

North Carolina State University – Industry Cooperative Tree Improvement Program

46TH ANNUAL REPORT



Department of Forestry
College of Natural Resources
North Carolina State University

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Front Cover:

Many of our Coop members are now carrying out polycross breeding of third-cycle selections that have been grafted into the tops of older trees. These “top-grafts” flower much more quickly than grafts made on small rootstock. Here, only one year after top-grafting, female strobili from a third-cycle selection are at “stage 5”, the optimum time for controlled pollination, when the opening between bracts and scales is at a maximum (photo courtesy Brad Kuegel, Weyerhaeuser Corp.)

EXECUTIVE SUMMARY

BREEDING, TESTING, AND SELECTION

During this past year, Cooperative members screened another 166 tests, bringing the total number of tests screened to about 970. About 87% of the 1,118 progeny tests established in the second cycle of breeding have now been measured, screened and selection completed. The past year's efforts yielded 114 new 3rd-cycle selections, bringing the total to 892.

The pace of 3rd-cycle breeding picked up in 2002. Our first priority is still to create the checklot seed needed for the next round of testing. For those parents that had extra flowers, polycrossing with the regional pollen mix and controlled crosses among parents within surlines were initiated. Well over 1,000 bags were pollinated this spring.

Details of the Coop's progeny testing plan for the third cycle were finalized. A complementary mating design is being used, with polycross progeny tested in single-tree plots in a randomized complete block designs at several sites, and block selection plots of full-sib crossed established at two sites.

PROGRESS REPORTS FOR RESEARCH

Analysis of wood cores collected from 10-year-old seedlings and rooted cuttings from 9 full-sib families established at two test locations showed no significant effect of plant propagation method on specific gravity. While genetic effects for specific gravity were strongly additive, greater gains are possible by deploying selected full-sib families or clones.

Quick wood density assessments using the Resistograph® were compared with specific gravity determined from 12-mm cores using the more conventional, but time-consuming, gravimetric method. The Resistograph measurements were as reliable as the conventional method for family-level selection and parental GCA estimates. Individual breeding values were similar, and culling the worst one-third of individuals on the basis of Resistograph measurements rarely resulted in the loss of an individual ranking in the top third by conventional specific gravity determination.

A re-evaluation of a resistance screening study established at 28 sites revealed that the families that are most resistant to fusiform rust are also the least stable. Nevertheless, a bulk seedlot of the 6 most resistant families would provide stable and predictable performance across a range of sites.

A genetics demonstration plot at the University's Schenck Memorial Forest provided an opportunity to conduct thinning trials "on paper", to determine the value of residual trees in both unimproved and genetically improved plots. Trees from improved seed sources grew faster and had better stem qualities such as better straightness and less forking. Having fewer cull trees should make thinning operations more flexible and increase the value of the residual stand.

A computer simulation study explored the possibility that clonal replication of progeny tests might eliminate the need for a polycross test to estimate breeding values. Clonal replication gave better results, even when the cost of propagation was five times higher than that for seedlings. Work on this project continues with additional simulations.

SEED PRODUCTION

The 2001 seed collection for the Cooperative provided 25.5 tons of loblolly pine seed (last year's crop was 36.9 tons), and for the first time we have measurable yields from two, third-cycle seed orchards! Seed yields in pounds per bushel were also less than last year (1.21 vs. 1.30 in 2000). About 70% of the total seed came from second-generation orchards and about 85% of the collection was from the Coastal source.

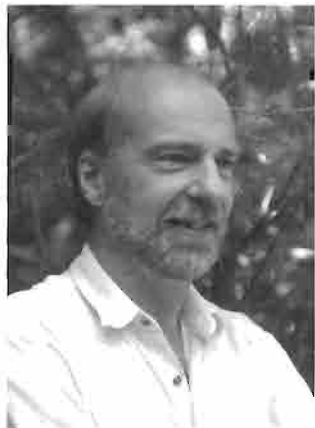
ASSOCIATED ACTIVITIES

All Cooperative staff on faculty were active in delivery of important courses in Forest Genetics, to students at both the undergraduate and graduate levels. During the past year, 10 graduate programs were under development or were completed in association with the Tree Improvement Cooperative. The Coop also had 2 post-docs working with us this year, and two visiting scientists from Sweden. The Tree Improvement Coop's founding director, Bruce Zobel celebrated 60 years of marriage to Barbara. As of May 2002, membership in the Coop stood at 16, with 6 state agencies and 10 private corporations.

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INTRODUCTION



“So, Tim, what’s been the key to the Coop’s longevity?”

That question came from the lips of Dr. Larry Nielsen, Dean of the College of Natural Resources here at NCSU. Dr. Nielsen had assumed his new duties as Dean in August 2001. I’d given him a couple of months to drive the alligators back into the swamp and find the men’s room, then I’d asked for an audience – figured it was time for “the new Dean” to get his introduction to NCSU’s longest-running university-industry research cooperative.

I’d arrived for my appointment, curious to know how this fellow would react to the story I was about to tell him of 46 years of university-industry cooperation. I didn’t have to wait long – he was right on time. Walking into his office, I noticed he had a rather large sign sitting by his desk that reads: “*The only rule is to break all the rules.*” Hmm, there must be a story behind that, I thought, but I could like this guy.

It was just me and the Dean. We exchanged some small talk and commiserated on the State budget woes that were (and still are) hitting the College hard, along with the rest of the University. Then I opened up my laptop and started into the briefing presentation I’d prepared. I went through our history, how it all began, the gains we realized through our first two cycles of breeding, our plans for the third, how are membership and staff have changed over the years, how industry had changed over that period, the impact of the restructuring of industry in recent years, the internal review we’d just completed to figure out how best to cope with these changes. As we moved through my presentation, Dr. Nielsen listened attentively, asking a question from time to time to clarify his understanding, but leaving me to deliver my material in the way I’d planned.

Then, as I finished up and sat back to get into a less formal discussion, Dr. Nielsen comes out with his key-to-longevity question. “Well, Larry”, I started, figuring that we must by now be on a first-name basis, “it can’t be me, ‘cause I’ve only been here a little more than a year.” It was obvious I was buying time, but Larry just smiled and waited for a more thoughtful answer.

“So you told him the key was having the best damn Coop staff in the world, right?” – that suggestion later from a long-time staffer who shall remain nameless, but he does have a point. People DO make a difference; ours are tremendously dedicated and their accomplishments truly inspirational.

What I answered to Larry was that the Tree Improvement Program had a clearly identifiable deliverable that made a real difference to our members: genetically improved trees. You can see them. You can touch them. You can know their quality and put a real value on them. The main deliverable of our program is truly owned by our members. Only our members know exactly its value and they control its use. We’ve been improving the quality of that deliverable for 46 years, and our members have done the research themselves to know it will get even better. The research and teaching we do at the university continues to have a direct impact on the genetically improved seedlings deployed by our members and sold to their customers. We’ve not gone to our members looking for support of our research. We’ve treated them as clients, offered them a service and delivered a product.

Ultimately, the key *is* the trees, and they keep getting better.

Tim Mullin, Director

BREEDING, TESTING AND SELECTION

THIRD-CYCLE BREEDING UPDATE

With the third cycle selection work almost complete, subline groups have been formed for breeding to begin. Since 1999, Cooperators have collected pollen to send to NCSU to be used for pollen mixes for the Northern, Coastal, and Piedmont testing zones. Pollen from 20 clones in each zone is to be mixed and used for polycross breeding in this next cycle. In 2002, we received enough pollen to make a mix of 2140 cc's for the Coastal zone, 1890 cc's for the Piedmont zone and 1490 cc's for the Northern zone.

The Cooperative's emphasis for the past few years has been twofold:

1) Collect more pollen from all three breeding zones to store for breeding. The designated pollen parents have also been assigned to backup organizations for grafting over the next couple years, assuring that we will not lose the pollen parent, and thus the PMX, if an orchard is lost.

2) Third-cycle breeding continued in a more lively fashion in 2002 than in the past couple of years. Our first priority is still to create the checklot seed needed for the next round of testing. Even though some seed has been collected, we still need quite a bit more to reach our goal. Ten clones were designated in each of the testing zones as female parents for checklot seed. These clones will be crossed with the PMX for that zone to create checklot seed. Our calculations show that total checklot seed needs for the three zones are as follows: Northern 600 seed per parent; Coastal 1500 seed per parent; and Piedmont 800 seed per parent. For the organizations who were ready to cross this spring, we suggested they pollinate about one-third of the total needed for the checklots. Any pollen left after pollinating the checklots was used in crossing sublines. Well over 1,000 bags were pollinated this spring.

THIRD-CYCLE SELECTION PROGRESS

During this past year, Cooperative members selected trees in another 166 tests, bringing the total number of tests screened to about 970. About 87% of the 1,118 progeny tests established in the second cycle of breeding have now been measured, screened and selection completed. The past year's efforts yielded 114 new selections, bringing the total to 892 for the third cycle. Of this total, 390 selections are for the Coastal program, 359 are for the Piedmont program, and 143 are for the Northern program.

New sublines were assigned in the last year and, in a few cases, previously formed sublines have been revised as conditions warrant. These assignments will facilitate breeding activities of the mainline breeding population. The progress of subline formation within the breeding programs is summarized in **Table 1**.

Third-cycle selection is reaching the final stages. The data for the final 18 diallel test series will be collected and analyzed in 2002, so that screening and identifying of the final selections will occur in 2003. All third-cycle selections are being grafted into breeding orchards and a few of the "truly elite" selections are being incorporated into new seed orchards.



Richard Bryant (left) and Greg Leach, both with International Paper, bag another 3rd-cycle selection.

Table 1. Summary of third-cycle breeding sublimes for the Coastal, Piedmont, and Northern breeding programs

| Program - region | Number of sublimes | Number of cooperators | Number of selections | Total % gain in height | Total % gain in volume |
|---------------------------|--------------------|-----------------------|----------------------|------------------------|------------------------|
| Coastal- Atlantic Coastal | 11 | 4 | 129 | 11 | 28 |
| Coastal- Florida | 2 | 2 | 27 | 18 | 60 |
| Coastal- Lower Gulf | 9 | 6 | 107 | 11 | 29 |
| Piedmont- GA and SC | 11 | 6 | 128 | 12 | 38 |
| Piedmont- Upper Gulf | 5 | 4 | 61 | 11 | 34 |
| Northern zone | 6 | 4 | 81 | 8 | 26 |
| TOTALS | 44 | | 533 | | |



2002 saw a strange mix of spring weather throughout the South, but that didn't slow down flowering at the North Carolina Forest Service seed orchard in Goldsboro, N.C.

EXPERIMENTAL DESIGN FOR THIRD-CYCLE PROGENY TESTS

As the Cooperative Tree Improvement Program progresses towards the beginning of the third-cycle breeding program, a comprehensive and cost-effective progeny testing plan is being finalized for the Cooperative breeding program. Progeny tests will follow the three general pollen mix zones as outlined in the third-cycle breeding strategy: 1) Virginia and northern NC, 2) Atlantic coastal plains and Lower Gulf, 3) Piedmont regions of SC and GA and Upper Gulf. The mating design for the mainline breeding is a complementary design that combines polycross mating to estimate breeding values and control-pollinated mating for within-family selection. Thus, different experimental designs will be used for the polycross and controlled crosses.

The polycross progeny tests are to estimate breeding values and rank selections among different sublines and within sublines in the breeding population. The polycross information is also useful to select the best parents for elite breeding populations for determining the composition of new seed orchards, and to determine the full-sib families from which selections will be made for the next round of breeding. Thus, it is important to have reliable breeding value estimates and efficient comparison of breeding values for all third-cycle selections in the mainline breeding population.

To determine reasonable experimental design for the polycross tests, age 4 data from the Cooperative's Plantation Selection Seed Source Study (PSSSS) polycross tests were analyzed to provide some guidelines. Briefly, the experimental design for the PSSSS trials was based on single-tree-plots, 24 blocks per location, 140 families plus 4 checklot seedlings. Eight PSSSS tests, with 80 families, from Coastal Plains of NC, SC, GA, FL, and AL were used for the calculations. Based on the combined analysis of variance, error variances of family means were estimated and the number of trees per family (e.g., locations and blocks within locations) was varied to determine the impact of different sample sizes on precision of family mean estimates. With the variance components estimated,

the error around a family mean was determined as the coefficient of variation of family mean (CVf). By varying the number of tests and reps (**Figure 1**), the effects of blocks and tests on precision can be examined. It is obvious that after 15 to 20 blocks per test, there is little improvement in test precision. As the number of tests increases, the error variance of family mean can be further reduced.

Given the various breeding population sizes for

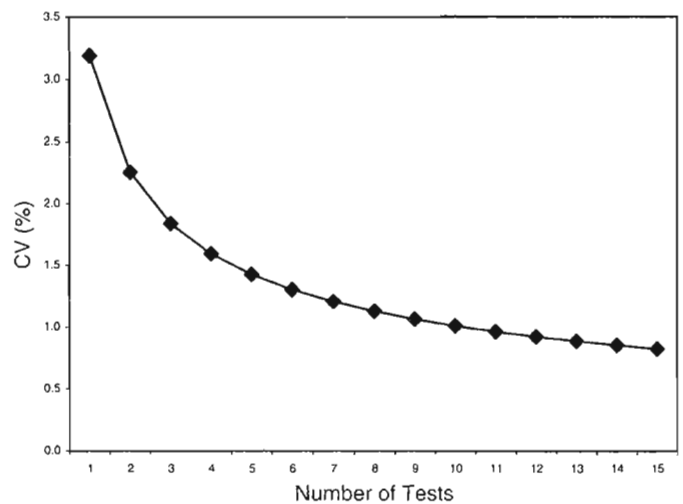


Figure 1. Impact of number of blocks per test on the precision of the estimate of family means for height at age 4 years. Vary the number of tests from 6 to 12 in this example

each geographic zone, if we set the number of blocks to a conservative number of 20, the expected impact on the precision of family means is given in **Figure 2**. The total number of tests varies by geographic zones due to different population size: Coastal: 15 tests, Piedmont: 10 tests, and Northern: 8 tests.

Based on the PSSSS results, the third-cycle breeding plan, and other practical consideration in breeding, a polycross test series will be established with 60 polycross families plus several checklots in multiple locations. The experimental design for each test will be a randomized completed block design with a single-tree plot design of 20 replications. The single-tree plot design is efficient for estimating family breeding values while providing an opportunity to test more selections together in a test series. Given a census population

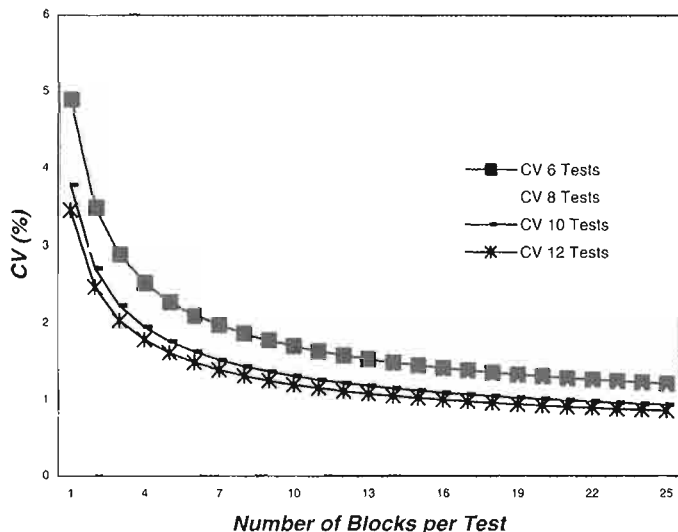


Figure 2. Impact of number of test on the precision of the estimate of family means for height at age 4 years. Assume that the number of blocks per tests is 20.

size of 300, about 6-8 test series are needed for a pollen mix zone, with some families overlapping in different test series. Assuming 60 polycross families, 5 checklots and 20 replications, a total of 1300 seedlings (plus borders) will be planted in each test.

A common checklot will be developed for each of the three polycross zones by using the same 20-parent pollen mix applied onto 10 of the selections in the mix. Several well characterized seedlots, good and average performers, from each zone will be included in each polycross test series. The common checks for and the special checklots are used for evaluating genetic materials among test series within a pollen mix zone and among zones. A separate test series that includes unimproved checklots and the common checklot will be established in each zone to establish the relationship between this common checklot and unimproved loblolly pine. This relationship will be used to estimate genetic gains in percent gain over unimproved check lots in the future breeding cycles. By planting well-characterized seedlots, it will provide the reliability to transform measurements to relative breeding values against "known" families, spanning a range of breeding values.

Tests in each test series will be evenly distributed across the geographic range within each testing zone, at least one test per member in its land holding and 3-4 tests in each of old test regions (as defined for the second-cycle progeny test). With the differences in each of three geographic testing zones and participating members, different numbers of tests will be established for each test series in different zones. To evaluate selections across the wide geographic range of the Coastal zone, the 15 tests will be established for each test series across the Atlantic coastal and Lower Gulf regions. In the northern zone, a much smaller geographic area, 8 tests per test series will be established. The Piedmont zone will require 10 tests per test series to cover the test region. Seedlings will be assessed at age 3 for height and fusiform rust, and these tests will be kept and measured again at 6-8 years for final assessment. These data will be very valuable for seed orchards establishment in different regions. Each cooperative member is encouraged to plant more tests on their own lands in different planting regions.

Within each subline, the controlled progenies will be generated from a modified half-diallel mating design for generating full-sib combinations for selection. With the information from polycross, the number of controlled pollination will be reduced by only making the most desirable crosses for forward selection. Because of the mating, more progenies from the top ranked selections will be available for selection. Seedlings from the controlled crosses will be planted in full-sib family blocks for within family selection. The family block plots can be established as soon as seeds are available. A block of 36 trees will provide adequate selection intensity for within family. While no replication is needed for within family selection, a second 36-tree block (plus border trees) will be planted on a different site by a different cooperator for insurance purposes. Selection of individual trees within full-sib families is expected to be more effective at a later age (i.e., age 5-6) than family selection based on polycross. The best phenotypes will be selected from the family block plots. Tests will be established on typical sites for rust infection level and conventional spacing is desired for late

measurements of elite breeding and deployment.

In summary, the polycross is to estimate breeding values to rank selections among different sublines and then followed by the diallel mating to provide the progeny for within-family selection. Polycross tests will be established with a single-tree plot design on different sites in 8-15 tests, while diallel crosses will be planted in full-sib family blocks for within family selection (36-tree block plot on two sites). Polycross tests will be evaluated at age 3 for among family selection and the best individuals will be selected from desirable family blocks at age 5-6. The new selections from each subline will constitute the new breeding population size for next cycle breeding.



Participants at the 2001 Tree Improvement Short-Course. The Tree Improvement Coop offers the course every 2 or 3 years, to bring the latest tree improvement techniques to the employees of our member agencies. In 2001, the short-course was held in Charleston, South Carolina, with field visits coordinated by MeadWestvāco (then Westvāco).

RESEARCH

GENETIC VARIATION OF SPECIFIC GRAVITY IN A CLONAL STUDY OF LOBLOLLY PINE

With an increased proportion of the wood supply in the south coming from intensively grown plantations, improvements in wood quality are becoming increasingly important. Genetic gains in wood quality may be achieved by controlled pollination and/or vegetative propagation. These strategies can increase the genetic quality of reforestation stock by eliminating pollen contamination and increasing selection intensity. While potential improvements have been estimated for growth and disease resistance (Frampton et al. 2000), information is limited on potential improvements in wood properties of loblolly pine. A clonally replicated trial provided an excellent opportunity to study the genetic variation of specific gravity. The specific objectives were to: determine if differences occur in wood density between seedlings and rooted cuttings; to determine the variation in wood density among and within families and across sites; and to compare uniformity between seedlings and clones for wood density.

Materials and Methods

The trial consisted of full-sib seedlings and rooted cuttings (clones) from a 3x3 factorial mating (6 parents and 9 full-sib families) planted in Nassau County, Florida (Rayonier), and Monroe County, Alabama. (Joshua Management). The experimental design was a split-plot with six replications on each site. Within each replication, seedlings and clones were planted as whole plots, with two individuals per plot. For the seedling plots, there were 3 sub-plots per family per replication (six seedlings per family per replication). In rooted cutting plots, there were 5-9 clones per family per replication, with each clonal sub-plot consisting of two ramets.

Increment cores were taken from surviving trees on each site during March and May of 2001. Wood

specific gravity was determined by the volumetric method. Growth measurements of tree height and DBH were also recorded to determine correlations between growth and wood density. Wood cores were then scanned using an X-ray densitometer (University of Georgia and USFS) for ring-density analysis.

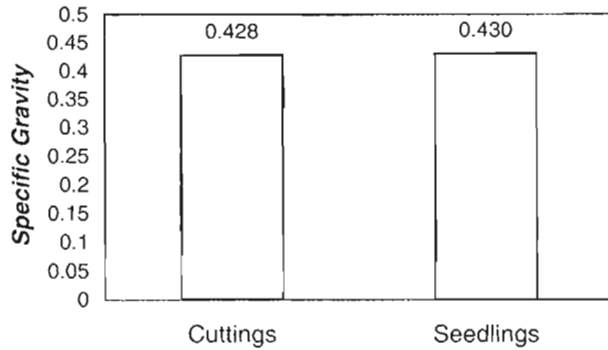
A linear model (GLM procedure in SAS) was used to analyze wood specific gravity of rooted cuttings and seedlings as a factorial mating design. The model included female parent, male parent, clone, replication, propagule type, site and their interactions, where applicable. Significant differences among means for propagule type, male parents and female parents were tested using Duncan's multiple range test within the GLM procedure. Individual, half-sib family mean, full-sib family mean, and clone mean heritabilities were estimated separately for rooted cuttings and seedlings. Linear models were analyzed separately for each propagule type using the GLM procedure. Variance components were then partitioned using the VARCOMP procedure.

For measuring the uniformity of wood density from the whole-core data, a coefficient of variation was calculated for ramets within a clone for rooted cuttings and for seedlings within full-sib families.

Analyses have begun on the ring density data generated from X-ray densitometry. Latewood percent has been examined by individual rings to compare the transition age from juvenile to mature wood in seedlings and rooted cuttings. A model similar to that used in the whole core density analysis was used to test for significant differences in individual rings.

Preliminary Results

No significant difference was detected between seedlings and rooted cuttings for the average specific gravity (**Figure 3**). Seedlings and rooted cuttings had similar values for specific gravity at age 10.



However, there were significant genetic

Figure 3. Mean specific gravity of rooted cuttings and seedlings across two sites.

differences in specific gravity among parents, full-sib families and clones. GCA values varied among parents (**Figure 4**). For rooted cuttings, the effect of the female parent was significant, as was the female by male interaction, clone within families and the clone by site interaction. The analysis of seedlings also found a significant female effect and a significant female by site interaction.

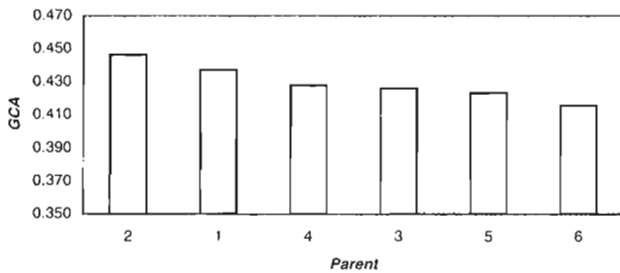


Figure 4. General Combining Ability of Parents for specific gravity.

Family means for specific gravity ranged from 0.409 to 0.457 while clone means range from 0.377 to 0.499. The site effect on specific gravity was significant for both rooted cuttings and seedlings. The CV for clone means (4.2%) is less than the CV for seedling means (5.7%), suggesting there would be greater uniformity of specific gravity in a monoclonal block than in a stand planted with

seedlings of a full-sib family.

Heritabilities were higher for rooted cuttings than seedlings for full-sib families, while the estimate for half-sib families was higher for seedlings. (**Table 2**). Clone mean heritability (H^2_C) was much greater than individual seedling heritability (h^2_i), due to less error variance in rooted cuttings (25.8%) compared to seedlings (52.9%). Replicating the genotype allowed for more variance to be partitioned into genetic components, leaving a smaller error variance.

Table 2. Heritability estimates for wood specific gravity

| Heritability estimate | Rooted cutting | Seedling |
|-----------------------|----------------|----------|
| h^2_i | - | 0.12 |
| h^2_{hs} | 0.86 | 0.94 |
| h^2_{FS} | 0.97 | 0.91 |
| H^2_C | 0.97 | - |

Wood specific gravity was found to have a strong negative family mean (phenotypic) correlation to tree volume (-0.79) in this study, which was significant; However, there were clones with moderate to good growth and higher density. This negative relationship may be due to the limited sampling of 6 parents and 9 full-sib families used in this study. In other loblolly pine studies with larger population sizes, growth and wood density showed no special relationship.

Preliminary results from individual ring analysis showed that ring density at years 3, 5, 6, and 7 were significantly affected by site. Only the density in ring 3 had a significant difference between propagule types (seedling and rooted cuttings). There were significant differences among female parents for rings 3 and 5, while the effect of male parent was not significant for any ring.

General trends of latewood percentage over time were similar for seedlings and rooted cuttings

on both sites (**Figure 5**), while sites appeared to differ. The Florida site showed that latewood percentage increased to the 40-50% range in rings 4 and 5, while in Alabama latewood percentage reached the same range later between rings 6 and 7.

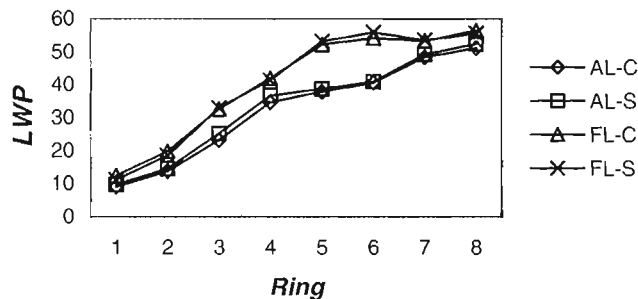


Figure 5. Plot of latewood percentage (LWP) against tree ring from the pith at DBH.

This study, though preliminary, is providing information on genetic variation of specific gravity within and among full-sib families of loblolly pine. Rooted cuttings appeared to be a viable alternative to seedlings for reforestation material, having no negative effect on specific gravity. This study also showed that while specific gravity was strongly additive, deploying full-sib seedlings or clones can capture greater gains in the improvement and uniformity of wood specific gravity in loblolly pine. The use of clonal rooted cuttings could reduce variation within plantations.

Reference:

Frampton, J., B. Goldfarb and B. Li. 2000. Early field growth of loblolly pine rooted cuttings and seedlings. *Southern J. Appl. For.* 24: 98-105.



M.S. student Patrick Cumbie sampled over 1,600 ten-year-old trees to estimate genetic parameters for wood density from seedlings and rooted cuttings of loblolly pine.

AN EFFICIENT METHOD FOR LARGE-SCALE SCREENING OF WOOD DENSITY IN PROGENY TESTS

As tree improvement and intensive silvicultural practices have decreased the rotation age of loblolly pine plantations, the quality of the wood has become an important issue in the South. Wood density is one of the most important characteristics that affects various end products; however, screening many trees from progeny tests for wood density has been very difficult with the methods available. Currently, wood density is most commonly determined by the volumetric method (dry weight per unit of green-wood volume). This method is generally reliable but time consuming for sample collection and laboratory processing, as well as expensive for large-scale screening. An efficient, reliable wood-density assessment method is needed.

The applicability of the Resistograph® to measure relative density of living trees has been investigated. The method is based on measuring the drilling resistance along a small needle path when inserted into a tree with a constant drive. The power consumption of the drilling device is measured electronically as a value of the drilling resistance, similar to a ring density profile (Figure 6). This tool can be used rapidly to drill many trees in a progeny test if a reliable relationship can be established with wood density. The objective of this study is to examine the relationship of Resistograph profiles and volumetric density of the same trees.

Materials and Methods

Fourteen full-sib families of loblolly pine, produced by a half-diallel mating design, were sampled from a test series at four test sites in South Carolina. A randomized complete block design with six replications was used in the field. Wood samples of 12-mm increment cores were taken from each tree at breast height (about 4.5 ft above ground level) using generator-powered drills. The number of increment cores included in the analysis ranged from 44 to 66 trees per family when all four sites were combined. The same trees were drilled at



Paula Zanker assists as Dr. Fikret Isik uses the Resistograph to produce a density profile for a mature loblolly pine.

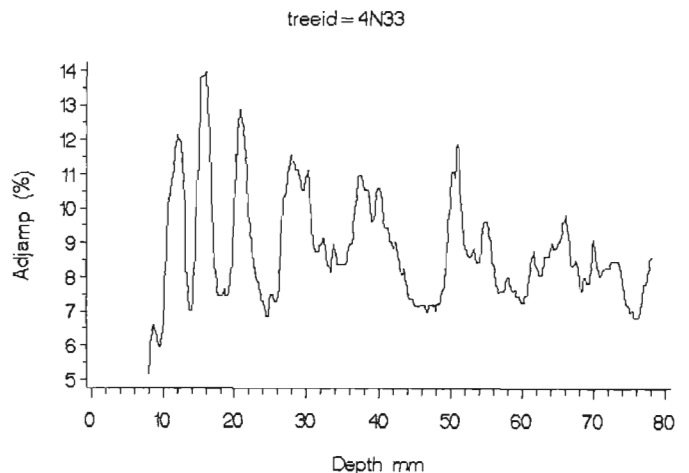


Figure 6. A Resistograph profile from the bark (left) to the pith (right) of a tree. The drilling was carried out at the breast height (4.5 ft) about 1.5 inches above the increment core's hole.

breast height using the B-400 Pro Resistograph, manufactured by IML Company. The drilling speed was set to 30 cm/minute (level 5) for all trees. The trees were drilled about 1.5 inches above the hole of the increment core, in order to reduce variation between the two data types. Wood density (kg/m³) was estimated by the standard method.

The Resistograph produced a profile when a tree was drilled, and the graphic file of each tree was converted to a text file of amplitude measures. The mean amplitude for each tree was estimated, and then wood density and amplitude (%) were then analyzed using an individual tree mixed model.

The genetic analyses were carried out using the mixed model for diallel tests (Xiang and Li 2001). Best linear unbiased prediction (BLUP) of breeding values of trees and genetic values of full-sib families for wood density and amplitude were estimated. Individual, family mean and within full-sib family heritabilities were estimated for wood density and amplitude using causal variance components.

Results and Discussion

Wood density and mean average amplitude of each tree had a moderate linear relationship (Figure 7). Average amplitude explained about 40% phenotypic variation in wood density ($R^2=0.40$). Phenotypic correlation between the two traits at each test site ranged from 0.52 (site 3) to 0.70 (site 4).

General combining ability (GCA) variance for wood density was about two times greater than that of amplitude (Table 3). Specific combining ability (SCA) variance was negligible and explained less than 1% of total phenotypic variance. For wood density, GCA x site interaction explained 1.5% of the variance, whereas this component was about 3% for amplitude. Plot-to-plot variances within sites were significant for both traits. Both traits had similar within-plot variances.

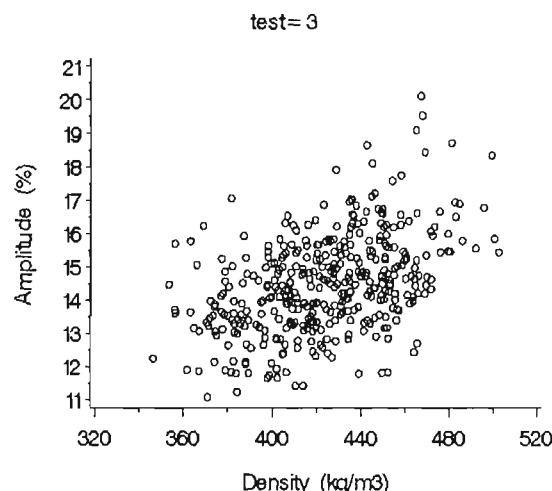


Figure 7. Relationships between average amplitude (from Resistograph) and gravimetric wood density at site 3 ($R^2=0.27$)

Table 3. Variance component estimates (\pm standard errors), percentage of variances in total phenotypic variance and heritabilities for wood density and amplitude.

| Source | Wood Density | | Amplitude | |
|------------|----------------------|------|----------------------|------|
| | Variance (\pm SE) | % | Variance (\pm SE) | % |
| G | 91.74 \pm 60.6 | 15.8 | 0.273 \pm 0.201 | 7.2 |
| S | 0.87 \pm 3.85 | 0.1 | 0.014 \pm 0.036 | 0.4 |
| GxT | 8.92 \pm 5.98 | 1.5 | 0.112 \pm 0.064 | 3.0 |
| SxT | 0.0 \pm 0.0 | 0.0 | 0 \pm 0.0 | 0.0 |
| Plot | 46.49 \pm 12.80 | 8.0 | 0.605 \pm 0.108 | 16.0 |
| E | 432.5 \pm 18.1 | 74.5 | 2.775 \pm 0.115 | 73.5 |
| h_i^2 | 0.63 \pm 0.35 | | 0.29 \pm 0.20 | |
| h_{hs}^2 | 0.82 \pm 0.01 | | 0.79 \pm 0.04 | |
| h_{fs}^2 | 0.94 \pm 0.04 | | 0.81 \pm 0.13 | |
| h_{wf}^2 | 0.38 \pm 0.25 | | 0.16 \pm 0.12 | |

G = GCA, S = SCA, GxT = GCA by site, SxT = SCA by site interaction, E is within plot variance. h_i^2 , h_{hs}^2 , h_{fs}^2 , h_{wf}^2 Individual, half-sib, full-sib family and within-full sib family heritabilities, respectively.

A very high percentage of phenotypic variance observed for wood density and amplitude was explained by genetic differences among the full-sib families. Individual heritability for wood density was about two times greater than that of individual heritability for amplitude (**Table 3**). For selections from diallel progeny tests, family and within-family heritabilities are more meaningful. Half-sib and full-sib family heritabilities of two traits were similarly close and high, suggesting that genetic gain from family mean selection based on either wood density or average amplitude will be efficient. Wood density within full-sib family heritability was more than two times greater than within full-sib family heritability of amplitude. Efficiency of within family selection for amplitude would be lower.

Genetic correlation analysis showed that wood density and amplitude had a surprisingly high additive genetic correlation ($r_g=0.94\pm0.05$), much higher than the phenotypic correlation. This suggests that genetic selection based on amplitude could be as efficient as direct selection on wood density. A strong relationship ($R^2=0.90$) between genetic values of 14 full-sib families estimated for wood density and amplitude indicated that ranking full-sib families for relative wood density based on resistograph measurement is reliable (**Figure 8**). Families having high genetic values for wood density also had high genetic values for average amplitude.

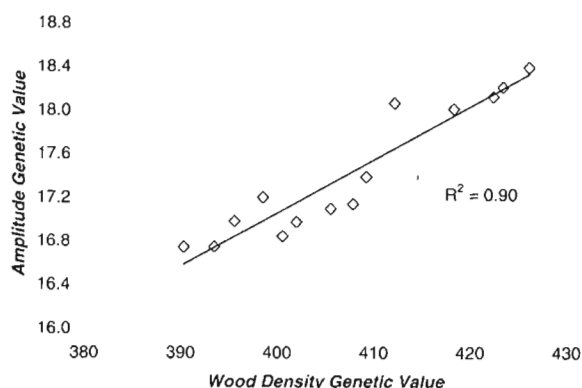


Figure 8. Relationships between genetic values of 14 crosses (full-sib families) for amplitude of the resistograph and wood density

Amplitude not only ranked the full-sib families precisely, but also ranked the parent trees correctly for their GCA values (**Figure 9**). For example, parent 1649 was distinctive from other parents by having the greatest GCA values for both wood density and amplitude. Parent 1634 had the lowest GCA value for wood density and lowest GCA value for amplitude. There was no rank change among the parent trees when amplitude GCA values were used.

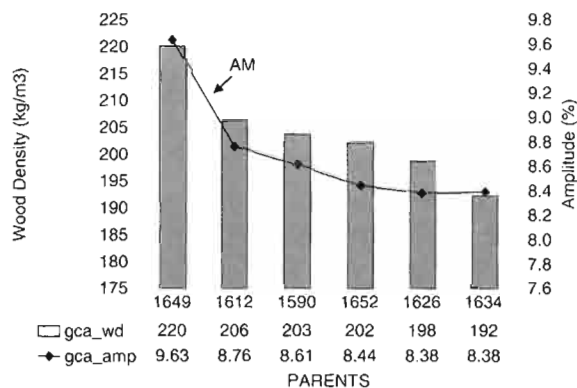


Figure 9. GCA values of parent trees estimated for wood density (bars) and amplitude (diamonds)

The relationship between individual-tree breeding values (IBV) for wood density and amplitude was linear (**Figure 10**). After removing fixed effects (site, replication within sites), the relationships between two traits increased from $R^2=0.27$ (Figure 2) to $R^2=0.64$.

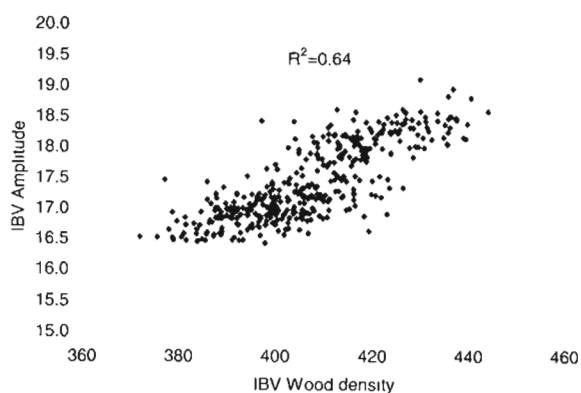


Figure 10. Relationships between individual tree breeding values estimated for wood density and amplitude at site 3

IBV from all sites for wood density were ranked and grouped into three categories as high, middle and low (Table 2). Percentages of correct classification, misclassification and serious misclassification of the trees for amplitude were calculated. Amplitude was highly successful in grouping the IBV as shown by the % of correct classifications (Table 4).

Table 4. Frequency and percent of trees classified for wood density by amplitude. The diagonal percentages are correct classification made by amplitude for each group of wood density

| | | Amplitude | | | Total |
|------|---------|-------------|-------------|-------------|-------|
| | Density | High | Middle | Low | |
| Freq | High | 385 | 81 | 13 | 479 |
| % | | 80.4 | 16.9 | 2.7 | 100% |
| Freq | Middle | 90 | 274 | 118 | 482 |
| % | | 18.7 | 56.9 | 24.4 | 100% |
| Freq | Low | 4 | 127 | 350 | 481 |
| % | | 0.8 | 26.4 | 72.8 | 100% |

Correct classification occurred when the trees from the high wood density group were placed in the high group of amplitude. Serious misclassification occurred when the best trees for wood density were placed in the lower group of amplitude. Amplitude correctly classified 80.4% of individual trees (regardless of families) in the higher group of wood density. Serious misclassification was only 2.7%. Percentage of trees in the higher group for wood density replaced in the middle group by amplitude was 16.9%. From a selection perspective, serious misclassification should be taken into consideration, because selection or culling would be placed on the tails of the distribution. However, if the family information of each individual can also be incorporated in this classification, the rate of correct classification is expected to be much higher.

Conclusions

The Resistograph can be used reliably and efficiently to access wood density breeding values of genotypes from progeny trials in a tree improvement program. Family-level selection and GCA estimates of parent trees using relative density (amplitude) are as reliable as direct measurement of wood density. Individual-tree breeding values for the two traits were also similar. Culling the lowest 1/3rd of the genotypes using resistograph measurement may end up about 3% of serious misclassification on individual tree level, while family culling should be much more reliable.

The method is more efficient compared to traditional gravimetric density estimations, because it only takes about 15 to 20 seconds to drill a 10-year-old loblolly pine. Additionally, the sampling is non-destructive to the progeny tests. The thin needle leaves a 1.5-mm wide hole in the tree that is barely visible. Data collection is much more simple and cheaper with a data recorder attached. With the further research of drilling profile and X-ray density profile, the tool may have potential to study ring-to-ring variation.

References:

- Xiang, B. and Li, B. 2001. A new mixed analytical method for genetic analysis of diallel data. *Can. J. For. Res.* 31: 2252-2259



The Resistograph

FAMILIES OF LOBLOLLY PINE THAT ARE MOST RESISTANT TO FUSIFORM RUST ARE ALSO THE LEAST STABLE

In a recent study of 43 open-pollinated families of loblolly pine across 28 diverse test sites in the southeastern US, we reported large family differences for rust infection (McKeand, S.E., B. Li, and H.V. Amerson. 1999. *Genetic variation in fusiform rust resistance in loblolly pine across a wide geographic range. Silvae Genetica* 48:255-260). Predicted rust levels for families at sites where 50% rust infection is expected (i.e., R-50 values) ranged between 22% and 75%. Although family by environment interaction was statistically significant, its overall variance was small compared to the family variance. Much of the G x E was due to heterogeneous regressions of families across different sites and not family rank change. One interesting finding was that for some families, the regression of family means on site rust infection means had low r^2 values (i.e., the confidence intervals of R-50 values of some families were wide). We speculated that this instability may be due to interactions of the family's susceptibility / resistance and specific pathogen avirulence / virulence among sites.

Upon closer inspection of the relationship between rust resistance and stability of resistance, it appeared that the most resistant families were the ones that were least predictable for performance. The Good General Combiner (GGC) Trials that were planted in the mid 1970's were analyzed in more detail to assess the nature of the instability of resistant families.

In the GGC trials, open-pollinated families from diverse origins were established in a series of tests throughout the South. Test trees were evaluated at age 8 years for presence or absence of galls on stems and/or branches. There were 43 open-pollinated families established in 28 test locations in the Coastal Plain from southeast Louisiana to eastern North Carolina used for the rust analyses.

The stability of families across site types was assessed using the traditional methods of Finlay and Wilkinson (1963 *Aust. J. Agric. Res.* 14:742-754) and Eberhart and Russell (1966 *Crop. Sci.* 6:36-40). Rust infection across the tests averaged 39%, ranging from 17% to 74%. The family mean at each site for percent galled trees was regressed on the site means (mean of all families and checklots planted at the site, average of 42 seedlots per test) to estimate the slope (b) and coefficient of determination (r^2) for each family. A slope of $b = 1.0$ plus small deviations from regression (i.e. a high r^2) indicate that a family has an average stability value (b) and responds in direct proportion to site changes. A slope $b > 1$ indicates that a family is "unstable" and responds to site changes more than most families. When $b < 1$, the family is more stable and does not respond to site changes. When r^2 is low, the reliability of the regression is low, indicating possible interactions of genetic entries and sites. High r^2 means that the performance of a family is predictable across sites.

To simulate the performance of a bulk seedlot of resistant families, individuals from the six resistant families were pooled. There were two tests, site 9 and T, where only one or two of the resistant families were planted. These sites were dropped from the analyses of the bulk seedlot. The mean of this resistant bulk at each site was regressed on the site means as was done for the individual open-pollinated families

As we reported in 1999, highly significant family differences ($h^2_F = 0.84$) for rust infection across the 28 tests were found. Predicted R-50 values ranged from 22.4% for the most resistant family to 75.3% for the most susceptible family. Slopes of the mean family rust percentage regressed on the mean site rust percentage did not differ significantly from $b=1.0$ ($p \leq 0.05$) for 34 of the 43 families indicating average stability for 8-year rust resistance for about 80% of the families. The relatively high coefficients of determination (r^2) and examination of residuals indicated that the linear models fit the data well. The highly significant GxE interaction sum of squares from the analysis of

variance was contributed by about 20% interacting families.

Although rust resistance for most families was predictable (average $r^2 = 0.78$), the r^2 values for the regression equations for predicting rust resistance were substantially lower than for predicting stem volume in the same tests where average $r^2 = 0.94$. The relatively low r^2 values for the rust regressions suggest more important genotype by environment interactions for rust than for volume. We speculated that for some families, the points that deviate the most from the regression line could be due to the interaction of a family's susceptibility / resistance and specific rust avirulence / virulence that may differ from site to site (e.g., Kuhlman et al. 1995 Plant Dis. 81: 597-600).

Upon more detailed evaluation of the family regressions, it appeared that the most resistant families were also those that had the lowest r^2 for the regression. We looked at the relationship between the stability parameter b (the slope of the regression line when family means for % galled are regressed on the site means for % galled) and the predictability of rust resistance. The correlation between b and r^2 was for the 43 families in the 28 tests was 0.74 (Figure 11); in other words, the most resistant families had the poorest regressions. Considering the relationship between stability and r^2 , the reliability of the "resistant" families (e.g. those with low b values) is poor.

From the 1999 paper, there were five families that had slopes significantly ($p \leq 0.10$) less than one. These families were considered the most resistant to fusiform rust: 10002, 10006, 11010, 11016, and 15042. A sixth family, 10005, was included since it had a relatively low slope ($b=0.75$) and is known to be a resistant family from other trials (data from N.C. State University-Industry Cooperative Tree Improvement Program). The average stability parameter for these six families was $b = 0.62$ with an average $r^2 = 0.58$, compared to the overall mean of all 43 families where the average $b = 1.05$ and the average $r^2 = 0.78$ (Figure 11).

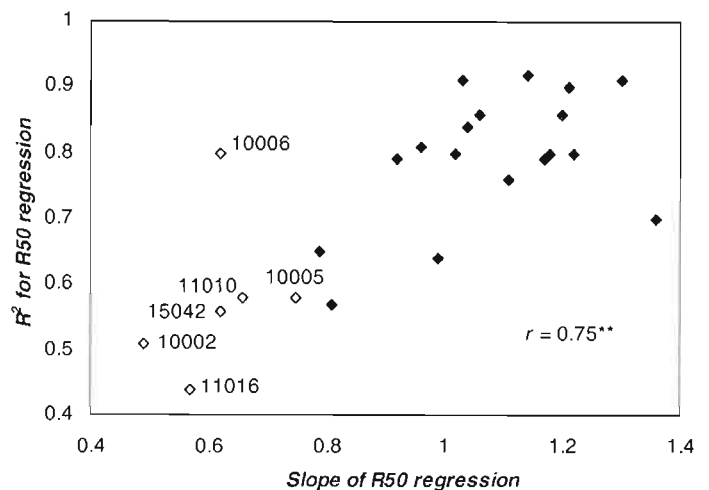


Figure 11. Relationship between rust stability (slope) and predictability (R^2)

When regressions for these six families are carefully evaluated, it is rare that the rust infection for any of these families exceeds the test mean (e.g., family 15042 illustrated in Figure 12). Foresters are still relatively well off deploying these families as opposed to families with average resistance. However, on some sites the performance will be substantially better or worse than predicted. That these six resistant families tend to have low coefficients of determination and relatively unpredictable rust performance.

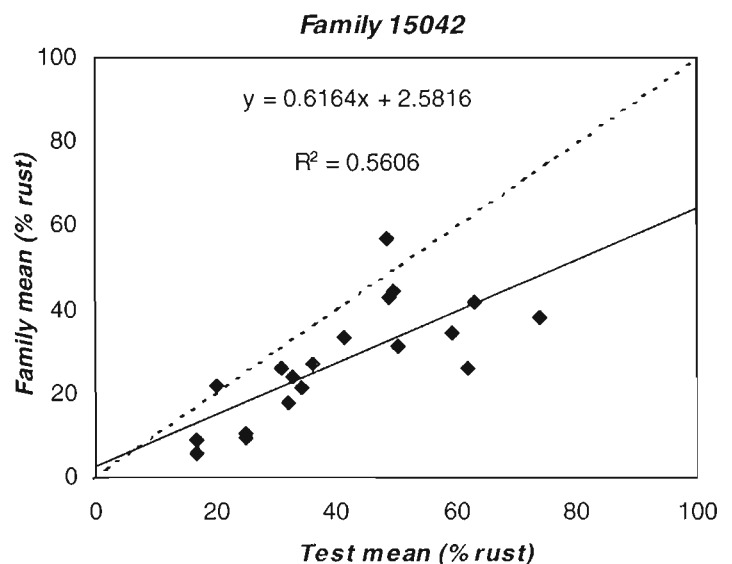


Figure 12. Typical regression of family mean on site means for one of the six resistant families

It is interesting that in two of the six resistant families (10005 and 10006), major gene resistance (MGR) to fusiform rust has been identified. We speculate that the instability of resistance for all six families is due to interactions of the family's susceptibility and specific pathogen virulence among sites. Interactions of major genes and different rust genotypes has been common in greenhouse screening trials and may well explain the instability in the field trials.

Although the predictability of the individual resistant families was relatively low (average $r^2 = 0.58$ for the six families), if these families were bulked into a resistant seedlot, they do perform in a more predictable manner (**Figure 13**). The coefficient of determination for the bulk is $r^2=0.74$ and is very similar to the average r^2 of all 43 families which was 0.78.

There appears to be some tests where the bulk

does not do well (Figure 3). Tests in Ware County, GA; McIntosh County, GA; and Baldwin County, AL, respectively, seem to be hot spots of virulence. At these three tests, there are 4 to 6 families making up the bulk. If these three tests are dropped from the regression analysis, the intercept is 1.56 and the slope is 0.52 (e.g. the family bulk is more resistant) with an $r^2=0.91$, so it is very predictable on the other 23 sites. One of our future research questions will be to determine if what might be causing this reaction on these three test sites.

We are still confident that resistant genotypes of loblolly pine can be identified and deployed on high rust hazard areas. But, from these analyses, it is clear that the predicted performance of the most resistant families will less reliable than for all the families. Bulks of 4 to 6 highly resistant families appears to be a good solution to obtaining stable and predictable performance across a range of sites.

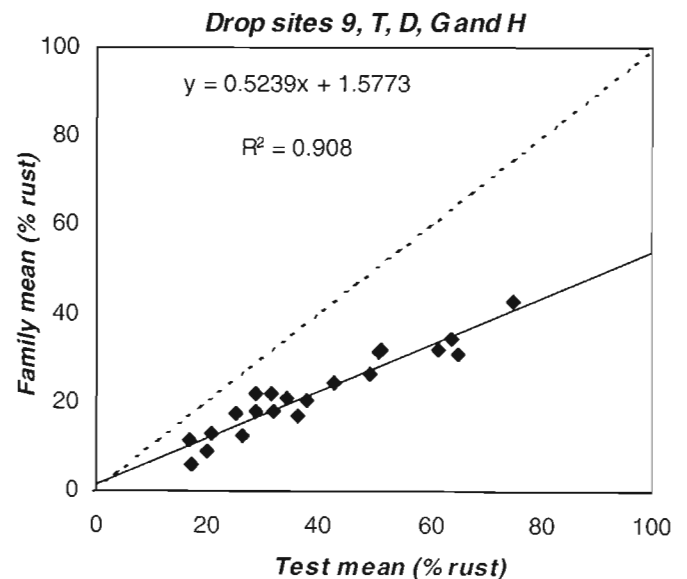
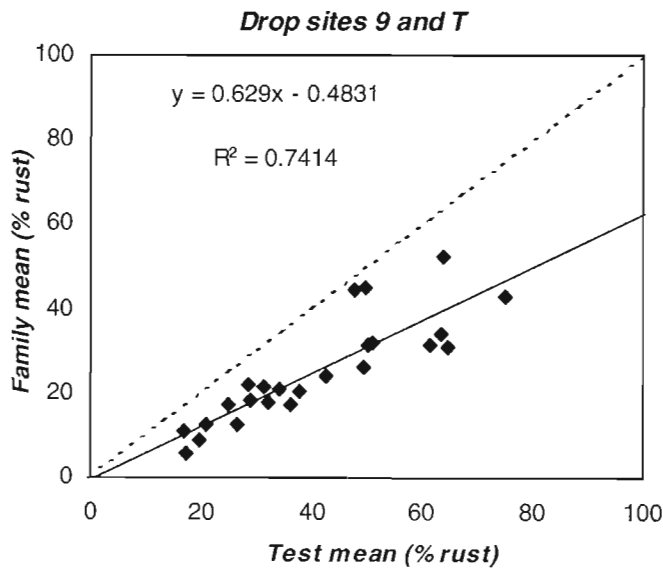


Figure 13. Regression of 6-family bulk lot rust performance against site mean, considering (a) all sites with exception of two (9 and T) that had only 1 or 2 of these families, and (b) all sites but 9, T, and three additional sites where the bulk lot did poorly.



Education of both graduate and undergraduate students is important to the Cooperative. Courses in forest genetics and tree improvement are available to both groups. Upon occasion, an outstanding undergraduate student becomes interested in tree improvement and wants to get involved in a research project. Carmen Barnes, a senior in Forest Management, participated in the University's honors program and was the recipient of one of the College's Undergraduate Research Awards. Her project for the Undergraduate Research Symposium is described below.

PROJECTED ROTATION STAND VALUES OF IMPROVED LOBLOLLY PINE FOLLOWING SIMULATED THINNING

Genetically improved loblolly pine not only grows faster but also has better stem quality than unimproved or wild loblolly pine. When unimproved, mid-rotation stands of loblolly pine are "thinned from below" to open up the stand and leave the most valuable crop trees for final harvest, the only residual trees available are often poor quality (forked, crooked, or cankered). If this is common, then the residual stands are poorly stocked and the site is not optimally occupied, or poor quality trees are left. With these conditions, fewer options are available for thinning.

The Cooperative staff established a Genetics Demonstration on the NCSU Schenck Memorial Forest in 1987. Plots of specific crosses that

exhibited varying levels of improvement were established. Most of the plots have 25 trees, but four larger plots were also planted with 85 or 90 trees per plot. Two of these large plots were unimproved loblolly pine from a mix of wild seed from the Piedmont of NC (Unimproved Checks), and two plots were a mix of seedlots originating from first-generation seed orchards in NC (Seed Orchard Mix).

Stem height, DBH, forking, ramicorn branching, and straightness were measured on all trees at age 15, and the number of trees/acre, average height, basal area/acre and volume/acre calculated. Once the measurements were obtained, the plots were "thinned on paper" and stand volume and values were projected for various levels of thinning. The thinning regimes included "no thinning", where the means describe the existing stands. A Timber Stand Improvement (TSI) cut that removed only the cull trees, those with forks, broken tops, and very poor straightness (i.e., those with straightness scores of 5 or 6 on a 1 to 6 point scale). The other thinning regimes also included a thin to 100 ft²/acre BA and 50 ft²/acre BA, where, in addition to the cull trees, the smallest diameter trees were removed until the desired basal area was reached.

The response of the four seedlots to the various thinning options was simulated using a Growth and Yield Model (Version 3 NCSU Managed Pine Plantation Growth and Yield Simulator, Hafley and Smith 1983). To run the model, the following inputs were used: site index 80 base age 25, Piedmont/upland height-age curves, stems/acre and basal area/acre at a known point in time, and reports were obtained at age 35. In addition, the volume equation and units (cords pulpwood, mbf sawlogs) were specified.

With the projected yields, evaluations were made for pulpwood, sawlogs, and total stand value. Average prices per ton for the region were found from Timber Mart-South in the 4th quarter 2001 issue (sawlogs \$46.93/ton and pulpwood \$6.68/ton). Analyses of variance were performed to determine

if the current means and projected values were significantly different between the improved and unimproved seedlots.

Height, DBH, individual tree volume, % forking, and straightness were better in the improved plots (**Table 5**). Due to small sample sizes, these results were not significant for any trait. Much larger sample sizes would be required to detect differences of this magnitude. In other trials of first-generation loblolly pine, similar gains (4.5% difference in height, 7.3% in stand volume) have been estimated and were highly significant (e.g., Talbert et al. 1984, *J. For.* 83:162-166).

In most trials, the largest and most consistent difference between improved and unimproved loblolly pine is the form of the trees. When compared to many families in genetic tests, the unimproved check seedlots almost always rank at the bottom for stem straightness and forking. For the TSI thinning regime, only trees that were considered as culls were thinned. We had originally intended to remove smaller diameter trees to bring these plots to a residual basal area of 150 ft²/acre. After the cull trees were removed, the estimated basal areas were 148.5 ft²/acre for the improved plots and only 106.3 ft²/acre for the unimproved plots (this difference was significant at $p = 0.09$). There were significantly more cull trees in the unimproved plots compared to the improved plots (**Table 6**). With fewer cull trees per acre, thinning operations should be more flexible, since more trees remain after the cull trees are removed. Foresters could leave larger trees at more desirable spacing in improved stands compared to unimproved stands.

When stands were thinned to approximately 100 ft²/acre of basal area, the improved plots had fewer but larger trees remaining (**Table 6**). There were 205 trees per acre with 1998 ft³/acre volume for the improved versus 254 trees per acre with 1910 ft³/acre volume for the unimproved plots.

Similarly, when stands were thinned to approximately 50 ft²/acre of basal area, the improved plots had fewer but larger trees remaining. There were 85 trees per acre with 951 ft³/acre volume for the improved versus 99 trees per acre with 926 ft³/acre volume for the unimproved plots. The volume differences at the heaviest thinning regime were statistically significant at $p = 0.02$.

When simulated thinnings were performed and stand volumes projected to age 35 years, the improved seedlots always had higher sawlog volumes and more value (**Table 7**). In addition, at 50 ft² and 100 ft²/acre basal area, pulpwood volumes were higher in the unimproved stands (Table 3). The unimproved trees did not grow as fast to reach the sawlog product class size. Thinning to 100 ft² BA, produced the greatest amount of sawlog volume and stand value. Differences in bold in Table 3 were marginally significant at $p \leq 0.15$ (sawlog volume and total volume for 100 ft² BA). Since the volume and value of the stand dropped when the seedlots were thinned to 50 ft²/acre BA, the optimum BA to which the stands should be thinned lies somewhere in between the two basal areas.

Genetics had played a key role in increasing forest productivity in these small demonstration plots. Overall, trees from the genetically improved seed sources grew faster and had better stem qualities such as better straightness and less forking. These traits would make subsequent silvicultural operations more feasible. Having fewer cull trees should make thinning operations more flexible. Foresters can leave larger trees at more desirable spacing in improved stands. Better-quality trees should result in an increase in volume and value at rotation age. Therefore, investing money in mid-rotation silvicultural manipulation of improved stands appears particularly valuable.

Table 5. Trait means for genetically improved and unimproved plots of loblolly pine at age 15 years. Two plots (either 85 trees or 90 trees) of each seedlot type were planted at 9'x9' spacing. There were no significant differences ($p \leq 0.10$) between the seedlots for any trait.

| Seedlot | Height (ft) | DBH (in) | Individual Tree Vol. (ft ³) | % Forking | Straightness Score | % Survival |
|------------|-------------|----------|---|-----------|--------------------|------------|
| Improved | 53.6 | 8.45 | 7.86 | 17.0 | 3.06 | 93.5 |
| Unimproved | 51.3 | 8.30 | 7.21 | 22.4 | 3.72 | 95.0 |

Table 6. Summary of current stand characteristics following simulated thinning of genetically improved and unimproved plots of loblolly pine at age 15 years. Trees were planted November 1986 at 9'x9' spacing (538 trees per acre). Estimated site index (base age 25 years) is 80 feet based on projected height of dominant and codominant trees (i.e., the top 50% of trees in the stand) at age 15 years.

| Thinning Regime ¹ | No. Residual Trees/acre | | Average Height (ft) | | Residual Basal Area (ft ² /ac) | | Residual Volume (ft ³ /ac) | |
|------------------------------|-------------------------|------------------------|---------------------|------------|---|--------------------------|---------------------------------------|-------------|
| | Improved | Unimproved | Improved | Unimproved | Improved | Unimproved | Improved | Unimproved |
| No Thinning | 518 | 520 | 53.6 | 51.3 | 206.9 | 199.2 | 3953 | 3685 |
| TSI | 363⁺ | 281⁺ | 54.0 | 51.5 | 148.5⁺ | 106.3⁺ | 2931 | 2005 |
| 100 (ft ²) BA | 205 | 254 | 55.7 | 52.2 | 99.2⁺ | 100.7⁺ | 1998 | 1910 |
| 50 (ft ²) BA | 85 | 99 | 56.9 | 53.7 | 46.3 | 47.7 | 951* | 926* |

⁺, * Means for a given trait in each thinning regime indicated in **bold face** are significantly different between the improved and unimproved seedlots at $p \leq 0.10$ and 0.05 , respectively.

¹ Description of Thinning Regimes Imposed:

No thinning: No trees removed. Means describe the existing stands.

TSI: Timber Stand Improvement - remove cull trees only [e.g., forked, broken tops, and trees with very poor straightness (scores of 5 or 6) trees]

100 (ft²) BA: Starting with the smallest diameter trees, remove stems until a basal area of 100 ft²/acre is obtained.

50 (ft²) BA: Starting with the smallest diameter trees, remove stems until a basal area of 50 ft²/acre is obtained.

Table 7. Summary of projected values at age 35 following simulated thinning at age 15 for genetically improved and unimproved plots of loblolly pine.

| Thinning Regime | Projected Value of Pulpwood (\$/acre) | | Projected Value of Sawlogs (\$/acre) | | Projected Total Stand Value at Age 35 Years (\$/acre) | |
|---------------------------|---------------------------------------|---------------|--------------------------------------|-----------------|---|-----------------|
| | Improved | Unimproved | Improved | Unimproved | Improved | Unimproved |
| No Thinning | 897.02 | 904.17 | 5,279.63 | 5,050.84 | 6,176.65 | 5,955.01 |
| TSI | 724.59 | 603.08 | 5,543.61 | 5,279.63 | 6,268.19 | 5,882.70 |
| 100 (ft ²) BA | 432.43 | 550.37 | 7,109.90 | 5,684.40 | 7,542.32 | 6,234.76 |
| 50 (ft ²) BA | 152.78 | 188.52 | 7,057.10 | 6,898.71 | 7,209.88 | 7,087.23 |

Figures in **bold face** were marginally significant at $p \leq 0.15$ (sawlog value and total value for 100 ft² BA) and $p \leq 0.08$ (pulpwood value for TSI).

CAN CLONAL REPLICATION IN PROGENY TESTS ELIMINATE THE NEED FOR POLY-CROSS TESTING? -- APPROACHING THE QUESTION THROUGH SIMULATION

“Forward selection” in our Coop breeding program is based on a combined index of family and individual performance. While the information on family performance is based on many observations, and is thus quite reliable, the individual performance is based on a single observation of the candidate phenotype, where the heritability is usually much lower. A polycross test is thus performed to get a good estimate of breeding value of the individual selections, normally before any decisions are made regarding which selections should be used for seed production.

One way to increase the precision of within-family selection is to establish clonally replicated tests, where one can make several observations of performance for an individual genotype. An obvious advantage would be that a polycross test would not be required to derive a good estimate of breeding value, so it might be eliminated, saving time and resources. On the other hand, establishing clonally replicated tests would require an extra propagation step, adding time, complexity and cost, and fewer genotypes could be evaluated.

To study these tradeoffs, Milan Lstibůrek (Ph.D. candidate) has performed some preliminary computer simulations using POPSIM (Mullin and Park 1995). For this first study, we considered a simple breeding population of 50 trees. We simulated a random, double-pair mating design so that each parent was mated twice, giving 50 crosses. Selection to the next generation was entirely within family, with the best single progeny from each cross going forward. The best 6 of these were to be used as orchard parents.

We used two approaches to field-testing of the crosses: (1) a seedling test (300 seedlings per each cross), followed by polycross assessment of breeding value of the best individual phenotype in each cross (100 seedlings per selected phenotype), and (2) a clonally replicated test, using the clone means to select the best tree without a polycross

test. In the former, the 6 trees for the orchard were selected on the basis of breeding values estimated from the polycross, while for the latter the orchard trees were chosen on the basis of clone means. The total number of plants tested in the seedling-based approach was 20,000. For the clonally replicated tests, the number of test plants was adjusted by relative cost of cloning versus seedling production, so that resources per cycle were kept constant.

For clonal replication scenarios, we tested different ratios of genotypes and number of ramets per genotype, to find the optimum balance. Only integer values were evaluated. **Figure 14** illustrates various ratios with an optimum peak corresponding to the maximum cumulative gain in a 5th-cycle seed orchard. The simulation assumed a metric growth trait with a variance structure typical for a northern conifer as well as constant environmental conditions across all sites and generations. C-effects were assumed to be absent. The cumulative genetic gain realized from the seed orchard was evaluated through 5 cycles of breeding.

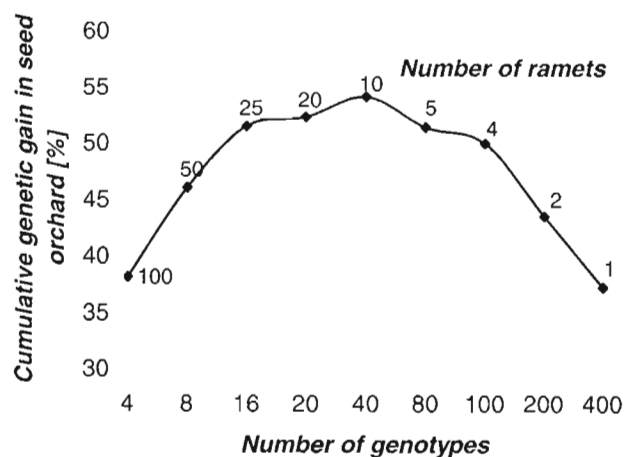


Figure 14. Cumulative genetic gain in a 5th-cycle seed orchard for different ratios of tested genotypes and clonal replicates. The optimum here corresponds to 40 tested genotypes, each tested by 10 ramets.

An example of the results is given in **Figure 15**, where the strategy with clonally replicated tests was superior to polycross progeny testing. Furthermore, clonal replication gave better results, even when the cost of propagation increased as

much as five times, reducing the number of test plants that could be established under constant resource levels.

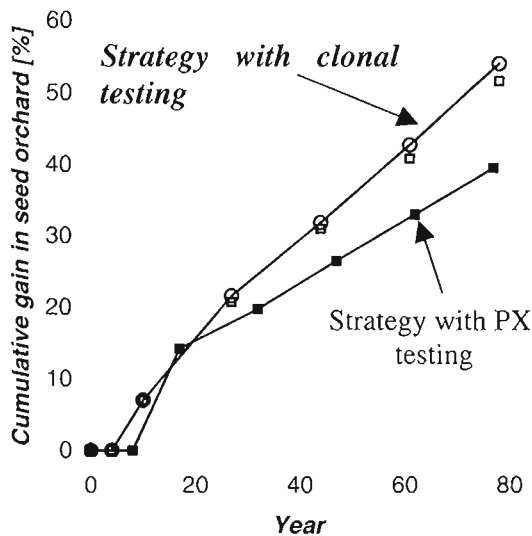


Figure 15. Cumulative seed-orchard gains over 5 breeding cycles, comparing a strategy with clonal replication as opposed to polycross testing of candidates. The broken line represents the case when cost of one plant in the clonal test is five times higher than that of a seedling.

The simulations lend support to some of our ideas on the utility of clonal propagation as a tool in genetic testing, even when normal deployment to plantations is with stock raised from seed. Work on the project is continuing, as we attempt to make the simulation more comparable to the current breeding strategy for loblolly pine.

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EXTERNALLY FUNDED RESEARCH GRANTS 2001-2002.

Chang, H.-m., J. Kadla, R. Sederoff, D. O'Malley, B. Li and B. Goldfarb. USDA-IFAFS: \$3,000,000. Wood and fiber quality of juvenile pine: characterization and utilization. 08/16/01-08/15/05.

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.

Chang, H.-m., J. Kadla, D. O'Malley and B. Li. N. C. Biotechnology Center: \$249,387. Exploiting genetic variation of fiber components and morphology in juvenile loblolly pine. 11/01/99-12/31/01.

Li, B., B. Liu, R. Weir and R. Sederoff. DOE Agenda 2020: \$424,417. Search for major genes using progeny test data to accelerate the development of genetically superior loblolly pine. 01/10/98-9/31/01.

Mullin, T.J., Rosvall, O., and Lindgren, D. Föreningen Skogsträdsförädling (Swedish Association for Tree Breeding): SEK 320 000 (approx. \$31,183). Optimal recruitment populations and hierarchical population structures. 01/01/01-12/31/02.

Mullin, T.J., Karlsson, B., and Högberg, K.-A. Föreningen Skogsträdsförädling (Swedish Association for Tree Breeding): SEK 70 000 (approx. \$6,821). Non-additive effects in a clonal test series of Norway spruce. 01/01/01-10/31/02.

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. 06/01/01-05/30/02.

SEED AND CONE YIELDS

The 2001 seed collection for the Cooperative provided 25.5 tons of loblolly pine seed (last year's crop was 36.9 tons), and for the first time we have measurable yields from two, third-cycle seed orchards!

Seed yields in pounds per bushel were also less than last year (1.21 vs. 1.30 in 2000). About 70% of the total seed came from second-generation orchards and about 85% of the collection was from the Coastal source (**Table 8**).

Four orchards produced above the 2.0 lbs./bushel mark this year, with the top three owned by MeadWestvāco; MeadWestvāco's 2.0 Virginia orchard had a yield of 2.4 lbs./bushel, followed by the SC 2.0 Coastal orchard with a yield of 2.32 lbs./bushel and in third place was the 1.0 Virginia orchard at 2.1 lbs./bushel. The fourth orchard is owned by Rayonier, 2.0 Coastal, with a yield of 2.03 lbs./bushel. The rest of the top ten producers are shown in **Table 9**

Table 8. Comparison of 2001 seed and cone yields with previous year's.

| Provenance | Bushels of Cones | | Pounds of Seed | | Pounds per Bushel | |
|--------------|------------------|--------|----------------|--------|-------------------|------|
| | 2001 | 2000 | 2001 | 2000 | 2001 | 2000 |
| Coastal 1.0 | 9,336 | 12,561 | 16,158 | 17,462 | 1.44 | 1.39 |
| Coastal 2.0 | 26,213 | 23,798 | 27,411 | 30,744 | 1.05 | 1.29 |
| Coastal 3.0 | 22 | 0 | 18 | 0 | 0.82 | 0 |
| Piedmont 1.0 | 457 | 859 | 764 | 1,155 | 1.67 | 1.35 |
| Piedmont 2.0 | 10,015 | 16,120 | 12,686 | 19,848 | 1.27 | 1.23 |
| Totals | 47,412 | 53,338 | 57,037 | 69,209 | 1.21 | 1.30 |

Table 9. Top ten production loblolly orchards in 2001.

| Organization | Orchard Type | Age | Lbs./Bushel | Orchard Manager |
|---------------------|--------------|-----|-------------|-------------------|
| MeadWestvāco | 2.0 Virginia | 15 | 2.40 | Dave Gerwig |
| MeadWestvāco | 2.0 Coastal | 16 | 2.32 | Dave Gerwig |
| MeadWestvāco | 1.0 Virginia | 31 | 2.10 | Dave Gerwig |
| Rayonier | 2.0 Coastal | 21 | 2.03 | Early McCall |
| MeadWestvāco | 1.0 Coastal | 36 | 1.86 | Dave Gerwig |
| Weyerhaeuser | 1.0 Coastal | | 1.80 | Drew Dunnam |
| Rayonier | 2.0 Piedmont | 26 | 1.76 | Early McCall |
| Weyerhaeuser | 2.0 Piedmont | | 1.74 | Franklin Brantley |
| International Paper | 2.0 Piedmont | 19 | 1.72 | Maxie Maynor |
| Weyerhaeuser | 2.0 Coastal | | 1.70 | Drew Dunnam |

MeadWestvāco's 2.0 Coastal Seed Orchard near Summerville, South Carolina



Then (1986) ...



... and now (2001)

As you can see, much has changed in 15 years ... but some things haven't! That's the same tree, the same jacket, and, yes, the same Dave Gerwig.

ASSOCIATED ACTIVITIES

TEACHING AND GRADUATE RESEARCH

The Cooperative continues to benefit from our involvement in teaching and graduate research in the Department of Forestry. All Cooperative staff on faculty were active in delivery of important courses in Forest Genetics, to students at both the undergraduate and graduate levels. Steve McKeand taught the graduate-level course FOR 725, Forest Genetics, and together with Tim Mullin, co-taught the undergraduate course FOR 411, Forest Tree Genetics and Biology. This was Tim's first time doing the course, as he stepped into the slot vacated by Professor Emeritus Bruce Zobel, who announced his retirement from teaching this year. Bailian Li

co-taught FOR 728, Quantitative Forest Genetics Methods, with Gary Hodge (CAMCORE). Bailian also assisted J.B. Jett in teaching FOR 727, Tree Improvement Research Techniques.

The education of graduate students and the research they conduct as part of their degree programs continues to be an important activity of the Cooperative. During the past year, 10 graduate programs were under development or were completed in association with the Tree Improvement Cooperative. Four were directed towards a Masters degree and six were involved in Ph.D. programs. 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

Patrick Cumbie, M.S. (In association with Rooted Cutting Program, NCSU).

Genetic variation in wood properties in clonal and seedling loblolly pine.

Daniel Gräns, Ph.D.

Project not yet determined, new student from Sweden on Nicholson Fellowship.

James Grissom, Ph.D.

Growth and physiology of loblolly pine seedlings as affected by genetics of the root system.

Josh Handest, M.S. (In association with the Nutrition Coop, NCSU). ***Completed Fall 2001.***

Effects of nutrient amendments and genotype on stand productivity and crown characteristics in loblolly pine (*Pinus taeda* L.).

Dominic Kain, Ph.D. (With Australia National University).

Inheritance of wood properties in slash by Caribbean pine hybrids.

Hua Li, Ph.D.

Major gene resistance in fusiform rust.

Ruyu Li, Ph.D.

Major gene detection using molecular markers.

Milan Lstibůrek, Ph.D.

Optimal population structures for forest tree breeding.

Paul Shannon, M.S.

An evaluation of the financial returns for different seed orchard establishment options.

Rob Sykes, M.S.

Genetic variation in tracheid and wood chemical properties in loblolly pine.

POST DOCTORAL FELLOWS

Dr. Fikret Isik continued working as a post-doctoral research associate with the Tree Improvement Cooperative. He is funded through external research grants (DOE and USDA) that study genetic variation of wood quality traits in loblolly pine. He has published several joint papers related to provenance variation (Forest Genetics), genetic variation in shoot morphology (Tree Physiology), genetic parameter estimates of clonal trails (Forest Science) and comparison of selection efficiency using seedling vs. cutting (summary in this report).

Dr. Bin Xiang was hired as a post-doctoral research associate after he finished his Ph.D. last year. Bin did the detailed analysis of Coop's Early Diallel Measurement Study of loblolly pine for his Ph.D. dissertation and submitted several papers for publication from this study. For his post-doctoral research, Bin is funded through a DOE grant working on the computer simulation and diallel genetic data analysis for major gene detection in loblolly pine.

VISITING SCIENTISTS

Dr. Gunnar Jansson, a forest geneticist with the Forestry Research Institute of Sweden (SkogForsk), spent 6 months in Raleigh on sabbatical leave with the Tree Improvement Program. He was partially supported by the Nicholson Fund for his research work here and worked closely with the Coop staff on several joint research projects. As a well-established forest geneticist, Gunnar has contributed a lot to our research program during his stay here. He has worked on genetic data analysis, early selection efficiency, breeding strategy optimization, and several other research projects. Gunnar's interactions with faculty, post-docs and graduate students have been beneficial to our research program.

Professor Dr. Dag Lindgren from the Swedish University of Agricultural Sciences in Umeå also paid a short visit to Raleigh this past winter. Dag is co-supervising Milan Lstibůrek's Ph.D. program, and has been involved in collaborative research with Coop staff. This collaboration will continue next winter, as Dag plans to return for a 6-month sabbatical.

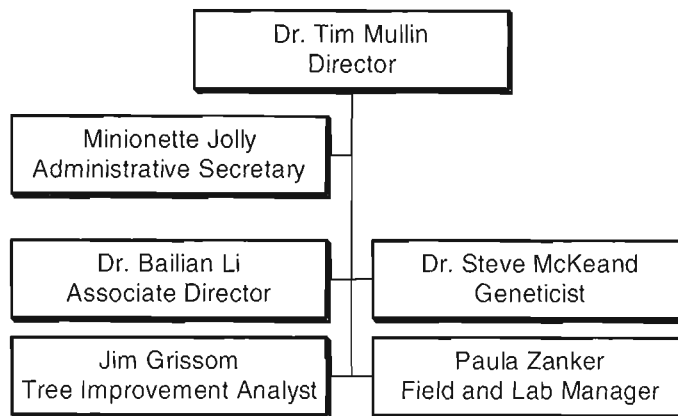


On sabbatical from SkogForsk, Gunnar Jansson found time to land this 5 lb., 24-inch "rock" bass.

PROGRAM STAFF

In last couple of Annual Reports, we've had many staff changes to report with retirements and new hires. The team has been rather more stable this year – thankfully! We did, however, celebrate two significant and very happy events this year. Steve McKeand became a grandfather – again – as daughter Theresa gave birth to a lovely little girl, Emily Louise Workman! Bruce Zobel, who comes to the office every week and continues to provide inspiration to us all, celebrated 60 years of marriage to Barbara.

NCSU-Industry Cooperative Tree Improvement Program Staffing Chart - May 2002



Team NCSU Tree Improvement – 2002. From left to right: Bailian Li, Ruyu Li, Milan Lstibůrek, Paula Zanker, Bin Xiang, Pat Cumbie, Mini Jolly, Rob Sykes, Steve McKeand, Jim Grissom, Hua Li, Tim Mullin, Fikret Isik. Those who missed the photo: Bruce Zobel, J.B. Jett, and Daniel Gräns.

MEMBERSHIP OF THE TREE IMPROVEMENT COOPERATIVE

The Coop continues to deal with the restructuring and consolidation of the US forest industry. Plum Creek completed its purchase of The Timber Company, and now operates under the name, Plum Creek Timber Company. Bowater completed its acquisition of fellow Coop member, Alliance Forest Products. Bowater then reduced its corporate landholdings through the sale of its Catawba, South Carolina management unit. Former members Mead Coated Board and Westvaco Corp. also completed a merger of their two companies to form MeadWestvaco Corp. As a result of these transactions, as well as the internal restructuring of other member agencies that continues from earlier acquisitions, we have been saddened that several of our tree improvement colleagues have had their jobs affected. As of May 2002, membership in the Coop stood at 16, with 6 state agencies and 10 private corporations:

Alabama Forestry Commission
Bowater, Inc.
Georgia Forestry Commission
Gulf States Paper Corp.
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.



Technology transfer to the tree improvement staff of member agencies is an important objective of the Tree Improvement Coop. Here, Steve McKeand explains the routine for screening candidates for 3rd-cycle selection at the 2001 Tree Improvement Short-course.

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 (21 articles published or in-press), as well as in conference proceedings and other technical publications (19 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.

- 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. Publ. 48, Southern Forest Tree Improvement Committee.
- 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.
- Frampton, J., B. Li, and B. Goldfarb. 2000. Early field growth of loblolly pine rooted cuttings and seedlings. South. J. Appl. For. 24(2): 98-105.
- Grissom, J.E. and S.E. McKeand. 2000. Growth efficiencies of diverse *Pinus taeda* families as affected by genetics of the root system. Abstract in: Proceedings of the North American Forest Biology Workshop-2000, Merida, Mexico. July 17-20, 2000.
- 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., Lindgren, D., and Mullin, T.J. 2001. Prediction of genetic gain and gene diversity in seed orchard crops under alternative management strategies. Theor. Appl. Genet. . 103: 1099-1107.
- 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 88:000-000 (in press).
- 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., Li, B. and Frampton, J. 2002. Additive, dominance and epistatic genetic variance estimates from a replicated clonal test of loblolly pine. For. Sci. (In Press)
- 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. 2000. Situation and results of breeding and plantation forestry in the American Southeast. Proceeding of the XXI IUFRO World Congress, Kuala Lumpur, Malaysia. Aug. 7-12, 2000. p. 38.
- Li, B. 2001. Hybrid aspen heterosis and breeding. In: P. Pulkkinen , P. M.A. Igerstedt and V. Raija (eds.), Aspen in paper making. P. 8-25, University of Helsinki Press, Helsinki, Finland.
- Li, B., S.E. McKeand and R.J. Weir. 2000. Impact of forest genetics on sustainable forestry - results from two cycles of loblolly pine breeding in the U.S. J. Sustainable For. 10: 79-85.
- Li, B. and R. Wu. 2000. Hybrid vigor and genetic mechanism of heterosis in aspen hybrids. Hybrid Breeding and Genetics (Eds M. Dieter and H. Dungey). Queensland Forestry Institute, Australia.
- Li, B. and W. Zeng. 2001. Invited: Detecting major genes of quantitative traits using phenotypic data. Abstract, IUFRO Tree Biotechnology Symposium: Tree Biotechnology in the New Millennium, Skamania Lodge, Stevenson, WA. July 22-27, 2001
- 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., J.E. Grissom, J.A. Handest, D.M. O'Malley, and H.L. Allen. 2000. Responsiveness of diverse provenances of loblolly pine to fertilization - age 4 results. J. Sustainable For. 10: 87-94.
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- McKeand, S.E. and E.M. Raley. 2000. Interstock effects on strobilus initiation in topgrafted loblolly pine. For. Genet. 7: 179-182.

- Mullin, T.J. 2000. Monograph on *Picea glauca*. In: Forestry Compendium Global Module on CD-ROM. CAB International, Wallingford, Oxon, UK.
- Mullin, T.J. 2000. Monograph on *Picea sitchensis*. In: Forestry Compendium Global Module on CD-ROM. CAB International, Wallingford, Oxon, UK.
- Mullin, T.J. 2000. Monograph on *Pinus strobus*. In: Forestry Compendium Global Module on CD-ROM. CAB International, Wallingford, Oxon, UK.
- 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 outcrossing 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* (in press).
- 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 four families from two diverse provenance of loblolly pine (*Pinus taeda* L.). *Can. J. For. Res.* (in press).
- Rojas, J.C., H.L. Allen, B. Li, and J. Wright. 2002. Clone and environmental effects for growth and foliar nutrient concentrations in *Eucalyptus urograndis* hybrid. *Silvae Genetica* (In press).
- 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.* (in press).
- Wu R., J.E. Grissom, D.M. O'Malley, and S.E. McKeand. 2000. Root architectural plasticity to nutrient stress in loblolly pine. *J. Sustainable For.* 10: 307-317.
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- Xiang, B. and B. Li. 2001. A new mixed analytical method for genetic analysis of diallel data. *Can. J. For. Res.* 31: 2252-2259.
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Any trees thinking of leaving the Virginia Department of Forestry's new seed orchard are hereby warned!

Back cover:

After the 2001-02 field season, we are at 87% of our third-cycle selection goal. Maxie Maynor (International Paper) has made many of these selections and is particularly proud of this high-scoring tree, 09-3516, selected in a progeny trial that he established in 1994, in Marion County, South Carolina. Thanks to Maxie, and to all the other dedicated employees of our member agencies, for making the Cooperative program such a success.

