.

Association genetics takes a population-level approach to complex traits to identify relationships between markers and phenotypic traits. Conifers are ideal for association genetic studies because they are generally large, unstructured populations, display rapid decay of linkage disequilibrium, are capable of precise phenotyping through clonal propagation, and allow haplotype determination from megagametophyte tissue¹.

Under the direction of Barry Goldfarb, a new association population was created in 2005 using first-generation selections from NCSU-TIP and WGFTIP to cover the natural range of Loblolly

pine. Hedges were grown from seed for over 600 seedlots. To date, cuttings have been successfully rooted for over 500 unrelated genotypes. Rooted cuttings will be produced over the next 4 years for collaborators to assess various phenotypes. In addition, clonal material will be produced to establish long-term field tests at several locations, providing a rich resource for the future.

In addition to propagating the association population, the team at NCSU will also be working on the association study for water relations and drought tolerance. This population also creates an opportunity to examine geographic variation in a clonally replicated experiment. The information will contribute not only to a greater understanding of the natural variation in loblolly pine, but will also provide information for breeders to aid in selection, breeding, and deployment of well adapted families and clones.

Collaborators on this project include David Neale (Project leader) and Chuck Langley at the University of California at Davis; Gary Peter, John Davis, Dudley Huber, Matias Kirst, and George Casella at the University of Florida; Carol Loopstra and Tom Byram at Texas A&M University; and Barry Goldfarb and Bailian Li at North Carolina State University. Patrick Cumbie is a PhD student whose dissertation research will focus on the project.



Patrick Cumbie (right) and crew (L→R Ben Smith, Tori Brooks, and Mike Aspinwall) take cuttings for the ADEPT2 project.

¹ Neale, D.B. and O. Savolainen. 2004. Association genetics of complex traits in conifers. *Trends in Plant Sciences* 9(7): 325-330.

Summary of CAD Allelic Diversity Project¹

The discovery that selection 7-56 is heterozygous for a mutation in the CAD gene sparked an interest in whether progeny that inherit the mutant allele of CAD grow faster or have different wood properties than progeny that do not inherit the mutant allele (see Yu et al. 2006 and 2005 in list of publications).

Three descendents of 7-56 that inherited the mutant version of the CAD gene were included as selections in second-generation diallel tests. Funding was obtained from DOE to compare the growth and wood properties of trees bearing the mutant allele ("heterozygotes") and those with two normal copies of the CAD ("wildtypes"). Each of these selections was in 5 crosses, except for selection "A" that was in 10 crosses (5 crosses in each of 2 diallels).

Only one of the 20 full-sib families tested showed statistically significant differences between "heterozygotes" and "wildtypes" among the progeny; the "heterozygotes" had 44% greater volume and 8% higher wood density than the "wildtypes". A second full-sib family showed trends in the same directions, but the effect was not significant after adjustment for the multiple comparisons carried out as part of the analysis (Yu et al. 2006).

The observation that only one of twenty full-sib families showed a difference between "heterozygote" and "wildtype" offspring can be explained genetically as a specific combining effect between the two parents of that full-sib family. In other words, those parents have some genetic factor that is associated with the observed outcome. This genetic factor could be a single genetic locus, or a combination of multiple loci. If the factor that causes this difference between progeny is a single gene, it could be the CAD gene itself, or a different gene. If the genetic basis for the observed specific combining effect is found at the CAD gene itself, it is a reasonable hypothesis that the two parents that show the specific combining effect will have a combination of CAD

alleles that are not found in any of the 19 pairs of parents that did not show the same effect.

The CAD gene was divided into four different segments for analysis. The first segment of the CAD gene to be analyzed was the region that contains the known mutation first discovered in 7-56; this is segment 3 in our terminology. This segment of the CAD gene was amplified from haploid megagametophyte DNA samples obtained from seeds of the two parents of the full-sib family where the "heterozygote advantage" was detected, and from two other individuals that were parents of families where no "heterozygote advantage" was detected. Each parent contains two alleles of the CAD gene, which could have the same DNA sequence in each segment of the gene or could have different DNA sequences. In the diagram below, parent "A" is an offspring of 7-56 that is known to have inherited the mutant allele of the CAD gene. Parent "B" is a "wildtype" parent and is the other parent of the full-sib family that showed significant differences between "heterozygotes" and "wildtypes" among the progeny. Selections "C" and "D" were also crossed to "A" but did not show any significant differences among progeny.

Heterozygote Parent	Wildtype Parent		
	B (w_1, w_1)	C (w_2, w_3)	D (w_1, w_1)
A (m, w_1)	Significant mutant effect	Non-significant mutant effect	Non-significant mutant effect

Analysis of megagametophyte DNAs from parent "A" confirmed that there are two different alleles of CAD with different DNA sequences in segment 3, one which is the mutant allele from 7-56 (allele m) and the other which is a normal functional allele (w_1). Parallel analysis of parents "B", "C", and "D" showed that parents "B" and "D" have alleles of the CAD gene with identical DNA sequences in segment 3, and that sequence is identical to that of the normal allele from parent "A" (w_1). Parent "C" carries two alleles of CAD that differ in DNA sequence from each other and from the normal allele in parent "A" in segment 3, shown above as w_2 and w_3 .

¹ This research was conducted by Dr. Ross Whetten in conjunction with the Cooperative's effort in the CAD Project.

The results of this comparison show that parents “A” and “B”, which show a specific combining effect, carry wildtype alleles at the CAD gene with identical DNA sequences in segment 3. The same combination of alleles also occurs in parents “A” and “D”, which did not show the specific combining effect. This finding does not support the hypothesis that the

genetic basis for the specific combining effect is in segment 3 of the CAD gene.

Research is still underway to test other segments of the CAD gene for DNA sequence differences among the parents of the different families. Results will be reported as they are obtained.

Microfibril Angle Variation in Loblolly Pine¹

Microfibril angle (MFA) and specific gravity are the most important indicators of wood quality in loblolly pine. MFA is the angle of the cellulose fibers from the vertical axis in the secondary cell wall of tracheids (Figure 1). Lower MFA has desirable effect on lumber strength, stiffness, and dimensional stability. Despite the significance of MFA on end products, this trait has not been fully utilized in loblolly pine improvement programs mainly because it is prohibitively expensive and time-consuming to measure. MFA in loblolly pine can be as high as 50 degrees in the pith and decreases rapidly towards the outer wood. If MFA can be reduced, the dimensional stability and strength of boards cut from plantation-grown loblolly pine can be dramatically improved.

MFA variation was investigated in a clonal trial of loblolly pine having 44 clones of nine full-sib families at two sites. Each clone had 7 to 12 wood cores, and a total of 369 trees were sampled across two sites. Average MFA for each ring was obtained on each 12 mm wood core using SilviScan-2 (Evans & Ilic. 2001. *Forest Products Journal* 51(3):53-57). The number of rings for each tree (on a wood core) ranged from 9 to 11 depending on the growth. Ring width and percent latewood of each ring were also obtained.

A decreasing quadratic model based on the rings explained much of the variation in MFA. The trend

was linear in the first few rings and became quadratic after ring 3. Latewood percentage, ring width and their interactions were significant, and they were included in the model as covariates (e.g. latewood percentage within ring and ring width had significant effect on MFA). As the ring width decreased and latewood increased from the pith to the outer wood, MFA decreased.

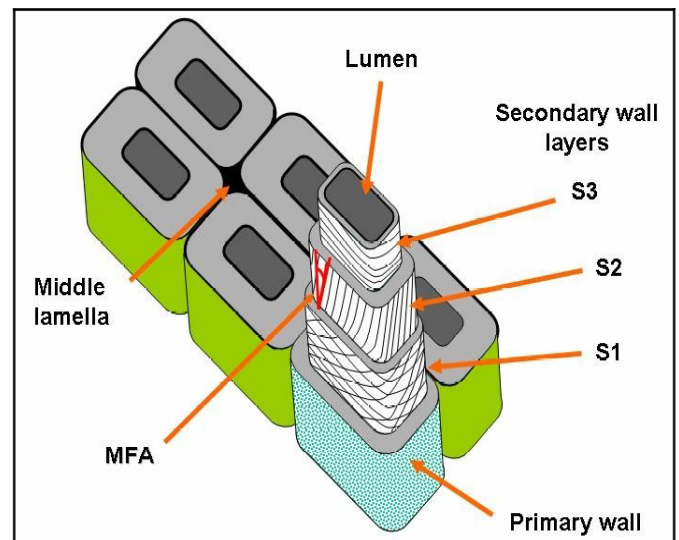


Figure 1. MFA is the angle of the cellulose fibers in the secondary cell wall (S2). Vertical fiber angles are favorably correlated with wood properties, including stiffness, strength, and stability. Picture is courtesy of Sandra Morgan, Forest Products Laboratory, Madison, WI. Used with permission.

Trees at the faster growing Alabama (AL) site had significantly greater MFA compared to the slow growing Florida (FL) site. MFA in the pith was about 32° at the AL site and it was about 29° at the FL site. The trend in MFA from the pith to the outer wood was quadratic for both sites, and the difference between the two sites decreased slightly from the pith to the outer wood (Figure 2).

¹ This project ‘Wood and Fiber Quality of Juvenile Pine: Characterization and Utilization’ is part of an external grant from the USDA-Agenda 2020 program to study wood characteristics of loblolly pine. Much of the research has been conducted by Dr. Fikret Isik.

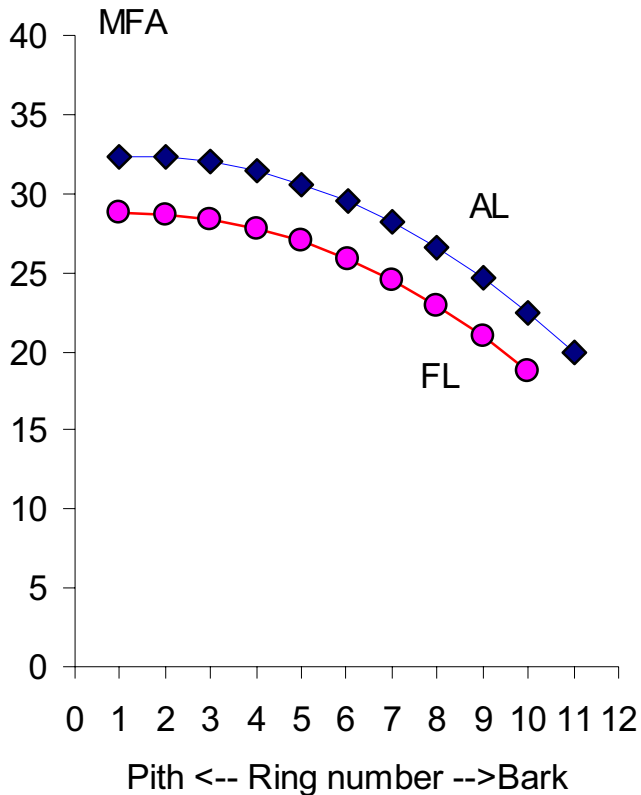


Figure 2. Predicted MFA for two sites. A significant difference in MFA between sites was observed. The same clones had greater MFA in the fast growing AL site than in the slow growing FL site.

Genetic differences in MFA were large, with family and clones within-families explaining 7.4% and 8.3% of overall MFA variation, respectively. Clone by site interaction contributed 2.7% to the overall variation. About 26% of the overall variation came from tree to tree variation and 55% from within-tree variation. Repeatability of full-sib family (0.75) and clones (0.70) were high, suggesting that improvement in MFA would come from combined selection of full-sib families and clones.

Families were stable across the two sites, but there was significant clone by site interaction (3%). Clonal stability across the sites is currently under investigation. Tree to tree variation and residual variation are due to environmental effects.

In conclusion, the change in MFA across growth rings within and between young loblolly pine clones was explained by a decreasing quadratic model. The moderately high repeatability values for MFA for full-sib families and clones suggests that selection will be effective to improve MFA. If efficient sampling methods can be developed, MFA may be considered for inclusion in our tree improvement programs to improve lumber quality.

EXTERNALLY FUNDED RESEARCH GRANTS 2005-2006

T.J. Mullin, Bailian Li, John F. Kadla, and Hasan Jameel. DOE Agenda 2020. \$1,125,410. Performance and Value of CAD-Deficient Pine. 05/03-05/06.

Robison, D., L.J. Frampton, R. Bardon, B. Goldfarb, G. Hodge, B. Li, J. Kadla, S. McKeand and S. Moore. USDA: \$266,005. Integrated Biotechnological and Genetic Systems for Enhanced Forest Productivity and Health. 06/01/03-05/30/05.

Amerson, H., S. McKeand, T. Mullin and B. Li. USDA-IPM. \$100,000. Development of Stable and Predictable Deployment Populations of Loblolly Pine to Minimize Fusiform Rust Impacts in Southern Pine Plantations. 2004-2005

Fikret Isik, Bailian Li, Bronson Bullock. USDA Forest Service Agenda 2020. \$169,125. Prediction of Whole-Stem Wood Quality of Superior Loblolly Pine Clones for Deployment. 05/05 - 05/07

Lauire Schimlek, Alex Clark III, Bailian Li, Fikret Isik. USDA Forest Service Agenda 2020. \$181,000. Rapid techniques for screening wood properties for genetic improvement of loblolly pine. 05/05- 05/07

SEED AND CONE YIELDS

Approximately 28 tons of loblolly pine seed were harvested in the Cooperative in 2005. This was about double the seed yield from 2004. Despite the barrage of record hurricanes the past 2 years, seed yields overall were strong, and the third cycle seed was at its highest level increasing from 377 pounds to 3,891 pounds (Table 1). Average seed yields also rose, from 1.38 to 1.55 pounds per bushel. Second-generation orchards contributed about 75% of total seed, and coastal orchards accounted for 69% of total seed production.

Table 1. Comparison of 2005 seed and cone yields with previous year.

Provenance	Bushels Of Cones		Pounds Of Seed		Pounds per Bushel	
	2005	2004	2005	2004	2005	2004
Coastal 1.0	5,389	3,414	8,156	5,098	1.51	1.49
Coastal 2.0	23,765	12,336	39,242	17,941	1.65	1.45
Coastal 3.0	3,231	382	3,638	377	1.13	0.99
Piedmont 1.0	305	19	375	33	1.23	1.74
Piedmont 2.0	3,375	3,060	4,863	3,772	1.44	1.23
Piedmont 3.0	330	na	253	Na	0.77	na
Northern 2.0	150	na	225	Na	1.50	na
Totals	36,545	19,211	56,752	27,221	1.55	1.38

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). Starting in the mid to late 1980's, cooperators were producing all the seeds needed for regeneration, so selective harvest of 20 to 40 tons of seed per year has been the norm.

From 1968 to 2005, over **2 million pounds of improved seed have been produced by Cooperative members** (Figure 2). At 15,000 seedlings per pound, this is enough seed to grow over 32 billion improved seedlings! This is quite an accomplishment for the Cooperative and its members, and we look forward to continuing this tradition of success.

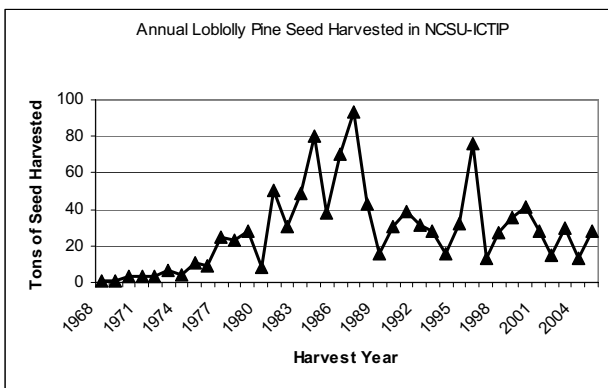


Figure 1. Annual seed yields from 1968 to 2005

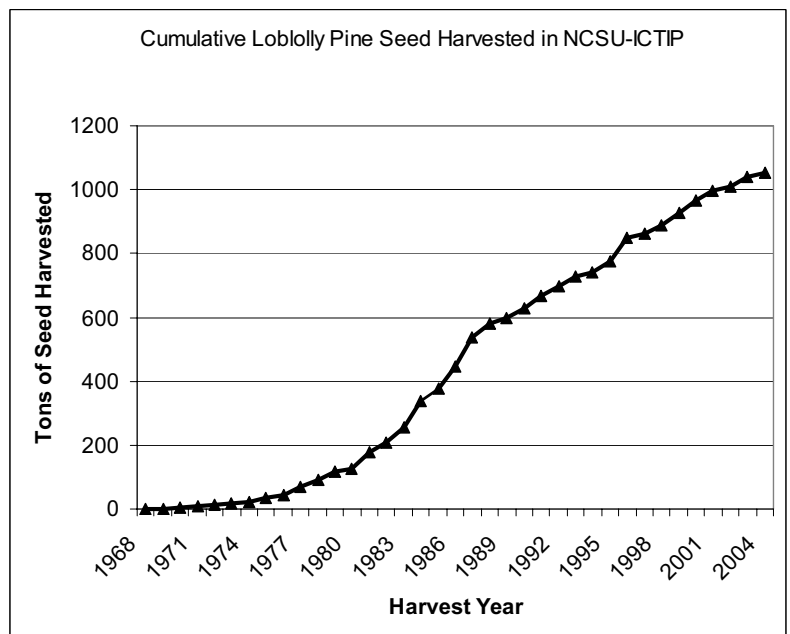


Figure 2 (right). Cumulative seed yields for the NCSU-ICTIP starting in 1968. An estimated 1,082 tons of improved seed harvested over the past 37 years.

Precocious Cone Production in the Piedmont – What Happens when Things are Done Right

Temple-Inland's first 10.3-acre production seed orchard block (A block) at the Brown Farm Tree Improvement Center northeast of Gadsden, AL near Centre was established in 2000. The Upper Piedmont region of the South has some distinct disadvantages for seed orchard establishment, chiefly ice storms and late spring freezes, but this was the land base available. The commitment was made to do things right in the late 1990's when Temple-Inland joined the Cooperative, and the early results speak for themselves.

In 1998, a 9-year-old loblolly pine plantation that had been established on an excellent agricultural site was cleared and intensively site-prepared for orchard establishment. The soil is a deep, well-drained, Holston loam over a clay-loam subsoil, ideal for an orchard. Twenty-five of the best tested 2nd- and 3rd-cycle Piedmont and Upper Gulf parents were included based on an index of breeding values for growth, rust resistance, and straightness. Scions were field-grafted onto 1-year-old rootstock at 21' X 21' spacing (~100 tpa) in a systematic orchard design in 10.3 acres. Graft mortality and the attrition of 5 severely incompatible clones has reduced stocking to 72 tpa in 2006.

Drip irrigation has been applied during the summer. Fertilization has followed standard orchard protocols to correct any nutrient deficiencies, along with judicious nitrogen applications to improve tree vigor in the early years of orchard development. To stimulate flower production, nitrogen was applied in late summer 2003 and 2004 at 150 lbs. per acre NH₄NO₃. In 2005, the rate was increased to 250 lbs. per acre. In 2004, an aerial spray program was initiated, alternating Capture 2EC[®] and Asana XL[®], to control cone and seed insects.

Temple-Inland has been blessed with early and abundant flowering in the breeding and production orchards at the Brown Farm. Favorable climatic conditions have played a major roll, but the excellent management by the tree improvement staff (John Hendrickson, Tree Improvement Manager; Dale Bates, Tree Improvement Technician; and Steve Raper, previous manager) has had a major impact. A qualitative survey of fecundity in A-block in the spring of 2003 revealed that 46% of 3-year-old ramets were producing pollen, and 76% of the trees were producing female flowers. As evident in the table below, Temple-Inland's concupiscent young pines have really started producing. Although seed yields are somewhat disappointing, this should improve as pollen production from within the orchard increases with age.

Year	Age	Bushels	Full Seed (Lbs.)	Yield (Lbs./Bu.)	Bushels / Acre	Lbs. / Acre
2003	4	3	1.3	0.43	0.3	0.1
2004	5	52	36.5	0.70	5.0	3.5
2005	6	260	199.0	0.77	25.2	19.3

Our precocious cone production at the Brown Farm is already making a significant contribution to Temple-Inland's regeneration program."



As John Hendrickson likes to emphasize, “The weighted average breeding values from this orchard serve to substantiate that this seed represents *The Best Pedigree in the Piedmont*.”

A-Block Breeding Values	
BV-HT	15.64
BV-VOL	44.47
R50	24.16
BV-STRT	20.88

Sometimes orchard management works so well, it gets obscene. Temple-Inland’s Brown Farm Tree Improvement Center has already been the site of spring shows like this.

ASSOCIATED ACTIVITIES

Regeneration on the Hofmann Forest

The 79,000 acre Hofmann Forest near Jacksonville, NC (<http://pff.ncsu.edu/hofmann.htm>) is a tremendous ecological, educational, research, and economic resource for the College of Natural Resources at NC State. Without the financial support from the Hofmann via the Forestry Foundation, our College, Department, and Cooperative would not function nearly as well. Over the past 5 years, the annual income from the Foundation has dramatically increased due to a more aggressive management plan that includes planting the best genetic material possible. In 2002, the Cooperative started advising the forest manager about what families should be planted each year. Since the management plan calls for intensive silviculture and sawtimber rotations, emphasis was put on selecting the best families possible for growth and form traits with moderate selection for fusiform rust resistance.

2005 and 2006 were major planting years to get caught up on regeneration backlogs. About 1500 acres were planted in 2005 and over 2000 acres this past winter. The best open-pollinated (OP) families available for the NC Atlantic Coastal Plain have been planted both years, and in 2006 over 500,000 full-sib families and clones were purchased and planted on the Hofmann. We estimate that the financial benefit of planting the Mass Control Pollinated seedlings and SE trees this year was over \$375,000. This benefit comes by simply planting the best genetic material possible as opposed to what might be available on the open market. Knowledge truly is power.

Benefit of Planting Outstanding Genetics at The Hofmann Forest

2005-06 regeneration ~2100 acres

~ 1200 acres with MCP and SE trees, ~ 900 acres with very good OP families

Conservative estimate of value of MCP & SE

... \$240 / acre

Conservative estimate of planting best OP families

... \$100 / acre

\$378,000 (today's \$\$\$)

Workshops

We have made a concerted effort over the past year to talk to landowners, foresters, extension agents, and the general public about the benefit of using the best genetic material possible for regenerating loblolly pine stands. Talks have been given to the foresters and rangers with the Georgia Forestry Commission, North Carolina Forest Service, and Virginia Department of Forestry. The Cooperative Extension Services in Virginia and North Carolina hosted a regeneration workshop in Danville, VA that was attended by over 100 foresters and consultants. Landowner groups such as the field trip hosted by Derek Dougherty in southern North Carolina help get our message directly to landowners. Together with colleagues such as Lee Allen, we have preached the benefits of genetics in conjunction with soil resource management to maximize stand value.



Dr. Lee Allen with the Forest Nutrition Cooperative professes the benefits of combining the best silviculture with the best genetics to reap maximum benefits from loblolly pine plantations.

2005 Southern Forest Tree Improvement Conference

The 28th Southern Forest Tree Improvement Conference was held in Raleigh, NC June 21-23, 2005 in cooperation with the Southern Forest Tree Improvement Committee and the Department of Forestry and Environmental Resources in the College of Natural Resources at NC State University. For three days over 100 foresters, tree breeders, scientists, and practitioners met and listened to the excellent presentations (proceedings can be downloaded at www.ncsu.edu/feop/sftic/proceedings.html). We all left Raleigh a bit more informed about what's happening in southern tree improvement and forest genetics than when we arrived.

We took this opportunity to celebrate the beginning of the Cooperative's 50th year of operation. Prior to the conference, we hosted a reception to honor our members, supporters, current and former students, faculty, and friends. A good time was had by all.



2005 Contact Meeting

This year's Contact Meeting was in Savannah, GA. Technical topics included presentations on the Rust IPM Study, Economic Benefits of Tree Improvement, the Performance Rating System, Fingerprinting Seedlots, Updates on Breeding, and Updates on Seed Orchard Insects. The field trip was hosted by Plum Creek Timber Company and emphasized clonal forestry and deployment options for the South.



Cooperators look on at a few million SE trees in the Plum Creek Nursery near Jesup, GA. “Big Red” is the CellFor planting machine in the background and is used to transplant the somatic embryogenic plugs into the nursery beds.



One stop on the Contact Meeting field trip brought back memories. This 22-year-old study was the early work of Henry Amerson (right) and Steve McKeand (left) when both worked with the Tissue Culture Program at NC State.

Interns and Visiting Scientists

This was a busy year for visitors with the Tree Improvement Cooperative. We had five visitors from around the world come to work and learn about Tree Improvement. Maria McDougall was an intern last summer from Alabama A&M and was here to learn about the Tree Improvement Program. She helped with various projects in the greenhouse and lab and was a big help with the densitometry scanning. She is currently employed as a Forester Trainee with the US Forest Service in Illinois.

Dr. Sükran Gökdemir is a researcher working for Central Anatolia Forest Research Institute, in Ankara, Turkey. Fazil Selek is a PhD candidate and researcher working for the Poplar Research Institute, in Izmit, Turkey. They were supported by the Ministry of Environment & Forestry in Turkey for three months of study at NC State. Dr. Gökdemir studied on analysis of provenance trials

using SAS software, while Mr. Selek focused on forest pests and research data management using SAS. They were a big help planting at the Hofmann Forest this winter.

Our last two visitors were Dr. Zhang Fangqui and Dr. Wang Chuanjia from China. Dr. Fangqui is a professor at the Guangdong Academy of Forestry and was here for 6 months learning about our Tree Improvement Program and working to develop a breeding plan for a native hardwood species. Dr. Chuanjia is a professor at Zhejiang Forestry Academy and joined Dr. Fangqui for a shorter time to learn about Tree Improvement here at NCSU. They were also happy to grab dibbles and help plant tests at the Hofmann Forest, all our visitors were a big help in our research endeavors and we hope that they learned as much from us as we did from each of them.



Dr. Wang Chuanjia



Dr. Sükran Gökdemir



Dr. Zhang Fangqui



Fazil Selek



Maria McDougall

GRADUATE STUDENTS

As from the beginning of the Cooperative, graduate education continues to be a focus in the program. Graduate students bring fresh ideas and facilitate collaboration with other faculty at NC State and at other institutions. We also help to educate and train the tree improvement leaders of tomorrow.

It is not coincidental that all these pictures are of students working in the field. Graduate student contributions to the Cooperative go well beyond their own research projects.

Mike Aspinwall joined us in January 2005 as a masters student. His project deals with wood properties and the inherent variation present up the boles of different genotypes. Mike has collected all his samples and is in the middle of assessments and analyses.



Jonathan (Tyler) Eckard started his MS in August 2005 and is funded by a research grant from the USDA Forest Service, Agenda 2020 Sustainable Forestry Research Program. He is working with Fikret Isik and Bronson Bullock, looking at development of prediction models for whole-stem wood quality of superior loblolly pine clones for deployment.



Patrick Cumbie returned to NC State in August 2005 to work on a PhD with Barry Goldfarb and Bailian Li in conjunction with the new NSF project on association genetics with loblolly pine. Patrick's focus is on genomics and tree breeding (see description in research section).





Jesus Espinoza is working on a project jointly funded by the Forest Nutrition and the Tree Improvement Cooperatives. Jesus received his MS with CAMCORE in 2003 then worked with Carton de Venezuela before returning for his PhD in January 2006. Jesus's project will focus on genetics and cultural effects on stem quality in loblolly pine.

Daniel Gräns's PhD research is looking at solid wood properties in loblolly pine and Norway spruce. Daniel's Nicholson Fellowship is supporting his work both in Sweden and with the Cooperative. His loblolly pine trial is a Genetics x Culture trial established by International Paper Company in Bainbridge, GA (see description in research section).



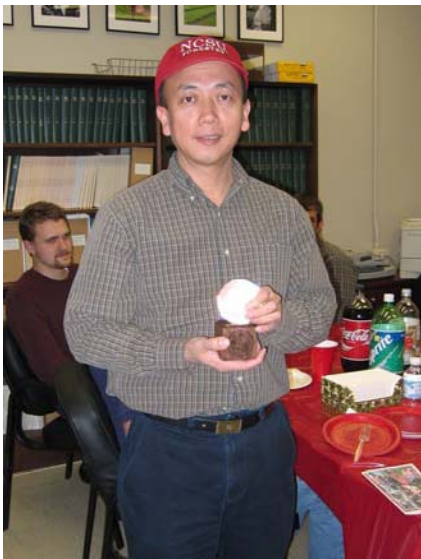
Ben Smith is a PhD student on a Hofmann Forest Graduate Fellowship. He started with the Cooperative in August 2005. Ben's interests are in forest biometry and tree improvement. His masters project dealt with diameter distributions at SETRES2.

Tracy Yu joined us in August 2004 as a PhD student with a departmental assistantship. She is working with Bailian Li and Ross Whetten on a marker-assisted breeding project in loblolly pine.



COOPERATIVE STAFF

Almost one year to the day that Bailian Li took over as co-director of the Cooperative, he decided to take a 16-month leave as Interim Vice Provost for International Affairs. That's the problem with having such talented people on our staff; they get stolen by our "colleagues" in other units on campus. We wish Bailian the best in his new position, and hope he will return in July 2007.



Dr. Bailian Li with his gift of a globe for his desk in Daniels Hall. We look forward to his return in July 2007

Steve McKeand continues as co-director and eagerly awaits Bailian's return. Jim Grissom continues as Tree Improvement Analyst with primary duties of managing and developing the Cooperative's data base and coordinating work activities for the Third cycle and other research programs. Mike Jett resigned as the Field and Laboratory Manager in September 2005, but continues working part time on computer and laboratory issues. We are very fortunate to have Tori Batista Brooks replacing Mike. Tori is no stranger to the program. She was a technician with the CAD Project for 2 years prior to joining the Cooperative staff full time. We were incredibly fortunate to have Tori step in and take over. Very little time and momentum were lost in the transition. Kathie Zink is our Administrative Secretary and makes sure the ship doesn't sink and the bills get paid.

For the next 16 months, Patrick Cumbie will be on board to help fill the hole that Bailian has left.

Patrick returned to Raleigh last August to pursue his PhD in genomics and tree improvement. Prior to that, he was a tree breeder with Weyerhaeuser Company, and prior to that was a masters student with the Cooperative. Starting in May, Patrick will take a detour from his PhD and work full time on various Cooperative projects until July 2007.

Saul Garcia (see picture on page 14) started in January as a research assistant with the Cooperative working with Henry Amerson and the fusiform rust gene discovery project. Saul also is no stranger to our program as he has worked with Henry since 1999. Saul's background in molecular genetics has been extremely valuable in our work to understand the genetics of the loblolly pine – fusiform rust pathosystem. Saul also works with Tori to fingerprint selections and seedlots, uncovering past mistakes and hopefully preventing future ones.

Dr. Fikret Isik still works very closely with us and is supported with funds from various research projects. Fikret has had primary responsibility for the IFAFS wood sampling project (see write up on previous pages). Fikret's contributions to our program go way beyond these research projects, and we really appreciate his input.

Dr. John King joined the FER faculty in summer 2005. John is a forest ecophysiologicalist and was previously on the faculty at Michigan Tech University in Houghton, MI. John has already jumped head first into the Hofmann GST, and we look forward to a long association with him to help understand the physiological basis of genetic improvement of loblolly pine.



Dr. John King joined us in the TIP office suite last summer.



Full-time Tree Improvement Cooperative Staff (L→R) Saul Garcia, Kathie Zink, Patrick Cumbie, Steve McKeand, Fikret Isik, Jim Grissom, Tori Brooks, and Bailian Li. This was Bailian's last staff meeting for a while and Patrick's first.

There are numerous collaborators in our College that contribute immensely to the success of our program. One in particular is Dr. Henry Amerson. Henry will be retiring at the end of 2006. Henry has worked with us for almost 30 years, first with the Tissue Culture Program and in recent years with the Forest Biotechnology Program and the Fusiform Rust Program. Henry's contributions to the Cooperative's research and breeding efforts will be sorely missed, but we wish him and his wife Ellen the best in his retirement.

Besides Henry Amerson's contributions to the Cooperative's research program, he is known as a pig cooking connoisseur. Henry has been a mentor and friend for many years.



Membership of the NCSU-Industry Cooperative Tree Improvement Program

The Cooperative is in the midst of the greatest period of change in membership in its 50-year history. This change is a reflection of the unprecedented shift in forest land ownership during the past 20 years, as well as budget limitations by many of the state forestry agencies. Since 1985, mergers and acquisitions within the industry have resulted in a significant decline in Cooperative membership (Figure 1). The amount of land and acreage reforested by members have not decreased, so the Cooperative's efforts in breeding, testing, and selection have not diminished.

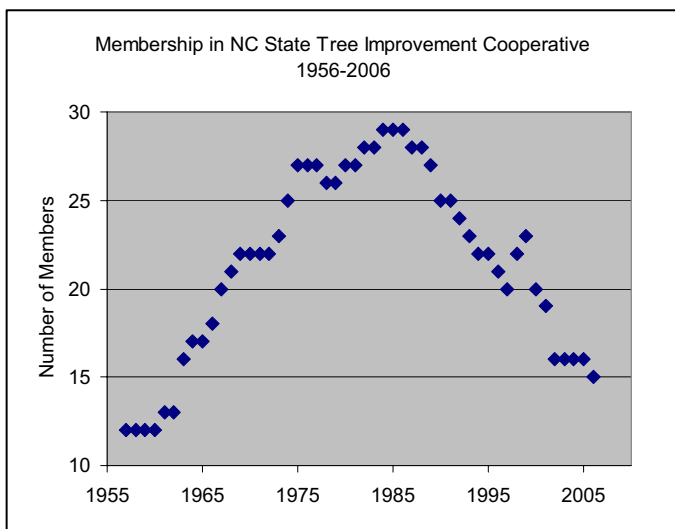


Figure 1. Number of members in the Cooperative over the past 50 years.

We currently have 15 full members and one research associate member; that includes 5 integrated forest products companies, 4 state forestry agencies, 1 nursery company, 1 landowner, 2 REITs (Real Estate Investment Trust), 1 TIMO (Timber Investment Management Organization), and 2 Biotechnology Companies (1 as an associate member). In 1985 at the height of membership in the program (Figure x), there were 29 members, with 24 integrated forest products companies, 4 state forestry agencies, and one nursery company (Table 1). Who would have predicted 20 years ago that our two newest members, CellFor as a full member and ArborGen as an associate member,

would be biotechnology companies? Back then, who knew what a TIMO or a REIT was? Our “traditional” players in tree improvement and forest management have changed and will likely continue to change in ways few can predict.

International Paper Company is one of the original members of the Tree Improvement Cooperative. In fact, they are the only original member whose corporate name has not changed in 50 years. In July 2005, IPCo announced that they would sell their forestland. At the time of this writing, Resource Management Service, LLC, Forest Investment Associates and other investors, and an investor group led by TimberStar will be acquiring the bulk of IPCo's southern U.S. forest land base. The Nursery and Orchard group is also up for sale, and the future of IPCo's huge genetic resource is a question. We wish our friends at IPCo the best in their very uncertain future. It's been a wonderful 50-year relationship.

In August 2005, Alabama Forestry Commission announced that they were leaving the Tree Improvement Cooperative. In 2004, the South Carolina Forestry Commission also withdrew from the program. With budget cuts in all the state forestry agencies, it has become increasingly difficult to justify nurseries and tree improvement to political leaders. Some state leaders feel that industry will provide landowners with all the seedlings that are needed and can manage the development of all the forest genetics resources without state aid. Given the turmoil in the forest products industry, this does not appear to be a prudent move. Most state forestry agencies are weathering today's storms and remain committed to forest genetics and tree improvement. The landowners and citizens of these states will benefit from the wise decisions made by their forestry leaders who understand the value and benefits from tree improvement.

The current membership of the Cooperative is 15 full members and 1 associate research member, with 4 state agencies, and 12 private corporations.

Membership of the NCSU-Industry Cooperative Tree Improvement Program

CellFor, Inc.
Georgia Forestry Commission
Gulf States Paper Corporation
Hancock Timber Resources Group
International Paper Company
Joshua Land Management, L.L.C.
MeadWestvaco Corporation
North Carolina Division of Forest Resources
Plum Creek Timber Company
Rayonier, Incorporated
Smurfit - Stone Container Corporation
Temple-Inland Inc.
Tennessee Department of Agriculture
Virginia Department of Forestry
Weyerhaeuser Company

Research Associate Member
ArborGen

PUBLICATIONS OF SPECIAL INTEREST TO MEMBERS

Our custom is to list the most recent three years of publications from NC State Tree Improvement Cooperative. This year we planned to list ALL the publications from 1956 to 2006 that have been in each annual report. The good news is that an incredible number of published manuscripts, books, theses, proceeding articles, and technical reports have emanated from this program. The bad news is that the list was so long that it would have more than doubled the length of the annual report. From our inception in 1956 to May 2006, there have been over 800 publications written by Cooperative Scientists, Students, and Associates. Below is a condensed list of 165 publications from the last 10 years. The entire list is available at our web site:

<http://www.cnr.ncsu.edu/for/research/tip/tip.html>

2006

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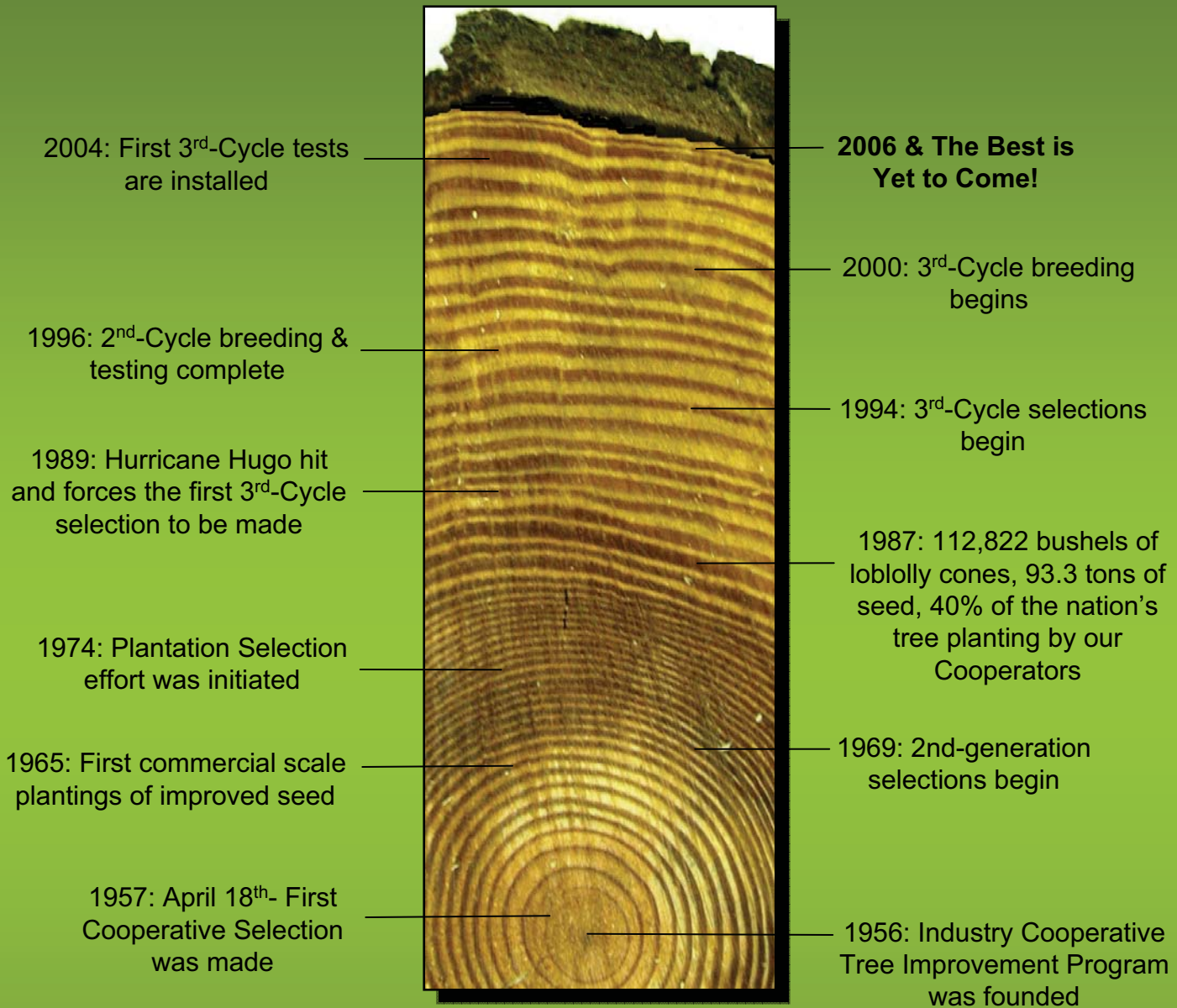
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