FORTY-SEVENTH

PROGRESS REPORT

OF THE

COOPERATIVE

FOREST TREE IMPROVEMENT

PROGRAM

By

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INTRODUCTION

The Western Gulf Region of the United States has experienced two consecutive years of drought. While the 1999 weather was not as severe as the drought of 1998, these dry years have had a cumulative effect on the tree improvement programs in the region. The drought has contributed to a short-term increase in demand for operational seedlings. Most of the region's nurseries are operating at near capacity and are projected to do so for the next few years. This is reducing seed inventories and placing greater pressure on seed orchards to increase seed production.

Demand for seed is increasing as the cooperative shifts from a dependence on older, rogued first-generation orchards to younger, advancing-front orchards. These younger orchards, while of higher genetic value, have more variable seed crops. The variability of the seed crop has also been exacerbated by the weather. Seed harvests were at record highs in 1996 and very good in 1998. However, the crop failed almost completely in 1997 and appears to be less than normal in 1999.

Drought related mortality resulted in the loss of some critical progeny tests, which delayed the completion of the first-generation breeding and testing program for some members. Despite this setback, five members have now completed loblolly pine first-generation field test establishment. Three more members will conclude this phase of the program if the 1999 fall planting season is successful. Diallel breeding in the slash pine program is also progressing rapidly. More slash pine selections were established in control-pollinated field plantings in 1999 than in any other single year. First-generation breeding has been completed in 90 percent of the loblolly pine diallels and 70 percent of the slash pine diallels. In many of the remaining groups, only a few crosses were uncompleted.

The drought also delayed orchard establishment in some programs because of poor rootstock development. This has been disappointing, as maximizing the genetic gain in an advancing-front orchard requires that older orchard blocks be replaced in a timely manner with genetically advanced material. Of the ten members with plans to graft new orchard blocks in 1999, five were able to do so. Six members have orchard expansion or replacement plans for 2000.

Orchard replacement efforts will take advantage of the record number of progeny tests evaluated in 1999. These tests are making continued gains possible in the orchard program by supplying information on previously untested parents. Because of renewed interest in wood quality and increased emphasis on mill profitability as a selection criteria, identifying individuals that combine both excellent volume growth and high wood density is becoming more important. Since these traits are poorly correlated, selecting parents with improvement in both characteristics requires screening a large number of individuals. This record amount of new data is valuable in designing new seed orchards with combinations of desirable traits.

The cooperative identified a total of 127 loblolly pine and six slash pine second-generation selections in 1999. This was the largest number of second-generation selections ever identified in a single season. Fourteen of the cooperative's sixteen members contributed to this endeavor by measuring and evaluating a record number of progeny tests. Almost all selections were identified during the test measurement season, and most were immediately top grafted to promote early flowering for advanced-generation breeding.

The cooperative continued to investigate the genetic relationships between growth, wood specific gravity, and microfibril angle. Preliminary results have suggested that microfibril angle is under genetic control. The cooperative has an excellent set of wood samples from several older control-pollinated progeny tests that can be used to investigate this trait and its relationship to other characteristics. This long-term study will be completed in stages and may have important implications for improving solid wood products as well as pulp mill profitability.

Several organizations are currently evaluating controlled mass pollination (CMP) programs with pilot scale studies and a few programs are beginning to supply seed in sufficient quantities for operational regeneration programs. However, the magnitude of losses from pollen contamination and poor pollination success is still uncertain. Two studies designed to answer these questions are described in this report. The first found that the level of pollen contamination could be minimized even when the installation and the removal of isolation bags were less than optimal. The second study examined the variation in seed cost with variable pollination success. This study found that the inexpensive seed produced in good years more than offsets the cost of expensive seed produced in years with crop failures.

The first Tree Improvement Short Course organized by the cooperative in 10 years was held in 1999. In the past, this type of training session was designed for new employees. However, as tree improvement changes from first-generation to advanced-generation breeding, breeding objectives and types of populations are proliferating. These changes made this year's session timely for tree improvement veterans as well as new workers.

The members of the Hardwood Tree Improvement program were active in natural stand management research in addition to the traditional tree improvement program in 1999. Temple Inland Forest collected first-year data on its intensive hardwood management program while Potlatch Corporation collected 15-year data from its hardwood natural regeneration study.

Activities in the traditional tree improvement program included test measurement and establishment. The members measured 25 progeny tests, which were either Nuttall oak or cherrybark oak with one exception. Two of the six Nuttall oak progeny tests planted during the 1997/98 planting season were lost because of the extended drought in 1998. The remaining tests will be used to determine which selections will be grafted into future seed orchards. The cherrybark oak tests measured this year were 15 and 20 years old. As with previously measured tests, outstanding families at younger ages continued to perform well in later measurements.

The last series of Nuttall oak progeny tests was planted in five locations in 1998/99. This brings the total number of active tests for this species to 22 with a total of 216 families represented. During the summer of 1999, the Arkansas Forestry Commission and Temple-Inland Forest also grew seedlings for the first second-generation sweetgum progeny tests in the cooperative. These tests are a collaborative project with the N. C. State University – Industry Hardwood Research Program and contain selections from both programs.

Data from the natural regeneration study collected by Potlatch Corporation indicated that site-preparation treatment significantly impacted the amount of oak stocking after 15 years. Any site-preparation treatment increased the amount of oak present in the new stand; however, the high shear treatment resulted in the largest amount of oak stocking.

WESTERN GULF FOREST TREE IMPROVEMENT PROGRAM

Highlights

- Five members grafted 74 acres of new loblolly pine seed orchard blocks in 1999. These included Louisiana Pacific Corporation's first orchard and the first advanced-generation orchard designed specifically for North Mississippi.

- The 1998 seed harvest was the second largest on record, behind only the excellent 1996 crop. These two outstanding years flanked the poorest harvest on record in 1997.

- Preliminary results from three unrelated families suggests that microfibril angle may be under genetic control. These families are part of a larger study to examine the relationships among growth rate, wood specific gravity, and microfibril angle.

- First-generation loblolly pine breeding is approaching completion: eight members have established all of the required field tests and breeding is completed for 90 percent of the diallels.

- A total of 127 loblolly pine and six slash pine second-generation selections were identified in 1999. Most of these selections were immediately top grafted to promote early flower production in support of the cooperative's commitment to shorten the next generation's breeding cycle.

- A Tree Improvement Short Course designed as an introduction to genetics, applied tree breeding, and seed orchard management was attended by more than 60 people. Excellent presentations from invited speakers were augmented by an outstanding field trip hosted by International Paper Company at their Nacogdoches Forest Tree Seed Laboratory and Seed Orchard Complex.

Seed Orchards

Ten organizations planned seed orchard expansions in 1999. However, poor rootstock development caused by the drought resulted in only five organizations grafting new seed orchards in 1999. Six members, including the five that had to delay their 1999 plans, will expand or replace orchard blocks in 2000. Four more members have planted rootstock for grafting in 2001. Only one member removed an orchard from production in 1999. The number of orchard acres within the cooperative increased slightly in 1999 and should continue to increase slowly over the next few years. This is the result of shortening the economic rotation age of orchards, increasing seed demands in some programs, and uncertainty surrounding cone and seed insect control methods.

Genetic fingerprinting using microsatellite DNA markers was used for the first time in the seed orchard program in 1999. By using these markers, it was possible to verify that ramets with different growth habits and appearance were, in fact, from the same ortet. This confirmed the correct composition of an advanced-generation seed orchard block and authenticated a control-pollinated seed lot.

One of the most significant events for the seed orchard program in 1999 occurred outside the cooperative. The federal pesticide use registration for Guthion®,[1] the standard for cone and seed insect control for many years, was temporarily lost. Without Guthion®, cone and seed insect control programs would be forced to depend solely on synthetic pyrethroids, a class of chemicals known to cause secondary insect problems in seed orchards. The registration for Guthion® was restored with a significant reduction in application rates and improved worker safety standards. This negotiation and other activities of the Seed Orchard Pest Management Subcommittee are described later in this report.

Orchard Establishment and Roguing

Five organizations grafted a total of 74 acres of new loblolly pine seed orchard blocks in 1999. This included 24 acres of advanced-generation loblolly pine seed orchard established by Louisiana Pacific Corporation, the cooperative's newest member (Figure 1). The Louisiana Pacific Corporation's first attempt at grafting was extremely successful, establishing 95 percent of the seed orchard with minimal transplanting. This was despite an attack of coneworms that occurred immediately after grafting. This insect, which had not been previously observed on new grafts, tunneled through the stems close to the graft union causing most of the early mortality.[2] Louisiana Pacific Corporation also established an additional 24 acres of rootstock for grafting in 2000. This accelerated orchard establishment program will make the company self-sufficient for improved seed as quickly as possible.

The Mississippi Forestry Commission grafted the first block of advanced-generation orchard for North Mississippi (Figure 2). Establishment of this orchard had been delayed until it could be designed using the results from regional progeny tests. The

[1] Mention of trade names is solely to identify material and does not imply endorsement by the Texas Forest Service or the Western Gulf Forest Tree Improvement Program, nor does it imply that the discussed uses have been registered.

[2] The cooperative is grateful to Don Grosman for the quick diagnosis of this problem.

Figure 1. Greg Garcia and one of Louisiana Pacific Corporation's grafts in their new 24 acre orchard block.

Figure 2. Robert Stewart examines the excellent first-year growth for the Mississippi Forestry Commission's first block of advanced-generation North Mississippi loblolly pine orchard.

Mississippi Forestry Commission is the only member testing in this region; however, this orchard also includes the best selections from central Mississippi, North Louisiana, and Arkansas.

Other members grafting new orchard blocks in 1999 included Oklahoma Forestry Services, Weyerhaeuser Company, and The Timber Company. Genetic gain for the most recently established orchard blocks averaged 29.9 percent above the unimproved local checklot (Figure 3). Continued genetic improvement in the production population was possible because of the quick incorporation of new information from the record number of five-year-old progeny tests measured during the 1998/99 season.

Boise Cascade and Champion International rogued first-generation and advanced-generation orchards in 1999. The Louisiana Department of Agriculture and Forestry also rogued a longleaf pine seedling seed orchard. The unplanned thinning caused by Hurricane Georges in one of the other longleaf pine seed orchards was highlighted in the 46^{th} Progress Report of the Cooperative Forest Tree Improvement Program (p. 8). This orchard, at the Mississippi Forestry Commission's Craig Seed Orchard, continued its recovery (Figure 4). Genetic data from the long-term progeny tests will be used for the next scheduled roguing.

Only one orchard in the cooperative was removed from production in 1999. This was a 18.2 acre drought-hardy loblolly pine seed orchard maintained by the Texas Forest Service at their Magnolia Springs Seed Orchard Complex. Seed from this orchard had not been collected for several years, and the land was needed to expand the East Texas advancing-front seed orchard. Irrigation has been installed on ten acres of this area for rootstock establishment this fall with grafting planned for 2001. Currently, the cooperative manages a total of 2,158 acres of seed orchard (Figure 5). Of this number, 903 acres, or 42 percent, are advanced-generation orchards.

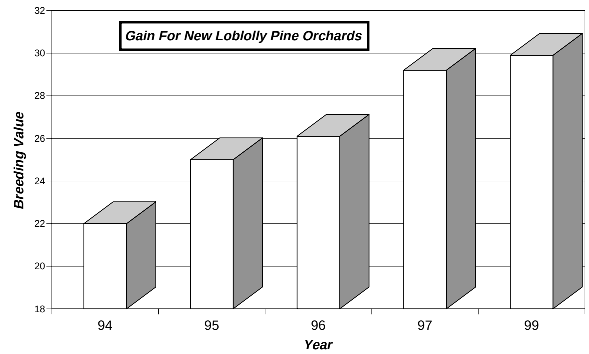

Figure 3. Gain in breeding value for volume growth averaged over new loblolly pine seed orchards by year of establishment.

Figure 4. The Mississippi Forestry Commission's longleaf pine seedling seed orchard one year after Hurricane Georges.

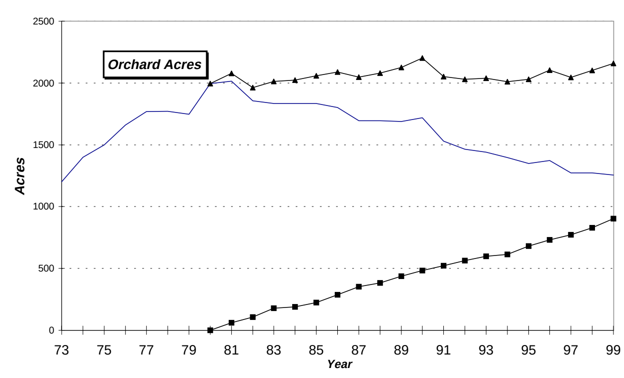

Figure 5. Seed orchard acres managed by the cooperative.

Orchard Yields

Seed orchard yields have been extremely variable over the last few years. The nearby complete failure of 1997 was followed by the second largest harvest on record in 1998 (Figure 6). The harvest totaled 53,761 pounds of loblolly pine seed and 5,063 pounds of slash pine seed. The excellent seed harvest in 1998 resulted from a very large cone crop, as seed yields were only moderate. The cooperative averaged 1.19 pounds of seed per bushel for loblolly pine and 1.08 pounds of seed per bushel for slash pine. Despite the average seed yields, excellent results were obtained in some orchards. One of the Arkansas Forestry Commission's orchards had the highest yield in the cooperative with 1.63 pounds of seed extracted per bushel. The Timber Company followed closely with an orchard yielding 1.57 pounds of seed per bushel. In all, eight orchards had yields in excess of 1.3 pounds of seed per bushel.

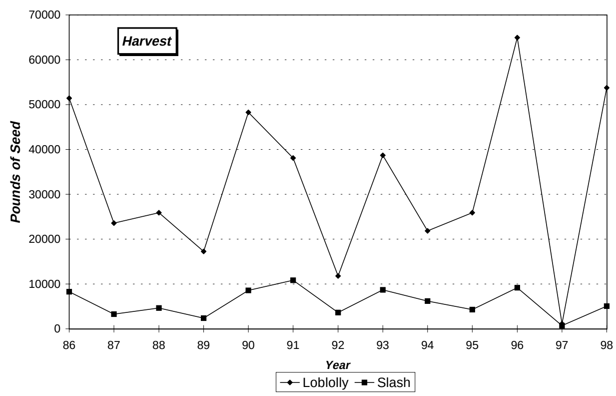

Figure 6. Pounds of seed harvested by the cooperative from 1986 to 1998.

Over 20,000 pounds of seed, nearly half the 1998 crop, were harvested from advanced-generation orchards. The advancing-front orchard program has progressed to the point where four organizations were able to collect their entire crop from these orchards. Most other programs harvested from both advanced-generation orchards and rogued first-generation orchards. The older, first-generation orchards are increasingly relegated to supplying seed from only selected open-pollinated families or for controlled mass pollination programs.

The 1999 cone harvest totaled 25,882 bushels of loblolly pine cones and 2,325 bushels of slash pine cones. Small amounts of shortleaf pine and Virginia pine were also harvested. Harvests of loblolly and slash pine were below expectations based on midsummer cone inventories due to a combination of factors. Contract labor and equipment used by the majority of our members for cone harvest were in short supply. The collection season was shorter than normal due to dry fall weather. However, the main reason for the disappointing crop was late-season damage to a significant number of this year's cones. A portion of this damage could be attributed to an August outbreak of coneworms. However, not all of the damage was clearly due to insects and other factors may have also been important. Early reports of seed extraction efforts from across the region indicate that seed yields may also be below normal.

Genetic Fingerprinting

Several members of the cooperative have provided Dr. Claire Williams' laboratory with foliage samples to aid in the development of DNA markers. These markers were put to practical use in the seed orchard program in 1999. Differences were reported in growth habit, needle color, and overall vigor among ramets of LSG-191 in several seed orchards. To determine if some of the ramets were mislabeled, fourteen foliage samples were collected by seven organizations. Four organizations each sent two foliage samples representing two morphological types of LSG-191. Two organizations sent only one foliage sample because all the ramets of LSG-191 in their orchards appeared to be from the same clone. One organization collected four samples, two each from two different morphological types of LSG-191. Unfortunately, it was impossible to sample the ortet as the original selection no longer existed.

DNA was extracted from the needles and microsatellite fragments were amplified using a technique similar to that used in human forensics. These techniques reveal differences in DNA sequences that vary widely even among closely related individuals. Six different highly variable markers were used, so the odds of different clones having the same marker genotypes would be infinitesimal. The results of the DNA fingerprinting indicated that all 14 samples were genetically identical and could only be ramets of the same ortet. Therefore, the apparent morphological differences must be attributed to environmental causes such as the early signs of graft incompatibility.

DNA fingerprinting is a powerful tool that will be used for tree improvement applications more frequently in the future. Isozymes and other biochemical methods have been used in the past to verify the genetic identity of clones in production orchards and the integrity of crosses in the breeding program. DNA markers will likely be used in the future for these applications because they are faster, less expensive, and less ambiguous. They also have other potential uses. Pedigree verification will become more important in advanced-generation breeding where the selection of a contaminating seedling in an inbred family would be a major liability. Fingerprinting deployment populations may also become more important because of the increased production costs associated with controlled mass pollination and vegetative propagation programs.

Super-Breeding Groups

Four more super-breeding groups, including the first for the Oklahoma Forestry Services, were designed in 1999 to bring the total number of these elite populations to 12. Because super-breeding groups use a backward selection procedure, their formation must be delayed until progeny test information is available for a majority of the parents in at least two breeding groups. Six members are now actively breeding to support this program, and two members have established block plots in which selections will be made.

The performance of super-breeding group selections will be compared to that of forward selections made simultaneously in the mainline breeding program. This will be accomplished by including polymix progeny from these selections in the advanced-generation polymix tests. This is another example of the important contribution that top grafting is making to tree improvement. The ability to substantially shorten the breeding cycle makes it possible to evaluate new breeding strategies so they can be appropriately integrated into the program.

Timing of Bag Application and Removal in Controlled Mass Pollination[3]

Controlled mass pollination (CMP) among outstanding parents is one way to increase genetic gains from traditional wind-pollinated seed orchards, but the economic success of CMP depends on both genetic gains and costs. CMP has been shown to be cost-effective even when costs were adjusted for risk.[4] These studies assumed that CMP was completely effective. That is, there was no pollen contamination during the CMP process that would reduce the expected gains from mating outstanding parents. This assumption is not always met under operational conditions due to variable strobilus development and the limited amount of time to conduct CMP. Therefore, it is important for producers of CMP seeds to know how much contaminated seed might be produced under operational conditions.

Eleven treatment combinations were applied to ramets of two female parents in Weyerhaeuser's grafted loblolly pine seed orchard in Lyons, GA. The first group of three treatments examined the effects of applying pollination bags at different female strobilus developmental stages. In this group of treatments, the bags were applied when female strobili were in stages 2, 3, and 4, respectively; no pollen was applied; and bags were removed ten days after maximum female receptivity. Thus, any seeds produced from these treatments could have occurred only from contaminating pollen. The second group of three treatments was the same, except that controlled mass pollination was performed twice while female strobili were in stages 4.5 to 5.5. In this group of treatments, pollen from the desired parents competed with contaminating pollen that may have entered pollen chambers before bagging. A third group of treatments examined the effects of removing bags at different intervals following pollination. In the first, bags were applied at female strobilus stage 2, pollen was applied twice at stages 4.5 to 5.5, and bags were removed at one of three intervals: immediately, two, or eight days following the second pollination. A fourth group of two treatments was examined to test various operationally efficient combinations of the variables under study. In each of these treatments, the bags were applied at female strobilus stage 3 and removed two days after the last pollination. In one of these, a single pollination was made at maximum female strobilus receptivity (stage 5.0). In the other, two pollinations were made as before. Pollen from three different male parents was applied to each treatment combination that received artificial pollinations.

Cone and filled seed counts were made for each treatment. Selected treatments for two crosses (81069 x 81056 and 81069 x 71022) that produced sufficient numbers of filled seeds were sent to the National Forest Genetics Electrophoresis Laboratory (NFGEL) in California for paternity analysis to determine the level of contamination for each treatment. Seeds were genotyped at 22 isozyme loci for 1,013 megagametophyte/embryo pairs. Both unambiguous and cryptic contamination levels were estimated. Cryptic contamination levels (ambiguous genotypes with regard to male parentage) were extremely low (less than 1 percent) and are not reported here.

The level of contamination in one cross (81069 x 71022) was so high (all treatments had 30 to 50 percent contamination) we concluded that the pollen source was not pure. Therefore, the results from the electophoretic analysis are reported only for a single cross (81069 x 81056).

Examination of the first group of treatments, which received no artificial pollinations, showed that pollen contamination occurred if female strobili were bagged after stage 2 (Table 1). This group of treatments is useful only to illustrate that pollen contamination can occur if bagging is delayed. Since artificial pollinations were not made, contaminating pollen did not have to compete for space in pollen chambers with applied pollen.

Even though contaminating pollen may be present before bags are applied, adequate and timely artificial pollination may reduce the number of seeds produced from contamination to acceptable levels. Paternity analysis revealed that there were no seeds produced from contaminating pollen with two artificial pollinations even when bags were applied at female stro-

Table 1. Numbers of cones / filled seeds per cone after bagging at strobilus stages 2, 3, and 4 with no pollen applied. Bags were removed 10 days after maximum receptivity.

		Strobilus Developmental Stage When Bagged		
Female	Male	2	3	4
81028	71037	0	0	2 / 30
	81069	0	0	5 / 21
	81077	0	1 / 0	8 / 18
81069	71022	0	0	0
	81056	0	1 / 26	0
	91039	0	0	0

[3] Submitted by F.E. Bridgwater, D. L. Bramlett, and V. D. Hipkins. We wish to thank Weyerhaeuser Co. and their Lyons, GA seed orchard staff for their contribution to this study.
[4] Bridgwater, F.E., D.L. Bramlett, T. D. Byram and W. J. Lowe. 1988. Controlled mass-pollination in loblolly pine to increase genetic gains. The Forestry Chronicle. 74(2): 185-189 and Byram, T.D. and F.E. Bridgwater. 1999. Risk analysis of loblolly pine controlled mass pollination programs. Proc. 25th Southern Forest Tree Impr. Conf. New Orleans, LA. In press. Also see the next section 'Risk Analysis of Controlled Mass Pollination'.

bilus stage 4 (Table 2). When bags were removed two days after the second pollination (Treatment 3*), rather than ten days as in the other treatments, a small amount (2 percent) of contaminant seed was produced.

The results from the remaining treatments (Table 3) show that it is unwise to remove bags immediately following artificial pollination at maximum receptivity (Treatment 0) or to pollinate only once (Treatment 2*). Although these two options would be operationally desirable, they resulted in 41 and 66 percent seeds from contaminating pollen, respectively.

Although pollen contamination can occur when female strobili are bagged after developmental stage 3, adequate and timely artificial pollinations can reduce the proportion of contaminant seeds to inconsequential levels. High proportions of seed produced from contaminant pollen resulted from removing pollination bags sooner than two days following pollination at maximum female strobilus receptivity or making only one pollination.

Risk Analysis of Controlled Mass Pollination

The average production cost for controlled mass pollinated (CMP) programs has been estimated to be approximately $0.05 per seed. The cost per seed is influenced by several factors including the number of female strobili per isolation bag, the cost of CMP per bag, and the number of seed recovered from each flower pollinated. These factors may vary widely among crosses and years and are only partly under operator control. An assessment of risk can be made by determining the probability of exceeding internal break-even costs.

The number of female strobili per isolation bag and the cost of CMP per bag, which includes the cost of pollen collection, the installation and removal of the isolation bag, and the pollen application, are all under operator control and can be estimated from pilot-scale programs. Pollination success, the number of seed obtained per flower pollinated, is a complex trait that depends on the weather, clonal selection, pollen viability, the timing of pollination, and insect predation. The effect of each of these factors on seed cost can be estimated by evaluating pollination success based on past experience with controlled pollination for the production of progeny test seed.

Cost data and estimates of strobili per bag from two pilot-scale CMP programs combined with estimates of seed production per strobilus from three different controlled pollination programs were used to estimate CMP production costs.

Table 2. Numbers of cones / filled seeds per cone after bagging strobili at stages 2, 3, and 4. Two pollinations were made at stages 4.5 and 5.5 and bags were removed 10 days after the first pollination. Numbers in parentheses for cross 81069 x 81056 are the percentage of seeds produced from contaminating pollen.

		Strobilus Developmental Stage When Bagged			
Female	Male	2	3	4	3*
81028	71037	1 / 34	7 / 78	4 / 50	8 / 67
	81069	5 / 34	14 / 34	7 / 40	2 / 33
	81077	0	7 / 0.3	6 / 55	5 / 12
81069	71022	1 / 62	4 / 119	9 / 88	11 / 59
	81056	6 / 62 (0 %)	5 / 57 (0 %)	8 / 43 (0 %)	10 / 65 (2%)
	91039	4 / 45	5 / 41	3 / 66	5 / 106

* Same treatment as 3, but bag was removed 2 days after the second pollination.

Table 3. Numbers of cones / filled seeds per cone for bag removal 0, 2, or 8 days after the last pollination. Two pollinations were made at stages 4.5 and 5.5. Numbers in parentheses for cross 81069 x 81056 are percentages of seeds produced from contaminating pollen.

		Strobilus stage at bagging				
		2			3	
		Days after last pollination to bag removal				
Female	Male	0	2	8	2	2 *
81028	71037	1 / 70	0	1 / 65	8 / 84	4 / 72
	81069	0	0	2 / 45	13 / 52	3 / 9
	81077	2 / 0	0	1 / 2	9 / 3	2 / 6
81069	71022	2 / 58	6 / 100	8 / 64	4 / 84	5 / 104
	81056	5 / 79 (41%)	1 / 24 (0 %)	1 / 36	7 / 122	6 / 185 (66%)
	91039	4 / 40	-	8 / 57	6 / 47	-

* Same treatment as 2, but only one pollination at strobilus stage 5.0.

The average values for CMP cost, number of strobili per bag, and seed obtained per strobilus are shown in Table 4. The average seed yield per strobilus over the three programs that provided data for this project was 16.12 (1,140,299 seed / 70,717 strobili pollinated). This included parents that were difficult to cross and individuals that were sometimes located in scion banks with less than optimal insect protection. Females that consistently failed to produce seed were eliminated to more accurately reflect an operational CMP program. Annual CMP seed costs were then predicted using Monte Carlo simulation.

Annual CMP costs per seed differed substantially among the three scenarios (Table 5). Scenario 1, in which seed yields were relatively high and reliable, had an average annual CMP production cost of $0.0535 per seed. In Scenario 2, average seed yields per strobili were high but relatively unreliable, and the average annual CMP cost ($0.0594 per seed) was very similar. Scenario 1 and 2 differed primarily in the maximum cost of seed observed ($0.1844 vs. $0.41 per seed). Scenario 3 assumed lower and more unreliable yields of seed per strobili. In this case, average annual seed costs were higher ($0.1275) and the maximum simulated annual cost per seed was extremely expensive ($12.12). However, when seed costs were combined across years and weighted for total seed production, the cost of CMP seed was reasonable even for the scenario in which variation in seed yield per strobilus was the greatest.

Pollination success for this study was estimated from data on a large number of female parents, many of which would be unlikely candidates for a CMP program because of undesirable flowering or seed set characteristics. CMP programs will concentrate on a few selected parents for which pollination techniques can be optimized, suggesting that these results may be conservative.

Top Grafting

Data collection in the top grafting experiment at the Mississippi Forestry Commission's Craig Seed Orchard continued in 1999. This study was initiated in 1996 in cooperation with D. L. Bramlett (retired) and F. E. Bridgwater of the USDA Forest Service. This year, graft and conelet survival was evaluated for grafts made in 1997, and seed yields from polymix breeding were calculated for 1996 grafts on one interstock clone. First-year performance of 1997 grafts and second-year performance of 1996 grafts were reported in the 46th Progress Report of the Cooperative Forest Tree Improvement Program (p. 9). First year performance of the 1996 grafts was reported in the 45th Progress Report of the Cooperative Forest Tree Improvement Program (p. 8).

Second-year survival of grafts made in 1997 was basically unchanged from year one. Survival of grafts on both slash pine and loblolly pine interstocks remained high and did not differ significantly between interstock species (90 and 84 percent, respectively). There were 2.2 conelets per graft on the slash pine interstocks and 2.6 conelets per graft on the loblolly pine interstocks. The differences between interstock species for the number of conelets per graft was not statistically significant.

Strobili on five top grafts of the same scion source on one slash pine interstock clone were control-pollinated with polymix pollen in the spring of 1997. Flower retention for the top grafts was 79 percent compared to 68 percent for similar crosses in a traditional scion bank. This difference may not be meaningful and was most likely due to differences in sample size (49 flowers debagged on the top grafts compared to 400 flowers in the operational sample) and more careful handling of the top grafts. When strobili were counted in December

Table 4. Mean, standard deviation, minimum and maximum values generated by the simulation for use as input to calculate CMP seed costs.

	Mean	Standard Deviation	Minimum	Maximum
Cost/Bag	$3.86	$0.95	$2.00	$7.06
Strobili/Bag	4.38	1.4	2.4	9.9
Seed/Strobilus				
Scenario 1	18.62	2.47	11.04	27.06
Scenario 2	18.56	5.33	1.69	35.43
Scenario 3	16.01	7.66	0.17	39.38

Table 5. CMP costs per seed for three different scenarios.

Scenario	CMP Cost Weighted for Total Seed Production	Annual CMP Costs Per Seed		
		Average	Minimum	Maximum
1	$0.0473	$0.0535	$0.0119	$0.1844
2	$0.0475	$0.0594	$0.0112	$0.4129
3	$0.0555	$0.1275	$0.0096	$12.120

1997, 46 of the original 49 flowers on the top grafts remained. In the fall of 1998, 39 cones were collected which yielded an average of 60.3 sound seed per cone. This compared favorably to the 61.9 sound seed per cone from the cones collected from the same cross in the traditional scion bank.

Based on the results of this study, the members of the Western Gulf Forest Tree Improvement Program have aggressively incorporated top grafting into their standard breeding strategy. While all of the loblolly pine second-generation selections identified in 1998/99 were grafted into traditional scion banks for preservation, most were also top grafted for accelerated flower production. Top grafting should significantly shorten the length of the second-generation breeding cycle compared to that of the first generation.

Wood Quality

Improvement of wood quality traits has been a secondary priority in the breeding and deployment populations of the program compared to the major emphasis placed on volume growth. However, some improvements have been made in wood quality because of selection for improved form characteristics, such as straightness.

In the last few years, increased attention has been given to wood quality traits and their relationships to other important characteristics. Most of the emphasis has been on specific gravity and its relationship to growth and straightness. Economic weights have also been developed to optimize pulp mill profitability by selecting for both volume growth and specific gravity simultaneously. These weights are combined in an index to design deployment populations for pulp mills. These developments were discussed in the 46th Progress Report of the Cooperative Forest Tree Improvement Program (pp. 11-12).

The cooperative is expanding its wood quality research to investigate microfibril angle. Microfibril angle is an important trait in determining the strength and stability of solid wood products. A challenge cost-share grant was obtained from the USDA Forest Service to evaluate this characteristic in two control-pollinated progeny tests. Boise Cascade Company, Champion International Company, International Paper Company, Temple-Inland Forest, and The Timber Company contributed additional funding to complete the project. The staff is preparing the samples for Dr. Robert Megraw (Weyerhaeuser Company) who is using X-ray diffraction to determine the microfibril angle.

In this project, microfibril angle is determined on both earlywood and latewood samples from juvenile and mature wood. Rings four and five from the pith represent the juvenile wood sample and rings 19 and 20 from the pith represent the mature wood sample. The analysis for three unrelated crosses has been completed (Table 6). Significant differences among crosses were detected for the earlywood and latewood samples from the juvenile wood and in the earlywood samples of the mature wood. The analysis of the remaining samples will be completed next year. This data set will also provide an opportunity to study the genetic relationships between microfibril angle and other traits of importance to the cooperative.

First-Generation Breeding and Progeny Testing

The six members of the slash pine tree improvement program dramatically increased the number of partial-diallel progeny tests established in 1999. Breeding has been completed in 70 percent of the cooperative's first-generation slash pine diallels, leaving only fourteen unfinished. The Louisiana Department of Agriculture and Forestry was the first member to complete first-generation slash pine breeding. The Texas Forest Service and Weyerhaeuser Company each have breeding efforts to complete in only one diallel. Regional cooperation has proved advantageous for establishing field plantings of this species. Because several members have had one or two diallels ready for field planting simultaneously, a few large joint plantings have been established, rather than many smaller and less efficient field tests.

The first-generation breeding and progeny testing efforts are approaching completion for loblolly pine at the same time efforts are increasing for slash pine. If the 1999 fall planting season is successful, eight of the cooperative's sixteen members will have established all of their required first-generation loblolly pine progeny tests. Breeding has been completed in 90 percent of the loblolly pine diallels, and there are only a handful of unfinished crosses left in most of the remaining groups. Because the single-tree plot field design has made it possible to include many families in each test, members are working together to complete progeny test establishment. Generally, this

Table 6. Microfibril angle (degrees from vertical) for three unrelated crosses in a progeny test. [1]

Cross	Microfibril Angle (°)			
	Juvenile [2]		Mature [3]	
	Early - Wood	Late - Wood	Early - Wood	Late - Wood
A	41a	44a	26 b	23a
B	39a	42a	29a	24a
C	37 b	39 b	22 c	19a

[1] Means followed by the same letter are not significantly different at the 10 percent level by Duncan's New Multiple Range Test.

[2] Juvenile wood-rings four and five from the pith.

[3] Mature wood-rings 19 and 20 from the pith.

cooperation takes the form of pooling crosses from several diallels, one organization growing the seedlings, and several members establishing field tests.

Slash Pine

In 1998/99, a record 100 slash pine parents were established in control-pollinated progeny tests. All of these parents have shown increased resistance to fusiform rust infection in greenhouse screenings at the USDA Forest Service - Resistance Screening Center. Significant gains for field resistance are anticipated for crosses among these parents as well. Four of the six members active in the slash pine tree improvement program established eight field plantings (Table 7). These tests were designed with 50 replications of single-tree plots and each included selections from three or four different diallels. A total of 29 diallel by location combinations were represented.

A severe outbreak of the pine colaspis beetle was observed in a Texas Forest Service slash pine progeny test this year (Figure 7). This beetle has only one generation per year and does minimal long-term damage. However, the damage symptoms include brown and curling needles, which closely resemble herbicide damage and can cause momentary concern over the possible misapplication of chemicals.

Figure 7. Pine colaspis beetle damage in a Texas Forest Service slash pine progeny test.

Genetic Mechanisms for Disease Resistance. Understanding the genetic basis for disease resistance is crucial to ensure a successful breeding and deployment strategy for slash pine. One type of resistance in several plant species results from a major gene effect that causes resistance to specific pathogen strains but not to others. Some of these genes are at different locations within the genome and can be differentiated through genetic mapping. Other resistance genes are closely clustered and can be distinguished only through their interaction with different disease strains. If this model applies to slash pine, it will be important to identify a panel of disease strains that can be used to screen candidates for new resistance genes. It will be equally important to identify a panel of slash pine parents that can be used to monitor the pathogen population for new strains of the disease.

A cooperative project to screen for host-pathogen interactions is being conducted with Hank Steltzer (formerly USDA Forest Service, now with Champion International) and Rob Doudrick (USDA Forest Service). Seedlots obtained by crossing 28 parents with rust-susceptible polymix pollen were subjected to artificial inoculation with four strains of fusiform rust. Fourteen seedlots were chosen because they exhibited high levels of disease resistance in previous greenhouse screenings with bulked inoculum while the other 14 had shown susceptibility.

The genetic interactions within this experiment are complex. Each maternal parent can be either homozygous or heterozygous for any disease resistance genes present. The

Table 7. Progeny tests established during the 1998/1999 planting season.

Cooperator	Number of Tests	Number of Diallels
<u>First-Generation Loblolly Pine Tests</u>		
Boise Cascade	2	6
Bosch Nurseries	3	12
Oklahoma Forestry Services	2	3
The Timber Company	3	11
Willamette Industries	1	2
Loblolly Pine Total:	**11**	**34**
<u>First-Generation Slash Pine Tests</u>		
Louisiana Department of Ag and Forestry	3	9
Mississippi Forestry Commission	1	4
Temple-Inland Forest	3	12
Weyerhaeuser	1	4
Slash Pine Total:	**8**	**29**

pathogen can also segregate for virulence genes, as the spores collected to start the disease strains are diploid (dikaryon) but the infectious form is haploid. However, if the major gene model is valid, there should be some slash pine parents previously judged as resistant which are susceptible to some strains of the pathogen. Conversely, some susceptible slash pine parents should be resistant to pathogens that are virulent on other slash pine parents.

When the complete data set was analyzed, there were no significant slash pine family by inoculum interactions. However, when individual families were examined, the type of interaction predicted by the major gene model of resistance was observed (Table 8). A-2-54, classified as a resistant parent, was susceptible to infection with rust strain 8-7 while D-2-4, a susceptible parent, showed resistance to this strain. MFCS-111 was susceptible to inoculum 8-7 but almost completely immune to infection with rust strain LT3, which was highly virulenet on slash pine parent D-2-4.

The effort to understand the resistance/virulence gene interactions suggested by this data is just beginning. However, developing methods of classifying resistance in the host and monitoring changes in pathogen virulence will affect the breeding program for this species and have important uses in planning deployment strategies. This could be especially important if vegetative propagation is used to deploy slash pine clones.

Slash Pine Demonstration Plantings. Five-year measurements were taken on four slash pine demonstration plantings in 1999. These plantings were the second in a series of plantings designed to demonstrate differences in fusiform rust resistance among three different sources of slash pine, including WGFTIP improved slash pine and unimproved commercial checklots from South Mississippi and Georgia. The WGFTIP improved slash pine source consisted of ten families selected for high rust resistance. The Mississippi source was the slash pine checklot used in all WGFTIP slash pine progeny tests. The Georgia source represented material that was widely planted in the Western Gulf region before seed from local seed orchards became available.

Boise Cascade Company maintained one location in Beauregard Parish, LA, the Louisiana Department of Agriculture and Forestry maintained one location in East Feliciana Parish, LA, and Temple-Inland Forest maintained one location in Hardin County, TX and one location in Vernon Parish, LA. All four of these demonstrations were repeats of plantings established in the spring of 1993 and measured last year. Five-year data from the older plantings were reported in the 46th Progress Report of the Cooperative Forest Tree Improvement Program (p. 14). Each planting consisted of two replications of large block plots (80 or 100 trees) for each source.

Five-year data from all eight locations were analyzed together. Significant differences among sources were found for volume growth, incidence of fusiform rust and height, but not for survival (Figure 8). Volume growth was highest for the improved slash pine source (6.2 dm^3/planted tree), followed by the WG slash and GA slash checklots at 5.2 and 4.4 dm^3/planted tree, respectively. The gains made by selection for rust resistance are evident when comparing the improved slash source to either of the unimproved sources. Fusiform rust infection for the ten families included in the improved source averaged 20.8 percent, approximately half that of the infection level in the WG slash checklot (40.5 percent) and the GA slash checklot (40.0 percent). The improved slash source had the highest average height (4.8 m) which was significantly greater than both the WG slash checklot (4.5 m) and GA Slash checklot (4.4 m), which were not significantly different from one another. Survival over all locations averaged 80 percent and was similar for all sources. Survival is likely to change: previous experience has shown that a great deal of rust related mortality occurs between five and 10 years of age.

These plantings were established as demonstration plantings, not as replicated trials designed to allow inference and the detection of statistically significant differences. Nevertheless, these plantings clearly demonstrate the benefits of selection for rust resistance achieved in only one round of selection and testing. These plantings will be measured again at ten years of age.

Loblolly Pine

A sufficient number of first-generation loblolly pine progeny tests were established in 1999 to evaluate 142 parents, 111 for the first time (Figure 9). A total of 2,261 loblolly pine

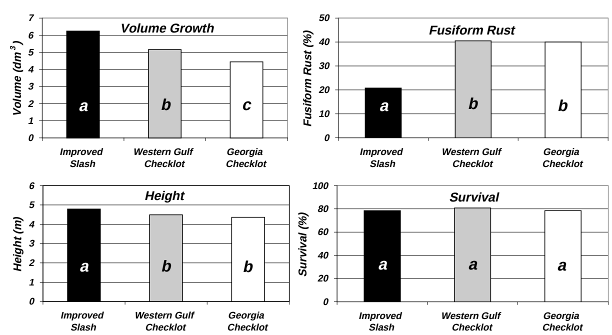

Figure 8. Results from the slash pine demonstration plantings averaged over 8 locations. Bars with the same letters are not significantly different at the 10 percent level by a Duncan's New Multiple Range Test.

Table 8. Percent of seedlings infected when artificially inoculated with four different strains of fusiform rust. RSC Classification is based on previous trials with a bulked inoculum.

		Inoculum			
Slash Pine Parent	RSC Classification	8-7	CCA-2	LT3	WLP10-2
A-2-54	Resistant	62	28	14	28
D-2-4	Susceptible	15	38	60	40
MFCS-111	Resistant	62	43	5	33

parents have now been planted in balanced control-pollinated progeny tests designed to estimate parental general combining abilities, and an additional 693 parents have been evaluated in open-pollinated progeny tests. First-generation loblolly pine breeding efforts are coming to a close as shown graphically in Figure 9. There has been a steady decrease in the number of parents established in progeny tests since the 1994/95 planting season.

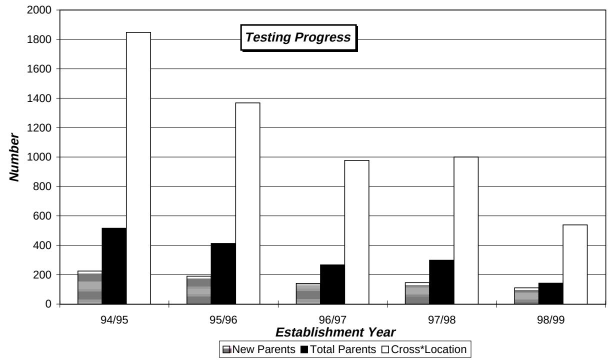

Figure 9. The number of loblolly pine crosses (total number of cross by location combinations), the total number of parents, and the number of parents established in tests for the first time in each of the last five years.

Five members collectively planted a total of 11 progeny tests prior to the 1999 growing season (Table 7) and an additional 10 progeny tests are scheduled to be planted this fall. If the 1999/00 planting season is successful, Boise Cascade Company, Louisiana Department of Agriculture and Forestry, and Oklahoma Forestry Services will be the most recent members to complete the establishment of all of their first-generation field tests. Louisiana Pacific Corporation, the newest member of the cooperative, has assumed responsibility for testing four loblolly pine breeding groups bred by other members of the cooperative. Because others have been willing to transfer the control-pollinated seed from these groups, the Louisiana Pacific Corporation has been able to move into the progeny testing program simultaneously with orchard establishment (Figure 10).

The cooperative now has a large number of ten-year-old tests, planted at a 6 by 8 foot spacing, approaching 10 years of age (Figure 11). After the 10-year measurements are taken in these tests, two of the three duplicate plantings will be abandoned and the third location will be thinned. However, reduced spacing to minimize replication size, combined with increased emphasis on site preparation and better herbaceous weed control, has resulted in unanticipated growth rates. Some of these plantings are requiring a considerable effort to thin in a manner that will maintain the integrity of the planting as a genetic test. Despite these difficulties, the improved efficiency of the progeny testing program resulting from smaller replication sizes and better maintenance has been well worth the effort.

Figure 10. One of two sites Louisiana Pacific Corporation prepared for planting of their first progeny tests.

Figure 11. Mike Lee of the Mississippi Forestry Commission standing between thinned and unthinned portions of an 11-year-old loblolly pine progeny test located in Lauderdale Co., MS. A 50 percent thinning removed 27.8 cords per acre.

Lower Gulf Elite Breeding Population. The cooperative established five polymix tests of the Lower Gulf Elite Breeding Population in 1998. This population is a collaborative effort among the three southern tree improvement cooperatives intended to combine the best selections for the Lower Gulf Region of the U.S. This collaboration is possible because the three tree improvement cooperatives have considerable overlap in their deployment populations for this region. Three of the five tests established by WGFTIP were planted in East Texas and southwest Louisiana and were intended to evaluate the western movement of this seed source. Unfortunately, two of these tests did not survive the summer drought of 1998 and were re-sown for fall planting in 1999. The three tests that did survive the first year had an average survival of 97.4 percent and excellent initial growth (Figure 12).

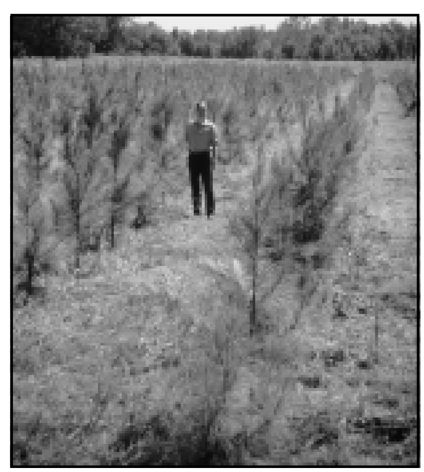

Figure 12. Jim Phillips in the Mississippi Forestry Commission's two-year-old planting of the Lower Gulf Elite Breeding Population polymix test.

Test Measurement and Second-Generation Selection Activity

The record number of crosses established in progeny tests in 1994/95 (Figure 9) translated into a record number of tests to measure and evaluate in 1998/99. In the loblolly pine program, the cooperative measured 45 five-year-old tests (Figure 13) and 15 older plantings. The five-year-old tests provided information on 515 parents, 225 for the first time. These tests contained 1,847 cross by location combinations, all potential candidates for second-generation selections. Five slash pine progeny tests were also measured in 1998/99. The rapid incorporation of this new data into the cooperative database was a major factor in obtaining continued improvement in the production population as well as the source for new advanced-generation selections for the breeding program.

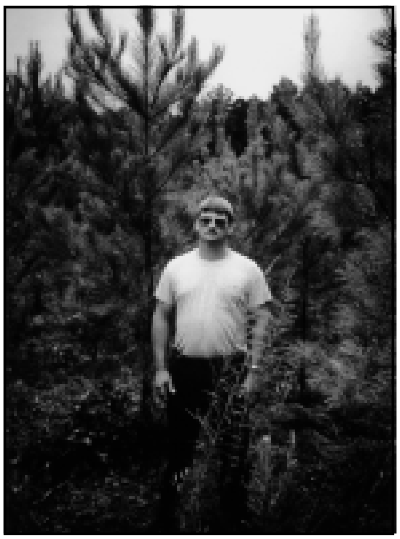

Figure 13. Tim Bosch in a five-year-old progeny test measured in 1999.

The cooperative is making the transition to advanced-generation breeding by identifying new selections, reconstituting breeding groups, and using top grafting to shorten the breeding cycle. In 1999, twelve members of the cooperative identified a total of 127 loblolly pine second-generation selections in 29 different breeding groups (Figure 14). This

Figure 14. A second-generation selection identified in 1998 before a 50 percent thinning was performed at age twelve in 1999. This test is maintained by the Mississippi Forestry Commission in Lauderdale County, Mississippi.

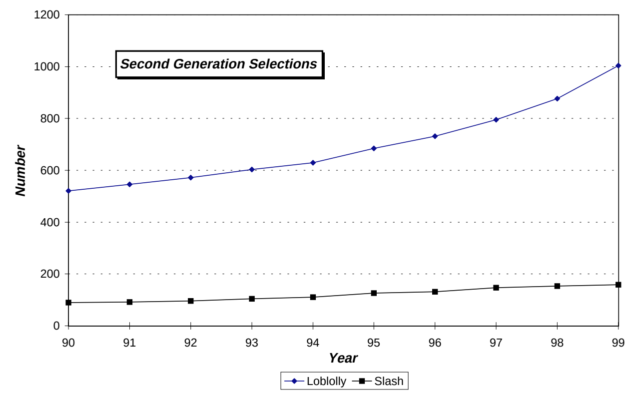

Figure 15. The number of second-generation selections in the cooperative.

included three second-generation selections identified by Willamette Industries in their first progeny test to reach age five. Willamette will contribute to the North Louisiana advanced-generation breeding population with the 11 diallels they now have in the field, each of which is planted in three locations. Six second-generation slash pine selections were also identified in 1999. This year's selection effort was followed by a concerted effort to top graft all of the loblolly pine selections. The cooperative has now identified a total of 1,004 loblolly pine selections and 115 slash pine selections. (Figure 15).

Second-Generation Breeding and Testing

Twelve of the cooperative's sixteen members are actively breeding to produce polymix seed for evaluating second-generation selections. The remaining members have either elected to concentrate on the completion of first-generation breeding or are just now beginning to identify second-generation selections. Four members, the Arkansas Forestry Commission, International Paper Company, the Mississippi Forestry Commission and Weyerhaeuser Company, have used top grafts in their breeding programs. Seven members are also producing controlled pedigree crosses that will serve as the source for third-generation selections.

No second-generation progeny tests were field planted in the 1998/99 season. However, four loblolly pine polymix tests were grown in the greenhouse for field planting in the fall of 1999. These plantings will evaluate a total of 140 selections from East Texas. Three plantings were grown as a collaborative effort and contained second-generation selections from three members. Temple-Inland Forest grew the test seedlings. The Texas Forest Service, Temple-Inland Forest, and Champion International provided the seed and each planted one location.

Additional Activities

Contact Representatives' Meeting

The 1999 Contact Representatives' Meeting was held in Pineville, LA, and co-hosted by representatives from the USDA Forest Service - Alexandria Forestry Center. James Barnett (Project Leader – Ecology and Management of Even-aged Southern Pine Forests) welcomed the members and provided an overview of USDA Forest Service research at Pineville. Les

Groom (Wood Technologist - Utilization of Southern Forest Resources) introduced the forest product research program at the Center. Highlights of the field trip hosted by Dr. Barnett and Dr. Groom included a tour of the wood technology research facilities and silvicultural research plots maintained by the USFS Southern Research Station. (Figure 16).

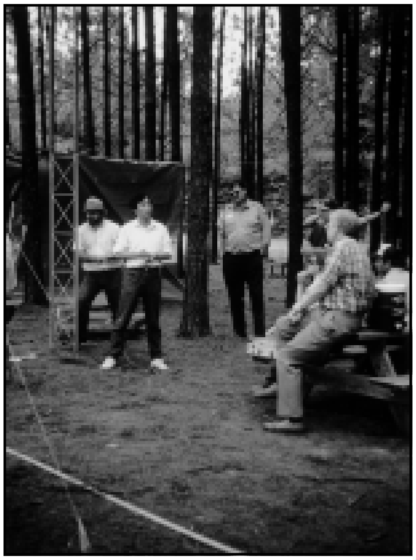

Figure 16. Dr. James Barnett looks on as Dr. Jamie Tang from LSU explains growth and environmental monitoring in a USDA Forest Service research plot to the attendees of the 1999 Contact Representatives Meeting.

The cooperative has always had an interest in the role of tree improvement in the supply of raw material for solid wood products. This interest has only increased as the industry becomes more dependent on small logs. Bob Megraw (Weyerhaeuser Company) contributed to the discussion of wood properties by describing the measurement and impact of microfibril angle. Les Groom expanded on the solid wood products theme by giving an overview of the types of manufactured solid wood products and panels now available. Solid wood properties are difficult to measure, and their economic value is not always defined in a market with many diverse needs. However, the cooperative will continue to devote time and effort to this topic because improving wood properties may be an important future breeding objective.

One possible method to exploit genetic variation in wood properties and growth rate is clonal forestry. Barry Goldfarb (NC State University) addressed this topic by describing recent progress in the production of rooted cuttings. Other possible technologies that may be used in the future include DNA markers and accelerated breeding. Claire Williams (Texas A&M University) described her work in developing microsatellite markers and outlined some practical applications. George Rheinhardt (Arkansas Forestry Commission), Mike Lee and Robert Stewart (both of the Mississippi Forestry Commission) discussed their experiences with top grafting for accelerated breeding.

John Taylor and Alex Mangini (both of the USDA Forest Service) completed the program by providing updates on the Food Quality Protection Act and on insecticides for control of cone and seed insects and tipmoth. The areas of pesticide regulation and registration are changing so rapidly that these updates have become a vital and regular feature of the Contact Representative's Meeting.

Web Sites

Information on the Western Gulf Forest Tree Improvement Program is now available on two different web sites. A brief description of the program and a list of members are available at http://txforestservice.tamu.edu/tfshome/aboutus/forscila.htm. The 46th and 47th Progress Reports of the Cooperative Forest Tree Improvement Program and the recently published brochure entitled "Tree Improvement and Genetic Diversity of Loblolly Pine" can also be downloaded from this site in Adobe Acrobat Reader (pdf) format.

The cooperative's listing on the National Germplasm Resource Information Network can be accessed at http://www.ars-grin.gov/misc/wgftip/index.html. This site lists all of the first-generation loblolly pine selections currently preserved by the cooperative. The county and state of origin and information about the type of stand in which the selections were originally identified are provided. This listing is intended to make the status of the cooperative's ex situ gene preservation efforts available to a wide audience interested in genetic diversity and conservation. This effort has recently been described in an article "Western Gulf Forest Tree Improvement Program Gene Conservation Plan for Loblolly Pine" in the FAO publication Forest Genetic Resources (see Publications).

Western Gulf Tree Improvement Short Course

A Tree Improvement Short Course, attended by more than sixty people, was held in Nacogdoches, TX, in August (Figure 17). The objective of this training session was to be an introduction for new staff members and a refresher course for long time employees on topics ranging from basic genetics to applied tree improvement programs. Several organizations have recently added new staff members. Additionally, all programs are in the transition from first-generation to advanced-generation breeding. This has resulted in changes to breeding and progeny testing programs, including the adoption of complementary mating, single-tree plots, large regional progeny tests, and block plots for selection. Production populations are also changing. Controlled crosses are now supporting the production population, through the creation of super-breeding groups and controlled mass pollination

Figure 17. Randy O'Neal of the Arkansas Forestry Commission examining a subsoiler on display during the Tree Improvement Short Course.

programs. Rapidly changing technology is also adding to the complexity of the tree improvement programs. These changes range from simple innovations, such as top grafting, to more complex operations like vegetative propagation.

All of these innovations have been discussed at previous Contact Representative Meetings. However, the Tree Improvement Short Course was the first time that the cooperative had the opportunity to discuss all of these changes at one meeting. As with most of our training sessions, we relied on a mixture of invited speakers and contributions from our members. Guest speakers and their subjects included Jimmie Yeiser, genetic principles; J. P. van Buijtenen, geographic variation and tree improvement populations; Jerry Tuskan, selection, heritability and application of biotechnology; and Bill Dvorak, biodiversity and germplasm conservation. Practical tree improvement topics were addressed by Lee Allen, fertilization; Alex Mangini, insect protection; and Dave Bramlett, pollen handling and top-grafting. We were also able to utilize the considerable expertise of our members. Members contributing talks on orchard management included Larry Miller, orchard site selection; Bill Jacobs, grafting; I.N. Brown, cone harvesting; Joe Weber, controlled mass pollination; and John McRae, seed extraction and seedling production. Breeding and progeny testing topics were discussed by Bob Purnell, controlled pollination; Richard Bryant, progeny test site selection and preparation; Ron Campbell, test establishment; and George Rheinhardt, test maintenance and measurement.

The quality of the shortcourse was considerably enhanced by the excellent field trip hosted by Jim Tule, Kay McCuller, and the staff at the International Paper Company Forest Seed Center (Figure 18). Attendees were divided into small groups and were able to see and discuss operations ranging from seed handling to orchard management and progeny test measurement. The degree to which tree improvement has always been a cooperative effort was certainly evident at this year's training session.

Seed Orchard Pest Management Subcommittee

The Seed Orchard Pest Management Subcommittee (SOPM) continued to be an important source of information and ideas on the biology and regulatory issues surrounding the control of cone and seed insects. By providing an informal forum, seed orchard managers, entomologists, and policy experts have been able to effectively leverage their individual efforts to support tree improvement programs and to further research. The tree improvement community has benefited from this cooperation by making significant progress toward the use of chemical controls as part of integrated pest management systems. The importance of this collaboration was certainly apparent in 1999.

Figure 18. Willie J. Brown of the International Paper Forest Seed Center demonstrating some of the steps in seed processing to the attendees of the Tree Improvement Short Course.

The Environmental Protection Agency (EPA), under the auspices of the Food Quality Protection Act, is currently reviewing the status of most pesticides used in the United States. Seed orchards are especially vulnerable because of the limited number of chemicals labeled for cone and seed insect control, the high application rates necessary to protect large trees, and the small size of the market compared to agricultural crops. In 1999 the status of Guthion®, which has been the standard for cone and seed insect control since its introduction into seed orchards, was reviewed. Initially, the manufacturer decided to delete conifer seed orchard use from the label to protect larger, more profitable markets. This would have required seed orchards to depend on synthetic pyrethroids, a class of chemicals known to cause buildups of secondary insects.

Because of the Guthion® rate study organized by the SOPM subcommittee and conducted in seed orchards across the South, John Taylor (USDA Forest Service – Forest Health Protection) and others were able to respond rapidly to the concerns of the manufacturer and the EPA. Because there was evidence that Guthion® can be effective at lower rates than the maximum rates previously allowed and applied in ways that limit worker exposure, the seed orchard registration was maintained. The near loss of this important chemical dramatically underscores the need for continued support of regional research on cone and seed insect control.

The SOPM subcommittee is taking several steps to prepare for future pesticide reviews. The committee is compiling the results of a pesticide use survey intended to cover the last three management years. This empirical evidence is needed because the EPA calculates risk from exposure based on the maximum allowable application rates if actual use data is not available. To support this documentation, a crop profile is also needed to outline typical worker exposure patterns to those responsible for regulating pesticide use. Pesticide labels must be periodically reviewed to identify application rates that are excessive when compared to other crops.

All of this activity highlights two important needs. The first is to use good management practices when applying currently registered products. The tree improvement community has too few available chemicals to risk losing a control option as a result of a preventable accident. The second need is to continue the development of efficacy and rate information based on operational application methods. To this end, a southwide rate study for Asana® is planned in 2000. These regional studies are expensive both in terms of labor and seed lost due to inadequate protection in some treatment blocks. The tree improvement community must make this investment or risk losing cone and seed insect control options as chemicals are scheduled for regulatory review.

There were several other developments in the SOPM subcommittee this year. Larry Barber (USDA Forest Service) retired in 1999. He will be greatly missed by the tree improvement community. The USDA Forest Service, in response to the SOPM subcommittee policy statement published in the Journal of Forestry and the participation of committee members in the Southern Research Station's Technical Assistance Visits, appointed Dan Miller as a research entomologist. He is stationed at Athens, GA, and his job duties include continuing USDA Forest Service cone and seed insect research.

The SOPM subcommittee is supporting registration efforts for two new chemicals, Imidan® and Warrior T®. Imidan® is an organophosphate pesticide that Gary DeBarr (USDA Forest Service) and others have shown to be nearly as effective as Guthion® in control of coneworms and seedbugs. Warrior T® is a third generation synthetic pyrethroid. DeBarr and Alex Mangini (USDA Forest Service) are just completing efficacy studies on single tree treatments using this chemeical. Early data indicates that Warrior T® may offer exceptional levels of control. Manufactures of both chemicals have indicated a willingness to pursue seed orchard registration.

Formal Reviews

The cooperative conducted five formal reviews in 1999. These program assessments consist of a field review of progeny tests, seed orchards, and facilities followed by an office evaluation of long range objectives. One of the major benefits of this process has been increased interaction between those within an organization responsible for shaping strategic objectives and those responsible for implementing the program. The formal reviews have proved equally valuable to the cooperative staff by providing regular input from the members on how well the cooperative is meeting their needs. The three-year cycle for these reviews has proven to be very useful given the current rate of change in the forest industry, natural resource management, and tree improvement.

Forest Genetics Research at Texas A&M University[5]

Two novel protocols for construction of low-copy DNA libraries are being used to search for microsatellite markers in gene-rich regions of the pine genome. Libraries, lines of bacterial cells into which a segment of foreign DNA has been inserted, can be propagated to preserve and greatly increase the amount of foreign DNA available for study. Because the pine genome is so massive, cloning random fragments of pine DNA is likely to be ineffective. To overcome this problem, two techniques that enrich the DNA fragments for low copy sequences prior to cloning have been developed. These techniques are based on reassociation kinetics and isolation of under-methylated DNA.

Microsatellite markers isolated from these libraries have proved to be highly variable and useful for a number of purposes. Some microsatellite DNA sequences are currently available at http://forestry.tamu.edu/genetics and others will be released as they are validated. To date, these markers have been used to estimate genetic diversity, allele numbers, genetic differentiation, and possible founder effects in a rangewide survey of loblolly pine. They have been used to detect genes contributing to reduced genetic fitness in loblolly pine. The markers are also being placed on genetic maps so they can be used for QTL detection and candidate gene detection.

HARDWOOD TREE IMPROVEMENT PROGRAM

Highlights

- The last series of Nuttall oak progeny tests was established in 1999. Currently, the members maintain 22 Nuttall oak progeny tests that include seedlings from 216 selections.

- The Arkansas Forestry Commission and Temple-Inland Forest, in a collaborative project with the North Carolina Hardwood Cooperative, grew the first second-generation sweetgum progeny tests with a total of 130 families.

- Potlatch Corporation collected 15-year data from their hardwood natural regeneration study. Shearing significantly increased the oak stocking as compared to the roller chop, injection, and control treatments.

Tree Improvement

Progeny Testing

The members of the Hardwood Cooperative have been active in developing improved genetic varieties of selected hardwood species. The first species selected for testing were sycamore, sweetgum, and green ash. Progeny testing for cherrybark oak, water/willow oak and yellow-poplar was initiated at a later time. Recently, the members began a testing program for Nuttall oak. To accomplish their goal, the members have established a total of 189 progeny tests. The tests are abandoned after 20-year data have been obtained. In 1999, the members maintained 40 progeny tests (Table 9). The newest species selected for study, Nuttall oak, comprised the majority of these tests (22).

Table 9. Active progeny tests in the Hardwood Tree Improvement Program.

Species	Number of Tests
Cherrybark oak	9
Green ash	1
Nuttall oak	22
Sweetgum	1
Sycamore	4
Water/Willow oak	3

[5] Submitted by Dr. C. G. Williams

Figure 19. Matt Lowe, Temple-Inland Forest, with their second-generation sycamore progeny test in the second growing season.

Figure 20. George Rheinhardt in the Nuttall oak progeny test maintained by the Arkansas Forestry Commission during the sixth growing season.

Temple-Inland Forest collected first-year data on their second-generation sycamore progeny test established in cooperation with the N. C. State University –Industry Cooperative Hardwood Research Program (Figure 19). This test includes 40 open-pollinated families from both cooperatives. Survival after the first growing season averaged 98 percent. Height ranged from 1.4 m to 1.8 m with an average of 1.6 m. Third-year data from this study will be used to determine which seed orchard clones will be used for operational seed collection. This data will also be shared with the North Carolina Hardwood Cooperative to evaluate the importance of a genotype by environment interaction for planted sycamore in the lower coastal plain.

Fifth- and first-year data were collected from the oldest and youngest series of Nuttall oak progeny tests. The oldest test series includes 42 open-pollinated families collected in Arkansas, Mississippi, and Texas and was planted by the Arkansas Forestry Commission, the Louisiana Department of Agriculture and Forestry, the Mississippi Forestry Commission, and Potlatch Corporation. Average survival ranged from 75 to 96 percent among the four tests. Height growth averaged 1.8 m and ranged from 1.4 m to 2.5 m. The best growth was obtained in the test maintained by the Arkansas Forestry Commission (Figure 20). At age five, there were no differences indicating that seed collected from any state performed better than seed collected from any other state. Families collected from each of the three states were represented in the best performing 20 percent of all families in each test. The ten-year data from these plantings will be used to select clones for seed orchard establishment.

Six Nuttall oak progeny tests were established during the 1997/98 planting season. The 1998 drought caused severe mortality in two of the tests, which had to be abandoned. The tests established by the Arkansas Forestry Commission, the Louisiana Department of Agriculture and Forestry, the Mississippi Forestry Commission, and Temple-Inland Forest survived the drought and had an average first-year survival of 88 percent. Survival varied from 77 to 97 percent among the tests.

The last test series for Nuttall oak was planted at five locations during the 1998/99 planting season. Even though another drought occurred in 1999, survival appears to be adequate in all of these tests. This series completed the selection of the genetic base for this species. The members are maintaining 22 Nuttall oak progeny tests that contain 216 selections from Arkansas, Louisiana, Mississippi, and Texas.

Fifteen- and twenty-year data were collected from eight cherrybark oak progeny tests. The average plantation performance for the 20-year tests is shown in Table 10. The test in Clark County, Arkansas, had the best growth. This test was maintained by International Paper Company and had an average height of 17 m. Family performance ranged from 16 m to 18 m in height. As with the past tests, families in which second-generation selections were made at earlier ages continued to perform well. Average volume for these families

Table 10. Twenty-year data summary for cherrybark oak progeny tests.

Location	Plantation Average				Pct.[1]
(Co./Par., State)	Survival (%)	Height (m)	Diameter (cm)	Volume (dm^3)	Vol. Imp.
Angelina Co., TX	71	13.8	16	81.6	19.5
Clark Co., AR	79	16.9	21	165.5	9.9
Montgomery Co., TX	69	7.4	9	23.5	20.4
Sabine Par., LA	79	16.2	16	98.3	15.0
St. Landry Par., LA	70	11.4	9	32.4	11.6
Tyler Co., TX	75	14.9	17	98.3	22.3

[1] Percent volume improvement is the mean of the selected open-pollinated families compared to the plantation average.

was 16 percent greater than the test means at age 20. Similar results were obtained for the 15-year tests. Because second-generation selections had previously been identified in the best families in these tests, no additional selections were made this year. The 20-year tests have been released to the cooperators and will not be measured in the future.

Temple-Inland Forest obtained first-year data from their project to evaluate the performance of selected hardwood species grown under intensive management for fiber production. Pure blocks (100 trees) of cottonwood, sycamore, and sweetgum were planted in a replicated field design. Ten-tree rows of either ten clones (cottonwood) or ten families (sycamore and sweetgum) were established in each plot. A block comparing the performance of several other species was also established. The entire study was intensively managed with fertilization and irrigation (Figure 21).

Table 11 presents the first-year data for the pure blocks. Survival was excellent for the three species ranging from 92 percent for cottonwood to 98 percent for sycamore and sweetgum. Cottonwood had the best growth of the three species, and sycamore had the second fastest growth. Survival and height growth did not differ among the cottonwood clones or sycamore families included in the test; however, significant differences in diameter were detected among families within both species. Significant differences in height were also detected among the sweetgum families included in the study.

Table 11. Species comparisons at one year for Temple-Inland Forest's intensive management hardwood species trial. [1]

Species	Survival (Percent)	Height (m)	Diameter (cm)
Cottonwood	92 b	2.2a	1.3a
Sycamore	98a	1.4 b	0.7 b
Sweetgum	98a	1.0 c	0.4 c

[1] Means followed by the same letter are not significantly different at the 10 percent level by Duncan's New Multiple Range Test.

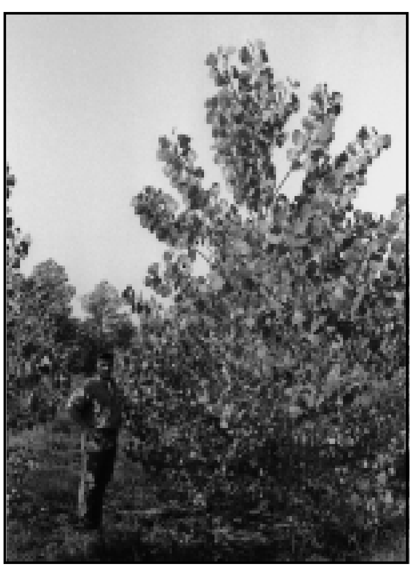

Figure 21. The hardwood intensive management project shown by Matt Lowe is maintained by Temple-Inland Forest.

In a cooperative project with the North Carolina Hardwood Cooperative, second-generation sweetgum progeny tests will be established with selections from both programs. The Arkansas Forestry Commission and Temple-Inland Forest grew tests in 1999 that will be planted in Arkansas and Texas (Figure 22). These tests contain 130 families of which 37 are from the Western Gulf Program. Members of the North Carolina Hardwood Cooperative will establish tests east of the Mississippi River, and the data will be shared between both programs. Plant material will also be exchanged for use in future seed orchards.

Figure 22. A second-generation sweetgum progeny test grown by Temple-Inland Forest.

Selections and Seed Orchards

The members have made a total of 334 second-generation hardwood selections among the six species shown in Table 12. Five new water oak second-generation selections were identified in two progeny tests during the past year. All of these selections have been preserved by grafting into scion banks. Seed orchards for selected species continue to be established by the state agencies. The Arkansas Forestry Commission, the Mississippi Forestry Commission, and the Texas Forest Service are grafting cherrybark oak seed orchards (Figure 23). Because of the increased demand for cherrybark oak seed, the Mississippi Forestry Commission is planning a seed orchard expansion for this species.

The Texas Forest Service continued grafting first-generation Nuttall oak selections in 1999. Grafting has been successful for Nuttall oak with an average 85 percent graft take in 1999. To date, the Mississippi Forestry Commission and the Texas Forest Service have preserved 185 of the 216 first-

Table 12. Number of second-generation selections in the Hardwood Tree Improvement Program.

Species	Number of Selections
Cherrybark oak	62
Green ash	61
Sweetgum	84
Sycamore	70
Water/Willow oak	45
Yellow poplar	12

Figure 23. The cherrybark oak seed orchard established by the Arkansas Forestry Commission.

generation Nuttall oak selections in scion banks (Figure 24). These scion banks will produce the scion for seed orchard establishment after progeny tests have identified the best selections. Grafting efforts will be continued in 2000 to complete the preservation of the selections for this species.

The expanded bottomland hardwood reforestation programs have created an increased demand for hardwood seedlings. Many of the older seed orchards are producing sufficient quantities of seed to help meet this demand in the members' nurseries (Figure 25). To ensure that only the highest genetic quality seed is used in the nurseries, the members are considering establishing open-pollinated progeny tests to evaluate the selections in the seed orchards. The data from these tests will be used to rogue seed orchards and determine which clones will be used for seed collection. The Texas Forest Service started open-pollinated seed collection by family for this purpose from their sycamore, sweetgum, and green ash seed orchards in 1999 (Figure 26). The membership will determine which species will be evaluated in progeny tests at the Executive Committee Meeting in 2000.

Figure 24. A Nuttall oak graft in the Texas Forest Service scion bank.

Figure 25. The Texas Forest Service sweetgum seed orchard at Hudson, TX.

Natural Regeneration

Potlatch Corporation collected 15-year data on their hardwood natural regeneration study. Four site preparation treatments (control—no treatment, high shear, roller chop, and inject) were established in a randomized-complete-block design with three replications. The study was installed in 1983 following an operational clearcut. Within the study, 192 permanent 0.1-acre circular plots were established. Diameter at breast height (DBH) and species class were determined on all stems greater than two inches DBH in each plot. Height was also measured on the tallest two trees (excluding residuals) in each plot for the following species classes: 1) cherrybark oak, 2) water/willow oak, 3) sweetgum, 4) miscellaneous desirable species and 5) undesirable species.

At age 15, site-preparation treatment had a significant effect on stocking (Table 13). Average stocking in the study was 398 trees per acre. The control treatment had fewer new trees greater than two inches diameter than any of the other treatments. The effect of site-preparation treatment was most pronounced for the amount of oak regeneration present in the study. The shear treatment averaged 125 oak trees per acre. This was significantly greater than any of the other site-preparation treatments. The inject and chop treatments averaged 82 oak trees per acre, which was significantly greater

Figure 26. Green ash seed collected by open-pollinated family from the Texas Forest Service seed orchard.

Table 13. Data summary at age 15 by site-preparation treatment for all species (excluding residuals) in Potlatch Corporation's hardwood natural regeneration study. [1]

Treatment	Trees/ Acre	DBH (cm)	Height (m)	Basal Area (feet^2/acre)
Control	260 b	11a	13.6a	35.9a
Insect	423a	9a	12.5a	37.3a
Chop	430a	10a	13.2a	40.2a
Shear	481a	10a	12.8a	48.3a

[1] Means followed by the same letter are not significantly different at the 10 percent level by Duncan's New Multiple Range Test.

Table 14. Data summary at age 15 by treatment for all species (including residuals) in Potlatch Corporation's hardwood natural regeneration study. [1]

Treatment	Trees/ Acre	DBH (cm)	Height (m)	Basal Area (feet^2/acre)
Control	304a	13.7a	13.7a	69.4a
Insect	431 b	9.6 b	12.0a	41.6 b
Chop	436 b	10.4 b	12.5a	49.4 b
Shear	482 b	9.7 b	12.5a	48.6 b

[1] Means followed by the same letter are not significantly different at the 10 percent level by Duncan's New Multiple Range Test.

than the control treatment which averaged 49 oak trees per acre. The site-preparation treatment did not have a significant effect on the stocking of any of the other species classes.

Site-preparation treatment did not have a significant impact on average DBH (10 cm), height (13.0 m), or basal area (40.4 square feet per acre) when all of the species were considered. Site-preparation treatment did have a significant effect on the diameter growth of the undesirable species class. The undesirable trees in the control treatment had a larger diameter (9 cm) than any of the other treatments (7 cm).

The control treatment averaged 44 residual trees per acre with a basal area of 33.5 square feet. The other treatments averaged 5 residual trees per acre with 4.6 square feet of basal area. When the residuals were included in the analysis, the control treatment had the largest average DBH and the most basal area (Table 14). The frequency of the residual trees was not sufficient to have a significant effect upon stocking, so the control treatment still had the fewest number of trees per acre. No stem quality measurements were taken at age 15. The impact of the residual stems on overall stem quality will be evaluated in later measurements.

PERSONNEL

Jennifer Myszewski joined the College Station staff in 1999 as a graduate student pursuing a doctoral degree. Support for her assistantship is provided by the USDA Forest Service, and she is working for both the USDA Forest Service and the WGFTIP programs. Myszewski has an MS degree from the University of Idaho and a strong interest in applied tree improvement. Mary Trejo, who served as the cooperative's secretary for the last year, left the cooperative to pursue other opportunities within the Texas Forest Service. There were no other changes in the Western Gulf Forest Tree Improvement Program or the Texas Forest Service tree improvement staffs in 1999.

The staff now includes the following:

W. J. Lowe .... WGFTIP Geneticist
T. D. Byram .... Assistant WGFTIP Geneticist
G. D. Gooding .... Assistant WGFTIP Geneticist
J. G. Hernandez .... Research Specialist
J. H. McLemore .... Aide to Specialist
J. H. Myszewski .... Graduate Student
G. R. Lively .... Research Specialist
I. N. Brown .... Research Specialist
D. M. Travis, Jr. .... Aide to Specialist
G. F. Fountain .... Aide to Specialist

PUBLICATIONS

Bridgwater, F.E., D.L. Bramlett, and V.D. Hipkins. 1999. Timing of bag application and removal in controlled mass pollination. Proc. 25th Southern Forest Tree Improvement Conference, New Orleans, LA. July 12-14 (in press).

Byram, T.D. and F.E. Bridgwater. 1999. Risk analysis of loblolly pine controlled mass pollination programs. Proc. 25th Southern Forest Tree Improvement Conference, New Orleans, LA. July 12-14 (in press).

Byram, T.D. and W.J. Lowe. 1998. Seed orchard production: Its potential and its limitations. National Proceedings: Forest and Conservation Nursery Associations. LaFayette, LA. July 13-15. pp. 10-13.

Byram, T.D., W. J. Lowe, and G.D. Gooding. Western Gulf Forest Tree Improvement Program gene conservation plan for loblolly pine. Forest Genetic Resources (in press).

Byram, T.D., J.W. Richardson, and W.J. Lowe. 1999. Predicting seed yields from loblolly pine seed orchards: A simulation approach using TImpSim. Southern Journal of Applied Forestry 23(2): 117-123.

Elsik, C.G., V.T. Minihan, S.E. Hall, A.M. Scarpa and C.G. Williams. Low-copy microsatellite markers for *Pinus taeda* L. Genome (in press).

Gooding, G.D., F.E. Bridgwater, D.L. Bramlett and W.J. Lowe. 1999. Top grafting loblolly pine in the Western Gulf region. Proc. 25th Southern Forest Tree Improvement Conference, New Orleans, LA. July 12-14 (in press).

Gwaze, D.P., F.E. Bridgwater, T.D. Byram, J.A. Woolliams and C.G. Williams. Predicting age-age genetic correlations: a case study with *Pinus taeda* L. Theoretical and Applied Genetics (in press).

Ji, Y.-F., D.M. Stelly, M. De Donato, M.M. Goodman and C.G. Williams. 1999. A candidate recombination modifier gene for *Zea mays* L. Genetics 151(2):821-830.

La Farge, T., F.E. Bridgwater and M. N. Baez. 1999. Performance of loblolly pine seed sources in Argentina. Proc. 25th Southern Forest Tree Improvement Conference, New Orleans, LA. July 12-14 (in press).

Lowe, W.J., T.D. Byram and F.E. Bridgwater. 1999. Selecting loblolly pine parents for seed orchards to minimize the cost of producing pulp. Forest Science 45(2): 213-216.

Myszewski, J.H., L. Fins, J.A. Moore, W.C. Rember, M. Rust, and P. Mika. 1999. Variation in root bark chemistry of Douglas-fir. Proc. 25th Southern Forest Tree Improvement Conference, New Orleans, LA. July 12-14 (in press).

van Buijtenen, J.P. 1999. Genomics and quantitative genetics. Paper presented at the Gene Namkoong Symposium, Vancouver, BC. July 23-24.

Williams, C.G., R.D. Barnes and I. Nyoka. 1999. Embryonic lethal load for a neotropical conifer, *Pinus patula* Schiede and Deppe. Journal of Heredity 90(3): 394-398.

Williams, C.G., C.G. Elsik and R.D. Barnes. Microsatellite analysis of *Pinus taeda* L. in Zimbabwe. Journal of Heredity (in press).

COOPERATIVE TREE IMPROVEMENT PROGRAM MEMBERS

Western Gulf Forest Tree Improvement Program

Pine Program

Full members of the Western Gulf Forest Tree Improvement Pine Program include the Arkansas Forestry Commission, Boise Cascade Company, The Bosch Nursery, Inc., Champion International Corporation, Deltic Timber Company, Inc., International Paper Company, Louisiana Department of Agriculture and Forestry, Louisiana-Pacific Corporation, Mississippi Forestry Commission, Oklahoma Forestry Services, Potlatch Corporation, Temple-Inland Forest, Texas Forest Service, The Timber Company, Inc., Weyerhaeuser Company, and Willamette Industries, Inc.

Associate members include International Forest Seed Company, Louisiana Forest Seed Company, Pacific Millennium Corporation, and Robbins Association.

Hardwood Program

The WGFTIP Hardwood Program includes the Arkansas Forestry Commission, Champion International Corporation, Louisiana Department of Agriculture and Forestry, Mississippi Forestry Commission, Potlatch Corporation, Temple-Inland Forest, and the Texas Forest Service.

Urban Tree Improvement Program

Membership in the Urban Tree Improvement Program includes the following municipalities and nurseries: Aldridge Nurseries (Von Ormy), Altex Nurseries (Alvin), Baytown, Burleson, Carrollton, Dallas, Dallas Nurseries (Lewisville), Fort Worth, Garland, Houston, LMS Landscape (Dallas), Plano, Rennerwood (Tennessee Colony), Richardson, Robertson’s Tree Farm (Whitehouse), and Superior Tree Foliage (Tomball).

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