



**North Carolina State University –
Industry Cooperative Tree
Improvement Program**

48TH ANNUAL REPORT

**Department of Forestry
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Front Cover:

Sampling for our new "CAD" project was in full swing the past year – details inside this report. Over 2,000 descendants of the cad-n1 progenitor were sampled for genotyping at the CAD locus and assessment of growth and wood properties. A major effort was at the SETRES2 site in Scotland County, NC. Depicted on the cover are Tori Batista and Qibin Yu assessing a ten-year-old tree for stem sweep in one of the control plots at SETRES2. Kudos to Tori and Qibin for all their hard work in collecting samples from around the South the past year.

EXECUTIVE SUMMARY

BREEDING, TESTING, AND SELECTION

All 925 third-cycle selections have now been assigned to sublines for breeding. A total of 77 breeding sublines were formed for the three breeding regions, i.e., 32, 31 and 14 sublines for the Coastal, Piedmont and Northern regions, respectively. The third-cycle breeding is progressing well. Following the Coop's 3rd-cycle breeding strategy, a complementary design with polycross mating for among-family selection and full-sib mating for within-family selection, a total of 13,103 bags of flowers have been pollinated for polycross, most of which were done in the last two years. Full-sib controlled crossing also started in the last year.

With the seeds harvested in the fall of 2003, we have now sufficient quantity of seeds to establish a set of polycross (PMX) tests for the Coastal region. Nine cooperative members will be responsible to establish these 15 progeny tests this coming winter.

PROGRESS REPORTS FOR RESEARCH

Our extensive database of diallel test for over 3,000 parent selections continues to provide valuable information. Analyses of 500+ diallels in 250 test series has led to several conclusions: (1) planting tests on uniform sites is essential if reasonable levels of heritability and genetic gains are to be achieved; (2) in general, genetic differences in height are not related to site index; and (3) genotype by environment interaction effects are relatively minor both for half-sib and full-sib families.

A study of 14 full-sib families tested on two sites in South Carolina has evaluated genetic variation of several juvenile wood properties of loblolly pine: α -cellulose content, average fiber length, coarseness, and lignin content. The families differed significantly for all the chemical and morphological wood properties on both sites. Preliminary results suggested that loblolly pine families might not be stable across different site conditions for some of the wood properties.

What is the relative advantage for the deployment strategy using only the best full-sib families available within disconnected diallels? How much additional gain, if any, might be achieved if full-sib families could be generated within the whole region based on GCA, with or without SCA adjustments? A study evaluating strategies for deploying full-sib families suggested that selection for full-sib families in loblolly pine based on GCA from all parents within a geographic region would result in a higher genetic gain. The relatively small importance of SCA in this population of loblolly pine, compared to GCA, gives little merit to exploitation of SCA.

One of our fastest-growing loblolly pine selections, whose progeny are widely planted in many southern states, is also the only known natural carrier of a rare gene, *cad-n1*. We are now in the early stages of a study, supported by the Department of Energy through Agenda 2020, with the objective of characterizing the performance and value of CAD-deficient wood, arising from descendants of the selected partially CAD-deficient founder. Preliminary results demonstrated a significant effect *cad-n1* allele on most of wood density traits, especially for latewood percentage. The higher wood density in CAD-deficient trees was most likely related to high proportion of latewood.

SEED PRODUCTION

The 2003 seed collection for the Cooperative provided approximately 28 tons of loblolly pine seed, almost double last year's 15.1 tons. About 75% of the total seed came from second-generation orchards and about 65% of the collection was from the Coastal sources.

ASSOCIATED ACTIVITIES

Two graduate students completed their programs, and one new student began studies for his Masters. A Tree Improvement Short-Course was offered again to personnel from member agencies. The focus of this year's contact meeting in Asheville, North Carolina, was on the fusiform rust disease. Several changes occurred in Program Staff, and we said a reluctant goodbye to long-time Field and Lab Manager, Paula Zanker. Membership in the Coop stands at 16, with 6 state agencies and 10 private corporations.

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FROM THE DIRECTOR



I was wrong.

Several years ago – OK, it was more like 20 – I was a forester who had gone back to grad school for a Masters degree in Tree Improvement, and figured I had a pretty realistic view on what genetics could and couldn't do for forestry. I was at a forest genetics conference where I felt the biotechies were just a little too full of themselves with promises of revolutionizing forestry and growing the "perfect" tree. With several witnesses present, I pronounced that, during the span of my career, we would never be able to select trees for valuable genes by directly reading their nucleotide sequence and identifying superior alleles. Oh sure, we might select for "genetic markers", sequences occurring somewhere on the chromosome "near" the putative superior allele (so-called "marker-aided selection"), but these are not necessarily inherited together. In unrelated trees, the superior allele might be present without the marker, and visa versa.

As I said, I was wrong. Like most people, I usually don't like admitting my errors, but this time I couldn't be more thrilled. During the past year, the NCSU-Industry Cooperative Tree Improvement Program has been working on a research project where we've been "reading" the ACGT alphabet of loblolly pine DNA, looking for a particular variant of base-pair sequences, a so-called Single Nucleotide Polymorphism, or SNP (*snip*). A SNP can serve as a marker WITHIN the sequence of the gene itself, but unlike a conventional marker sequence, it is always inherited.

The allele we sought codes for lower-than-normal production of an enzyme, cinnamyl alcohol dehydrogenase, or CAD. CAD has been recognized for some time for its role in the formation of lignin. One of our best first-generation selections was known to be deficient in the production of CAD and experiments had shown this was due to a non-functioning "null" allele at the CAD locus, named *cad-n1*. Until recently, our only way to identify the presence or absence of this rare allele was with conventional genetic markers, but these were not reliable in all populations or pedigrees. Then, a team of clever genomics researchers at the Institute of Forest Genetics at UC-Davis discovered a SNP in the CAD gene that could identify the *cad-n1* allele (Gill *et al.* 2003).

Over the past year, we've collected tissue samples from over 2,000 trees in progeny tests, and used this SNP to confirm the presence or absence of *cad-n1*. It then becomes a relatively simple statistical test to determine the magnitude of the effects on growth, wood properties and other traits, conferred by this gene (you can read more about this project beginning on page 17). Over the coming years, research on the loblolly pine genome will almost certainly associate more and more SNPs with variation in important traits. The more associations we confirm, the more we will do our selection of superior genotypes by directly "reading" a tree's genetic code. It's a moment I never thought would come in my career, and I'm pretty excited by it.

Sometimes, it's not so bad to be wrong!

Tim Mullin, Director

Reference:

Gill, G.P., Brown, G.R., and Neale, D.B. 2003. A sequence mutation in the cinnamyl alcohol dehydrogenase gene associated with altered lignification in loblolly pine. *Plant Biotechnology Journal*, 1: 253-258.

SELECTION, BREEDING, AND TESTING

THIRD-CYCLE MAINLINE BREEDING PROGRESS

All third-cycle selections were completed in 2003 for our mainline breeding program. To date, the census numbers of 3rd-cycle selections for the three general breeding regions are: Northern = 178, Piedmont = 362, and Coastal = 385 (**Table 1**).

All selections have been assigned to sublines within each of the three regions. A total of 77 breeding sublines were formed for the three breeding regions, i.e., 32, 31 and 14 sublines for the Coastal, Piedmont and Northern regions, respectively (**Table 1**). All sublines have been assigned to cooperators within each breeding region. Most sublines have 8 to 15 selections, except one with 22 selections. Subline selections have been grafted into breeding orchards or top-grafted on to larger trees for breeding by each of cooperators.

The third-cycle breeding is progressing well. Following the Coop's 3rd-cycle breeding strategy, a complementary design with polycross mating for among-family selection and full-sib mating for within-family selection, a total of 13,103 bags of flowers have been

pollinated for polycross, most of which were done in the last two years. The numbers of crosses that have been completed or are being done are summarized in **Table 1**. Crossing was much more active in the Coastal region than Piedmont and Northern regions.

Cooperative members within each region have grafted all parents selected for generating the pollen mix (PMX) for polycross mating. Three, 3rd-cycle check lots are being created for each of the three regions by using the same 20-parent PMX applied to 10 of the selections in the mix (**Table 1**). Checklot crossing progressed well last year, especially in the Coastal region. Sufficient quantities of checklot seeds have been produced for both the Coastal and Piedmont regions for establishing the PMX tests. More seeds will be needed to support more test series that will be established in the future.

In addition to the 3rd-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 comparisons. Seeds from some common family checks have been collected for each zone.



Greenhouse Breeding by International Paper Co.



Outdoor breeding by International Paper Co.

Table 1. Summary of third-cycle breeding progress, as of winter 2003-04.

Type of cross	Breeding region	# cooperators	# sublimes	Total # crosses	# crosses producing Seed	# crosses with Sufficient Seed	% crosses complete
Polycross	Coastal	10	32	385	170	79	20.5 %
	Piedmont	9	31	362	75	35	9.7 %
	Northern	6	14	178	30	10	6.0 %
Polycross PMX check	Total	16	77	925	275	124	13.4 %
	Coastal	10	--	10	10	9	90 %
	Piedmont	9	--	10	10	6	60 %
PMX CF	Northern	6	--	10	8	3	30 %
	Coastal	10	--	7	4	4	57 %
	Piedmont	9	--	7	2	2	28 %
Full-Sib	Northern	6	--	7	2	1	14 %
	Coastal	10	32	235	21	13	5.5 %
	Piedmont	9	31	186	0	0	0.0 %
Full-Sib	Northern	5	13	81	3	1	1.2 %
	Total	16	76	502	24	14	2.2 %

Key:

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 check: Polycrossed checklot mixes

PMX CF: Polycrossed common family checks

Full-sib controlled crossing also started in the last year (**Table 1**). It is expected that the numbers will rise for full-sib crossing in the next few years, as the 3rd-cycle selection trees produce more flowers. These full-sib crosses will generate seeds for within-family selection for the next breeding cycle.

THIRD-CYCLE PROGENY TESTING

With the seeds harvested in the fall of 2003, we have now sufficient quantity of seeds to establish a set of polycross (PMX) tests for the Coastal region. Seeds were distributed to members this spring for establishing 15 tests for the first PMX test series in the Coastal region. The tests will be distributed across the whole Coastal region from North Carolina in Atlantic coastal plains to lower gulf coastal plains in Alabama. Nine cooperative members will be responsible to establish these 15 progeny tests this coming winter.

As we continue to make progress for the Piedmont and Northern breeding programs, it is expected that the first test series for Piedmont should be established in 2005.



A pollination bag with 32 female strobili

RESEARCH

VARIANCE COMPONENT ESTIMATES FROM 6-YEAR-OLD DIALLEL TESTS OF LOBLOLLY PINE

Cooperators in the NCSU Tree Improvement Program started breeding and testing over 3000 plantation selections and over 700 second-generation selections in the early 1980's. The breeding and testing has been completed, and progeny test results are now available for over 3000 parent trees. Selections were bred in 6-parent disconnected half-diallels, and the resulting progeny were planted in balanced test series (4 tests per series) each comprised of progeny from two or on occasion three diallels. Each full-sib family was planted in a 6-tree plot and is replicated 6 times in each of the 4 tests. At age 6 years, height, diameter at breast height (DBH), presence or absence of fusiform rust, and survival were assessed. The mixed linear model of Xiang and Li (2001) was used to analyze the data within a test series. Variance components were estimated for all the random effects, and heritabilities and type B genetic correlations were computed (see Xiang and Li 2001, and Xiang et al. 2003 for details).

As of 2003, measurement and analyses have been completed in 250 test series at age 6, with over 500 separate diallels represented (**Table 2**). Overall, trees averaged 20.6 feet in height and 0.87 cubic feet in volume at age 6 years. Fusiform rust infection levels were 33.5%, and survival was generally very good, averaging 91%. The range of values for individual test series means across the Coop was large. Height varied from 12.6 feet to 31.4 feet, volume from 0.24 ft³ to 02.80 ft³, rust from 0 to 87%, and survival from 76% to 99%. As expected, there were regional differences for almost all the traits and variance components estimated. The largest trees were in the Coastal Plain of South Carolina and the smallest in the northern tests in Virginia and northern North Carolina. Fusiform rust ranged from a low of 17% in Virginia to the highest average of 49% in tests in the Lower Gulf Coastal Plain. Survival did not vary by region.

The individual-tree narrow-sense heritability for height averaged 0.18 and varied from a low of 0.13 in northern test and a high of 0.23 in the Lower Gulf trials. The highest h^2 estimate for height in a test series was 0.62 and the lowest was 0. Type B genetic correlations estimate the correlation of genotypic performance in different environments and give an indication of the

importance of genotype by environment interaction. If the correlation is high (generally > 0.6), then GxE is considered to be of minimal concern in breeding and deployment programs. We estimated type B genetic correlations for both GCA (the most common type of estimate) and for SCA. Overall the correlations were high ($r_{GCA}=0.77$ and $r_{SCA}=0.70$ for height), indicating a relative lack of GxE both for additive and non-additive genetic effects. For the r_{GCA} , there were regional differences. Families were most stable in the Lower Gulf and Upper Gulf tests and slightly more interactive in the other regions. As found in numerous other trials, GxE appears to be of minimal concern for this large sample of families.

Broad-sense heritabilities were slightly higher than narrow-sense heritabilities due to the presence of non-additive genetic variation for height. Overall, the ratio of non-additive to additive genetic variation was 0.60, so the h^2_{BS} was 0.25 or 40% greater than the h^2_{NS} for height. There was relatively little regional variation in h^2_{BS} , but the highest heritability was found in the Lower Gulf region.

A common measure used to evaluate test quality and precision is the rep by family coefficient of variation. Values below 8 to 10% are very acceptable and indicate high precision in a testing program. Overall, the average CV was 6%, with a low of 5.3% in the Upper Gulf and a high of 7.3% in the Coastal Georgia and Florida trials. Cooperators put a tremendous amount of effort in finding uniform test sites for the 2nd-generation and plantation-selection testing program. The high-quality data and breeding values that we have today is a direct result of these efforts.

Relationships between Genetic Parameters and Test Characteristics

Several informative relationships among progeny test characteristics and estimates of genetic parameters have been identified. The estimates for narrow sense heritability (individual-tree basis) ranged between 0 and 0.62. Reasons for this large range of estimates include: 1) Genetic sampling; there were real differences in the genetic variance included in the 500+ samples of 6

parents comprising the diallel mating groups. 2) The variation in heritability estimates were at least partially explained by variation in test precision (as measured by the CV based on rep x family effects) and by average test survival. If test precision was relatively low (CV > 8%) then the likelihood of having a high heritability was relatively low (**Figure 1**).

Survival had little effect on h^2 estimates, probably because the overall survival was very high (91%), and the range in survival was fairly small. The poorest survival in a test series was 76%, but only 5 of the 250 test series had survival below 80%. Additionally, if survival in a rep was below 75%, we discarded it before analyzing the data. Therefore, the poorest surviving tests are not included in this analysis.

Although these relationships are not extremely strong, they illustrate the value of maintaining minimum standards for survival and environmental uniformity in a progeny testing program. While high survival and good test precision do not guarantee that heritability estimates will be high, since the effect of genetic sampling in the small diallels and test series can always be influential, and sometimes there are no genetic differences among the 12 parents in a series, and $h^2 = 0$. However, in tests that have large environmental variance and/or poor survival, genetic effects will very often be masked, and heritability will be low.

Overall, there was a small, but significant, correlation ($r=0.17$) between average height in a series and the estimate of narrow-sense heritability for height (**Figure 2**). There is a confounding effect in the correlation for h^2_{NS} since the heritabilities and average heights both vary across regions. For example, both h^2_{NS} and average height were high in the Lower Gulf and low in the northern tests. When the relationship between test height and h^2_{NS} was assessed by region, there was generally no significant correlation. One exception to this was in the Coastal South Carolina trials. There was a moderately strong positive correlation between height and h^2_{NS} ($r=0.60$). Why this significant correlation occurred in SC is not clear, but in general we are just as likely to see strong genetic effects on low site index sites as on high site index sites. The average height for a test series varied by almost 150% (from 12.6 feet to 31.4 feet) at age 6 years. When establishing progeny tests, the use of agricultural fields was encouraged, not because of the potential for fast growth, but because in general the site uniformity on agricultural fields is much better than what is routinely encountered on cut over sites.

Genotype by Environment Interactions

In past studies, we have seen very little evidence for important genotype by environment interaction (GxE) for height or volume at the half-sib family level. For example, in the Good General Combiner trials, half-sib families were remarkably stable across a wide range of sites that encompass large differences in site productivity. In the diallel tests, we have the opportunity to assess GxE for both half-sib and full-sib families. Full-sib families are expected to display a higher degree of interaction with the environment than half-sib families since there is less genetic variance within each family (i.e., a lower level of buffering to environmental variations), and more of the genetic variance exists among families. Additionally, GxE at the full-sib family level is caused by both additive and non-additive effects.

One way to evaluate the importance of G x E is to relate its magnitude to the magnitude of genetic variance. The ratio of the genotype x environment variance over the genetic variance may be referred to as the K-statistic (very similar to the type B genetic correlations):

For half-sib families:

$$K = \frac{1}{4}\sigma^2_{AxE} / \frac{1}{4}\sigma^2_A$$

For full-sib families:

$$K = (\frac{1}{2}\sigma^2_{AxE} + \frac{1}{4}\sigma^2_{NAxE}) / (\frac{1}{2}\sigma^2_A + \frac{1}{4}\sigma^2_{NA})$$

This is a useful measure of genotype by environment interaction when the environments are considered to be a random sample from a larger set. For this case, the K-statistic may be interpreted as the proportional amount by which the expected genetic variance within environments exceeds the genetic variance measured over environments.

The average K-statistic for half-sibs was 0.34 meaning that the GxE variance was only about one-third the genetic variance. As we have found in other trials, the family by environment or specifically the additive genetic by environment variance is of little practical concern. Interestingly, the GxE for full-sibs is essentially the same as for half-sibs ($K = 0.36$). Since the non-additive by environment interaction is small (e.g. the large r_{SCA} in **Table 2**), full-sib families should be as stable and predictable across sites as half-sib families.

In summary, the analyses of 500+ diallels in 250 test series has led to several conclusions:

1. Planting tests on uniform sites is essential if reasonable levels of heritability and genetic gains are to be achieved.
2. In general, genetic differences in height are not related to site index.
3. Genotype by environment interaction effects are relatively minor both for half-sib and full-sib families.

Analyses of our diallel data will continue to more thoroughly investigate GxE, correlations among traits, and estimates of genetic gain for different traits. This is a unique data set because of the size of the genetic sample (over 3000 parent trees) and the high quality of the test information.

Table 2. Means for 250 diallel test series at age 6 years in different geographic regions in the NCSU-ICTIP Cooperative. Means within a column followed by the same letter are not significantly different at $p \leq 0.05$.

NCSU Test Area	# Series	Height (ft)	Volume (ft ³ /tree)	% Rust	% Surv.	RxF CV Height(%)	h^2_{NS} Height	h^2_{BS} Height	r_{GCA} Height	r_{SCA} Height
1-VA & North.NC	35	18.4 ^d	0.61 ^d	17.0 ^c	92.5 ^a	5.8 ^{bc}	0.13 ^c	0.23 ^b	0.70 ^c	0.73 ^a
3-Coast SC	35	23.5 ^a	1.30 ^a	36.1 ^b	91.4 ^a	6.0 ^b	0.15 ^{bc}	0.20 ^b	0.73 ^{bc}	0.77 ^a
4-Coast GA&FL	20	18.9 ^{cd}	0.64 ^{cd}	33.3 ^b	91.4 ^a	7.3 ^a	0.18 ^{abc}	0.24 ^b	0.72 ^c	0.73 ^a
5-Lower Gulf	45	21.5 ^b	0.93 ^b	49.1 ^a	89.7 ^a	6.3 ^b	0.23 ^a	0.32 ^a	0.84 ^a	0.72 ^a
6-Upper Gulf	35	21.8 ^b	1.00 ^b	21.6 ^c	90.6 ^a	5.3 ^c	0.19 ^{ab}	0.25 ^b	0.83 ^{ab}	0.69 ^a
7-Pied. SC&GA	80	19.6 ^c	0.76 ^c	36.0 ^b	90.4 ^a	5.8 ^{bc}	0.19 ^{ab}	0.25 ^{ab}	0.78 ^{abc}	0.64 ^a
Overall	250	20.6	0.87	33.5	90.8	6.0	0.18	0.25	0.77	0.70

RxF CV Height (%) = rep by family coefficient of variation for height

h^2_{NS} = individual-tree narrow-sense heritability for height

h^2_{BS} = individual-tree broad-sense heritability for height

r_{GCA} = type B genetic correlation (GCA) for height across test sites within a series

r_{SCA} = type B genetic correlation (SCA) for height across test sites within a series

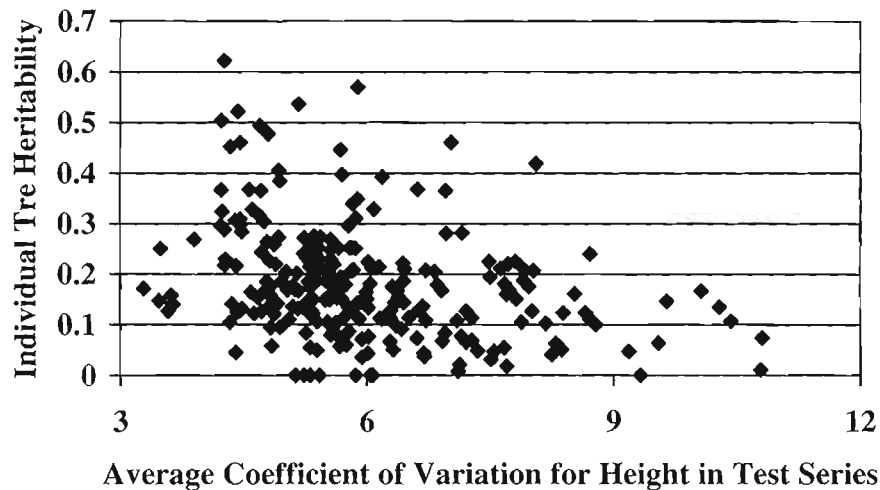


Figure 1. Relationship between estimates of individual-tree narrow-sense heritabilities for height at age 6 years and estimates of pooled coefficients of variation (rep x family mean basis) for height. The correlation was $r = -0.32$ and significant at $p \leq 0.01$.

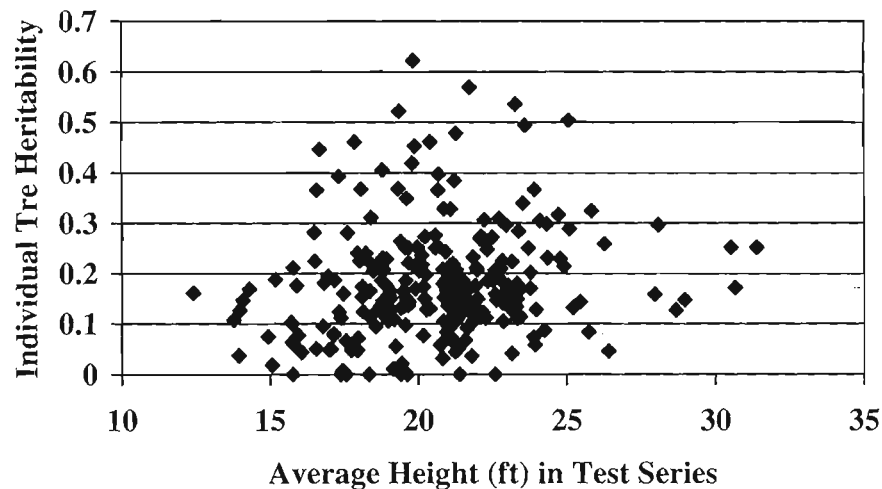


Figure 2. Relationship between estimates of individual-tree narrow-sense heritabilities for height and average test height at age 6 years for all 250 test series. The correlation was $r = 0.17$ and significant at $p \leq 0.01$.

GENETIC VARIATION AND GXE INTERACTION OF JUVENILE WOOD PROPERTIES IN LOBLOLLY PINE

Large genetic variation has been found in some wood properties in a loblolly pine test (Sykes et al. 2003) for α -cellulose content, average fiber length, and lignin content. But there is little information on how those chemical wood properties interact with the environments, e.g., genotype by environmental interactions (GxE). The information of GxE interaction is important for tree breeders and practical foresters on how to design breeding programs and deploy genotypes with desirable wood traits over different environments. This study evaluated genetic variation of several juvenile wood properties of loblolly pine, α -cellulose content (ACY), average fiber length (FLW), coarseness (COA), and lignin content (LIG), on two test sites and examines GxE interaction.

MATERIALS AND METHODS

Fourteen full-sib families generated by a 6-parent half-diallel mating design were tested on two sites in the Piedmont of South Carolina. Randomized complete block design with six replications was used in the field. Each full-sib family was laid out in 6-tree row-plots in each replication. Wood core samples were collected when the trees were 11-year-old. Increment cores (12 mm) were taken from each tree at breast height using generator-powered drills. Chemical analysis was done using microanalytical techniques developed by Yokoyama et al. 2002, which allow the rapid characterization of fiber components and morphology of loblolly pine in a large number of samples. Briefly, the techniques involved are extractive removal, holocellulose preparation, α -cellulose and lignin content determination, and average fiber length and coarseness analyses. Within-core samples were taken from ring 3 and ring 8 to study chemical properties of juvenile wood (ring 3) and transition wood (ring 8), respectively. Thin wafers from ring 3 earlywood, ring 3 latewood, ring 8 earlywood, and ring 8 latewood were taken using a microtome. At least 300-500 mg of each sample were taken from the earlywood and latewood of each ring. Each sample was oven-dried for 12 hours.

Analysis of variance was conducted, and juvenile wood and transition wood were compared for micro wood traits using paired T-tests. A general linear mixed model was used to estimate variance components for each site and for combined sites using the method

developed for diallel analysis. The mixed model used for combined sites analysis was an extension of the model given by Sykes et al. 2003 with additions of site, site by general combining ability and site by specific combining ability. Using variance components from the mixed model, individual-tree (h^2_i), half-sib family (h^2_{hs}), full-sib family (h^2_{fs}), and within full-sib family (h^2_{wfs}) heritabilities were estimated following Sykes et al 2003. Product-moment correlations were estimated among the morphological wood traits and their relationships with growth traits. Approximate genetic correlations among traits were calculated using individual-tree breeding values of each trait that were estimated according to Xiang and Li 2001.

RESULTS

Latewood and earlywood of ring 3 and 8 showed similar differences on both sites (**Figure 3**). Latewood had significantly greater ACY, FLW and COA than earlywood in ring 3 (juvenile wood) on both sites. The differences between latewood and earlywood for ACY, FLW, and COA were generally greater in ring 8 compared to ring 3. Earlywood and latewood of ring 3 were not different for LIG on site 2 but they differed on site 3. Earlywood and latewood of ring 8 were not significantly different for LIG on both sites. Site 2 had considerably greater COA in latewood of ring 8 than site 3. Transition wood had higher ACY, FLW, COA, and a lower LIG content than juvenile wood.

Juvenile wood at ring 3 appeared to be less desirable for chemical wood traits compared to ring 8, which is considered as a transition wood rather than mature wood for loblolly pine. There was an increasing trend of cellulose content, fiber length and coarseness from juvenile wood to transition wood. Genetic selection based on latewood of the growth ring could be an effective way to manipulate chemical and morphological wood properties. The results based on combined two sites from this study were parallel to results by Sykes et al. 2003 that were based on one site.

Families differed significantly for all the chemical and morphological wood properties on both sites (**Figure 4**). Variation among families for all the traits was greater on site 3 than on site 2. Families had generally greater ACY, COA, FLW and lower LIG

means on site 3 than on site 2. Although there was significant variation among families for lignin, the range of families was small. Families showed some rank changes for all four chemical wood traits between two sites, especially for COA and FLW (**Figure 4**).

Significant differences among families for morphological wood traits imply efficiency of family selection. Genetic improvement for cellulose content and coarseness may be realized based on transition wood because heritabilities were higher for transition wood than juvenile wood. Transition wood heritabilities may be more meaningful than those of juvenile wood, as they are closer to the age (age 6) where most selections are made for the Cooperative. The heritabilities based on combined sites were unbiased and lower than those from Sykes et al. 2003, due to removing the site term from the denominator of the heritability equations.

The mixed diallel analysis showed no significant site by general combining ability interaction for all traits, but site by specific combining ability interaction was significant for most traits. Variance components for site by general combining ability ranged from 0 to 12.5% of the total phenotypic variation, while site by specific combining ability interaction variance ranged from 0 to 22.4% of the total phenotypic variance. The site by specific combining ability interaction variances was greater for ring 3 than ring 8.

The noticeable GxE interaction for these traits was mainly due to non-additive genetic effects by environment interaction. These results were preliminary and based on the limited samples from only two test sites. There were differences in magnitude of genetic variances between two sites. Site 3 had much higher SCA variation than site 2. Site 3 was established near a river bottom and had large tree size, while site 2 was established on a piedmont site, and had relatively smaller tree size than site 3 and was less uniform. Due to cost restraints, the sample size on site 3 was approximately 60% of the sample size on site 2. The different sample size may have also contributed to the GxE interaction.

Genotype by environment interactions may play a larger role as the trees mature and need to be investigated further. Our preliminary results suggested that loblolly pine families might not be stable across different site conditions for some of the wood properties. Two additional sites will be analyzed in this study to more accurately assess the GxE component for these wood properties. If site by specific combining ability continue to be important interaction, as shown in these two tests, breeding for full-sib families and family deployment strategy may be considered for wood quality improvement.

Moderate phenotypic and genetic correlations were observed among the morphological wood traits, stem straightness, volume, height and rust infection (**Table 3**). Stem straightness had favorable (negative) correlations with ACY, COA and FLW in the combined analysis. ACY was positively correlated (favorable) with FLW and COA. There was a slightly negative correlation of rust infection with ACY, COA and FLW. There was a negative correlation of FLW with volume and height. There was a moderate negative correlation between volume and LIG.

The favorable correlations of stem straightness with ACY, COA and FLW indicated that selection for straight trees could improve these wood traits in a breeding program. Since volume and height can be measured more effectively, and has already been incorporated into the tree improvement programs, improving height or volume may decrease lignin content. Increasing cellulose content may result in the production of more paper per cubic meter of wood.

References

- Sykes, R., Isik, F., Li, B., Kadla, J., and Chang, H. M., TAPPI 2(12): 3-8. (2003).
- Yokoyama, T., Kadla, J. F., and Chang, H-M., Journal of Agricultural and Food Chemistry 50(5):1040 (2002).
- Xiang, B., and Li, B., Canadian Journal of Forest Research 31:2252 (2001).

Table 3. Genetic correlations based on individual-tree breeding values for juvenile wood (above diagonal) and transition wood (below diagonal) among fiber length (FLW), α -cellulose yield (ACY), coarseness (COA), lignin content (LIG), height at age 6 (HT6), volume at age 6 (VOL6), fusiform rust infection at age 6 (RUST6), and straightness at age 6 (STRT6) for combined sites

	ACY	COA	FLW	LIG	HT6	VOL6	RUST6	STRT6
ACY		0.54***	0.58***	¹	0.13**	-0.07	-0.19***	-0.34***
COA	¹		0.70***	-	0.16***	-0.01	-0.38***	-0.53***
FLW	0.26***	-		-	-0.19***	-0.44***	-0.30***	-0.69***
LIG	-0.14**	-	-0.23***		-	-	-	-
HT6	0.11*	-	-0.37***	-0.56***		0.92***	0.11*	-0.24***
VOL6	-0.12**	-	-0.53***	-0.52***	0.92***		0.22***	-0.03
RUST6	-0.30***	-	-0.53***	0.37***	0.11*	0.22***		0.07
STRT6	-0.48***	-	-0.43***	0.31***	-0.24***	-0.03	0.07	

*, **, ***: Correlations are significant at 0.05, 0.01, and 0.001 level respectively. Number of observations used ranged from 515 to 550

¹ Genetic correlation was not estimable due to zero GCA variance

Figure 3. Site 2 (left) and Site 3 (right) means and 95% confidence intervals of α -cellulose content, lignin content, weighted fiber length, and coarseness for ring 3 earlywood (3e), ring 3 latewood (3l), ring 8 earlywood (8e), and ring 8 latewood (8l). Bars having the same horizontal line are not significantly different.

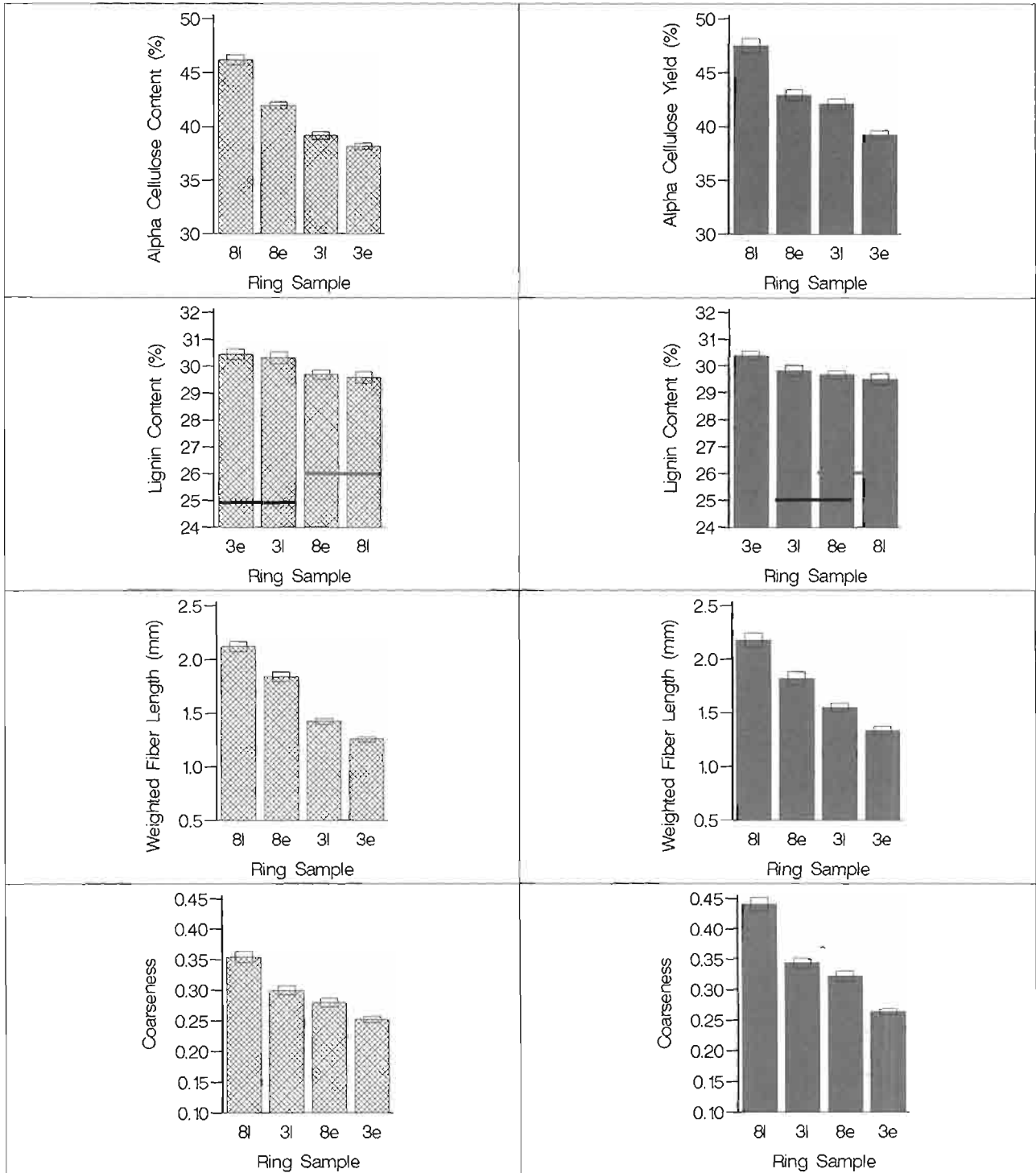
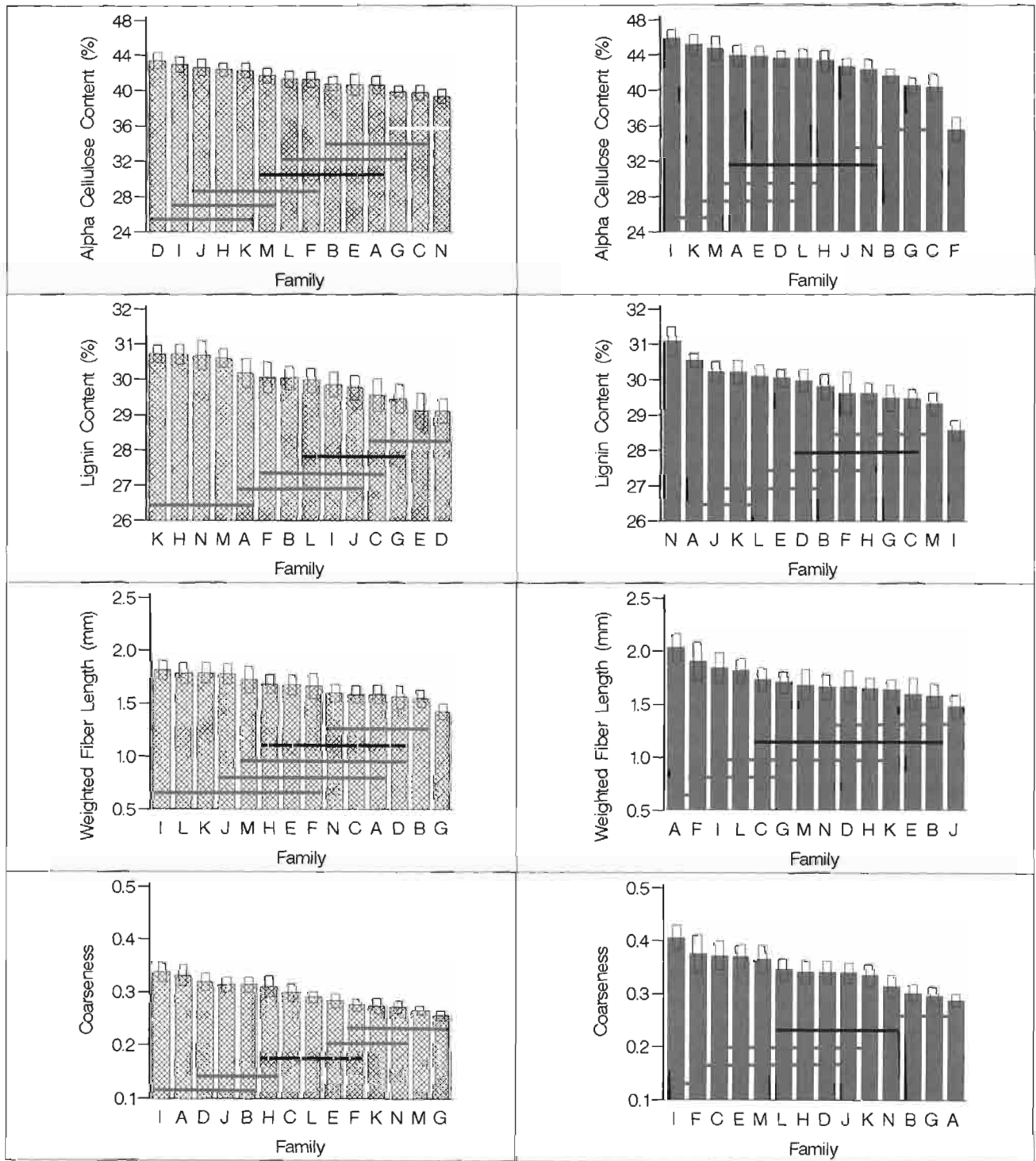


Figure 4. Family means and 95% confidence intervals for α -cellulose content, lignin content, weighted fiber length, and coarseness at Site 2 (left) and Site 3 (right). Horizontal lines across bars are not significantly different for a given trait.



POTENTIAL GENETIC GAINS OF FULL-SIB FAMILIES FROM 2ND-CYCLE BREEDING

Deployment of open-pollinated seeds, capturing genetic gain primarily from additive genetic variance, have been utilized for the two cycles of breeding (Li et al 1999). But the low efficiency of wind-pollinated seed orchards may have limited the genetic gain due to high levels of pollen contaminations in the open-pollinated seed orchards, low selection intensity in typical seed orchards and inability to utilize the non-additive genetic variance.

The Cooperative 2nd-cycle loblolly pine breeding used a disconnected half-diallel mating with 6 parents and 15 crosses per diallel, i.e. each parent is represented by 5 full-sib crosses. This design was chosen to limit the number of crosses per parent, but still yield information on general combining ability (GCA) for all parents in the breeding population. Disconnected diallels were used to maximize the number of unrelated families for selection in the breeding population. This design has shown the efficiency for breeding and testing because parents can be grouped into diallels based on their reproductive maturity, crosses can be made in a short period of time, and progeny tests can be established quickly and at manageable size. While this design has generated useful GCA values for all parents, many parents among disconnected diallels were never crossed. Thus the SCA estimates were only made for full-sibs within each diallel, and no direct SCA estimates for other possible full-sibs among diallels. Although the non-additive genetic component for SCA was often found to be less important than the additive genetic variance for growth traits of loblolly pine, dominance variance estimated from disconnected diallels accounted for 20% to 40% of the total genetic variance (Li et al., 1996; Xiang et al., 2003a). Based on the genetic variance component estimates, Xiang et al. (2003b) demonstrated that additional and significant genetic gain can be achieved by partially capturing the non-additive genetic component through mass production of the best full-sib families, or all of the non-additive component through either clonal deployment or through vegetative propagation of the best individual trees in the best full-sib family. For example, full-sib selection based on total genetic component had the greatest genetic gain, followed by mid-parent full-sib selection (additive variance) and half-sib family selection; full-sib selection produced 40% genetic gain over selection on half-sib families (Xiang et al., 2003b).

With all breeding value estimates are available now from the Cooperative database, genetic gains could be directly estimated for full-sib families within the disconnected diallels and then compared with the potential gains that would be expected if we select parents for full-sib families based on GCA values for all parents across diallels within a breeding program. This study focused on several key questions. What is the relative advantage for the deployment strategy using only the best full-sib families available within disconnected diallels? How much additional gain, if any, might be achieved if full-sib families could be generated within the whole region based on GCA, with or without SCA adjustments? These are important considerations for maximizing genetic gains when making controlled crosses and full-sib deployment in loblolly pine. This study evaluated strategies for deploying full-sib families for highest gain in the loblolly pine breeding program. We compared genetic gains obtained from tested full-sib families with the potential gain obtainable from full-sib families based on good GCA estimates within the region.

Material and methods

We evaluate 111 progeny tests with 338 parents in 60 disconnected diallels in the Coastal region of loblolly pine for this study. The best linear unbiased predictions (BLUP) of GCA, SCA and variance components were estimated using the mixed linear model and analytical method (Xiang and Li, 2001). Briefly, mixed model equations were used to predict GCA for all parents, and SCA for full-sib crosses, for 6-year height and volume, resulting in best linear unbiased predictors (BLUP). Genetic gains were estimated and expressed in percentage gain compared to local commercial unimproved check lots (Xiang et al., 2003b). Since the same check lot was used for all disconnected diallels within a region, a fair comparison can be made among disconnected diallels within the Coastal region.

Using the appropriate gain formula for estimated gains, gain was predicted, based on genetic parameters, for: (1) selecting X full-sib families from the approx. 1000 tested in diallels, (2) selecting X crosses based on mid-parent selection (i.e. additive value) from all possible combinations of 338 parents, or (3) selecting X full-sib families (i.e. total genetic value) using all possible combinations of the 338 parents. The gains are

in scenarios (1) and (3) based on the broad-sense full-sib mean heritability, and in (2) on narrow-sense full-sib family mean heritability.

Based on the BLUP estimates of GCA and SCA of 6-year volume from the database, the genetic values of full-sib crosses (B) were calculated based on GCA only: $B_{ij} = GCA_i + GCA_j$, or based on both GCA and SCA: $B_{ij} = GCA_i + GCA_j + SCA_{ij}$, where GCA_i and GCA_j are the general combining abilities for the first and second parent, respectively, and SCA_{ij} is the special combining ability between parent i and j .

Several selection scenarios for deployment were evaluated for comparing genetic gains on various selection intensities, 1 to 25 full-sib families:

1. Selection of tested full-sib families based on total predicted genetic value, *i.e.* GCA and SCA of crosses made within diallels.
2. Selection of full-sibs based on mid-parent values across disconnected diallels within the whole geographic region. Gain calculated from all possible combinations of crosses based on parental GCA only.
3. The same selection scenario as above (2), but restricted to allow the same parent only in 1, 2, 3, 4 or 5 crosses, respectively. Limiting the number of crosses per parent increases the number of required parents; thus reducing the selection intensity.
4. Linear deployment where parents ranking 1-5 were allowed in 4 crosses, parents ranking 6-10 in 3 crosses, parents ranking 11-15 in 2 crosses and parents ranking 16-20 only in one cross.

Results

Selection of the best 10 families based on genetic parameter estimates gave a gain of 53% for selection based on mid-parent value, and a gain of 45% for the 10 best full-sib families of 1000 crosses (**Figure 5**). The potential gain increased to 63%, *i.e.* by 10%, when selecting among all possible full-sib families compared with selection based on mid-parent value only.

Gain in volume growth from selection based on high GCA parents (middle-parent values) among disconnected diallels within the whole region produced significantly higher genetic gains than selection based on tested full-sib families made within diallels (total genetic value) (**Figure 6**). Selection of the 10 best field-tested

full-sib families within diallels resulted in a gain of 45%, compared to 54% gain, when the best GCA parents across disconnected diallels within the whole region were mated. The difference in gain was small at high selection intensity but increased as more full-sib families were selected. When selecting 5, 10, or 25 full-sib families, respectively, the difference in gain from selection based on high-ranking GCA parents, compared to full-sib families selected within disconnected diallels, was 8%, 9%, and 11%, respectively (**Figure 6**).

From the ranking of families with tested full-sibs it was obvious that those crosses with high GCA values also often had small positive SCA effects. The total gain, based on ranking for GCA was therefore nearly the same as ranking for total value, *i.e.* GCA+SCA. This was probably due to the low relative importance of the SCA (15%) variance component in this study. When SCA was included, the gain increase was 1%, and 2%, respectively, compared to the gain calculated only from GCA when 10, or 25, full-sib families were selected, respectively.

The potential gains from different selection scenarios for the Atlantic Coastal region, based on selection of good GCA parents (**Table 4**), showed that restricting the number of full-sib crosses per parent to 1, 2, 3, 4, and 5 crosses resulted in decreased gain as the number of full-sib crosses per parent decreased. In other words, the gain with 5, 10 and 25 selected full-sib crosses was 47%, 40%, and 31% when the same parent was restricted to only one full-sib cross, while the corresponding gain increased to 56%, 53%, and 47%, respectively, when the same parent was allowed in 5 full-sib crosses.

The gain from disconnected diallels with 5, 10 and 25 selected full-sib crosses was 49%, 45%, and 40%, respectively (**Figure 6**). Restricting the number of full-sib crosses per parent from two to one reduced the gain more than restricting the number of full-sib crosses per parent from five to four. Compared with the scenario of only using crosses within disconnected diallels, there is still room for restricting the number of crosses per parent and also get a higher gain than selecting only within the disconnected diallels. The gain with linear deployment (**Table 4**) from selecting 5, 10, and 25 full-sib crosses, respectively, resulted in a gain of 56%, 53%, and 44%, respectively. By using the best parents in more full-sib crosses, high genetic gain was achieved, while at the same time the genetic diversity in the production population was higher.

Discussion

As the additive genetic variance from the second generation progeny tests was more important than dominance variance, 60% to 80% of the total genetic variance (Xiang et al. 2003a, Xiang et al. 2003b), selection based on realized gains of full-sib families within disconnected diallels only gave a small increase in gain compared to selection based on parents that had high GCA values within the same diallels. Gain based on GCA from all parents within a geographic region resulted in a higher gain than selection limited to tested full-sib families. One reason for the low increase in gain may be a consequence of a limited number of available full-sib crosses when selection is done only within diallels. The difference in gain increased with increasing number of selected crosses (**Figure 6**). When selecting between 5 and 25 full-sib families the potential gain could increase as much as 8% to 11%.

Given the moderate levels of SCA and the limited number of crosses that were tested, maximum gains may be attainable by selecting for parental GCA in a breeding program for loblolly pine. Greater genetic gains should result from large-scale production of the best general combiners, rather than selection and multiplication of best full-sib families.

SCA can be captured through mass controlled pollination or vegetative propagation techniques such as rooted cuttings or somatic embryogenesis. Full-sib families may be produced on a large scale, and this may also increase the motivation to look for non-additive effects. Xiang et al. (2003b) showed that additional and significant gain can be achieved by keeping all of the non-additive component through vegetative propagation of the best individual trees in the best full-sib families. Sometimes, it may be safer to use a tested full-sib family for deployment than an untested one. This should be considered together with the potential gains that can be obtained by using the best GCA parents. The average of GCA of two parents should be a very conservative estimate of the full-sib performance. But additional crossing among parents and planting their full-sib progenies in field trials is needed.

In conclusion, results suggest that selection for full-sib families in loblolly pine based on GCA from all parents within a geographic region would result in a higher genetic gain. The relatively small importance of SCA in this population of loblolly pine, compared to GCA, gives little merit to exploitation of SCA. The specific crosses are very important for achieving higher

genetic gains in a loblolly pine breeding program. But they were not due to the SCA contribution as expected, and rather the highest GCA of parents is most important in creating the full-sib crosses. So the strategies for clonal deployment are still very attractive based on the data from this study.

References

- Li, B., McKeand, S. E., and Weir, R. J. 1999. Tree improvement and sustainable forestry - impact of two cycles of loblolly pine breeding in the USA. *Forest Genetics* **6**: 229-234.
- Xiang, B., and Li, B. 2001. A new mixed analytical method for genetic analysis of diallel data. *Can. J. For. Res.* **31**: 2252-2259.
- Xiang, B., Li, B., and Isik, F. 2003a. Time trends of genetic parameters in growth traits of *Pinus taeda* L. *Silvae Genetica* **52**: 114-121.
- Xiang, B., Li, B., and McKeand, S. 2003b. Genetic gain and selection efficiency of loblolly pine in three geographic regions. *For. Sci.* **49**: 196-208.

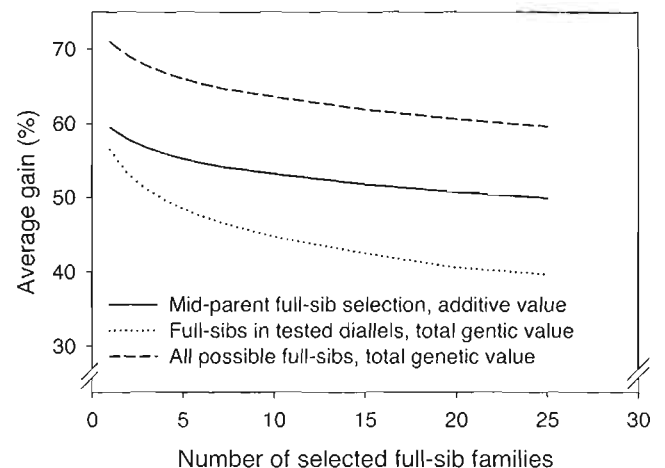


Figure 5. Predicted gains calculated from genetic parameters. Comparison of (1) selection of full-sib families based on total genetic value from full-sib families tested in diallels, (2) selection of full-sib families based on mid-parent value using all possible combinations of the parents, and (3) selection based on all possible full-sib families. Gains in volume (%) are presented in comparison to local commercial seed lots.

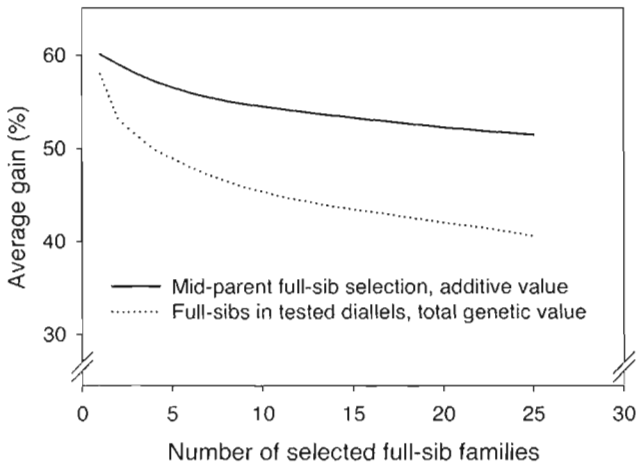


Figure 6. Comparison of selection of tested full-sib families within diallels based on total genetic value, and selection based on full-sibs of parental GCA across diallels within the Atlantic Coastal region. Gains in volume (%) are presented in comparison to local commercial seed lots.

Table 4. Gain in volume (%) for different selection scenarios, restricted selection (one parent involved in 1-5 crosses) and linear deployment, in Atlantic Coastal region of loblolly pine. Gains were estimated for different selection intensities and expressed as the % superiority over to the local commercial seed lot.

No. of selected full-sib families	Selection based on good GCA parents, within whole region					
	Restricted selection					Linear deployment ¹⁾
	1	2	3	4	5	
1	60	60	60	60	60	60
2	55	59	59	59	59	59
3	51	57	58	58	58	58
4	48	54	57	57	57	57
5	47	52	55	56	56	56
10	40	47	50	52	53	53
15	36	43	47	49	51	49
20	34	40	44	47	48	46
25	31	38	42	45	47	44

¹⁾ Linear deployment where parents ranking 1-5 is allowed in 4 crosses, parents ranking 6-10 in 3 crosses, parents ranking 11-15 in 2 crosses and parents ranking 16-20 only in one cross.

EFFECT OF A RARE MUTANT GENE ON GROWTH AND WOOD PROPERTIES IN LOBLOLLY PINE

One of our fastest-growing loblolly pine selections, whose progeny are widely planted in many southern states, is also the only known natural carrier of a rare gene, *cad-n1*. This allele codes for deficiency in an enzyme, cinnamyl alcohol dehydrogenase (CAD), which catalyzes the last step in the biosynthesis of lignin precursors. Breeding work based on this selection has produced two types of mutant trees: partially and totally CAD-deficient. Totally CAD-deficient trees (inbred and homozygous for *cad-n1*) produce wood that is much more easily delignified, but is not ideal raw material, as pulp yields are relatively low in comparison to “normal” pine and the inbred nature of these trees results in poor growth. On the other hand, in a preliminary study, partially CAD-deficient loblolly pines (heterozygotes) produced 14% more debarked volume at age 4, compared to normal trees (Wu et al. 1999). Furthermore, kraft cooks of 4- and 6-year-old trees have resulted in “kappa numbers” (lignin content) that were significantly lower for partially CAD-deficient wood versus normal pine (Dimmel et al. 2001). Estimates indicate that between 15% and 25% less H-factor (energy) is needed to reach a 30-kappa pulp in the case of the partially CAD-deficient wood.

To date, the evidence for differences in the growth and wood quality of partially CAD-deficient wood is limited. However, the energy-saving implications suggest that *cad-n1* may be a particularly valuable gene when deployed on a large scale into forest plantations. We are now in the early stages of a study, supported by the Department of Energy through Agenda 2020, with the objective of characterizing the performance and value of CAD-deficient wood, arising from descendants of the selected partially CAD-deficient founder. In this first report, we present early results on growth and wood quality for open-pollinated progeny of a single, partially CAD-deficient seed parent, growing in a replicated field test under two levels of nitrogen nutrition.

MATERIALS AND METHODS

Field Sampling and Data Collection

The field test reported on here is located in Scotland

County, NC, adjacent to the U.S. Forest Service / N.C. State University SETRES (Southeast Tree Research and Education Site). The soil is very infertile and somewhat excessively drained. The existing 10-year-old loblolly pine stand was carefully removed in 1993, before this trial was established in November, 1993 with container-grown seedlings from 10 open-pollinated families. Two fertilizer sub-plots consisting of 100 measurement trees were established in each of 10 family plots, and replicated over 10 complete blocks. The trees were planted at 1.5 x 2.1 metre spacing, with a 12-metre buffer around each treatment plot, to minimize the influence of one nutrient treatment on another. All trees were measured annually (except for year 7) up to age 8 for height and, starting in year 3, for breast-height diameter and total volume. Fertilizer was applied annually to maintain an “optimum” supply of all nutrients in fertilized plots.

The seed parent for one of the 10 open-pollinated families is partially CAD-deficient and is a progeny of the partially CAD-deficient founder. In August 2003, a total of 200 healthy, undamaged trees were systematically sampled from 5 blocks (20 trees from each fertilizer sub-plot). A 12-mm core was sampled from each tree at breast height for wood quality analyses, and cambium tissue collected for DNA extraction.

Genotyping

DNA was isolated from each cambium sample and genotyped for the CAD locus by Polymerase Chain Reaction (PCR) with a labeled primer allowing the products to be detected by fluorescence. Completed PCRs were resolved and detected on an ABI 3100 Genetic Analyzer. Peak sizes (PCR fragment lengths in base pairs) were determined with GeneScan[®] and labeled with Genotyper[®] software (Applied Biosystems). Homozygous wild-type trees produce a single peak, while heterozygous trees produce two peaks—the same peak as the homozygote plus a peak 2 base pairs (bp) longer (**Figure 7**). These results are consistent with CAD sequence data where *cad-n1* contains a 2 bp insertion compared to the wild-type allele (Gill et al. 2003).

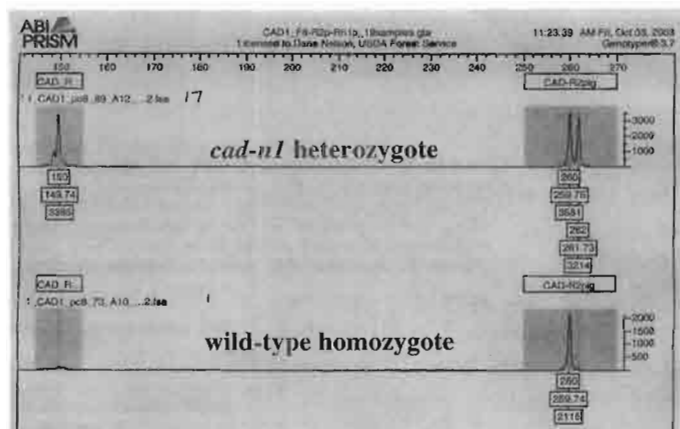


Figure 7. CAD-deficient trees, heterozygous for the mutant *cad-n1* gene, can be identified by a characteristic second peak when PCR products are analyzed.

Wood density measurements

Wood density was measured using X-ray densitometry. A radial segment from pith to the bark was taken from each core. Each strip was scanned from pith to the bark in an air-dried condition on a QMS Tree Ring Analyzer[®] (Model Qtrs-01x, Quintek Measurement Systems, Inc.). The last growth ring from 2003 was excluded due to missing latewood on cores collected in mid-summer. For each ring scanned, the following intra-ring wood density and ring-width characteristics were determined: earlywood width, latewood width, latewood percentage, average ring density, earlywood density, latewood density, and cambial age.

RESULTS

CAD genotype data were available for 197 trees. The segregation ratio of 107:89 for the *cad-n1* and wild-type alleles of the seed parent was not significantly different from the expected 1:1 ratio ($\chi^2=1.6531$, $p = 0.1985$).

Height growth response to fertilization was very large and significant (**Figure 8**). The *cad-n1* heterozygotes were slightly taller than wild-type genotypes in most years on both fertilized and control treatments, although these differences were not statistically different at any age.

The effect of CAD genotype was not significant for any growth traits in year 8 (**Table 5**). Height and DBH were 50% and 47% greater in fertilized plots, respectively, and the volume growth difference was even more dramatic (**Table 6**). The effect of CAD genotype

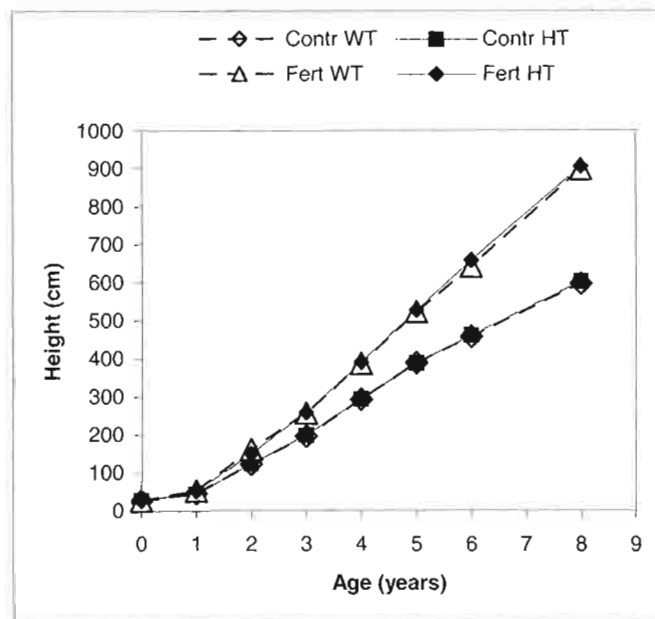


Figure 8. Mean tree height during the first eight growing seasons for *cad-n1* heterozygotes (HT) and wild-type (WT) trees in fertilized and control plots.

was significant for all wood traits, except for latewood density. Fertilization effects were highly significant for all wood traits. CAD genotype-treatment interactions were not significant for any growth or wood density traits.

The latewood percentage for *cad-n1* heterozygotes was 7.2% higher than wild-type trees. The heterozygotes were also 2.1%, 2.9%, 1.6%, and 0.2% higher for weighted wood density, ring density, earlywood density and latewood density, respectively. Weighted wood density, ring density, latewood density, earlywood density and latewood percentage were 9.3%, 10.3%, 4.5%, 7.4% and 18.5% lower, respectively, in fertilized plots. Weighted wood density in year 9 was 2.6 % and 1.3 % greater for *cad-n1* heterozygotes than for wild-type trees on control and fertilized treatments, respectively, but these differences were significant only for the control plots ($p < 0.002$), and not in fertilized plots ($p < 0.501$) (**Figure 9**). Ring densities in most years were significantly different between *cad-n1* heterozygotes and wild-type trees in the control treatment, but not in the fertilized treatment.

The mean weighted average wood density on fertilized sub-plots was 9% less than that on controls. For both partially CAD-deficient and wild-type trees, the increase in radial growth under fertilization was due primarily to an increase in earlywood width. As an effect of greater earlywood, the proportion of latewood decreased correspondingly in both *cad-n1* heterozygote and wild-type trees.

Table 5. Significance of main effects from the ANOVA of the linear model for growth traits at 8 years and wood density traits at 9 years (*p* values less than 0.05 shown in **bold type**).

Main effect	df	Level of significance (<i>p</i>)							
		Height	DBH	Volume	Mean density	Ring density	Latewood density	Earlywood density	Latewood percent
Fertilizer (F)	1	0.0021	0.0005	0.0018	0.0002	<.0001	0.0160	0.0002	0.0001
CAD genotype (G)	1	0.3971	0.9566	0.7684	0.0292	0.0017	0.9654	0.0302	0.0184
GxF	1	0.9215	0.9071	0.9288	0.3338	0.3102	0.8173	0.9164	0.1907

Table 6. Average effect (\pm standard error) of CAD genotype (wild-type = WT, *cad-n1* heterozygote = HT) and fertilizer treatment for growth traits at age 8 years and wood density traits at 9 years.

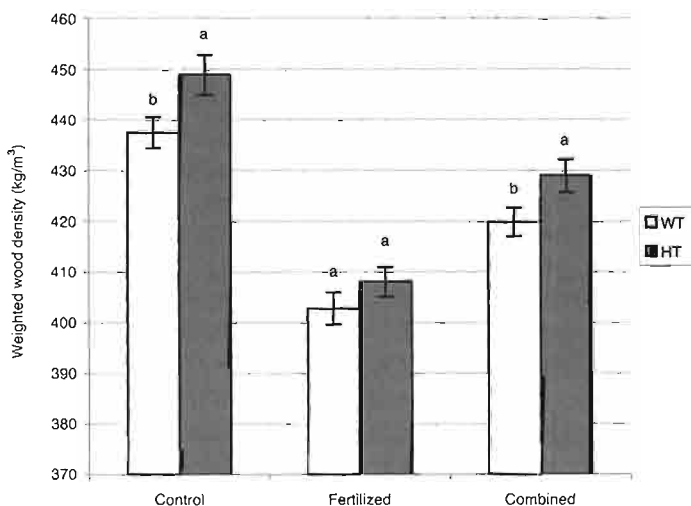
	N	Height (cm)	DBH (mm)	Volume (dm ³)	Mean density (kg/m ³)	Ring density (kg/m ³)	Latewood density (kg/m ³)	Earlywood density (kg/m ³)	Latewood percent
CAD Genotype									
WT	107	749.4 \pm 17.1	96.4 \pm 2.3	36.03 \pm 1.8	419.9 \pm 2.8	423.1 \pm 2.8	602.7 \pm 2.66	332.6 \pm 1.90	31.9 \pm 0.61
HT	89	753.5 \pm 18.7	96.2 \pm 2.6	36.12 \pm 2.0	429.0 \pm 3.4	435.3 \pm 3.5	604.2 \pm 3.33	338.1 \pm 1.97	34.2 \pm 0.75
Fertilizer treatment									
Control	98	600.9 \pm 7.9	78.0 \pm 1.6	21.46 \pm 0.75	442.8 \pm 2.5	449.4 \pm 2.6	616.7 \pm 3.00	347.08 \pm 1.65	35.7 \pm 0.73
Fertilized	98	901.6 \pm 10.3	114.6 \pm 1.5	50.68 \pm 1.44	405.1 \pm 2.2	407.8 \pm 2.1	590.0 \pm 2.18	323.07 \pm 1.38	30.2 \pm 0.50

DISCUSSION

The segregation ratio for *cad-n1* and wild-type alleles agreed with the expectation of 1:1. This suggests that the *cad-n1* allele is selectively neutral for survival and adaptability, and that our sampling strategy was random. To-date, no CAD-deficient trees have been identified outside of the pedigree of the original selected founder.

While Wu et al. (1999) found that *cad-n1* heterozygotes appeared to grow more quickly, we have not found a significant correlation between CAD genotype and growth. These conflicting results likely arise from different sample size and age of the trees. In the present study, the total of 197 trees was rather small compared with 869 seedlings in the earlier report. We suspect that the effect of *cad-n1* on growth may be small, requiring a larger sample to detect statistical significance.

Wood density is an important wood characteristic that is related to the yield and quality of pulp and sawn products. In this study, the *cad-n1* allele demonstrated a significant effect on most of wood density traits, especially for latewood percentage. The higher wood density in CAD-deficient trees was most likely related to

**Figure 9.** Means of weighted wood density for *cad-n1* heterozygotes (HT) and wild-type (WT) trees in the 9th year on fertilized and control plots, and for all plots combined.

high proportion of latewood.

Silvicultural treatments, including fertilization, can affect wood density by modifying the environmental conditions controlling tree growth. McKeand et al. (2000) earlier found that fertilizer amendment in this trial had increased the uniformity of growth over all 10 families in fertilized plots. In this analysis, we also found that trees from the single *cad-n1* segregating family grown in fertilized plots had greater uniformity for both wood density and growth, although the differences in uniformity for growth traits between fertilized and control plots were smaller than reported earlier over all 10 families. The coefficient of variance for height, DBH, volume and weighted wood density in control plots were 13%, 20%, 34% and 5.6 %, while those for fertilized plots were 11%, 12%, 28% and 5.4 %, respectively.

The effects of fertilization on wood properties are difficult to generalize. In this study, fertilization significantly decreased the mean wood density for both *cad-n1* heterozygotes and wild-type trees. The decrease was partly caused by a shift in the relative widths of earlywood and the proportion of latewood.

The general trend in the wood density of loblolly pine is an increase from pith outwards reaching a maximum value around ring 12. In this study, the mean ring density, as well as the earlywood and latewood density for both *cad-n1* heterozygotes and wild-type trees, followed a continuously increasing trend in both control and fertilized plots. This was expected due to the lack of a CAD genotype-fertilizer treatment interaction effect.

FUTURE WORK

In addition to the sampling at the Scotland County site, our team is also collecting growth data and wood samples from other 1st- and 2nd-generation progeny tests where *cad-n1* segregating descendants of the founder CAD-deficient selection have been evaluated up to age 28 years. Growth data and wood samples from these trees

will add to the information about the effects of the *cad-n1* allele in a wide variety of genetic backgrounds, replicated over a range of growth environments. Wood analyses will also be expanded to further characterize the lignin structure of CAD-deficient wood.

ACKNOWLEDGEMENTS

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References

- Beckwith, J.R., III, and Reines, M. 1978. Aerial fertilization increases volume and weight of planted loblolly pine. *Southern Journal of Applied Forestry*, **2**(4): 118-120.
- Dimmel, D.R., MacKay, J.J., Althen, E.M., Parks, C., and Sederoff, R.R. 2001. Pulping and bleaching of CAD-deficient wood. *Journal of Wood Chemistry and Technology*, **21**(1): 1-17.
- Gill, G.P., Brown, G.R., and Neale, D.B. 2003. A sequence mutation in the cinnamyl alcohol dehydrogenase gene associated with altered lignification in loblolly pine. *Plant Biotechnology Journal*, **1**: 253-258.
- Rockwood, D.L., Windsor, C.L., and Hodges, J.F. 1985. Response of slash pine progenies to fertilization. *Southern Journal of Applied Forestry*, **9**(1): 37-40.
- Wu, R.L., Remington, D.L., MacKay, J.J., McKeand, S.E., and O'Malley, D.M. 1999. Average effect of a mutation in lignin biosynthesis in loblolly pine. *Theoretical and Applied Genetics*, **99**(3-4): 705-710.

FUSIFORM RUST INFECTION LEVELS IN 6-YEAR-OLD DIALLEL TESTS

From the 250 diallel test series analyzed by the Cooperative, there were 973 individual tests of 2nd-generation or plantation-selection diallels where we have evaluated fusiform rust infection levels at age 6 years. We looked at percent infection of the control-pollinated families, the mean of the three unimproved checklots, and the seed orchard mix in each test across the different test areas. The 2nd-generation tests and the plantation-selection diallels were analyzed separately.

As expected, the level of infection varied significantly by geographic region. The mean of the checklots is the best indicator of the natural levels of fusiform rust to be expected in a region, since there was no selection for resistance in these checklots. Rust levels for the checklots were highest in the Lower Gulf (>50% at age 6) and about 14% in the Virginia and northern North Carolina tests (Table 7). In regions where the rust levels were fairly high and where selection for rust resistance is important (Areas 3, 4, 5, and 7), the diallel families had significantly lower rust infection compared to the checklots. In both the 2nd-gen and plantation selection tests, the families had from 5% to 10 % lower rust than the unimproved checks. Even in the plantation-selection tests where the parents had only gone through one round of phenotypic selection for rust (e.g. only rust-free trees in plantations were selected), rust levels for the CP families were lower than the checks. Many plantation selections were made in heavily rust-infected stands where mass selection of rust-free individuals should be most effective. This improvement in rust resistance in plantation selections is very different from what was found in the first-generation selection program in natural stands where no gain in rust resistance was found.

In Areas 3, 4, 5, and 7, the 2nd-generation diallel families had significantly lower rust infection than the plantation selection families, 30.0% vs. 39.7%, respectively. The extra round of selection that occurred for the 2nd-gen parents appeared to be effective compared to the phenotypic selection for the plantation selections.

Gain in rust resistance from the Cooperative's breeding efforts have been significant. These results simply show the population means in the Test Areas, but in each region, there are families that are extremely resistant to fusiform rust, and some that are more susceptible than the checks. The most rust-resistant, fast-growing families from the Coastal regions are being studied in the Integrated Pest Management Rust Study funded by the USDA (Development of Stable and Predictable Deployment Populations of Loblolly Pine to Minimize Fusiform Rust Impacts in Southern Pine Plantations) in conjunction with Henry Amerson in the Biotechnology Program. The study will investigate the stability and predictability of high-yielding families that appear putatively resistant, based on limited testing. These families will be tested across multiple inoculum sources in both greenhouse (GH) and field tests. Included are families from 7 loblolly pine clones that contain 8 different heterozygous fusiform rust resistance genes, tagged with genetic markers. Markers for genes, Fr1--Fr8, will be used to examine pathogen virulence variation across the range of the rust pathogen and as a possible explanation for the previously observed unpredictability of resistance. Research will be conducted in large GH tests funded by this proposal, while field studies will be funded and conducted by industry/state partners. Specific objectives are: to develop breeding and operational deployment strategies in high-hazard rust areas, utilizing those families having stable and predictable rust resistance; to identify stable (highly resistant) but unpredictable families for site-specific or regional deployment; to identify families that are likely sources for future Fr gene discovery and genetic marker investigations; to characterize patterns of geographic virulence variation (i.e., regional vs. site to site) for each of 8 Fr genes; and to characterize virulence variation against resistance, in non-Fr families in GH studies for correlation with field studies.

Table 7. Rust Means (% infection at age 6 years) in Diallel Tests.

Test Area	2 nd -Generation Diallel Tests				Plantation Diallel Tests			
	# Tests	Families	CC	SOM	# Tests	Families	CC	SOM
1-VA & NC	48	13.7	14.4	15.2	139	13.2	13.7	14.9
3-Coastal SC	33	21.5	30.7	30.6	91	39.8	47.0	45.1
4-Coastal GA & FL	16	14.2	28.5	27.2	59	35.6	46.3	39.3
5-Lower Gulf	8	46.0	51.8	53.2	175	46.6	53.6	52.0
6-Upper Gulf	11	20.3	22.2	18.7	121	20.3	22.6	18.7
7-Piedmont GA & SC	54	37.5	45.4	44.0	218	35.2	42.0	37.9
Average	$\Sigma=170$	24.8	31.0	30.3	$\Sigma=803$	32.2	37.6	35.1



The diallel test database has been and will continue to be an invaluable resource for the Cooperative. We have only begun to “milk” it for information on fusiform rust resistance and variation in infection levels.

EXTERNALLY FUNDED RESEARCH GRANTS 2003-2004.

Amerson, H., McKeand, S.E., Mullin, T.J. and Li, B. USDA-IPM. \$86,900. *Development of stable and predictable deployment populations of loblolly pine to minimize fusiform rust impacts in southern pine plantations.*

Chang, H.-m., J. Kadla, R. Sederoff, B. Li and B. Goldfarb. USDA-IFAFS: \$3,000,000. *Wood and fiber quality of juvenile pine: characterization and utilization.* 08/01-08/05.

Chang, H.-m., J. Kadla, D. O'Malley and B. Li. DOE Agenda 2020: \$585,000. *Exploiting genetic variation of fiber components and morphology in juvenile loblolly pine.* 07/01/01-06/30/04.

Mullin, T.J., Li, B., Chang, H.-m., and Jameel, H. DOE Agenda 2020. \$1,125,410. *Performance and Value of CAD-Deficient Pine.* 04/03-04/06.

Robison, D., L.J. Frampton, R. Bardon, B. Goldfarb, G. Hodge, B. Li, J. Kadla, D. O'Malley and S. Moore. USDA: \$266,005. *Integrated Biotechnological and Genetic Systems for Enhanced Forest Productivity and Health.* 06/01/03-05/30/04.



Trials such as this International Paper Company 1989 diallel test in Jay, FL have been extremely valuable to the Cooperative and collaborating scientists for innovative research. In cooperation with Jim Roberds and other scientists at the USDA Forest Service, the genetic control of resin flow and its association with resistance to southern pine beetle was examined in this and other IPCo trials (see Roberds et al 2003 in the publication list for more details).

SEED AND CONE YIELDS

The 2003 seed collection for the Cooperative provided approximately 28 tons of loblolly pine seed, almost double last year's 15.1 tons. This yield is more in line with previous year's harvests, even with one organization not reporting. Third-cycle seed collection continues to make good progress, increasing five-fold from 2002 to 2003.

Average seed yields in pounds per bushel were lower than in 2002 (1.25 vs. 1.42 in 2002). About 75% of the total seed came from second-generation orchards and about 65% of the collection was from the Coastal sources (**Table 8**).

Four orchards produced above the 2.0 lbs./bushel mark in 2003, with first place being held (again) by MeadWestvaco's 2.0 South Carolina Coastal orchard with a yield of 2.13 lbs./bushel. Second and third places were held by Plum Creek's 2.0 Coastal orchards in Oliver and Jesup, GA at 2.04 and 2.03 lbs./bushel respectively. Fourth place was Smurfit-Stone's 1.5 Coastal orchard in Brewton, AL at 2.02 lbs./bushel. The rest of the top ten producers are shown in **Table 9**.

Table 8. Comparison of 2003 seed and cone yields with previous year.

Provenance	Bushels of Cones		Pounds of Seed		Pounds per Bushel	
	2003	2002	2003	2002	2003	2002
Coastal 1.0	9,200	3,319	13,931	4,259	1.51	1.28
Coastal 2.0	16,250	10,284	23,116	15,156	1.42	1.47
Coastal 3.0	295	42	262	50	0.89	1.19
Piedmont 1.0	415	450	452	760	1.09	1.69
Piedmont 2.0	13,606	7,114	18,315	9,965	1.35	1.40
Totals	39,766	21,209	56,075	30,190	1.25	1.42

Table 9. Top ten production loblolly orchards in 2003.

Organization	Orchard Type	Age	lbs./bushel	Orchard Manager
MeadWestvaco	Coastal 2.0	19	2.13	Dave Gerwig
Plum Creek Timber Co.	Coastal 2.0	25	2.04	Lorin Clark
Plum Creek Timber Co.	Coastal 2.0	20	2.03	Lorin Clark
Smurfit Stone	Coastal 1.5	28	2.02	Chris Rosier
Smurfit Stone	Coastal 2.0	24	1.98	Chris Rosier
Weyerhaeuser Co.	Piedmont 1.0		1.94	Drew Dunnam
MeadWestvaco	Virginia 2.0	18	1.85	Dave Gerwig
Weyerhaeuser Co.	Coastal 1.0		1.71	Drew Dunnam
Weyerhaeuser Co.	Coastal 2.0		1.59	Franklin Brantley
Smurfit Stone	Coastal 1.0	34	1.57	Chris Rosier

ASSOCIATED ACTIVITIES

TEACHING AND GRADUATE RESEARCH

Coop staff continue to be active in the delivery of courses in Forest Genetics at both the graduate and undergraduate levels. Unfortunately, this has been a slow year for most courses, as changes filter through the undergraduate curriculum and graduate admissions are down. Meanwhile, our current “crop” of graduate students has kept us busy, with two students, Dr. Hua Li and Robert Sykes successfully defending their dissertations. Milan Lstibůrek, one of this year’s recipients of the prestigious Namkoong Family

Fellowship, successfully passed his preliminary exams and has moved to the hard-core research stage of his program. One new graduate student, Josh Sherrill, was admitted, co-supervised by Tim Mullin and Bronson Bullock (Forest Biometrics) to work on variation in stem taper and bark thickness in loblolly pine.

The graduate students working in association with the Cooperative, the degree to which each aspires, and the subject of their research project are listed below.

Student, Degree, Research Project

Daniel Gräns, Ph.D.

Variation in whole-tree wood properties in loblolly pine.

Hua Li, Ph.D.

Major gene resistance in fusiform rust **** completed ****

Milan Lstibůrek, Ph.D.

Optimal population structures for forest tree breeding.

Josh Sherrill

Genetic variation in stem taper and bark thickness in loblolly pine

Rob Sykes, M.S.

Genetic variation in tracheid and wood chemical properties in loblolly pine **** completed ****

TECHNOLOGY TRANSFER

Besides our involvement in structured academic programs, the Coop staff is also active in delivering technology transfer activities and participation in collaborative initiatives with other groups. This year was no exception.

Tree Improvement Short-course

Every two or three years, we organize a short-course for our cooperators, covering the basics of tree improvement and standards for technical operations under our breeding and research programs. New tree improvement staff working for our member agencies are expected to attend, while old-timers are encouraged to take the course as a refresher and to pass on some of their practical experience to others.

This year, the course was held in August at Pensacola Beach, Florida. Coop staff were joined once again by old-timer and orchard guru J.B. Jett, and presented a comprehensive curriculum of basic forest genetics and background on our current breeding plan and operations. International Paper's Greg Leach organized a spectacular field tour, illustrating excellent field testing technique, top-grafting, controlled mass pollination operations, pollen processing and accelerated breeding.



Loblolly "top-grafts" made in tops of slash pine at International Paper facilities in Jay, FL



Tree Improvement Short-course class of 2003

Contact Meeting – Asheville, N.C.

Our annual Contact Meeting is an opportunity for personnel from our member agencies to network, share their experiences, and receive up-to-date information on topics relevant to their interest in tree improvement. This year, our meeting was held in the mountains of North Carolina where the foliage around Asheville was in full autumn color.

Nearby Asheville, the Resistance Screening Center (RSC) of the US Forest Service offers screening services for disease resistance that have been used by many of our members for research and selection. With the focus of the meeting on fusiform rust and southern pines, the attendees visited the RSC for an informative tour hosted by Carol Young and her staff. NCSU's Henry Amerson was also in attendance to share his knowledge on this pathosystem and explain the applications of greenhouse screening to increasing our understanding of this important disease.



Attendees at the 2003 Contact Meeting saw first hand how families of loblolly pine are assessed for resistance to fusiform rust at the US Forest Service Resistance Screening Center near Asheville, NC. Above: trays of seedlings receive a measure dose of rust spores before entering an incubation chamber at high humidity to encourage infection. Left: Dr. Henry Amerson points out stem galls that have developed on seedlings, several weeks after inoculation.



New Genetics Demonstration at NCSU's "Schenck Memorial Forest"

In April 2004, a new demonstration planting at NC State's Schenck Memorial Forest was established to illustrate the results of decades of genetic improvement from the Cooperative. A combination of row plots and large block plots were planted with the very best and the very worst that loblolly pine has to offer. Containerized seedlings of the fastest growing and best-form families from the Piedmont and upper Coastal Plain of the Carolinas are being compared to unimproved loblolly pines from the same region.

Around the outside of the planting along the walking path, 21 paired row plots will illustrate extreme family differences as well as species differences (we planted longleaf, shortleaf, and Virginia pines at the ends of the plot). We also have 5 fast-growing clones from the Clonal Selection Study in conjunction with the Rooted Cutting Program. We hope to show the superior growth and uniformity that is possible with clonal propagation of superior individual clones. In the interior portion of the trial, 10 large block plots (36 to 100 trees depending on the location within the trial) will be available for forestry students to evaluate growth and yield and stand-level attributes of these family plots.

"A picture is worth a thousand words." We hear this all the time, and we are confident that the new demonstration planting will live up to our hopes. No matter what the numbers say or how sophisticated we get with genetic analyses, there is simply no substitute for seeing the genetic differences on the ground. The new 2-acre plantation will be a place where visitors and students can see the benefits of planting healthy, fast-growing, adapted, well-formed trees to meet the demands of consumers and landowners for forest products.



Graduate and undergraduate students, staff, and even some faculty all pitched in on a beautiful spring day in April to plant the new Genetics Demonstration Trial at the Schenck Memorial Forest.

PROGRAM STAFF

Some big changes in the Coop staff this past year. We were very sad to see Paula Zanker, our Field and Lab Manager, leave us after ten years of service. In a symbolic gesture, Paula's last official duty was to "surplus" our original X-ray densitometer – certainly no one else had the Paula's special combination of determination and patience to get the thing to cooperate, so it seemed fitting that its days should end on her departure. Paula became Director of Religious Education at her church in Burlington and is enjoying a much shorter commute to work.



Paula Zanker

Paula's departure left us without any technical support, just as we were beginning a large new project funded by the Department of Energy (DOE). The search was on! Fortunately, we managed to recruit Mike Jett, who's no stranger to these halls. Mike (yes, he's J.B.'s son) had spent many hours working in our lab as he was going through university, so he knew what he was getting into.



Michael Jett

Our second technical support slot was ably filled by Tori Batista, whose training in the Forest Biotech Group lab was excellent preparation for the molecular biology procedures required by our new DOE grant. Nothing can slow this gal down and she had a grueling schedule of field sampling organized in no time.



Tori Batista

Another new recruit for the DOE project was Dr. Qibin Yu, a native of China who came to us via Finland and Canada. Qibin's background in quantitative forest genetics and wood properties mad him an obvious choice as a post-doctoral fellow working on our DOE grant.



Dr. Qibin Yu

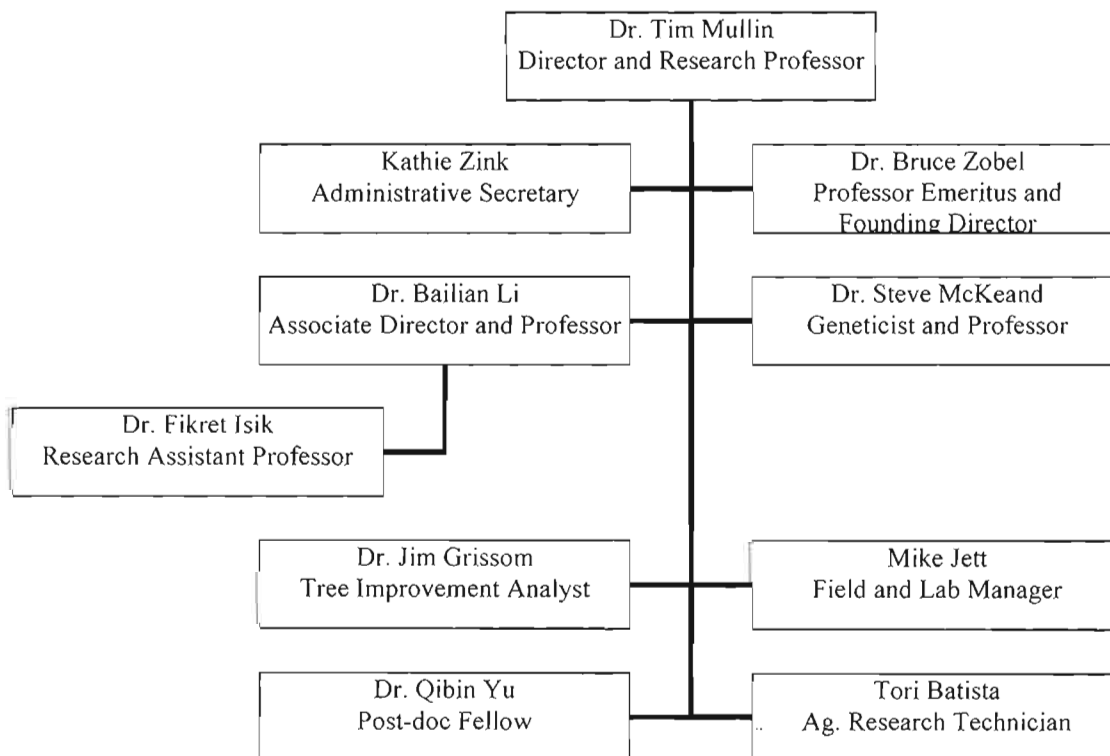
Robin Hughes was only with us a short time before Dean Nielsen decided he rather have her working in HIS office. Our loss was certainly his gain, but Robin still comes by often to make sure we're doing OK. While we set about to recruit a new secretary, Carl Schreiber came

on board part-time and kept the bills paid and the pay checks coming. At time of writing, we're getting ready to welcome Kathie Zink to our front office – Kathie comes to us from the Operations Research Program in the Department of Engineering at NCSU.



Carl Schreiber

**NCSU-Industry Cooperative Tree Improvement Program
Staffing Chart – May 2003**



MEMBERSHIP OF THE NCSU-INDUSTRY COOPERATIVE TREE IMPROVEMENT PROGRAM

As of April 2004, membership in the Coop stood at 16, with 6 state agencies and 10 private corporations:

Alabama Forestry Commission
Georgia Forestry Commission
Gulf-States Paper Corp.
Hancock Natural Resources Group
International Paper Company
Joshua Land Management L.L.C.
MeadWestvaco Corp.
N.C. Division of Forest Resources

Rayonier, Inc.
S.C. Commission of Forestry
Smurfit-Stone Container Corp.
Tennessee Forestry Division
Temple Inland Forest, Inc.
Plum Creek Timber Company
Virginia Department of Forestry
Weyerhaeuser Corp.



From his perch in a lift bucket, staff geneticist Steve McKeand grafts a scion from a 3rd--cycle selection into the top of a 30 foot tree. Grafted in this position, the cutting will produce flowers much earlier than grafts made on smaller rootstock, and will shorten the time to accomplish our 3rd-cycle breeding plan.

PUBLICATIONS OF SPECIAL INTEREST TO MEMBERS

Research and the dissemination of those findings continue to be a critical component of the Cooperative program. Over the past 3 years, program staff members have made a major scholarly contribution in refereed, peer-reviewed journals (38 articles published or in-press), as well as in conference proceedings and other technical publications (26 papers). The support from Cooperative members allows staff to maintain the highest scholarship expected of university faculty, while also directing the research effort towards today's questions and tomorrow's challenges.

- Alizoti, P., B. Li, and S.E. McKeand. 2003. Early performance and genetic parameters for Atlantic Coastal and Piedmont loblolly pine and their hybrids in the Piedmont. P. 138 In: Proc. 27th South. For. Tree Impr. Conf., Stillwater, OK.
- Byram, T.D., A.C. Mangini, and S.E. McKeand. 2003. Cone and seed insect pest research: the role of the southwide studies. P. 116-125. In: Proc. 27th South. For. Tree Impr. Conf., Stillwater, OK.
- Byram, T.D., Mullin, T.J., White, T.L., and van Buijtenen, J.P. 2004. Tree improvement: Alternative visions for the next decade. Invited presentation: IEG-40 Meeting, 17-19 September 2002, Wrightsville Beach, NC. South. J. Appl. For. (in press)
- Cumbie, W.P., Li, B., Goldfarb, Mullin, T., and McKeand, S. 2001. Genetic parameters and uniformity of wood properties of full-sib families and clones of loblolly pine. Pp. 104-106 In: Proc. 26th Southern Forest Tree Improvement Conference, Athens, GA, 26-29 June 2001. Edited by: J.F.D. Dean. Pub. 48, Southern Forest Tree Improvement Committee.
- Dimmel, D.R., J.J. MacKay, C.E. Courchene, J.F. Kadla, J.T. Scott, D.M. O'Malley, and S.E. McKeand. 2002. Pulping and bleaching of partially cad-deficient wood. J. Wood Chem. and Tech. 22:235-248.
- Dimmel, D.R., J. J. MacKay, C. Courchene, A. White-Hughes, J. Kadla, J. T. Scott, K. Holtman, H-m. Chang, D. M. O'Malley, and S. E. McKeand. 2001. Pulping and bleaching of partially cad-deficient wood. P. 33-36 In: Proc of the 11th International Symposium of Wood and Pulping Chemistry, Nice, June 2001, Vol.
- Grissom, J.E. and S.E. McKeand. 2001. Comparative physiology of contrasting genotypes of loblolly pine under dry field conditions. P. 122-124. In: Proc. 26th South. For. Tree Impr. Conf.
- Kang, K.-S., D. Lindgren, and T.J. Mullin. 2001. Prediction of genetic gain and gene diversity in seed orchard crops under alternative management strategies. Theor. Appl. Genet. 103:1099-1107.
- Kang, K.-S., Lindgren, D., and Mullin, T.J. 2004. Fertility variation, genetic relatedness, and their impacts on gene diversity of seed from a seed orchard of *Pinus thunbergii*. Silvae Genet. (in press)
- Kegley, A.J., S.E. McKeand, and B. Li. 2004. Seedling evaluation of Atlantic Coastal and Piedmont sources of loblolly pine and their hybrids for height growth. South. J. Appl. For. (In press).
- Lai, C.T., G.G. Katul, J. Butnor, M. Siqueira, D. Ellsworth, C. Maier, K. Johnsen, S. McKeand, and R. Oren. 2002. Modeling the limits on the response of net carbon exchange to fertilization in a southeastern pine forest. Plant, Cell, and Environment 25:1095-1119.
- Hu, X. and B. Li. 2001. Assessment of the ratio of pollen to seed flow in a cline for genetic variation in a quantitative trait. Heredity 87:400-409.
- Hu, X. and B. Li. 2002. Seed and pollen flow and cline discordance among genes with different models of inheritance. Heredity 88:212-217.
- Hu, X.S. and B. Li. 2002. Linking the evolutionary qualitative genetics to conservation of genetic resources in natural forest populations. Silvae Genetica 51:20-31.
- Hu, X.S., W. Zeng and B. Li. 2003. Impacts of one-way gene flow on genetic variance components in a natural population. Silvae Genetica 51:18-24.
- Hu, X.S. and B. Li. 2003. On migration load of seeds and pollen grains in a local population. Heredity 90:162-168.
- Isik, F., K. Isik, T. Yildirim, and B. Li. 2002. Using shoot growth patterns to select desired genotypes and understanding adaptation of *Pinus brutia*. Tree Physiology 22:51-58.
- Isik, F., S. Keskin, and S.E. McKeand. 2001. Provenance variation and provenance-site interaction in *Pinus brutia* Ten.: consequences on the defining of breeding zones. Silvae Genet. 49: 213-223.
- Isik, F. and B. Li. 2001. Use of resistograph for wood density measurements. Abstract (p. 120) Proc. 26th Southern Forest Tree Improvement Conference, Athens, GA, June 26-29, 2001.
- Isik, F., B. Li and J. Frampton. 2001. Additive and non-additive genetic parameters from replicated clonal tests of loblolly pine. P. 143-146, In: Proc. 26th Southern Forest Tree Improvement Conference, Athens, GA, June 26-29, 2001.
- Isik, F., B. Li, and J. Frampton. 2003. Additive, dominance and epistatic genetic variance estimates from a replicated clonal test of loblolly pine. Forest Science 49:77-88.
- Isik, F. and B. Li. 2003. Rapid assessment of wood density of live trees using the Resistograph for selection in tree improvement programs. Can. J. For. Res. 33:2426-2435.
- Isik, F., B. Li, J. Frampton, and B. Goldfarb. 2003. Efficiency of seedlings and rooted cuttings for testing and selection in *Pinus taeda*. For. Sci. 50:44-53.
- Jansson, G., B. Li, and K. Hannrup. 2003. Time trends in genetic parameters for height and optimal age for parental selection in Scots pine. For. Sci. 49:696-705.
- Landsberg, J.J., K. H. Johnsen, T.J. Albaugh, H.L. Allen, and S.E. McKeand. 2001. Applying 3-PG, a simple process-based model designed to produce practical results, to data from loblolly pine experiments. For. Sci. 47: 43-51.

- Li, B. 2003. Implementation of Third-Cycle loblolly Pine Breeding Plan – N. C. State University-Industry Cooperative Tree Improvement Program. Abstract, Proc. 27th Southern Forest Tree Improvement Conference, Stillwater, OK, June 24-27, 2003.
- Li, B. 2001. Hybrid aspen heterosis and breeding. In: P. Pulkkinen, P. M.A. Ingerstedt and V. Rajja (eds.), *Aspen in paper making*. P. 8-25, University of Helsinki Press, Helsinki, Finland.
- Li, B. and W. Zeng. 2001. Detecting major genes of quantitative traits using phenotypic data. Abstract, Invited Paper at IUFRO Tree Biotechnology Symposium: Tree Biotechnology in the New Millennium, Skamania Lodge, Stevenson, WA. July 22-27, 2001
- Li, H., H. Amerson and B. Li. 2003. Time Trend and Genetic Difference of Rust Infection in a Diallel Loblolly Pine Population Across Four Tests. Abstract. In: Proc. 27th Southern Forest Tree Improvement Conference, Stillwater, OK, June 24-27, 2003.
- Li, H., H. Amerson and B. Li. 2003. Complementary Pairs Interaction of Resistance Genes and Avirulent Genes in Loblolly Pine: Fusiform Rust Pathosystem using Diallel Data. Abstract. In: Proc. 27th Southern Forest Tree Improvement Conference, Stillwater, OK, June 24-27, 2003.
- Lstibůrek M. and Mullin T.J. 2003. Evaluation of an open-nucleus model for forest tree breeding. Pp. 77-80 In: Proceedings of the 27th Southern Forest Tree Improvement Conference, Oklahoma State University, Stillwater, Oklahoma, June 24-27, 2003.
- Lstibůrek, M., and Mullin, T.J. 2003. Can clonal replication of genetic tests for advance-generation selection eliminate the need for polycross assessment of breeding value? Pp. 60 In: Proceedings of the 28th Canadian Tree Improvement Association. Part 2.
- Lstibůrek M., T.J. Mullin, D. Lindgren and O. Rosvall. 2004. Open-nucleus breeding strategies compared to population-wide positive assortative mating. I. Equal distribution of testing effort. *Theoretical and Applied Genetics* (in press).
- Lstibůrek M., T.J. Mullin, D. Lindgren and O. Rosvall. 2004. Open-nucleus breeding strategies compared to population-wide positive assortative mating. II. Unequal distribution of testing effort. *Theoretical and Applied Genetics* (in press).
- McKeand, S.E., H.L. Allen, and B. Goldfarb. 2001. Tree improvement and intensive silviculture - productivity increases from modern plantation methods. Invited Paper. P. 99-112. In: Proc. 14th CAETS Convocation. World Forests and Technology. June 11-15, 2001. Espoo, Finland.
- McKeand, S.E., H.V. Amerson, B. Li, and T.J. Mullin. 2003. Families of loblolly pine that are most stable for resistance to fusiform rust are the least predictable. *Can. J. For. Res.* 33:1335-1339.
- McKeand, S.E., J.E. Grissom, R. Rubilar, and H.L. Allen. 2003. Responsiveness of diverse families of loblolly pine to fertilization: eight-year results from SETRES-2. P. 30-33. In: Proc. 27th South. For. Tree Impr. Conf., Stillwater, OK.
- McKeand, S., T. Mullin, T. Byram, and T. White. 2003. Deployment of genetically improved loblolly and slash pine in the South. *J. For.* 101(3): 32-37.
- McKeand, S.E., T.J. Mullin, and B. Li. 2003. Genetic diversity in intensively managed loblolly pine plantations. Invited Paper for Society of American Foresters National Convention. Buffalo, NY (In press).
- Mullin, T.J. 2002. Consensus document on the biology of *Pinus strobus* L. (eastern white pine). Series on Harmonization of Regulatory Oversight in Biotechnology No. 22, Organization for Economic Cooperation and Development, Paris. 50 pp.
- Mullin, T.J. 2002. Consensus document on the biology of *Picea sitchensis* (Bong.) Carr. (Sitka spruce). Series on Harmonization of Regulatory Oversight in Biotechnology No. 21, Organization for Economic Cooperation and Development, Paris. 53 pp.
- Mullin, T.J. 2002. Applied forest tree breeding in the Southeastern United States – quantitative genetics at work! Invited presentation. Pp. 19-20 In: “Quantitative Genetics and Efficient Plant Breeding”, Abstracts of presentations at the 28th Nordic Postgraduate Course in Plant Breeding, 21-26 January 2002, Marholmen, Sweden.
- Mullin, T.J. 2002. Analyzing breeding strategy options for out-crossing species and their impacts on gain and genetic resource management. Invited presentation. Pp. 21-22 In: “Quantitative Genetics and Efficient Plant Breeding”, Abstracts of presentations at the 28th Nordic Postgraduate Course in Plant Breeding, 21-26 January 2002, Marholmen, Sweden.
- Olsson, T., D. Lindgren, and B. Li. 2002. Balancing genetic gain and relatedness in seed orchards. *Silvae Genetica* 50:222-227.
- O'Malley, D., J. Scott, D. Harkins, J. Kadla, S. McKeand, and H-M Chang. 2001. Cinnamyl alcohol dehydrogenase (cad) and genomic approaches to manipulating wood properties in loblolly pine. P. 19-24. In: 7th Brazilian Symposium on the Chemistry of Lignins and Other Wood Components. Belo Horizonte, MG, Brazil.
- Retzlaff, W.A., J.A. Handest, D.M. O'Malley, S.E. McKeand, and M.A. Topa. 2001. Whole-tree biomass and carbon allocation of juvenile trees of loblolly pine (*Pinus taeda*): influence of genetics and fertilization. *Can. J. For. Res.* 31:960-970.
- Roberds, J.H., B.L. Strom, F.P. Hain, D.P. Gwaze, S.E. McKeand, L.H. Lott. 2003. Estimates of genetic parameters for oleoresin and growth traits in juvenile loblolly pine. *Can. J. For. Res.* 33: 2469-2476.
- Rosvall, O., D. Lindgren, and T.J. Mullin. 2003. Controlling parent contributions during positive assortative mating and selection increases gain in long-term forest tree breeding. *For. Genetics* 10(1):35-53.
- Rosvall, O., and Mullin, T.J. 2003. Positive assortative mating with selection restrictions on group coancestry enhances gain while conserving genetic diversity in long-term forest tree breeding. *Theor. Appl. Genet.* 107: 629-642.
- Rubilar, R., S.E. McKeand, and H.L. Allen. 2003. Dominance and stand structure analysis in a GxE interaction trial. P. 34-37. In: Proc. 27th South. For. Tree Impr. Conf., Stillwater, OK.

- Schmidtling, R.C., T.L. Robison, S.E. McKeand, R.J. Rousseau, H.L. Allen, and B. Goldfarb. 2003. The role of genetics and tree improvement in southern forest productivity. Chapter in: Rauscher, H.M. and K. Johnsen. Southern Forest Science: Past, Present, Future. Government Printing Office, Washington, DC. (In Press).
- Sierra-Lucero, V., D.A. Huber, S.E. McKeand, T.L. White, and D.L. Rockwood, D.L. 2003. Genotype-by-environment interaction and deployment considerations for families from Florida provenances of loblolly pine. *Forest Genetics* 10(2):85-92.
- Sierra-Lucero, V., S.E. McKeand, D.A. Huber, D.L. Rockwood, and T.L. White. 2002. Performance differences and genetic parameters for four coastal provenances of loblolly pine in the southeastern United States. *For. Sci.* 48:732-742.
- Sykes, R., F. Isik, B. Li, J. Kadla, and H-m. Chang. 2003. Genetic variation of juvenile wood properties in a loblolly pine progeny test. *TAPPI* 86(12): 3-8.
- Tang, W., Mullin, T.J., and Newton, R.J. 2003. Genetically engineering trees to enhance productivity, reduce disease, and improve wood properties. *Recent Res. Devel. Plant Sci.* 1: 209-230.
- Wu, R., B. Li., S. S. Wu, and G. Casella. 2001. A maximum likelihood-based method for mining major genes affecting a quantitative character. *Biometrics*, 57(3):764-768.
- Xiang, B. and B. Li. 2001. A new mixed analytical method for genetic analysis of diallel data. *Can. J. For. Res.* 31: 2252-2259.
- Xiang, B. and B. Li. 2003. Best Linear Unbiased Prediction of Clonal Breeding Values and Genetic Values from Full-sib Mating Designs. *Can. J. For. Res.* 33:2036-2043.
- Xiang, B. and B. Li. 2001. Optimal method for analysis of disconnected diallel tests. P. 107-111, In: Proc. 26th Southern Forest Tree Improvement Conference, Athens, GA, June 26-29, 2001.
- Xiang, B., B. Li, and S.E. McKeand. 2003. Genetic gain and selection efficiency of loblolly pine in three geographic regions. *For. Sci.* 49:196-208.
- Xiang, B., B. Li, and F. Isik. 2003. Time trend of genetic parameter estimates in growth traits of *Pinus taeda* L. *Silv. Genet.* 52:114-121.
- Zeng, W. and B. Li. 2003. Power and robustness of statistical tests for major gene detection in diallel progeny test data. *For. Sci.* 48: 268-278.
- Zeng, W., S. Ghosh and B. Li. 2004. Blocking Gibbs Sampling with a Mixed Inheritance for Major Gene Detection. *Genetical Research.* 84:1-12.
- Zobel, B.J. 2004. Biological improvement of wood properties. Chap. In *Encyclopedia of Forest Sciences.* Academic Press.



Keeping Coop Directors in-line is a tough job, but someone's gotta do it! As Field and Lab Manager, Paula Zanker worked with all three of the Coop's Directors, past and present, who turned out to express their appreciation for her valuable contributions at the farewell Pig Pickin' held in her honor. (from left to right: Bob Weir, Paula Zanker, Bruce Zobel and Tim Mullin)

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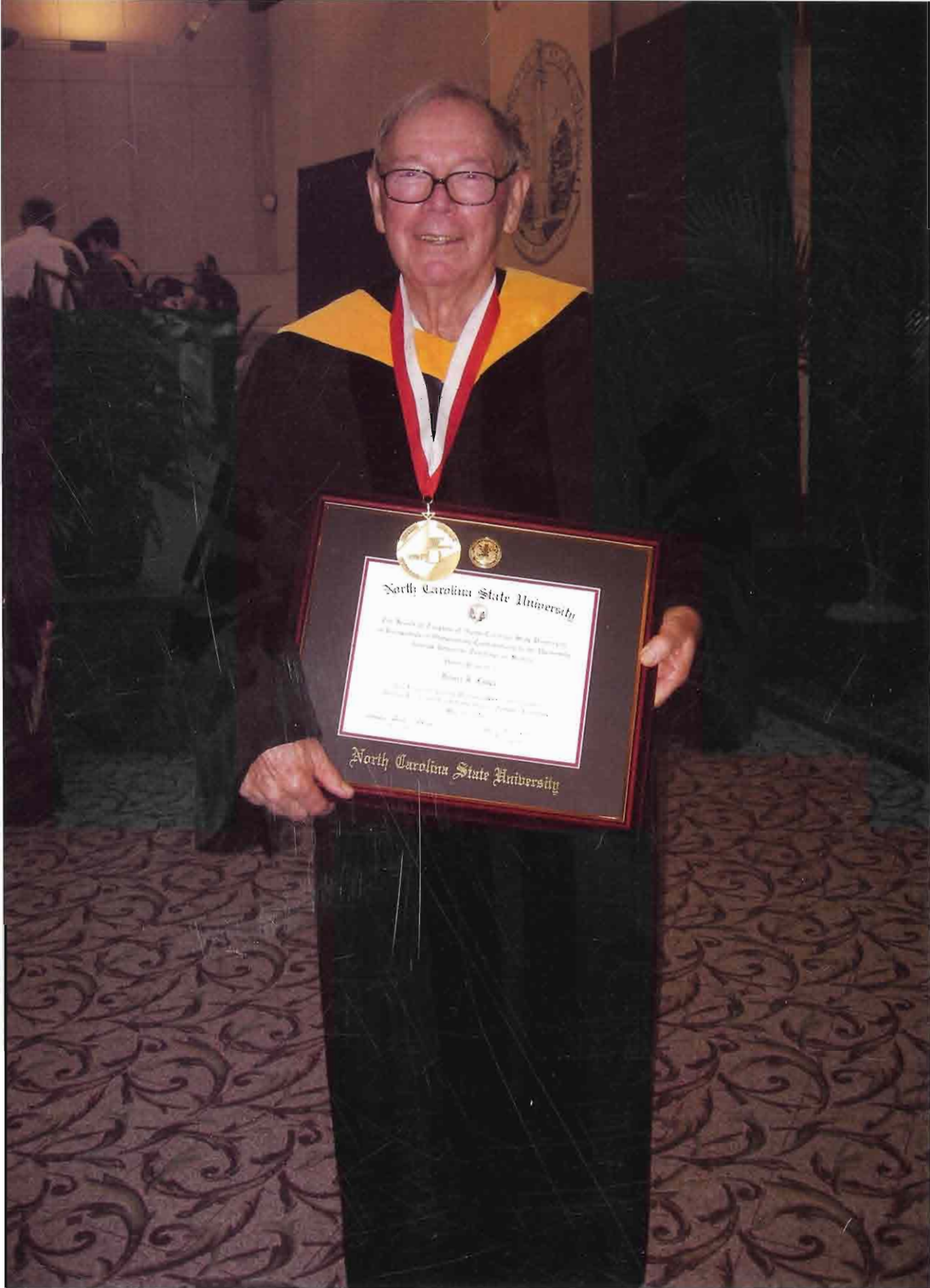
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Back cover:

The NC State University Board of Trustees awarded the Alexander Quarles Holladay Medal for Excellence to the Cooperative's founding director, Dr. Bruce Zobel, in recognition of his outstanding career at NCSU. The Holladay Medal is the highest honor bestowed on a faculty member by the trustees and the university.



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