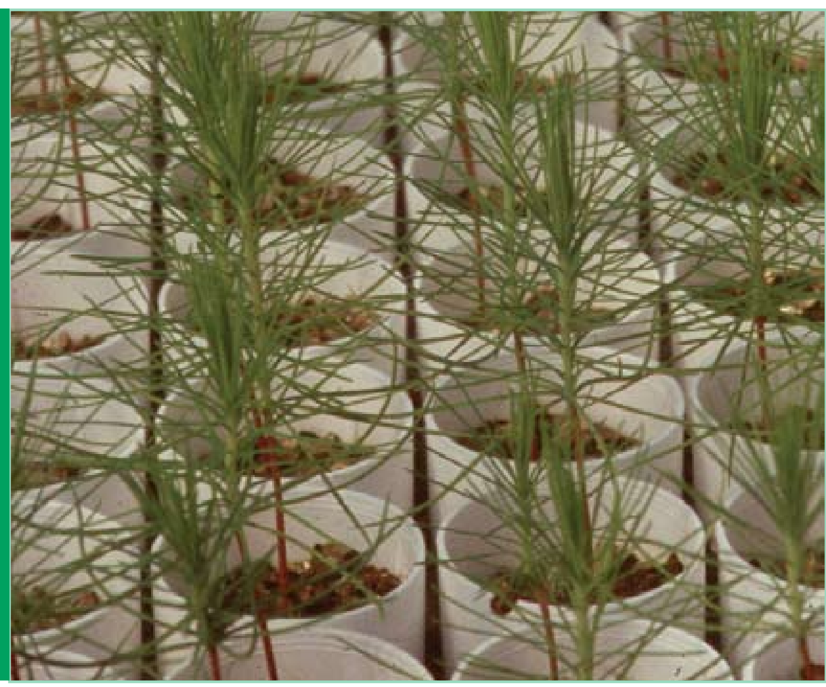
December 2009

55th PROGRESS REPORT OF THE COOPERATIVE FOREST TREE IMPROVEMENT PROGRAM



TEXAS FOREST SERVICE

FIFTY-FIFTH

PROGRESS REPORT

OF THE

COOPERATIVE

FOREST TREE IMPROVEMENT

PROGRAM

By

T. D. Byram, L. G. Miller, and E. M. Raley

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TEXAS FOREST SERVICE
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INTRODUCTION

The 55th Progress Report of the Cooperative Tree Improvement Program is being published after a hiatus of two years. A number of factors have contributed to this gap. During this period the program, while continuing to move aggressively in the area of breeding and progeny testing, has matured into a "business as usual" mode of selection and regular orchard replacement. Retrenchment within the forestry community continues to take its toll on our resources and has made it necessary for the staff to concentrate on program delivery. Because of outside collaboration on major research projects, the Progress Report has also become less significant for releasing research results. Members continue to receive reports through our internal channels and Progress Reports to the public will be made periodically.

The highlight of 2007 was the opportunity for the Western Gulf Forest Tree Improvement Program (WGFTIP) to co-host the first ever joint meeting of the Southern Forest Tree Improvement Conference and the Western Forest Genetics Association. The meeting held June 19-22 in Galveston, TX, was attended by 120 participants from 19 US states and 11 different nations (Figure 1). Featured speakers, from a range of professional backgrounds, focused on regional differences among tree improvement programs, novel applications for tree improvement tools, and the potential response of the forest genetics community to future challenges. Fifty-two volunteer papers covered topics as widely varied as classical forest genetics, ecophysiology, evolutionary biology, and molecular genetics.

A significant event in 2008/09 was the establishment of the first clonal line trials from the Wood Quality Elite population. While many of our members have been instrumental in developing family and clonal forestry deployment strategies, this represented WGFTIP's first attempt to use this technology as a tool for within-family selection. These clonally replicated trials should improve the accuracy of within-family selection. The objective is to identify individuals that combine improved wood specific gravity with growth rate, two traits that appear to have a slight but negative correlation in our mainline breeding population. This was an outstanding example of leveraging the efforts by many different organizations to accomplish a common goal. Five organizations made the crosses represented in these plantings, CellFor, Inc. initiated the lines, seedlings were grown by one of our members under contract with CellFor, and six members established plantings (Figure 2). Trials were established for both the Arkansas and Texas Wood Quality Elite populations.

Other major activities over the three-year period covered by this report centered on the cooperative's continuing response to our member's changing expectations and capabilities. A primary example of this was the restructuring of the breeding population required to accommodate International Paper Company's reconfiguration from an integrated forest industry into ArborGen, LLC, a supplier of genetically improved planting material, and the elimination of tree improvement, seed orchards, and nurseries by the state of Mississippi. Both programs were large and complex, supporting multiple breeding populations intended to supply geographically diverse planting zones. Over the last three years, as the cooperative scrambled to reorganize one program and close the other we have had to find homes for some outstanding selections that previously belonged to these two organizations.

Figure 1. Attendees at the Joint Meeting of the Southern Forest Tree Improvement Conference and the Western Forest Genetics Association held June 19-22, 2007 in Galveston, TX.

At the time the WGFTIP was organized in 1969, International Paper Company managed seed orchards, breeding, and progeny testing programs in three of the five states in our operating region. Over the following three decades through mergers and acquisitions, they acquired and then maintained three additional breeding programs that had previously been independently operated. As a result, International Paper Company had the largest breeding and progeny testing program in the cooperative and contributed a substantial number of tested selections to all of the advanced-generation orchards in the region. In addition, International Paper Company's South Arkansas breeding program generated the very first third-cycle selections in the WGFTIP. This level of activity was justified because of downstream profits from increased harvests on company lands and cash flows from outside seedling sales. Outside seedling sales could also be subsidized when necessary as they ensured a cheap and stable source of raw material for company mills.

Figure 2. Les Welsh of Deltic Timber Corporation with crosses made to support the Wood Quality Elite program.

Likewise, the Mississippi Forestry Commission had an extensive breeding and progeny testing program contributing to two breeding populations of loblolly pine, a slash pine population, a longleaf pine program, and hardwood programs for several different species. As with most publicly funded breeding programs, the State of Mississippi was motivated by the desire to have the best planting material distributed as widely as possible with the expectation that increased forest productivity would serve the public good as an economic driver. They also viewed improvement programs for minor species as a public service to support ecosystem restoration. As is common with most states, the tree improvement program was ultimately linked to seedling sales from state run nurseries.

An unpleasant but undeniable fact is that nursery sales capture only a fraction of the value added from genetic improvement, frequently leaving other parts of the business or public funds to cover the costs of tree improvement. In recognition of the operational constraint this places on the size of the breeding program, the cooperative originally agreed that International Paper Company should concentrate their efforts on their East Texas population. The International Paper Company Nursery and Orchard business was then acquired by ArborGen, LLC and combined with the MeadWestvaco program on the East Coast to create the largest supplier of improved genetic material for reforestation in the South. ArborGen, LLC, who had originally joined the cooperative as a Sustaining Member in 2006, agreed to support the program as a Full Member beginning in 2007. The Mississippi Forestry Commission (MFC), faced with economic constraints brought on by declining sales from state nurseries, opted to eliminate their programs for tree improvement, seed orchards and nurseries.

Outcomes from these two situations are vastly different for the cooperative. ArborGen, LLC is maintaining a sizeable proportion of the East Texas population that includes selections from three different former cooperative members. Furthermore, this is an important population to the cooperative because of its growth rate, wide adaptability, and fusiform rust resistance. In contrast, the Mississippi Forestry Commission decided to completely shutter their program. They were the only organization in the cooperative generating statewide performance data for the landowners in the Mississippi deployment zone. More importantly, the state program fed selections into two private programs within their breeding zone, supplemented the selection population in an adjacent breeding zone, and supported regionally important programs in slash pine, longleaf pine, and multiple hardwood species.

Figure 3. Robert Whitmire of ArborGen, LLC with grafts of International Paper Company elite selections that were transferred to the Arkansas Forestry Commission and the Louisiana Department of Agriculture and Forestry to be archived.

The MFC longleaf orchard is currently being managed by the USFS to support ecosystem restoration for this species. The remaining slash and loblolly pine orchards at the MFC Craig Seed Orchard complex, which had been decimated by Hurricane Katrina, were cleared and are being converted to an operational longleaf pine stand. The immediate reforestation needs of the landowners in Mississippi will be provided for by surplus seed production from the current cycle of seed orchards operated by others. It is uncertain whether future advanced-generation orchards will have sufficient capacity to supply all of the state's landowners. The ultimate cost, however, will be felt as the rate of gain in all of the programs that they supported through testing and selection will be adversely affected. The Mississippi Forestry Commission's contribution to the regional tree improvement effort will be sorely missed.

Figure 4. Louisiana Department of Agriculture and Forestry scion bank at Oberlin, LA where selections from the orphaned International Paper Company's North Louisiana population were archived. This facility was activated and the trickle irrigation installed solely for this purpose.

Both organizations attempted to ensure that valuable breeding material was not lost by making germplasm available to other members of the WGFTIP breeding programs. This was especially true for International Paper Company who went to great lengths to make sure that valuable breeding material in the South Arkansas and North Louisiana populations was transferred to other members of the cooperative. Through some heroic efforts on the part of Dan Morrow and Bob Purnell large numbers of selections were provided as potted grafts (Figure 3). In turn, the Arkansas Forestry Commission and the Louisiana Department of Agriculture and Forestry (LDAF) supplied an invaluable service to the larger tree improvement community by ensuring that these selections were preserved. The Louisiana Department of Agriculture and Forestry established the North Louisiana population in long-term scion bank at the decommissioned nursery site near Oberlin, LA (Figure 4). The Arkansas Forestry Commission maintained the selections from South Arkansas in pots and serially grafted individuals represented by low numbers (Figure 5). Most of these selections will ultimately be infused in several different breeding programs in both regions. The Mississippi Forestry Commission provided access to scion material for selections from both the loblolly and slash programs to other WGFTIP members. Campbell Timberland Management, Forest Capital Partners, the LDAF and Plum Creek Timber Company have gone above and beyond the call of duty to capture this material in their scion banks.

The WGFTIP germplasm conservation program also suffered. Because of the massive numbers of selections that had to be transferred in short order, priority was given to preserving selections with known performance or with progeny tests awaiting evaluation. Some first-generation selections representing the wild loblolly population that existed before domestication began were lost.

Figure 5. Randy O'Neal with the Arkansas Forestry Commission with some of the serial grafts he completed to help ensure there were adequate numbers of the orphaned International Paper Company South Arkansas population to be distributed to other members.

The Texas Forest Service (TFS) also closed its Indian Mound Nursery because of declining seedling sales. This was one of two nurseries operated by the Texas Forest Service and primarily provided bare-root pine seedlings for reforestation. The TFS West Texas Nursery at Idalou not only remains open but was recently expanded to provide planting material for windbreaks and ecosystem restoration projects in central and west Texas. Despite closing the pine seedling nursery, the TFS is taking a proactive approach to tree improvement. The TFS is doing this in recognition that the public tree improvement program continues to benefit the state's taxpayers by providing commercial partners with genetically improved material that can in turn be delivered to the landowners through privately operated seed orchards and nurseries. The TFS continues to breed and progeny test the proportion of the East Texas population for which it is responsible. The TFS is also continuing to support research projects that improve the overall efficiency of private programs. These activities include the development of elite breeding populations, development of new breeding tools such as molecular markers, and the evaluation of new pesticides for control of cone and seed insects. As an example of an activity in this last category, the TFS orchard was one of two locations testing the efficacy of a new pesticide in 2009.

Other developments that impacted the WGFTIP membership included the transfer of forest land from Temple-Inland Forest to Campbell Timberland Management, LLC and the divestiture of paper mills by both Potlatch Land and Lumber and Weyerhaeuser Company. As a result of these changes, no member of the cooperative currently operates a paper mill in our area. This is an astonishing turn of events given that paper mill operators were the prime movers in establishing the tree improvement program in the 1950s. The long-term significance of this change is unclear, but it will most likely drive the breeding program toward factors that improve stumpage values to the landowner by lowering production costs and improving the mix of high-value solid wood products. The western Gulf Coast of the US also experienced its third major hurricane in as many years. No orchards were lost to Hurricane Ike in 2008, but trees were downed and orchard capacity was detrimentally impacted in east Texas.

The current membership of the WGFTIP stands at 13 full members with breeding and progeny testing responsibilities. This includes the four state forestry agencies in the states of Arkansas, Louisiana, Oklahoma, and Texas, and nine commercial operations organized as integrated forest industries (2), real estate investment trusts (REITs) or timber investment management organizations (TIMOs) (5), and regeneration companies (2).

WESTERN GULF FOREST TREE IMPROVEMENT PROGRAM

Highlights

- The WGFTIP has 2,177 acres of loblolly, slash, longleaf, and shortleaf pine seed orchards, of which 1,329 acres are actively managed and 847 acres have been mothballed.

- Of the actively managed orchards, 48 acres are heavily rogued first-generation loblolly and slash pine orchards and 61 acres are minor species such as shortleaf and longleaf. The remaining 1,220 acres are advanced-generation loblolly and slash pine orchards with individuals selected for proven performance.

- Loblolly pine harvests totaled 6,375 pounds of seed in 2007, 16,459 pounds of seed in 2008 and 25,200 pounds of seed in 2009. Slash pine seed harvests were 792 pounds in 2007, 2,031 pounds in 2008 and 2,376 pounds in 2009. Members are self-sufficient for improved seed and are managing inventories by collecting only the highest gain families.

- The Louisiana Department of Agriculture and Forestry collected 1,672 pounds of longleaf pine seed from their seedling seed orchard in 2008 and 1,176 pounds of seed in 2009. The Arkansas Forestry Commission collected 134 pounds of shortleaf seed from a young four-acre orchard in 2009.

- In the last three years, the WGFTIP established 53 plantings in the progeny testing program. This included the last three first-generation control-pollinated loblolly pine progeny tests, 22 advanced-generation loblolly pine polymix progeny tests, three advanced-generation slash pine polymix tests, four Virginia pine Christmas tree plantings, eight wood quality elite clonal line trials and 13 locations of block plots for the selection population

- Newly established loblolly pine progeny will evaluate 524 advanced-generation selections from Arkansas/Oklahoma, North Louisiana, and Texas. The advanced-generation slash pine trials will evaluate 34 advanced-generation selections. The cooperative has progeny tests established to evaluate a cumulative total of 1,367 loblolly and slash pine selections or 61 percent of the current second cycle advanced-generation population.

- Four clonal line trials were established for each of the Arkansas and Texas Wood Quality Elite populations. These trials are to support within-family selection by providing multiple observations for each genotype in different environments.

- Over the last three years, 105 selections have been added to the loblolly pine second-generation selection population and 48 selections were added to the slash pine second-generation population. Realignments within the breeding population due to additions and deletions have resulted in a second-cycle advanced loblolly pine population size of 1,926 (status number = 745) across four breeding zones. The slash pine population stands at 298 (status number = 141) for a single breeding zone.

- The third-cycle advanced-generation population for loblolly pine now totals 105 selections (status number = 33) in 13 different breeding groups.

Seed Orchards

WGFTIP members have been reducing orchard acreage over the last few years in order to bring seed supplies in line with lower seed demands. There are several factors that have contributed to this situation. Ownership patterns continue to be volatile with land decoupled from mills and non-core lands fragmented into smaller parcels. Pulp mills are being closed and the poor housing market has driven down the demand for small logs used to manufacture products like oriented strand board. These factors, at least temporarily, favor silvicultural regimes that plant fewer acres, fewer trees per acre, and manage stands over longer rotations for higher value end products. The biomass/biofuels market represents a potential countervailing trend that would favor planting more trees per acre and harvesting or thinning on shorter rotations and would, therefore, drive an increase in seedling demand. This market is speculative at the moment and has not yet had an impact on regeneration programs. While longer rotations for higher value products or shorter rotations for biomass have the opposite effect on the number of seedlings needed, either option puts a premium on genetic improvement for productivity. The WGFTIP members are trying to balance seedling demand and the need for genetic quality when projecting the total number of orchard acres required and evaluating potential orchard replacement schedules.

In order to better plan for the future, the WGFTIP conducted a careful program wide review to distinguish between orchards that are being actively managed and those that have been mothballed. Several trends were apparent. The number of total orchard acres reported by the membership has remained more or less constant despite loss of membership and accelerated decommissioning of older orchards. This has unfortunately concealed the fact that the number of orchard acres under active management has been rapidly declining since 2004. This is in part due to lower seed demands. But it also due to the fact that maturing advanced-generation orchards have been highly productive. For the most part, these advanced-generation orchard blocks, which were established on high-quality orchard sites, are living up to expectations that they would be very productive at young ages. A second trend that became apparent from our review was that seed orchards have been increasingly decoupled from the geographic ownerships for which they were designed. This is a trend that offers both risks and rewards, but one that we have yet to deal with adequately.

In response to short-term overcapacity in the seedling market, two state organizations closed nursery programs and several organizations mothballed genetically obsolete orchards. There are several factors, however, that may counter lower short term demands and, in fact, promote an increase in seed demand. First and foremost, landowners continue to have many incentives to maximize growth and enhance stand value. Aggressive forest management, including the planting of the best available genetics continues to be one of the most cost effective means of meeting this goal. One indication that landowners understand this is the increased interest in full-sib family and clonal deployment strategies that emphasizes planting stock value rather than cost. Fewer forestland acres available for harvest could also favor more aggressive plantation management. In addition, many national priorities such as production of cellulosic ethanol and more efficient carbon cycling also point to more aggressive management of commercial forestland. Southern pines have a role to play in meeting these needs because of their wide adaptability and the fact that flexibility in allocation to final end uses reduces risk and adds value. In the face of this uncertainty, the cooperative continues to establish advancing-front orchard blocks as the best strategy for meeting the future with maximum flexibility.

One trend that the WGFTIP membership has yet to fully confront is that the rapid change in land ownership patterns has resulted in a situation where orchards are increasingly decoupled from the land base they were designed to supply. In some cases, this is because the seed orchards were not part of the land trades and have been separated from an internal customer. In other cases, this is because members have acquired large acreages in areas where they have had no previous ownerships. The danger in this situation is that organizations with no continuing internal use for particular seed sources will decommission or fail to replace orchards. Overall seed orchard capacity will decrease and the new landowners will be forced to settle for less than optimal seed sources. The opportunity in this situation is that a true market for seed will develop as organizations without internal seed supplies move to purchase appropriate seed sources. If prices then more closely reflect value rather than cost, organizations will have incentives to continue to establish advanced-generation orchards. While this seems unlikely, the result would be that the seedling supply/regeneration system would gain stability and be less perturbed by volatility in ownerships.

Despite the decrease in the number of orchard acres, the annual demand for local seed used by the cooperative remains between 20,000 and 25,000 pounds. Producing seed with outstanding genetic quality in these quantities remains a challenging task. The maxim that the best seed will always be in short supply is still as true today as when the cooperative was first formed.

Orchard Establishment and Acres Managed

During the 2007 grafting year 57.5 acres of loblolly pine seed orchard, 34 acres in south Arkansas and 23.5 acres in east Texas, were established. Twenty-two acres of slash pine seed orchard were grafted in 2007 and an additional 12 acres of slash pine seed orchard was established in 2008 (Figure 6). Four acres of longleaf seed production area were also developed for east Texas. No additional orchard acres were established in 2009.

Figure 6. Jim Tule and Glen Herr of Forest Capital Partners, LLC display two successful field grafts in their new orchard.

The western Gulf coast experienced three hurricanes in as many years, hurricanes Katrina and Rita in 2005 and Hurricane Ike in 2008. The three storms collectively caused extensive damage to orchard complexes in Mississippi, Louisiana, and Texas. Some orchards in Texas were damaged by both Rita in 2005 and again by Ike in 2008 (Figure 7). Storm damage ultimately contributed to the decommissioning of several orchards. The loss of all the slash pine seed orchards and the older loblolly orchards at the Mississippi Forestry Commission Craig Seed Orchard after Hurricane Rita contributed significantly to their decision to discontinue their nursery, tree improvement, and seed orchard programs. This resulted in a large loss of orchard capacity designed specifically to support regeneration in the state of Mississippi. The one orchard that remains at this complex is the longleaf pine seedling seed orchard that will be managed and harvested by the USDA Forest Service to support longleaf restoration efforts. Several other orchard blocks were mothballed following the storms although no other complexes were completely closed. Older orchard blocks with less genetic gain are being closed as they are replaced by maturing advanced-generation orchard blocks.

Figure 7. One of the hardest hit orchard blocks at the ArborGen, LLC Livingston Seed Orchard after Hurricane Ike.

As of 2009, the WGFTIP has 2,177 acres of orchard, but only 1,329 acres are under active management. The 847 acres of orchards that have been mothballed could be reactivated if demand for seed suddenly increased, but this would be at a cost of generally lower genetic improvement. Of active orchards, 48 acres are rogued first-generation loblolly and slash pine orchards, 61 acres are minor species such as longleaf and shortleaf and 1,220 acres are advanced-generation loblolly and slash pine seed orchards (Figure 8). The members of the cooperative have collectively targeted a production goal of 300 million seedlings per year. This goal can be met with 1,329 acres of mature orchard, but not with advanced-generation orchards under a five-year replacement/expansion cycle as implemented by the cooperative. This implies that the targeted goals are overstated and that actual seed harvests more accurately reflect needs.

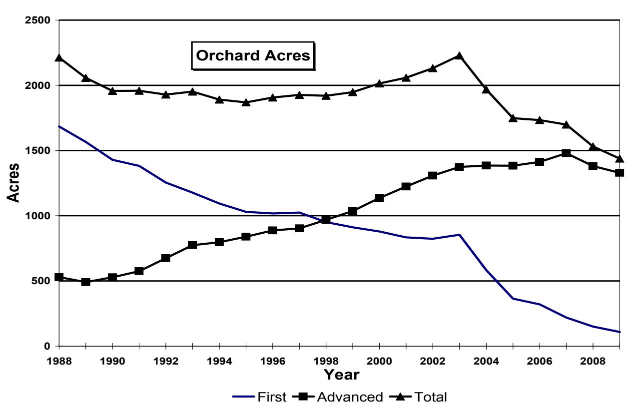

Figure 8. Seed orchard acres managed by the cooperative.

Orchard Yields

Loblolly pine seed harvest totaled 6,375 pounds in 2007, 16,459 pounds in 2008 and 25,200 pounds in 2009 (Figure 9). These totals are well below the 30,000 pounds per year that the program has historically been designed to support. The 2009 harvest year is probably more indicative of actual demand plus some additional allowance to replace previously depleted seed inventories. The 2009 cone crop was excellent, allowing most, if not all, members to selectively harvest only their best families.

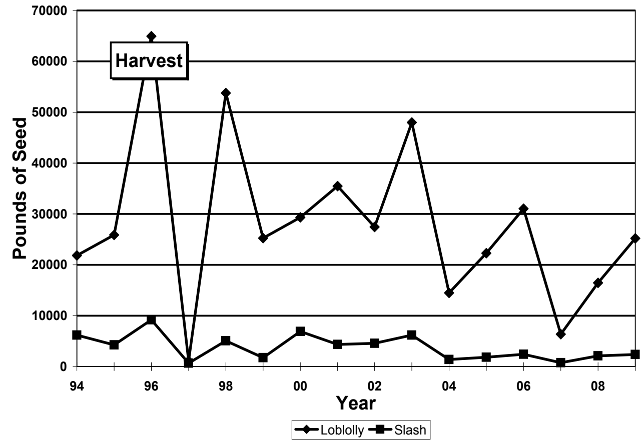

Figure 9. Pounds of seed harvested by the cooperative from 1994 to 2009.

Loblolly pine seed yields were 1.09 pounds of seed per bushel in 2007, 1.32 pounds of seed per bushel in 2008, and 1.37 pounds of seed per bushel in 2009. ArborGen, LLC held the record for the best loblolly pine yield in 2007 and 2008 achieving 1.46 pounds per bushel in 2007 and 1.61 pounds per bushel in 2008. In 2009, ArborGen, LLC had the orchard with the highest yield at 1.66 pounds of seed per bushel while Hancock Forest Management had the highest yielding orchard complex at 1.61 pounds of seed per bushel. These yields were achieved with large cone crops and represented outstanding insect control and cone handling procedures.

Slash pine seed harvest was 792 pounds in 2007, 2,100 pounds in 2008 and 2,375 pounds in 2009. Slash pine, while regionally important, is currently a minor crop for the cooperative with only five organizations having operational orchards. In the last three years, only four organizations collected seed for this species. Yields averaged 0.89 pounds of seed per bushel in 2007, 1.03 pounds of seed per bushel in 2008, and 1.07 pounds of seed per bushel in 2009. ArborGen, LLC set the high mark in 2007 with a yield of 1.0 pounds of seed per bushel while Campbell Timberland Management achieved 1.23 pounds of seed per bushel in 2008 and 1.20 pounds of seed per bushel in 2009.

With the loss of the Mississippi Forestry Commission, the only remaining member with a longleaf pine seedling seed orchard in production is the Louisiana Department of Agriculture and Forestry. The LDAF did not harvest this orchard in 2007 because of a poor cone crop. However, they collected 1,672 pounds of seed with an average yield of 1.03 pounds of seed per bushel in 2008 and 1,176 pounds of seed with an average yield of 0.93 pounds of seed per bushel in 2009. This 30-acre orchard was established with seedlings from families originating primarily in the western Gulf region that had outstanding survival, grass stage emergence, and brown spot resistance in short-term evaluations. It was later rogued on 10-year growth performance in a series of long-term progeny tests and the remaining families were thinned to the best tree per plot.

Breeding and Progeny Testing

One of the continuing success stories of the cooperative is the rate at which advanced-generation polymix progeny tests are being established. In 2006/07 and 2008/09 planting seasons, the cooperative established seven loblolly pine test series consisting of 22 separate locations. These plantings will evaluate 524 advanced-generation selections with approximately 63,000 test seedlings. In addition, three slash pine advanced-generation polymix plantings were planted in 2008/09 to evaluate 34 advanced-generation selections for this species. To date, 1,367 loblolly and slash pine selections or 61 percent of the second-cycle population has been established in polymix field trials for growth and form evaluation. The selection population for the third-cycle was further augmented with the establishment of 13 locations each containing multiple block plots.

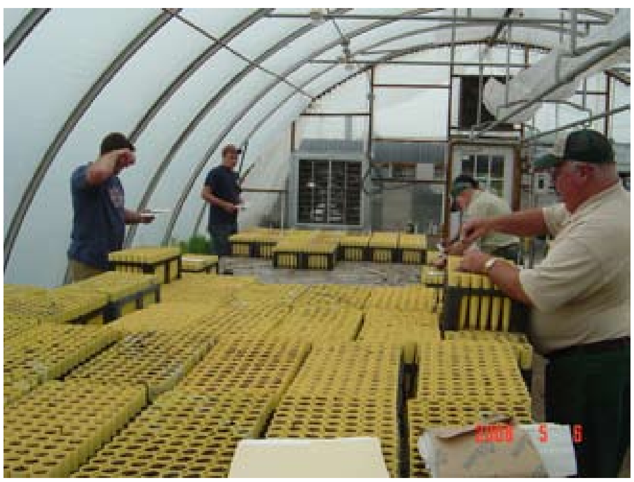

Figure 10. Keith Byrd of ArborGen, LLC helps the TFS crew sow the East Texas loblolly polymix test series grown in the College Station greenhouse in 2008. This test series included families belonging to all four members operating in the breeding zone.

This level of activity is possible only because of the outstanding collaboration of the entire membership (Figure 10). Each member is responsible for helping collect the pollen to create the regional polymix and then breeding the selections belonging to the breeding groups they own. Seed is consolidated by regions, one organization takes responsibility for greenhouse production of test seedlings, and multiple organizations establish field locations. In general, no organization establishes more than one location per test series so the testing load on each cooperator is minimized. Because there are multiple organizations involved at each step, the cooperative has been able to leverage individual contributions to make sustained progress at the regional level.

Supplemental to the mainline breeding and progeny testing program, four line trials from each of two Wood Quality Elite populations were grown in the greenhouse for planting in 2008/09. These tests will evaluate 208 lines from 8 different crosses to support within-family selection.

Test Measurement and Second-Generation Selection Activity

A total of 71 progeny tests were evaluated during the 2006/07 measurement season. Fourteen plantings were 5 years old and evaluated for the first time. Thirty-three plantings were 10 years of age or older and represented a reevaluation of results from a previous measurement cycle. Ten of the older tests were statistically significance for the first time and, therefore, contributed new information. This occurred primarily in slash pine tests with low rust infection levels at age five. As rust related mortality developing after age five contributes significantly to family rankings for this species, rust free slash pine progeny tests are not included in the growth database until age 10. The 2006/07 measurement season provided first-time evaluations on 51 slash pine parents and 89 loblolly parents.

Similar numbers of progeny tests were evaluated in the 2007/08 measurement season. A total of 75 progeny tests were measured, including 17 first-year survivals, seven (7) three-year height evaluations, 13 five-year growth and form evaluations and 36 older growth evaluations. These tests provided new information on a total of 70 first-generation parents from five different breeding groups and 39 slash pine parents from four different breeding groups.

In 2008/09, the cooperative was scheduled to evaluate 52 progeny tests and two (2) sets of block plots for third-cycle selection. This included 12 five-year-old plantings (four control-pollinated plantings and eight advanced-generation polymix plantings), 14 age-10 control-pollinated plantings, and 17 tests that were 15 and 20 years of age. Only 41 of these plantings were actually measured. None of the four 20-year-old tests were measured and test measurements were delayed for two five-year-old tests, two 10-year-old tests, and two 15-year-old tests. All of the older tests that were not evaluated had been previously measured and were already in the database with evaluations taken at earlier ages. The two five-year-old tests that were not measured were slash pine plantings with less than 30 percent rust and would not have been included in the family summaries until they reach age 10. In these particular cases, postponing test measurement could be done with little or no loss of data. However, these delays reflect the larger problem that cooperative members have had to prioritize test measurement schedules to accommodate continued budget constraints.

Figure 11. Larry Miller of the cooperative staff identifies a second-generation selection.

The 12 five-year-old tests evaluated provided new information on 41 first-generation loblolly pine parents and added a total of 19 second-generation selections to the program.

The first-generation progeny testing program continues to provide second-generation selections to reconstitute the population for the next cycle of advanced-generation breeding (Figure 11). These newest selections will not impact current orchard establishment plans, which rely on progeny tested material. They are, however, absolutely essential to the long-term viability of the breeding program by keeping reasonable effective population sizes and adding to the number of unrelated breeding groups in the population. In 2006/07, 38 loblolly and 15 slash pine second-generation selections were identified. In 2007/08, 23 loblolly pine and 33 slash pine second-generation selections were identified. This year, 2008/09, there were an additional 44 loblolly pine advanced-generation selections added. Actual numbers of individuals in the advanced-generation program reflect both the addition of the best first-generation parents moved forward to be reused and the deletion of some untested selections made necessary to accommodate reorganization within the cooperative membership. The second-cycle populations now consist of 1,926 loblolly and 298 slash pine selections (Figure 12).

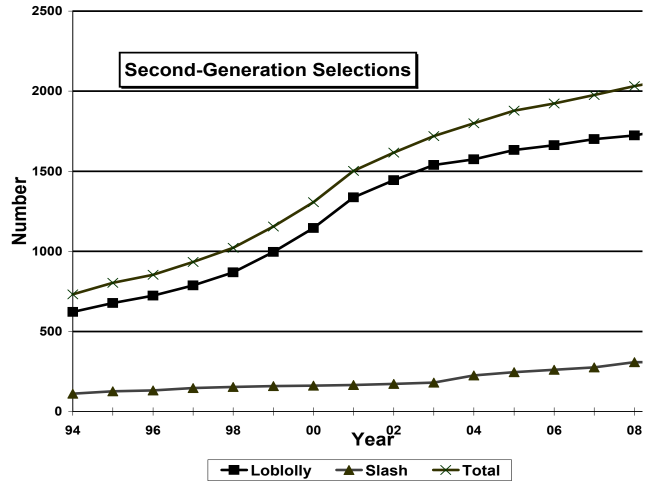

Figure 12. Cumulative number of WGFTIP second-generation selections.

Second-Generation Loblolly Pine Breeding and Testing

Nine of the WGFTIP's 13 members were involved in progeny test establishment in 2007/08 and every member contributed either control-pollinated seed, greenhouse space, and/or established field trials in 2008/09. This combined effort resulted in the establishment of eight different test series comprised of 22 loblolly pine progeny tests and three slash pine progeny tests. The loblolly pine progeny tests established for breeding programs in Arkansas/Oklahoma, North Louisiana, and Texas will evaluate 524 advanced-generation selections. The slash pine breeding program established three progeny tests to evaluate 34 advanced-generation selections. A sufficient number of advanced-generation progeny tests have been established to evaluate 1,361 loblolly and slash selections or 61 percent of the currently identified advanced-generation populations.

In 2007/08 the cooperative established two test series in Arkansas evaluating 140 parents and the first ever advanced-generation polymix test series for the North Louisiana breeding zone evaluating 102 parents. This effort used all of the polymix seed then in hand for those two breeding regions. Because the 2006 control-pollination season was so successful, a sufficient number of crosses were collected in the fall of 2007 to follow up with another outstanding planting season in 2008/09. In 2008/09 planting season, two Arkansas series were established to evaluate 121 families (Figure 13). In addition, a second round of advanced-generation tests was planted in the North Louisiana breeding zone evaluating 56 families. As a result of this effort, half of the currently identified North Louisiana advanced-generation breeding population was established in field trials in a two year period. An additional test series was planted in Texas to evaluate 105 families.

Figure 13. Jimmy Dale Camp and French Wynne with Potlatch Forest Holdings, Inc. with one of the new Arkansas/Oklahoma polymix progeny test series.

The slash pine breeding program is currently supported by four members in the South Louisiana/Southeast Texas deployment zone. Two of these members are the states of Texas and Louisiana who serve the nonindustrial forest landowner and two are commercial interests with extensive holdings in the coastal flatwoods. Breeding for this species has received less support over the last few years because a relatively small number of trees are used in the cooperative's regeneration program. Nevertheless, the cooperative recognizes that slash provides the only viable alternative to loblolly on many of the phosphorous-deficient, poorly drained, flatwoods sites in the lower coastal plain and are committed to the continued development of an advanced-generation population of fast growing, rust resistant sources. To support this goal, a test series to evaluate 34 advanced-generation parents was established in 2008/09. A sufficient number of advanced-generation tests have now been planted to evaluate 75 of the 298 advanced-generation selections for this species. In addition, extra seed created with a susceptible polymix are being banked for eventual submission to the USDA Forest Service Resistance Screening Center. This extra screening is necessary because weather conditions have contributed to low disease incidence over the last few years. As a result, many of these selections come from plantings that were insufficiently challenged by the rust pathogen to allow resistance to be adequately evaluated.

Selection Population Establishment for the Advanced-Generation and Elite Populations

Once evaluated in polymix tests, crosses are made within breeding groups (pedigree crosses) to form the selection population from which third-cycle selections will be made. These selections in turn will be evaluated in polymix tests for growth and form. The winners will support future deployment populations and be crossed to create the next cycle for selections. Simultaneously, crosses are also made among the best individuals across paired breeding groups (super breeding groups) and selections from this population following polymix evaluation will be used in the deployment population.

In 2007/08 22 selection blocks were established in south Arkansas by the Arkansas Forestry Commission and Potlatch Land and Lumber. Of these, 18 were for mainline advanced-generation selection while four will contribute to the elite super breeding group deployment population. In 2008/09, 25 additional control-pollinated crosses to support the selection population were established by Arkansas Forestry Commission and Oklahoma Department of Agriculture, Food and Forestry. The selection population in these block plots includes crosses for the mainline breeding population (crosses within breeding groups), crosses for the super breeding groups (crosses among parents from paired breeding groups), and the wood quality elite (crosses from the best parents in the region selected for wood quality regardless of breeding group). To further complicate the record keeping, some of these selection plots belong to more than one category.

Status of the Mainline Loblolly Pine Breeding Population

The WGFTIP loblolly pine breeding population was originally subdivided into 116 breeding groups within five different breeding zones. The intent was to reconstitute this population at each cycle by selecting 18 or more individuals from crosses made among parents within each group. To date, selections have been made in 91 groups (Figure 14). Of these, 59 breeding groups have 18 or more selections and are considered to be reconstituted for the next round of breeding. An additional 21 breeding groups have ten (10) or more selections and are well on the way to being reconstituted. Of the original 116 breeding groups, eight have been abandoned because they lacked material of sufficient quality to promote to the next generation and selections have not yet been started in 12 more breeding groups. Four groups in North Mississippi no longer have an owner with the withdrawal of the Mississippi Forestry Commission and the best selections will be infused in other groups. The process is underway to redistribute several additional groups previously belonging to International Paper Company to other organizations as infusions into existing groups. It is anticipated that this consolidation will result in a second-cycle population of between 80 and 90 breeding groups distributed over a total of four breeding zones.

The size and structure of the advanced-generation breeding population will ultimately determine the amount of genetic variation available to the breeding program. Within a closed breeding population with no future infusions, relatedness and inbreeding will increase within breeding groups. Therefore, the number of breeding groups that support a

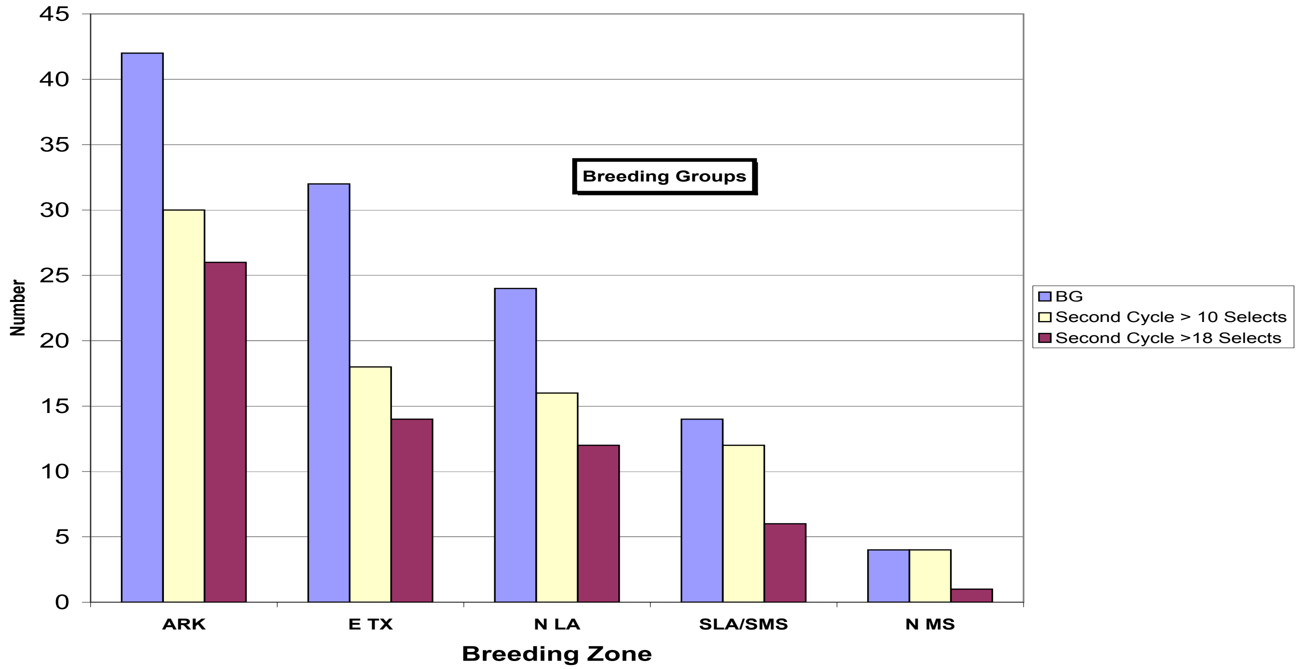

Figure 14. The number of completed breeding groups in each region showing the groups nearing completion with ten or more selections and the fully reconstituted groups with 18 or more selections.

deployment population ultimately determines how many unrelated individuals are available for open-pollinated orchard design. The Census Number is the actual count of individuals that need to be preserved and tested and, therefore, reflects the true workload. The Status Number measures effective population size or the number of individuals that would have the same diversity in an idealized "wild" or random mating population. Relative Status Number is the ratio of Status Number to Census Number and is useful to compare trends across populations of different sizes. These parameters are shown in Table 1.

At the breeding group level, these numbers reflect how fast inbreeding is building within the breeding population. This indirectly indicates how difficult future breeding is likely to be as loblolly pine expresses significant inbreeding depression and lower viability in related crosses. The average Census Number across all second-cycle breeding groups is 21.4 with an average Status Number of 8.7.

At the population level, these numbers reflect the rate at which diversity is being lost across the population. The WGFTIP population was selected to be much larger than simulation studies indicate necessary. This was done because multiple traits need to be improved simultaneously and there was little knowledge about how these traits would respond to selection. An unintended consequence of having such a large population is that inbreeding will be much easier to manage for a number of generations. The second-cycle Census Number is 1,926 with a Status Number of 745. The Relative Status Number is 0.38.

Table 1. The number of Breeding Groups (BG) and average Census Number, Status Number and Relative Status Number by breeding region for the current second-cycle and third-cycle populations.

	Breeding Zone					
Second Cycle	AR	TX	N LA	S LA/S MS	N MS	Total
No. of BGs	32	23	18	14	4	91
Census No./BG	22.9	23.9	19.4	16.3	16.5	21.4
Status No./BG	8.5	9.0	7.8	7.2	6.6	8.7
Total Census No.	734	549	349	228	66	1926
Total Status No.	271	208	140	100	26	745
Rel. Status No.						0.38
Third Cycle						
No. of BGs	10			3		13
Total Census No.	82			23		105
Total Status No.	25			8.2		33.5
Rel. Status No.						0.32

Third-cycle selections are being made in pedigree crosses established in block plots. Selections from two breeding zones, South Arkansas and South Louisiana/South Mississippi, have currently been identified. One-hundred and five (105) individuals with a Status Number of 33.5 have been selected in 13 breeding groups (Table 1). Selection efforts in three of these breeding groups, two from South Arkansas and one from South Louisiana, have been completed. It is too early in the third-cycle selection process to accurately compare changes in inbreeding levels across generations. However, it is possible to get some idea of trends by comparing the averages for the second-cycle breeding groups to the averages for the three third-cycle groups for which selection has been completed. Average Census Number and Status Number in the second cycle are 21.4 and 8.7, respectively. In the third-cycle these same population parameters are 21.3 and 6.2 (Figure 15). Relative status number has gone from 0.38 in the second-cycle to 0.29 in the third-cycle. This decline cannot be avoided in a closed breeding population. It can be controlled by changing the relative emphasis on family selection by including more parents in the pedigree. As desirable traits are combined in elite individuals it may be possible to reduce Census Numbers more rapidly, but doing so will contribute to a more rapidly declining Status Numbers. Having this information will facilitate tracking relatives in advanced generations. The cooperative will continue to monitor the population structure and make adjustments as needed to ensure that the future control-pollinated crossing program is successful.

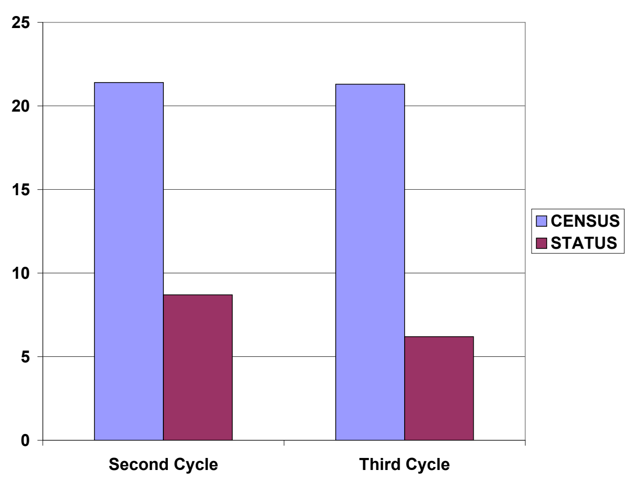

Figure 15. Average Census and Status numbers for the second generation and reconstituted third-generation breeding groups.

Wood Quality Elite Population

The Wood Quality Elite population was created to rapidly improve both growth rate and wood quality in selections to support the deployment population. Backward selection has been used to identify 62 individuals from four different breeding zones that combine high breeding values for these traits. Crosses are being planted in block plots for selection. To date, 19 block plots have been established and Potlatch Land and Lumber identified the first two advanced-generation selections for this population from a four-year-old set of plots. In addition to identifying candidates with good growth and form, the selection process is complicated by the need to sample wood cores to simultaneously evaluate wood specific gravity. These selections will be grafted, crossed with polymix pollen, and established in growth and form progeny tests. The winners will be used to support the deployment population and used for further breeding in the Wood Quality Elite population. In 2008/09 the Texas Forest Service established seedlings in eight additional block plots for this population. The Oklahoma Department of Agriculture, Food and Forestry grew seedlings in their greenhouse for another 12 crosses for field planting in east Texas and south Mississippi during the 2009/10 planting season.

A unique component of the Wood Quality Elite population is the collaboration with CellFor, Inc. to use clonal testing as a basis for selecting individuals within control-pollinated families. Conelets from ten (10) different crosses were submitted to CellFor, Inc. for the initial round of this project. Following line initiation and propagule development, Campbell Timberland Management produced the test seedlings and eight different members established clonal field trials. Trials, comprised of six replications of single-tree plots, were established in two breeding zones: South Arkansas and East Texas. A total of 125 lines will be evaluated in the Arkansas trials and 75 lines evaluated in the East Texas trials. The expectation is that having multiple observations on genetically identical individuals over multiple locations will allow a much more accurate evaluation of phenotypes and prediction of genotypes. This could be especially valuable in simultaneously improving a low heritability trait like volume and specific gravity which appears to have little or no correlation with growth at the population level.

Early indications are that the Texas series will have to be dropped due to mortality caused by droughty conditions experienced by much of southeast Texas/southwest Louisiana early in the spring of 2009. This is an unfortunate loss of time and investment, but unlike progeny tests established with seedlings, this is not a loss of plant material. All of these lines are stored in cryopreservation and somatic embryogenesis can be used to generate genetically identical seedlings for future testing. It is anticipated that these lines will be added to the crosses collected for line initiation in the summer of 2010.

Slash Pine

The first advanced-generation polymix tests in the slash pine program reached age ten in 2007 and the analyses of the data brought interesting and unexpected results. The system developed for summarizing performance in first-generation progeny tests predicts breeding value for planted tree volume at base age 15 by giving different weights to different traits at different measurement ages (38th Progress Report). Breeding value estimates for five-year-old trees are based solely on rust infection levels. Progeny test results are not included in our data summary unless there are significant differences among families for infection levels and the planting shows evidence of having been sufficiently challenged (30 percent or more infection in the stand or the unimproved checklot). Infection levels are used because rust related mortality between the ages of 5 and 10 impact family performance at age 15 more than any trait or combination of traits we have yet evaluated. At age ten, breeding value estimates for volume at age 15 are based on the target trait of volume performance on a planted tree basis. Survival incorporates rust related mortality, but height and diameter growth are also important. Because breeding value estimates are based on a different suite of traits at different ages, it is not unusual to see more shifting in breeding value estimates between ages five and ten in slash pine than normally observed in loblolly pine. Breeding values for slash pine can also be larger than those reported for loblolly because high rust susceptibility can result in very low volumes.

We fully expected the same set of equations that had been so useful in the first generation to work for the advanced-generation progeny testing as well. However, breeding value estimates between the top and bottom performers in the ten-year-old polymix progeny tests exhibited huge ranges, in some cases with spreads nearing 200 percentage points. These differences were not supported by observed survival or growth differences in the field and it seemed most likely that they were an artifact of the predictive equations. This situation could result because 1) selection has been successful and the first and second generations represent different populations with different genetic parameters, 2) the environment has changed making rust resistance less important, or 3) a combination of both genetic selection and temporary changes in pathogenicity of the fungus mediated by changes in the environment.

Current first-generation slash pine data summaries predict breeding values for average family volume and the variance among families for volume at base age 15. These differences are then standardized and expressed as a percent improvement in volume growth. In these formulas rust infection, either expressed directly or in combination with mortality, is a primary contributor to among family variance at age 15 for volume. The data set from which the original formulas were developed consisted of 14 progeny tests, all with extremely high rust infection levels. The slash pine parents contributing to the later first-generation progeny tests, as well as all of the parents for the second-generation population, have all been screened for resistance at the USDA Forest Service Resistance Screening Center (RSC). In addition, most of the younger slash pine progeny tests, with the exception of those planted in southern Mississippi, have had very low rust infection levels.

To investigate the combined impact of these factors, all available data with repeated measurements through age 15 were reanalyzed using the same methodology reported in the 38th Progress Report. The results were a new set of formulas (Table 2) that 1) raised the predicted stand level volume at age 15 because of an increase in site index and 2) lowered the predicted variance among family means for volume. The impact of these two changes was that when slash pine breeding values were standardized and expressed as a percent, they were slightly lower than previously reported and the ranges from the best to the worst families were reduced. Breeding value estimates for slash pine are now much more in line with those reported for loblolly pine and more reflective of the growth performance actually observed in the more recently established slash pine progeny tests.

All slash pine data sets were reanalyzed using the new predictive equations. Family performance summaries were completed in early 2008 and the Slash Pine Catalog distributed to the members mid-year.

Table 2. Predictive equations used in the slash pine data summarization programs. Original equations developed in 1983 and subsequently recalculated using all available data in 2008 with major changes bolded.

Trait	Age	Original Equation	R^2	New Equation	R^2
Site Index (SI)	5	37.695 + 5.805*HT5	0.82**	40.161 + 5.498*HT5	0.78**
	10	26.886 + 3.624*HT10	0.64**	27.388 + 3.657*HT10	0.78**
	15	0.930 + 4.334*HT15	0.99**	1.988 + 4.262*HT15	0.99**
Volume	5	-12.780 + 0.255*SI + 0.103*SUR5	0.74**	-15.349 + 0.240*SI + 0.127*SUR5	0.68**
	10	-15.043 + 0.277*SI + 0.102*SUR10	0.82**	-13.943 + 0.283*SI + 0.090*SUR10	0.57**
	15	-17.992 + 0.337*SI + 0.108*SUR15	0.88**	-15.386 + 0.320*SI + 0.090*SUR15	0.65**
STVOL+	5	-6.777 + 0.151*SI	0.71**	-5.776 + 0.107*SI **+0.030*PR5**	0.66**
	10	-9.883 + 0.268*PR10	0.83**	7.110 **– 0.064*SUR10**	0.71**
	15	-9.883 + 0.268*PR10	0.83**	5.410 **– 0.047*SUR15**	0.64**

+ = standard deviation of family mean volumes

** = significant F-value at the 10 percent level of probability (α=0.10)

Abbreviations: HTx = mean plantation height at age x
SURx = mean plantation survival at age x
PRx = mean plantation rust infection at age x

Virginia Pine

Data collection continues on the few remaining young Virginia pine polymix tests. At ages four to five tests are evaluated for growth and scored for Christmas tree marketability. The best individuals identified to date are currently being grafted by the Texas Forest Service.

Additional Activities

Contact Representatives' Meetings

The primary purpose of the WGFTIP Contact Representatives' meetings is technology transfer. Topics are chosen that relate to tree improvement, forest management or the larger socioeconomic context in which tree breeding participates. These meetings also serve as a chance to network and visit other member's operations.

In 2007 the Oklahoma Department of Agriculture, Food and Forestry hosted the Contact Meeting in Idabel, Oklahoma. Topics covered during the indoor sessions included migrant worker issues (Dan Bremer – AgWorks, Inc.), seed production technology (Dr. David South – Auburn University), population genomics and tree improvement (Dr. Kostya Krutovsky – TAMU) and an update of the seed orchard chemical residue study was given by Dr. Bob Krieger (UC-Riverside). Tours stops included the ODAFF seed processing and greenhouse facilities and a tour of the Forest Heritage Museum near Broken Bow. Attendees received 7 SAF Category I CFE credits (Figure 16).

Figure 16. Attendees at the 2007 Contact Representatives' Meeting held at the Oklahoma Forest Heritage Center, Beavers Bend State Park (Photo courtesy of Al Myatt).

The 2008 Contact Meeting was hosted by the WGFTIP in College Station, TX and included site visits to the USDA Southern Research Station Pecan Germplasm Repository (Figure 17), the USDA Forest Service Cytogenetics Laboratory and WGFTIP offices and greenhouse facilities. Topics covered during the indoor sessions included loblolly pine conservation (Dr. Bill Dvorak – CAMCORE), American chestnut preservation (Dr. Nurul Faridi – USDA Forest Service), carbon credits (Burl Carraway – TFS), and tip moth control in progeny tests (Dr. Don Grosman – TFS). Attendees were awarded 10 SAF category I CFE credits.

Figure 17. Drs. Tommy Thompson and L.J. Grauke host the field trip for the 2008 Contact Representatives' Meeting at the USDA Southern Research Station Pecan Germplasm Repository.

In 2009, the Contact Meeting was in Monroe, LA. Presentations at this meeting included cold tolerant eucalyptus (Dr. Mike Cunningham – ArborGen), wood gasification (Dr. Les Groom – USDA Forest Service), state assessments (Dr. Brad Barber – TFS), hardwood research (Dr. Randy Rousseau- Mississippi State University), regeneration research (Dr. Michael Blazier – Louisiana State University), pesticides (Dr. Don Grosman – TFS) and herbicides (Dr. Andy Ezell – Mississippi State University). Multiple sessions were devoted to the potential for marker-assisted breeding. These talks were presented by Dr. Dave Harry (Oregon State University) as part of the training made available to the cooperative through participation in the Conifer Translational Genomics Network Coordinated Agricultural Project (CTGN-CAP). This research program, targeted at making the use of molecular markers a reality in applied tree improvement programs, is supported by the USDA National Institute for Food and Agriculture (NIFA, formerly CSREES) and the USDA Forest Service. Attendees earned 8 SAF category I CFE credits.

The evening social provided an opportunity for the membership to wish Larry Miller well in his retirement from the WGFTIP. Larry has been an integral part of the staff since 2001 after a long career with Temple-Inland. Prior to moving to Texas in 1976, he had worked for Weyerhaeuser's regeneration program in Washington State. The membership benefited from Larry's extensive knowledge of applied tree improvement and nursery operations (Figure 18).

Figure 18. Larry Miller at the reception held in honor of his retirement from the WGFTIP cooperative.

Seed Orchard Pest Management – Coragen® Efficacy Study

The Texas Forest Service provided one of two orchards used in 2009 to evaluate the efficacy of Coragen®[1] for the control of coneworms and seedbugs. This study was done in cooperation with Dr. Don Grosman of the Texas Forest Service and Dr. Alex Mangini of the USDA Forest Service. Cone and conelet survivals were tallied in Coragen® treated trees and compared to an equal number of cones and conelets in untreated controls from the same clones. Treatment consisted of four applications at concentrations consistent with currently labeled rates. Identical study designs were used in the TFS orchard at Magnolia Springs and in the study installed by Dr. Mangini in the Plum Creek Hebron Orchard.

Coragen® (Rynaxypyr®) has proven highly effective against *Lepidoptera* spp. on a number of vegetable crops. It has a novel mode of action that is narrowly targeted to moths and related species, has very low mammalian toxicity, and its use requires very few safety precautions. Unfortunately, the efficacy against coneworms was less than satisfactory. While the percentage of cones at Magnolia Springs that were clearly damaged by coneworms was reduced, the percentage of cones classified as healthy did not improve. Over both orchards, there were no differences for the percentage of cones classified as coneworm damaged, other damage, or healthy. Seed bug damage was not tallied as the hope had been to find a chemical effective against coneworms.

While the results of this study were disappointing, it highlighted the need to have orchards and crews available to conduct this type of research. If orchard managers depended solely on result from other crops, this chemical would have appeared very promising indeed. Actual loses under operational conditions where seed yields naturally fluctuate are difficult to document and anecdotal at best. As a result, several seed crops might have been jeopardized before this mistake could be recognized and corrected.

[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 use has been registered.

Conifer Translational Genomics Network Coordinated Agricultural Project (CTGN-CAP)

The WGFTIP along with the tree improvement programs at NC State University, University of Florida, Oregon State University, and research programs at UC Davis and the USDA Forest Service Southern Institute of Forest Genetics are participating in a program aimed to bring molecular markers to applied tree breeding. This program is funded by the USDA National Institute for Food and Agriculture (NIFA, formerly CSREES) and the USDA Forest Service.

The overall methodology is to genotype as many trees as possible for commonly occurring SNPs (single nucleotide polymorphisms) derived from a library of expressed genes. The tree improvement programs are supplying the plant material and performance evaluations, UC Davis is extracting the DNA and contracting the genotyping. Oregon State University is providing theoretical support and is hosting the outreach component of the project. Each organization has its own research plan and will make a unique contribution to the overall project. The WGFTIP role is to characterize a multiple-generation breeding population from a single breeding zone as completely as possible. In the first two years, 2,000 foliage samples representing the East Texas breeding population were collected and submitted for genotyping. This included both first- and second-generation selections. In addition, progeny from separate groups of genotyped parents were sampled in a control-pollinated progeny test and a clonally replicated line trial. Objectives are to characterize structure in the breeding population and how this structure is changing over generations. Marker-trait associations will be evaluated and alternatives for using this information in the applied tree breeding program will be proposed.

Formal Reviews

Formal Reviews serve the dual purpose of benchmarking individual programs while providing feedback to the staff on member needs. This process has been invaluable as the cooperative has struggled to stay abreast of the emerging priorities in the rapidly changing business environment. Reviews were held for the Arkansas Forestry Commission, the Oklahoma Department of Agriculture, Food and Forestry, and Potlatch Land and Lumber in 2007. Formal reviews were also on the docket for ArborGen, LLC and the Texas Forest Service, but were postponed to allow internal reorganizations within each entity to be completed. In 2008, Formal Reviews were held for Campbell Timberland Management, Deltic Timber Corporation and Forest Capital Partners, LLC. CellFor, Inc. and Weyerhaeuser Company programs were reviewed in 2009. Future seed orchard expansion plans dominated these discussions because of the uncertainties in the land ownerships, changes in silvicultural practices, and unpredictable demands for planting material.

USDA Forest Service Southern Institute of Forest Genetics - Forest Tree Molecular Cytogenetics Laboratory

Evidence for a structural difference between American and Chinese chestnut trees at the major 18S-28S rDNA locus

Contributed by: Nurul Islam-Faridi[2], C. Dana Nelson[3], Paul H. Sisco[4] Thomas L. Kubisiak[3], Frederick V. Hebard[5], Robert L. Paris[5] and Ronald L. Phillips[6]

The American chestnut tree (*Castanea dentata*), once known as "The King of the Forest", after flourishing some 40 million years in much of eastern North America is no longer a dominant forest tree due to the chestnut blight disease, incited by an exotic, invasive fungus (*Cryphonectria parasitica*). The fungus was accidentally introduced in the late 1800s on Japanese chestnut nursery stock and stem cankers were first reported in 1904, killing the American chestnut trees in the Bronx Zoo, New York City. No control attempts (e.g., chemical treatments, clearing and burning of trees around infected areas) were successful in protecting the trees. In the following years, the disease was reported in neighboring states, and by the late 1920s, the disease was spread throughout the entire natural range of American chestnut. By 1950, almost the whole species was decimated by the fungus, except for sprouts originating at the trees' root collar and possibly advance regeneration.

In the 1920-1940s the U.S. Department of Agriculture (USDA) and the Connecticut Agricultural Experiment Station (CAES) attempted to restore the American chestnut by crossing the Asian chestnuts (Chinese and Japanese) onto American, but their efforts were largely unsuccessful. The main reason was that they did not have basic information on the inheritance of blight resistance, leading to a closure of the USDA program. The CAES program was suspended, for the most part, in 1963, but resumed in the 1980s. In 1983, a group of prominent scientists led by Dr. Charles Burnham and Dr. David French and interested lay persons, led by Mr. Phillip Rutter, established The American Chestnut Foundation (TACF) with a goal to restore American chestnut to its native range using interspecies backcross breeding. In this breeding method, blight resistance genes from Chinese chestnut are transferred into American chestnut by multiple generations of crossing and selection (Burnham et al. 1986). Wide crosses, such as interspecific hybrids, often uncover significant structural differences between the parental species' chromosomes, and previous genetic mapping work (Kubisiak et al. 1997, Sisco et al. 2005) suggested this might also be the case for these two species. To pursue this question more definitively, we formed a collaborative team to study the cytology and cytogenetics of chestnut.

Florescent *in situ* hybridization (FISH) is a powerful cytogenetic tool used to visualize gene(s)/marker(s) directly on chromosomes. We found that chestnut has two18S rDNA sites (i.e., loci) located on two different pairs of homologous chromosomes (Figure 19a). One of the sites has more rDNA sequence compared with the other as revealed

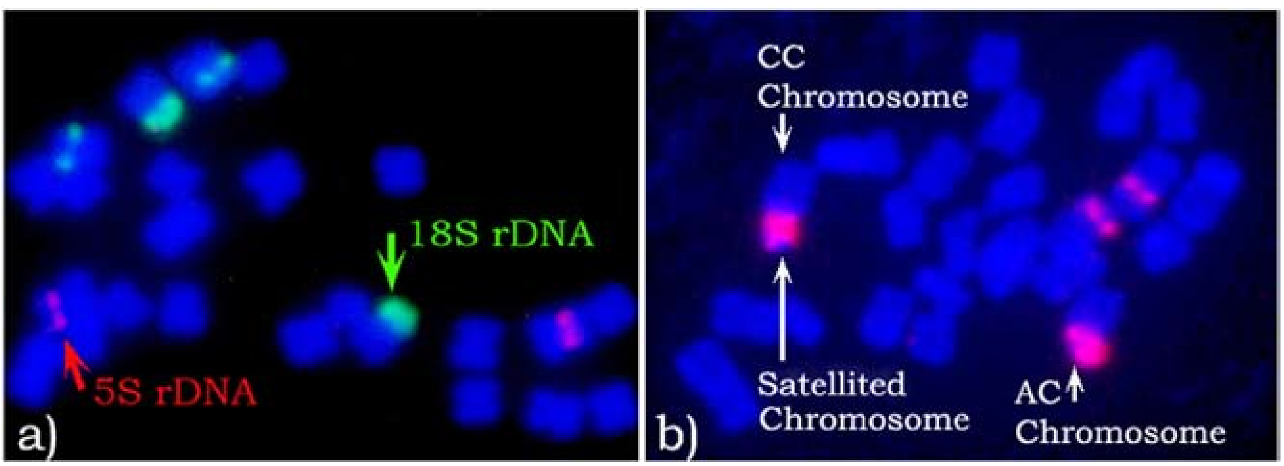

Figure 19. FISH with rDNA probes on metaphase chromosome spreads in chestnut; a) FISH with 18S rDNA (green signals) and 5S rDNA (red signals) probes in American chestnut, b) FISH with 18S rDNA probe in American chestnut (AC) X Chinese chestnut F1 hybrid. *Figure 19. FISH with rDNA probes on metaphase chromosome spreads in chestnut; a) FISH with 18S rDNA (green signals) and 5S rDNA (red signals) probes in American chestnut, b) FISH with 18S rDNA probe in American chestnut (AC) X Chinese chestnut F1 hybrid.*

[2]U.S. Forest Service, Southern Institute of Forest Genetics, Forest Tree Molecular Cytogenetics Laboratory, Department of Ecosystem Science and Management, Texas A&M University, College Station, TX 77843. [3] U.S. Forest Service, Southern Institute of Forest Genetics, Harrison Experimental Forest, 23332 Old Mississippi 67, Saucier, MS 39574. [4] The American Chestnut Foundation, 160-D Zillicoa Street, Asheville, NC 28801. [5] Meadowview Research Farms, The American Chestnut Foundation, 14005 Glenbrook Avenue Meadowview, VA 24361. [6]Department of Agronomy and Plant Genetics, University of Minnesota, 1991 Upper Buford Circle, 411 Borlaug Hall, St. Paul, MN 55108

by a much stronger FISH signal. One 5S rDNA site was identified in chestnut, and it is located on a third chromosome pair (Figure 19a). As expected, telomere probe signals were observed at the end of each chromosome arm. An additional early result of this research suggests that the major 18S rDNA chromosome of Chinese chestnut is structurally different than its homologous chromosome in American chestnut in that it contains a longer segment distal to the 18S rDNA signal (Figure 19b). Ongoing research will specify the chromosomal structural differences between these two species and guide tree breeders and genetic engineers in their efforts to produce blight-resistant American chestnuts that are capable of living and reproducing in the wild.

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HARDWOOD TREE IMPROVEMENT PROGRAM

Hardwoods are getting a lot more press lately with the promotion of biomass/biofuels. For the most part, however, these markets have not yet developed sufficiently to impact our members' reforestation programs. Until they do, the WGFTIP – Hardwood cooperative will continue to concentrate on evaluating and establishing orchards for various oak species used for timber, wetland restoration, and wildlife habitat. The cooperative also has selections from a number of fast growing, well adapted species that are suitable for short rotation management should the need arise (Table 3).

Cherrybark Oak

Cherrybark oak (*Quercus pagoda* Raf.) is an outstanding source of quality red oak timber. On the appropriate site it can be very productive, easily exceeding 100 feet in height. It favors the better drained hardwood bottoms and is frequently found on ridges and older alluvium within the major river drainages. The cooperative originally identified 237 parents for this species and evaluated open-pollinated families in replicated progeny tests. Sixty-two second-generation cherrybark oak selections from the best families were then grafted into advanced-generation orchards managed by the Arkansas Forestry Commission and the Texas Forest Service.

Table 3. Summary of the WGFTIP – Hardwood progeny testing and selection effort by species.

Species	Progeny Tests	Parents	Sec. Gen Selections	Sec Gen Selections in Progeny Tests
Green Ash	21	234	70	
Sweetgum	16	295	84	37
Sycamore	23	280	61	12
Cherrybark Oak	40	237	62	56
Water/Willow Oak	21	208	21	
Yellow Poplar	8	61	12	
Nuttall Oak	22	210	5	
Total	151	1,525	315	105

Progeny Testing

The WGFTIP – Hardwood cooperative has thirty-two active progeny tests. Seven of these are young cherrybark progeny tests intended to evaluate and rogue advanced-generation orchards for this species. Older plantings include 22 Nuttall oak progeny tests being used to select parents for new orchards, two advanced-generation progeny tests for sweetgum and one advanced-generation progeny test for sycamore.

Seed was collected from a total of 56 orchard parents in 2006/07 and 2007/08. Two series of progeny tests were planted in a total of seven locations, each designed as randomized complete blocks with 30 replications of single-tree plots. The remaining six parents that were not included in these tests were represented by a limited number of orchard ramets and did not produce sufficient seed for testing. They will not be evaluated and will eventually be removed from the orchards.

Table 4. Mean first-year survival and range among family means for first-year survival in the advanced-generation cherrybark oak progeny tests.

Year Planted Cooperator - County/Parish, State	Survival (%)	Among Family Significance Level	Range among Family Means (%)
2007/08 Series 638			
AFC - Pulaski, AR	95.6	ns	82-100
MFC – Tallahatchie, MS	92.3	Pr > F =0.001	77-100
TFS – Jasper, TX	98.0	ns	90-100
LFSC/LDAF – Rapides, LA	97.7	ns	90-100
2008/09 Series 639			
AFC Pulaski, AR	98.6	ns	93-100
MFC – Tallahatchie, MS	99.7	ns	97-100
TFS – Jasper, TX	71.3	ns	60-83

First-year survivals were outstanding for all plantings with the exception of the TFS Series 639 planting location in Jasper Co., TX. Survival at this location was 71 percent while survivals at the other six locations were all above 90 percent (Table 4). This test, planted at the Magnolia Springs Seed Orchard complex on part of the site that was too wet for pine seed orchards, is not a bottomland site. Establishment survival among families differed at only one location, indicating that evaluations can be based on subsequent survival and growth. Ultimately, this information will be used to eliminate the poorer performing parents in these orchards resulting in the creation of well evaluated seed source for this important species.

Nuttall Oak[7]

Nuttall oak (*Q. texana* Buckl. formally Q. *nuttallii* Palmer) is a red oak with a natural range restricted to the bottomlands of the Gulf Coastal Plain of the southern US (Figure 20, Filer 1990). It is the most tolerant of the red oak species to heavy, poorly drained, alluvial clay soils and is, therefore, favored for bottomland planting. It exhibits good survival on a range of sites, is relatively fast growing, produces high quality sawtimber, and is beneficial to wildlife producing large acorn crops at young ages. Like most oaks, it is shade intolerant and planting open areas following harvesting is a viable method of stand restoration. The WGFTIP – Hardwood members collected seed from 210 individuals, preserved the parents in scion banks, and are establishing seed orchards with backward selections from the best of the progeny tested parents.

Five Nuttall oak test series were established, each comprised of a different set of families. All test series were planted as randomized complete blocks with ten replications of four-tree row plots. Height, diameter, and survival were measured at five-year intervals. As nothing was previously known about geographic variation within the species, selections were organized into provenances or seed sources within the range primarily delineated by river drainages for analysis (Figure 21, Table 5). Geographic differences were analyzed to inform decisions on wild seed collection while among-family differences were quantified to guide orchard establishment.

Previous analysis from the first three test series measured through age ten indicated that provenance effects were moderate at best. However, wild seed collected toward the center of the range (northern Louisiana or southern Arkansas) should be favored when purchasing wild seed for use in the central Mississippi Delta (northern Louisiana, south Arkansas and adjacent areas in Mississippi). The better performing provenances tended to be from the Ouachita and Red River basins while the poorer provenances tended to be from the Western Region. It should be noted that sources from Alabama and southern Louisiana were not included in this evaluation. There were also outstanding parents from all of the provenances regardless of the average performance for the seed source.

All five test series have now been measured through age ten and the first series has been measured through age 15 (Table 6). As the WGFTIP- Hardwood members establish seed orchards from individual selec-

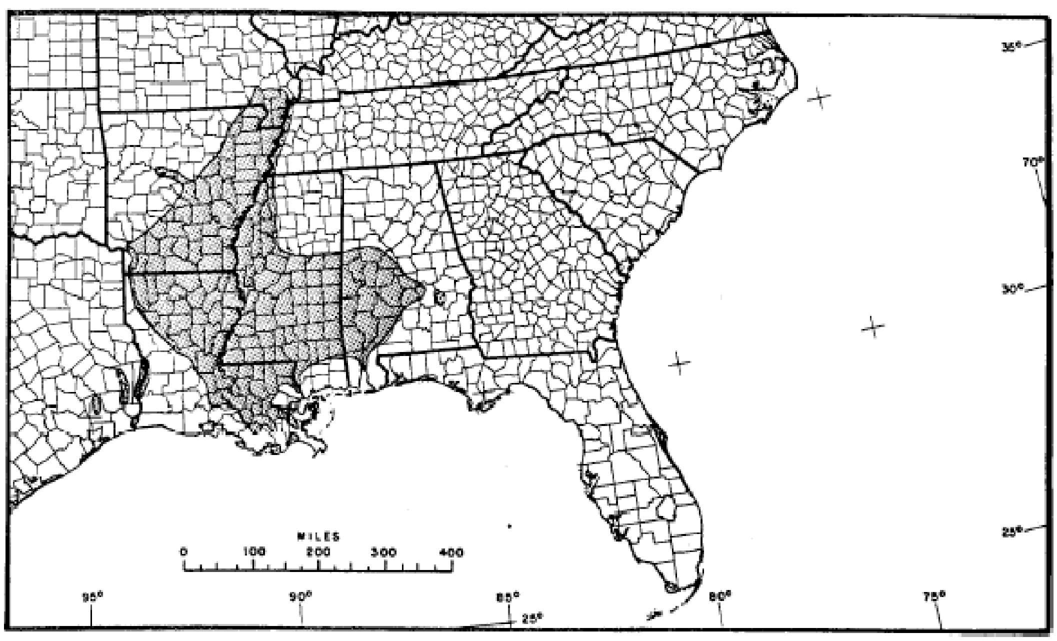

Figure 20. Natural distribution of Quercus texana *Buckl. (formally* Q. nuttallii *Palmer) (Filer 1990).*

[7] See Byram, T.D., E.M. Raley, and D.P. Gwaze. 2007. Performance of Nuttall oak (*Quercus texana* Buckl.) provenances at age 10 in the Western Gulf Region. Proc. 29th S For Tree Imp. Conf.: Joint Meeting of the Western Forest Genetics Association and the Southern Forest Tree Improvement Committee, Galveston, TX, June 19-22. pp. 28-38.

Table 5. The number of open-pollinated families representing each provenance by test series

	Provenance	Test Series 1	2	3	4	5	Total
1	Western Region	15	3	12	2		32
2	Black- White Rivers		20	5	13	38	76
3	Ouachita River	6	2	6	18	10	42
4	Mississippi River	12	3	4	9	2	30
5	Red River			12	1		13
6	Tallahatchie – Yalobusha	8			3		11
	Other					6	6

tions, a number of questions need to be answered. How much gain could be expected from selecting the top parents for inclusion in the new seed orchards? How reliable are performance estimates across different sites? How early can selections be identified? To answer these questions, volume per live tree was analyzed with ASREML®, individual heritabilities estimated, and gains calculated. Site to site variation in performance was estimated with Type b genetic correlations and age-age additive genetic correlations were calculated for Series 1.

Table 6. Fifteen-year means and ranges among family averages for Nuttall oak Series 631 by planting.

Cooperator – County, State	Survival (%)	Height (m)	Diameter (cm)	Volume (dm^3/ planted tree)
MFC Sharkey, MS	80 48-95	10.2 8.8-11.3	13.3 10.5-14.8	41.6 18.9-60.4
AFC Lonoke, AR	90 70-100	12.1 10.9-12.9	14.2 11.0-17.3	62.0 34.6-98.3
Potlatch Dehsa, AR	72 48-92	10.1 8.9-11.1	11.3 9.3-12.7	29.3 13.8-41.6

Individual-tree heritabilities for live tree volume ranged from 0.03 to 0.88 when calculated on a test by test basis. Combining tests within a series which incorporates site to site variation produced estimates that ranged from 0.08 to 0.51 (Table 7). The lowest combined heritability estimate was from a tests series which includes a highly variable test in Mississippi (individual h^2=0.03). Removing this test raised the combined heritability estimate to 0.15. The numerical average for heritability across test series was moderate (h^2 = 0.29). Type b genetic correlations which reflect the amount of agreement across locations varied from 0.43 to 0.76. Type b genetic correlations in this range are considered moderate and indicate that selections will perform with reasonable predictability across sites. Low to moderate Type b correlations suggest that either different deployment populations should be designed for different sites or a sufficient number of families should be included in a single deployment population to minimize risk of less than optimal site assignment. As the factors causing the site to site variation in this study are not understood, it would be impossible to wisely design multiple deployment popula-

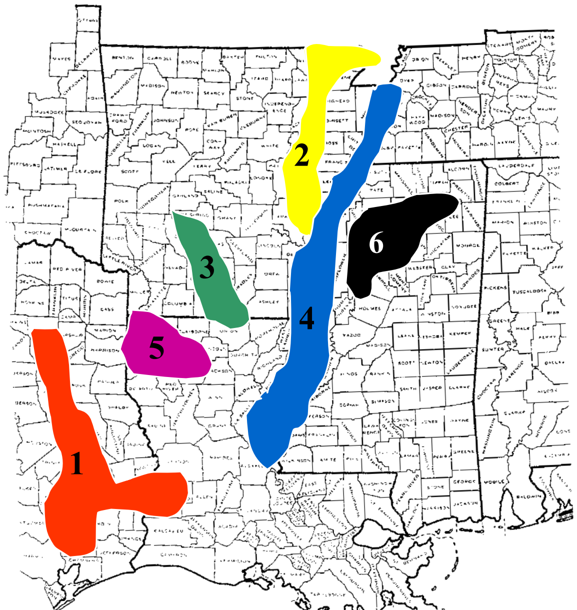

Figure 21. County/Parish locations of families used in the study are: Western Region (Provenance 1), Black -White Rivers (Provenance 2), Ouachita River (Provenance 3), Mississippi River (Provenance 4), Red River (Provenance 5) and Tallahatchie-Yalobusha Rivers (Provenance 6).

tions. Separate deployment zones for a minor species are also economically prohibitive. Therefore, the cooperative has adopted the second strategy of having a single broadly based deployment population. This assumes that while some trees may perform poorly when off site, the stand value will be near optimal because better adapted neighbors

Table 7. Age-10 heritabilities, Type b correlations and standard errors for volume from five Nuttall oak progeny test series. Gain is predicted change in volume growth expected from selecting the top 20 percent of the population.

Series	Cooperator	h^2 ± S.D.	Type b corr. ± S.D.	Gain (%)
631				
	MFC	0.25 ± 0.08		
	AFC	0.62 ± 0.14		
	Potlatch	0.12 ± 0.05		
	Combined	0.16 ± 0.06	0.43 ± 0.12	14.0
632				
	MFC	0.82 ± 0.20		
	AFC	0.37 ± 0.12		
	Potlatch	0.64 ± 0.17		
	Combined	0.38 ± 0.11	0.76 ± 0.09	31.2
633				
	MFC	0.35 ± 0.10		
	AFC	0.49 ± 0.12		
	Potlatch	0.42 ± 0.11		
	Combined	0.24 ± 0.07	0.60 ± 0.11	29.0
634				
	MFC	0.03 ± 0.04		
	AFC	0.38 ± 0.10		
	TEF	0.42 ± 0.11		
	Combined	0.08 ± 0.04	0.36 ± 0.16	
	Combined (AFC & TEF)	0.15 ± 0.06	0.59 ± 0.20	18.0
635				
	MFC	0.88 ± 0.15		
	AFC	0.56 ± 0.12		
	Combined	0.52 ± 0.11	0.73 ± 0.08	35.2

will fill in the available growing space. An additional factor is that Type b correlations are a population parameter and it is possible to select individuals with stable performance over a range of sites.

Gain estimates based on these population parameters indicate that selecting the top 20 percent of the parents for inclusion in seed orchards should produce seedlings that perform ~ 25 percent better than unimproved planting stock for live tree volume. This will add considerable value to plantings established with this stock.

The second question that we hoped to answer was how early in the testing process could we identify outstanding performers? Age-age genetic correlations showed good agreement between ages 5 and 15. The agreement between ages 10 and 15, however, was almost perfect (Table 8). The orchards for this species will be based on age 10 data.

Table 8. Age-age genetic correlations for Nuttall Oak test series 631.

Age	10	15
5	0.80	0.70
10		0.97

Sweetgum

Several years ago, the WGFTIP - Hardwood cooperative and NC State Hardwood Cooperative joined forces to evaluate their second-generation sweetgum (*Liquidambar styraciflua* L.) selections. Families from both programs were grown by the NC State Cooperative and multiple test locations were established across the South. The Arkansas Forestry Commission and Temple-Inland Forests planted one test each in the western gulf region. Both of these tests have large amounts of environmental variation caused by an off target herbicide application at the Temple-Inland location and drainage problems that developed below a large farm pond at the Arkansas location. Because of these problems neither site has given unambiguous results.

The Arkansas Forestry Commission recently completed the ten-year evaluation on the 130 families in the test located in Lonoke County, AR (Table 9). Excessive environmental variation and patchy survival problems have made it necessary to drop19 replications from the original 35 established. When the remaining 16 replications were analyzed, there were no significant family differences at either age 5 or age 10 for volume production. However, there appear to be some trends emerging. There were significant differences among families for survival at age 10 and the differences among families for height was very nearly so (Pr > F = 0.12). Interestingly, the significance level for differences among families for volume production has improved from a Pr > F =0.82 at age 5 to a PR > F=0.12 at age 10 for the same cohort of trees. If this trend continues, it would appear that genetic differences will eventually become apparent despite the considerable environmental noise.

Results from this single location showed no meaningful differences in survival or growth rate for performance between eastern and western seed sources.

Table 9. Age-10 averages for the Arkansas Forestry Commission second-generation sweetgum progeny test in Lonoke Co., AR

	Survival (%)	Height (m)	Dbh (cm)	Volume (dm3)
Test Ave.	65.8	7.4	9.1	11.8
Family Range	37-94	6.4-9.1	7.5-11.0	5.1-18.9

Seed Orchards

Arkansas, Louisiana, Texas, and the Louisiana Forest Seed Company are taking the lead in establishing improved seed sources for a number of different hardwood species. The Arkansas Forestry Commission and the Louisiana Department of Agriculture and Forestry supply seedlings for reforestation, wetland restoration, and wildlife habitat for bottomland sites associated a number of major river drainages. While the Mississippi River is the most famous, the region also includes many other very significant rivers and swamps with extensive bottomland forests. Among these rivers are the Arkansas, the White, the Ouachita, the Red and the Atchafalaya along with many smaller rivers and streams. While most of the actual hardwood planting is in these river drainages, upland hardwoods also make up a considerable portion of the forests in all of the states participating in the cooperative.

Figure 22. Van Hicks with the Louisiana Department of Agriculture and Forestry checking inventories in their baldcypress orchard at Monroe, LA.

The Arkansas Forestry Commission began establishing what will eventually be an 18-acre Nuttall oak orchard adjacent to the Baucum Nursery near Little Rock, AR in 2006. In the first year they established 147 positions on a 40 by 40 foot spacing (5.4 acres). In 2008 they added another 105 positions or 3.8 acres to fill half of the available area. In that same year they also planted 42 positions in a water oak (*Q. nigra*) orchard and 31 positions to a willow oak (*Q. phellos*) orchard. A second-generation cherrybark orchard is already in production at this location. The Louisiana Department of Agriculture and Forestry continues to be aggressive in establishing hardwood seed orchards at a number of their facilities (Figure 22). They currently have orchards for sweetgum, baldcypress (*Taxodium distichum*), Nuttall oak, water oak, willow oak, live oak (*Q. virginiana*) sycamore (*Platanus occidentalis*), green ash (*Fraxinus pennsylvanica*), and cherrybark oak. The Louisiana Forest Seed Company, another key player in the production of improved hardwood seed, is aggressively developing improved seed sources for green ash and cherrybark oak. The Texas Forest Service has 41 acres of hardwood seed orchards for both the state's Urban Tree Improvement Program and for timber species developed as part of the WGFTIP (Table 10). The Texas Forest Service expanded this list in 2009/10 by planting 114 positions in a new Nuttall oak seed orchard.

Table 10. Hardwood orchards managed by the Texas Forest Service for the Urban Tree Improvement Program and for the TFS/WGFTIP reforestation program.

	Species	Acres
Urban	Baldcypress	2.7
	Bur Oak	1.6
	Cedar Elm	2.2
	Chinkapin Oak	1.0
	Live Oak	6.0
	Magnolia	1.6
	Shumard Oak	4.5
	Sweetgum	3.8
WGFTIP/TFS	Cherrybark Oak	2.7
	Green Ash	5.3
	Nuttall Oak	1.4
	Sweetgum	3.4
	Sycamore	2.2
	Water /Willow Oak	2.1

Joe Hernandez and Larry Miller retired from the Texas Forest Service and the Western Gulf Forest Tree Improvement Programs in 2009.

PERSONNEL

The Texas Forest Service tree improvement program was fully staffed in 2007 for the first time in several years with the hiring of Dyrle Ann Joiner. Ms. Joiner, stationed at the Arthur Temple Sr. Research Area, wasted no time in proving herself to be a valuable addition to the team. Unfortunately, this situation was too good to last and she left the program in 2008. The Tree Improvement Program was fortunate to acquire the services of two long-term TFS employees during the final phase out of the Indian Mound Nursery in 2008 through the remainder of the 2009 fiscal year. Lee Thacker and Willie Thacker assisted Gerald Lively at the Arthur Temple Sr. Research Area prior to their retirement in August of 2009. Scott Taylor was hired at the end of 2009 to fill the Resource Specialist position at the Arthur Temple Sr. Research Area. Hubert Sims retired from Magnolia Springs Seed Orchard at the end of 2009, leaving I.N. Brown and Walter Burks at that facility. The Texas Forest Service East Texas field crew for tree improvement stands at four.

There were also several changes in College Station. Joe Hernandez retired in January 2009 from the Resource Specialist position with the Hardwood and Urban Tree Improvement Programs. Joe has been an integral part of the staff since 1975. He made many of the first- and second-generation selections for the hardwood programs and developed innovative techniques in hardwood grafting that set the standards for the industry. He is currently working part time as a retire/rehire to help hold the program together while we attempt to reorganize. A second crucial loss to the College Station staff occurred when Larry Miller left the program in May, 2009. He joined the WGFTIP staff in 2001 after a long and influential career in industry. His knowledge of applied tree improvement programs is sorely missed. Duties from Joe Hernandez's Resource Specialist position and Larry Miller's Assistant WGFTIP Geneticist specialist position will be combined and reorganized into a new position to be titled Silviculturist. This position was briefly filled by Marvin Lopez before he left to rejoin a previous employer. In addition, several people were given new titles in order to make their job descriptions equivalent to other positions within the agency requiring similar levels of seniority and professional expertise. The Texas Forest Service and WGFTIP staff for the period included the following people:

T. D. Byram …....………………… WGFTIP Geneticist
L. G. Miller ...…… Assistant WGFTIP Geneticist (Ret.)
E. M. (Fred) Raley ......… Assistant WGFTIP Geneticist
P. V. Sowell ………………….............. Office Associate
J. G. Hernandez …Resource Specialist IV (Ret. and now working part time)
M. S. Lopez Sr. ..……........ Silviculturist I (Resigned)
G. R. Lively ……...…………… Resource Specialist IV
I. N. Brown …………………… Research Specialist II
H. Sims ………...……… Resource Specialist IV (Ret.)
W. Burks …………….........…… Resource Specialist III
D.A. Joiner ....……… Resource Specialist I (Resigned)
Scott Taylor ……………......……… Resource Specialist I
L. E. Thacker ……………...... Resource Specialist II (Ret.)
W. E. Thacker ……….....…. Resource Specialist II (Ret.)

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COOPERATIVE TREE IMPROVEMENT PROGRAM MEMBERS

Western Gulf Forest Tree Improvement Program Membership

Pine Program

Full members of the Western Gulf Forest Tree Improvement Pine Program in 2008/2009 include ArborGen, LLC, Arkansas Forestry Commission, Campbell Timberland Management, CellFor, Inc., Deltic Timber Corporation, Hancock Forest Management, Forest Capital Partners, LLC, Louisiana Department of Agriculture and Forestry, Oklahoma Department of Agriculture, Food and Forestry, Plum Creek Timber Company, Potlatch Land & Lumber, LLC, Texas Forest Service, Weyerhaeuser Company.

Associate members include International Forest Seed Company and Louisiana Forest Seed Company.

Hardwood Program

The WGFTIP Hardwood Program includes the Arkansas Forestry Commission, Campbell Timberland Management, Louisiana Department of Agriculture and Forestry, Louisiana Forest Seed Company, Potlatch Land and Lumber, LLC, and the Texas Forest Service.

Urban Tree Improvement Program

The Urban Tree Improvement Program has received past support from 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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