

*College of Forest Resources
N.C. State University
Raleigh, North Carolina*

May, 1989



THIRTY-THIRD ANNUAL REPORT

NORTH CAROLINA STATE UNIVERSITY - INDUSTRY
COOPERATIVE TREE IMPROVEMENT PROGRAM

EXECUTIVE SUMMARY

Progress reports for nine research studies reveal that:

- In vitro agar germination testing of loblolly pine pollen is extremely sensitive to pollen moisture content and, consequently, a 2-hour rehydration period for pollen lots below 15% moisture content is necessary to assure reliable germination results.
- In a study comparing several in vitro pollen germination methods, the agar germination (48 hours) and respiration were highly correlated with pollen germination in vivo ($r^2 = .82$ and $.70$, respectively).
- Loblolly pine pollen viability as measured by pollen germination on an agar medium, or by pollen respiration rate combined with pollen age, accurately predicted the percent filled seed per cone.
- In a study of seed quality variables in Fraser fir, early results indicate that pollen quantity is one of the key components in determining seed quality.
- Diagnoses made using the summer DRIS norms developed for Fraser fir indicated the DRIS technique does offer the ability to identify nutritional limitations upon yield.
- In a turf management study with a James River seed orchard, two Oust/Roundup treatments showed promise of reducing growth of fescue turf without destroying the fescue and the protection it affords the orchard site.
- Family stability for wood specific gravity is generally good; however, on a provenance level there are differences in wood specific gravity that could prove to be important.
- An important Cooperative Research study was initiated this past year; the Verification Trial for Early Selection of Loblolly pine. The objective of this study is to evaluate the use of stem elongation for early selection among families from five provenances of loblolly pine. The ability to identify selections which could be dropped from the breeding population before expensive controlled crossing begins would be very valuable.
- Breeding for the Plantation Seed Source Study was continued in 1989 but due to poor flower crops, additional crossing will be needed in 1990.

The 1988 harvest of cones and seeds was the smallest since 1982.

- Cooperative members harvested 42.7 tons of improved loblolly pine seed, a 53% decline over the 1987 record of 90.3 tons.
- Second generation loblolly seed orchards produced 5 tons of seed. The percent of total production from second-generation orchards continues to rise.
- Seven seed orchards in the Cooperative exceeded two pounds of seed per bushel of cones harvested in spite of the poor year.

Good progress continues to be made in the breeding and testing of the Cooperative's 3300 plantation and 720 second-generation selections. Presently about 67% of the crossing is complete and about 15% of the testing.

A total of 14 graduate students worked in association with the Cooperative on M.S. and Ph.D. programs.

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INTRODUCTION

The North Carolina State University-Industry Cooperative Tree Improvement Program has completed 33 years of activity. Genetic gains of 7 to 12 percent for growth, with comparable improvements in quality traits, have been realized in seed harvests sufficient to reforest 13 million acres of commercial forest land. In the last ten years we have expanded and strengthened our commitment to research conducted in support of the Tree Improvement Cooperative. The research activity contributes to a greater understanding of loblolly pine genetics, which in turn translates into improved methodology, gains in efficiency, and therefore value returns. Effort and accomplishment with breeding and testing has been outstanding. We are now preparing for the "big push" in the next four years to establish the majority of the genetic tests that will form the resource base for a third cycle of selection and improvement.

Much of the research initiated in recent years is long term, which is an unavoidable characteristic of genetic variation and seed orchard management experimentation. We need to maintain the current strong commitment in the years ahead if meaningful results are to be realized. We have recognized that improved understanding of genetic variation can be enhanced by researching the physiological basis for differences we have traditionally evaluated through empirical studies. We are currently working to develop a research initiative that focuses on the physiology/genetics interface. The Department of Forestry has recently strengthened forest physiology research through new faculty hiring. We are seeking to develop linkages with the physiology faculty, and anticipate a mutually beneficial association for years to come.

Throughout the world of forest genetics research and development, there is increased activity on vegetative propagation, tissue culture, and more recently genetic engineering. As we complete the strategic planning work for the Cooperative, it is important to pay particular attention to options that can allow for incorporation of the "new genetics" in the future. With the continuation of a strong foundation built on traditional plant breeding and quantitative genetics, the possibility of new technology being an integral part of our future is both challenging and exciting. It is a challenge we accept with confidence as we strive to continue **To economically increase forest productivity through genetic manipulation of forest tree populations.**

RESEARCH

Important results were obtained during the past year with several Cooperative studies. J. B. Jett's Pollen Rehydration Study and Ray Moody's research on correlation of *in vitro* to *in vivo* pollen germination and seed set resulted in three publications. Other studies yielding interesting results include: Evaluation of Seed Quality Variables in Fraser Fir; Foliar Mineral Nutrient Diagnosis with DRIS for Identifying Nutritional Influence on Female Cone Production in Fraser Fir; Suppression of Turf Growth in a Loblolly Pine Seed Orchard; and Stability of Wood Specific Gravity in Open-Pollinated Families of Loblolly Pine. Another very important study, A Verification Trial for Early Selection of Loblolly Pine, was initiated in the spring of 1989.

Impact of Rehydration on In Vitro Germination of Loblolly Pine Pollen

Frequently, the viability of pollen lots is tested prior to their use. Pollen germination and subsequent tube growth in these tests can be erratic unless dried pollen is rehydrated. Although Goddard and Matthews suggest southern pine pollen be rehydrated prior to conducting *in vitro* germination tests, a precise understanding of the sensitivity of southern pine pollen to rehydration has been lacking.

The objectives of these studies were to quantify the relationships between:

- 1) percent germination and percent moisture content
- 2) percent moisture content and rehydration time
- 3) percent germination and rehydration time of loblolly pine pollen during *in vitro* testing

In study 1, percent germination *in vitro* showed a pronounced sensitivity to pollen moisture content (Figure 1). For example, increasing the pollen moisture content from 8 to 10% resulted in an increase in percent germination from 15 to 42.7%. Above approximately 15% moisture content, percent germination was relatively insensitive to changes in moisture content. The need to rehydrate loblolly pine pollen prior to conducting agar tests has been well understood, but these results clarify the necessity of rehydration. Even small shifts in moisture content may substantially distort test results and interpretation with respect to the use of a given pollen lot. All agar germination tests should be preceded by rehydration.

In Study 2, the relationship between pollen moisture content and length of rehydration was highly significant. Pollen moisture content rapidly increased for the first two hours in rehydration and increased slowly thereafter (Figure 2). Although not shown in Figure 2, after 16 hours of rehydration, moisture content had reached 63.5% and was still slowly increasing.

After 0.62 hours of rehydration, the percent germination began to increase rapidly and peaked after about 1.3 hours reaching an average germination level of 84.2% (Figure 3). This peak level of germination occurred at a moisture content of approximately 20% (Figures 2 and 3). These results indicate that the 16 hour rehydration period commonly used before germination testing of dried pollen lots is longer than necessary. Rehydration for two hours appears to be adequate to insure peak germination.

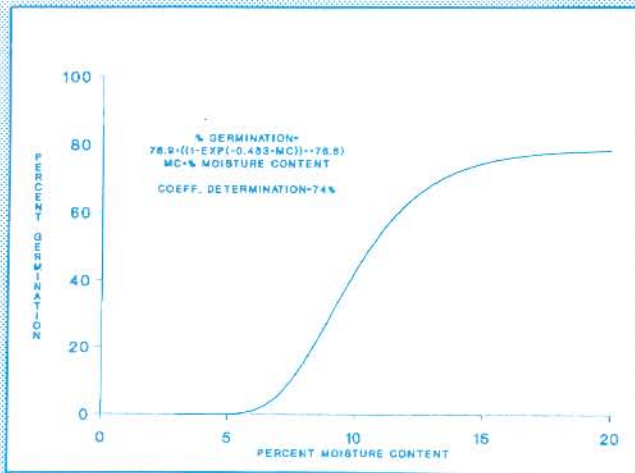


Figure 1: Predicted percent germination *in vitro* for loblolly pine pollen at different moisture contents.

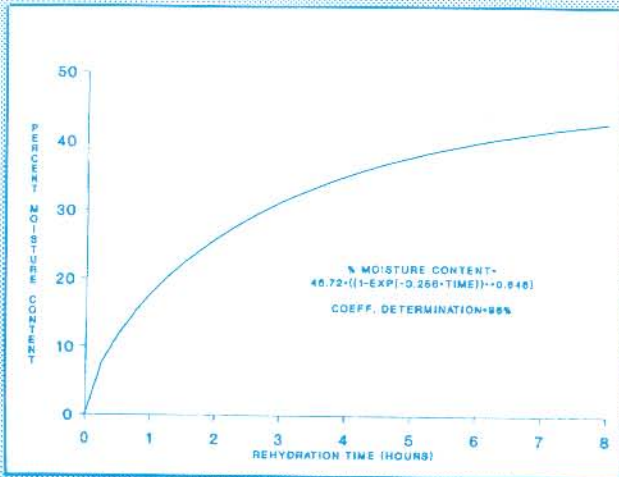


Figure 2: Predicted percent moisture content of loblolly pine pollen for different rehydration times.

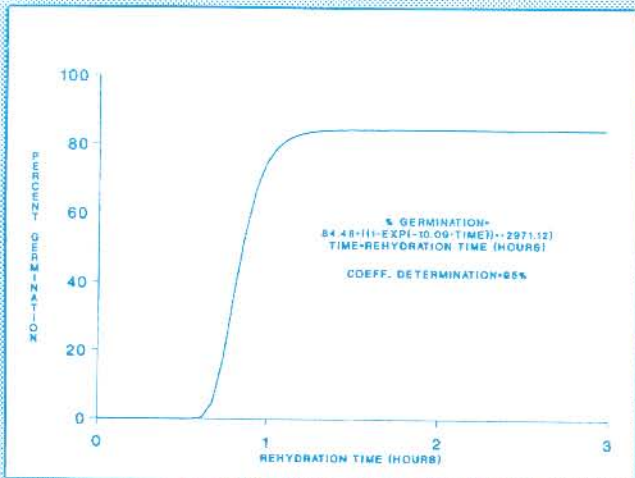


Figure 3: Predicted percent germination *in vitro* of loblolly pine pollen for different rehydration times.

In conclusion, *in vitro* agar germination testing of loblolly pine pollen is extremely sensitive to pollen moisture content. Between 5 and approximately 15% moisture content, pollen germination can vary widely, therefore rehydration of pollen lots with moisture contents below 15% is critical. Small samples of pollen for viability testing do not require a prolonged rehydration. Approximately two hours of rehydration proved adequate to give maximum germination *in vitro*. This shortened length of rehydration represents a considerable time savings over the standard 16 hour rehydration time and allows laboratories to process greater numbers of pollen lots in a given time period.

A Comparison of In Vitro and In Vivo Pollen Viability in Loblolly Pine

Graduate student Ray Moody compared *in vitro* and *in vivo* viability in loblolly pine. Several *in vitro* testing procedures were investigated: agar germination test, pollen respiration, UV absorbance and electrical conductivity.

Correlations between *in vitro* pollen germination methods tested and *in vivo* pollen germination are shown in Table 1. Both leachate analyses, UV absorption and electrical conductivity, were poorly correlated ($r^2 = .03$ and $.21$ respectively) with percent germination *in vivo*. In contrast agar germination (48 hours) and respiration rate were highly correlated with pollen germination *in vivo* ($r^2 = .82$ and $.70$ respectively).

Both the 48 hour agar germination and respiration rate were good predictors of pollen germination *in vivo*. The 48 hour germination test on an agar medium produced a good measure of pollen viability and required fewer replications than the 24 hour tests. The measurement of pollen respiration rates was less time consuming and easier to

Table 1. Relationship between *in vitro* pollen germination and *in vivo* pollen germination in loblolly pine.

In Vitro Test	Relationship ¹ to In Vivo Pollen Germination
Electrical Conductivity	$r^2 = .21$
Ultraviolet Absorbance	$r^2 = .03$
Agar Germination - 24 hours	$R^2 = .73$
Agar Germination - 48 hours	$R^2 = .82$
Respiration Rate - 25°C	$R^2 = .70$
Respiration Rate - 30°C	$R^2 = .70$

¹ Coefficient of determination for linear regression = r^2
 Coefficient of determination for multiple linear regression = R^2

calculate when conducted at 30°C although the results were essentially the same. The measurement of respiration rates also has the advantage of being a one-step operation with considerable time savings as well as being less susceptible to operator bias than the agar germination test. The respiration method has the disadvantages of high initial costs and, until a universal respiration standard is developed, the results cannot be directly compared between laboratories.

Prediction of Percent Filled Seed in Loblolly Pine by Pollen Viability Tests and Pollen Age

The *in vivo* determination reported above only gives a partial measure of pollen vigor. Since the ability of pollen to set seed is the ultimate measure of pollen vigor, the relationship of *in vitro* tests to seed set was also investigated. It was found that pollen viability, as measured by the agar germination and pollen respiration rate, is a good predictor of total seed. When combined in models with pollen

age, the percent filled seed per cone was reliably predicted.

Results also showed that the percent first year aborted ovules was inversely related to both pollen germination *in vitro* ($R^2 = .84$) and to respiration rate ($R^2 = .75$). The number of total seed is reduced directly by the number of first year ovule abortions (Figure 4). Age of pollen was another important factor affecting seed yield, as percent

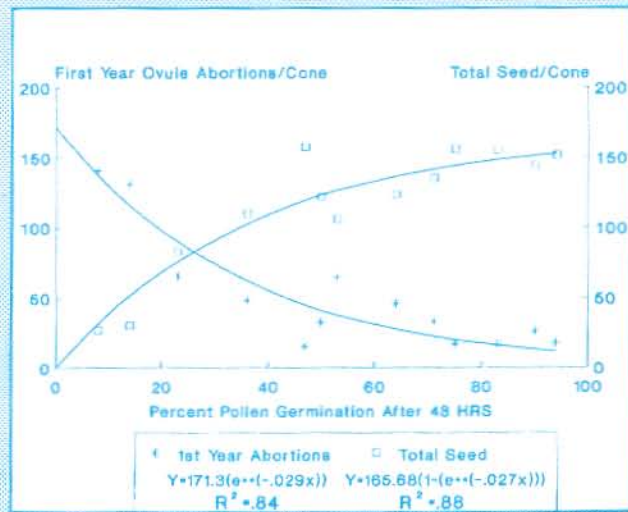


Figure 4: Number of first year aborted ovules per cone and number of total seed per cone of loblolly pine versus percent germination on an agar gel after 48 hours.

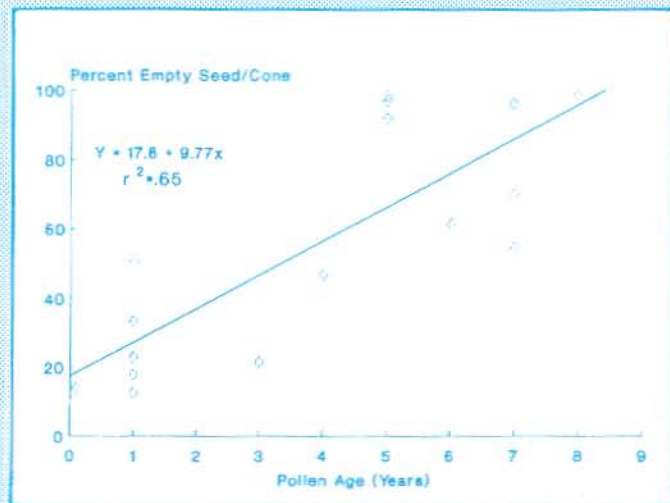


Figure 5: Percent empty seed per cone versus the age of loblolly pine pollen in years.

empty seed was correlated to the age of pollen in storage ($r^2 = .65$, Figure 5). Under the operational pollen storage condition used by the Cooperative, pollen age influenced the ability of the pollen to complete fertilization in the last weeks of seed development (Figure 5). Whether or not stored pollen retains the ability to set seed may be related to storage conditions.

Based on the results of these studies, operational tree improvement programs can utilize these measures of pollen viability to evaluate pollen samples. However, these results further indicate that even though pollen may test well in viability tests, the use of pollen that has been stored without vacuum drying and freezing may result in low seed yields, even if pollen viability is high. Therefore, tree breeders may want to replace pollen as it gets older. If it is necessary to use "old" pollen, the number of pollinations should be increased to give the desired number of seed.

Evaluation of Seed

Quality Variables in Fraser Fir

In the state of N. C., Fraser fir is the foundation of an estimated \$55 million/year Christmas tree business. Availability of Fraser fir seed production areas is restricted, with a single natural stand on Roan Mountain accessible for commercial seed collection. A five acre seedling seed orchard established by the N. C. Forest Service in the 1960's provides the bulk of the seed for the State's Fraser fir seedling production. Erratic seed yields coupled with routinely low seed quality from both the seed orchard and natural stands has caused concern for the State's Christmas tree industry.

In 1986 a study was initiated by graduate student Anne Margaret Hughes to evaluate factors contributing to the seed quality of Fraser fir in both a seed orchard and a natural stand. Factors investigated were: insects, pollen quality and quantity,

flower phenology, and the role of selfing. The seed orchard utilized in this study was the N.C. Forest Services' seedling seed orchard located at Crossnore, NC (3400 feet elevation) which is isolated from any natural fir populations by several miles. The natural stand was located on Roan Mountain, NC at an elevation of 5500 feet. Results of the study were:

1. Pollen quantity was evaluated by pollen trapping and ovule dissection techniques. At the Crossnore seedling orchard, pollen flight peaked between April 27-30, 1986, coinciding with maximum receptivity of the female flowers. On Roan Mountain the peak pollen flight was apparently missed but observations revealed the pollen flight was still underway during receptivity. These results do not agree with literature reporting a lack of synchronization between male and female flowers for many firs.
2. Ovule dissections revealed that in 1987, open pollinated ovules from Roan Mountain averaged 6.6 pollen grains as opposed to 2.4 grains per ovule for the seed orchard trees at Crossnore. It is suspected that this large difference was due to local weather conditions. There was also a large difference between years for pollen grains per ovule on Roan Mountain, with means of 6.6 for 1987 and 1.5 for 1986. This difference could be due to the late summer drought of 1986, or to more favorable weather conditions at pollination time.
3. An x-ray analysis of seed was used to examine the impact of pollen quality and quantity on sound seed set. Cones from which pollen was excluded did not abort and appeared normal in both cone and seed characteristics except that none of the seed contained embryos as confirmed by x-rays. Open pollinated cones produced a significantly higher number of normal full sized seed than did control pollinated cones.



Growth and development have been excellent following four growing seasons in the field for the Fraser fir Provenance Study. Shown is the planting located at Crossnore, NC on the N.C. Forest Service Nursery site.

4. In both 1986 and 1987 open-pollinated cones from Roan Mountain produced a greater number of normal sound seed per cone than did trees in the seed orchard. A greater amount of pollen per ovule at Roan Mountain was probably the reason for the difference in seed set in both years, though the higher pollen count was only confirmed in 1987.
5. There were no significant differences between locations for the number of fertile scales per cone. However, the seed orchard cones were significantly larger (2.5 inches) than the Roan Mountain cones (2.2 inches). This difference was probably due to the better nutrition and more favorable growing conditions at the orchard site.

Though the project is not yet complete, pollen quantity seems to be one of the key components in determining seed quality in Fraser fir.

Foliar Mineral Nutrient Diagnosis with DRIS for Identifying Nutritional Influence on Female Cone Production in Fraser Fir

Cone productivity of individual trees in any one year is related to the proportion of axillary buds initiated, which differentiate from cone buds the previous year. Bud differentiation is influenced by balance and supply of nutrients, hormones, and assimilates available during the determination period. It is during this period that nutritional factors may have their greatest influence on cone productivity.

Nitrogen is the only individual mineral nutrient consistently reported to have significant and direct influence on cone production in coniferous trees. Cone production is generally enhanced by appropriate nitrogenous fertilizer application. Effects

of other mineral nutrients on cone bearing in conifers are not well known. However, deficiencies of any mineral nutrient, if sufficiently severe, will reduce cone production.

One method for assessment and diagnosis from tissue analysis, is the Diagnostic and Recommendation Integrated System; DRIS. This system uses nutrient ratios rather than individual nutrient levels to characterize limits to yield. The objectives of the study conducted by graduate student, Roger Arnold were: 1) to develop preliminary sets of DRIS norms and indices for female cone production based on nutrient levels at the time of cone bud initiation and during the previous dormant season; 2) to evaluate differences in the norms obtained from different periods in the growing season; 3) to evaluate DRIS diagnosis of internal nutrient level limitations on female cone production.

The results of the study can be summarized as follows:

- 1) Variations of nutrient level ratios in Fraser fir were found to provide nutritional discrimination between high and low yielding subpopulations. Ratios among the nutrients assessed (N, P, K, Ca and Mg) differed significantly from winter to summer, with the summer values providing markedly superior discrimination.
- 2) Diagnoses made using the summer DRIS norms indicated the DRIS technique does offer the ability to identify nutritional limitations on yield.
- 3) The nutrient most directly linked to cone yield in other coniferous species, N, was diagnosed to rank only second (Mg was first) in the order of nutrient limitations on yield for low yielding Fraser fir subpopulations.
- 4) Variations in the assessed nutrients explained

only a small proportion of yield variation. The factors most influential on 1988 yield were not identified in this study.

- 5) The DRIS technique appears to have potential in Fraser fir and other forest tree species to refine seed orchard management for higher yields. Base population data obtained can be progressively supplemented to continually refine the norms and their diagnostic abilities.

Suppression of Turf Growth in a Loblolly Pine Seed Orchard

As seed orchard management costs have increased over the years, managers are increasingly interested in alternative turf management strategies, particularly ones which reduce frequency of mowing. With up to six or seven mowings per season, the annual per acre cost of mowing can be substantial. Additionally, the costs of mowing are not limited to the costs of personnel and equipment, but extend to the indirect but real costs associated with soil compaction and injuries to the tree stems and roots.

The use of growth retardants and sub-lethal doses of selected herbicides to reduce the required number of mowings has been successful on highway right-of-ways, fruit tree orchards, and landscaped areas. It may also be possible to obtain similar savings in southern pine seed orchards.

During April of 1988 a study was established in the James River Corporation's loblolly pine clone bank near Butler, Alabama. The ground cover in the study area consisted primarily of tall fescue with a very minor component of broad-leafed weeds. Six treatments were installed in this study:

1. Control. No herbicides.
2. 11.78 g Oust/25 gallons of water applied in April.
3. 11.78 g Oust + 179 ml Roundup/25 gallons water applied in April.
4. 11.78 g Oust + 179 ml Roundup/25 gallons water applied in April and repeated in late June.
5. 11.78 g Oust + 717 ml Roundup/25 gallons water applied in April.
6. 11.78 g Oust + 717 ml Roundup/25 gallons water applied in late June.

Treatment plots, 30 x 15 feet in size, were replicated four times and located between tree rows. The effectiveness of treatments in reducing turf growth was evaluated by mowing two 15-foot strips each month in each treatment plot using a standard walk behind mower with a grass catching attachment. The fresh weight of the grass clippings were weighed and a total clipping weight yield was obtained for each sample strip each month beginning one month after the initiation of the study and continuing until cone harvest season.

Changes in species composition were monitored in each treatment each month by taking three 1 ft. sq. sample plots in each of the two strips selected to be mowed each month. The species sample plots were taken just prior to mowing the sample strips.

There were significant treatment differences ($P < .0001$) for clipping weight each month as well as significant treatment differences when data were combined over months. Treatments incorporating the heavy rate of Roundup apparently overwhelmed the fescue. In July there was no clipping weight for treatment 5 indicating that height growth had stopped (Figure 6). A month later the height growth of treatment 5 continued to be very slow. Treatment 6 was applied in late June and by the August evaluation also resulted in strongly suppressed growth (Figure 6). On-site evaluation of these two treatments indicated that both had essentially killed the fescue (Figure 7). In a practical sense, fescue was not a component of the turf in treatment 5 for the months of July and August even though it was

initially a major component of the study area. At the August measurement, the frequency of occurrence for fescue was significantly less in the treatment 5 plots than in the other treatments (Figure 7).

Treatment 4, which consisted of the Oust and "low" rate of Roundup (179 ml/25 gallons of water), appears to have effectively reduced growth without the mortality caused by treatments 5 and 6 (Figure 6 and 7). Treatment 3 seems to offer some advantage over the control in July and August (Figure 6) and did substantially alter the fescue component of the turf (Figure 7). Treatment 2 did not appear to offer any particular promise as a turf growth reducing treatment (Figure 6). It was only better than the

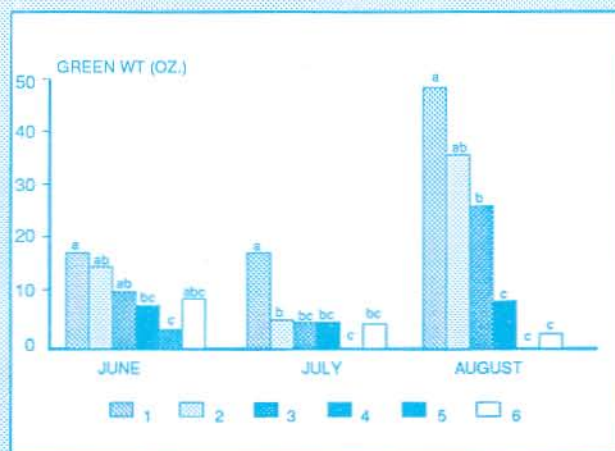


Figure 6. Mean clipping weight (ounces)/25 sq. ft. for herbicide treatments in the James River Turf Study

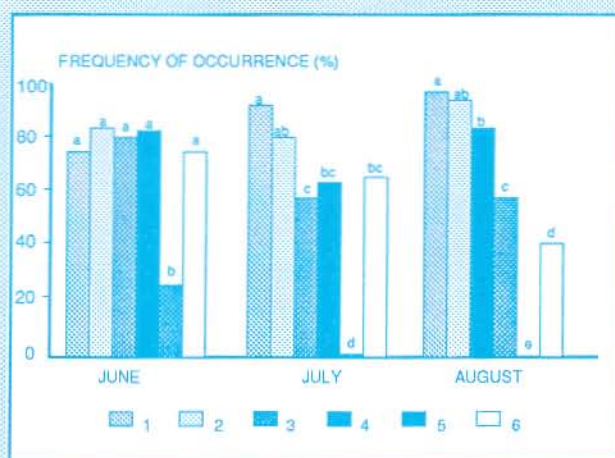


Figure 7. Mean frequency of occurrence (%) of fescue for herbicide treatments in the James River Turf Study

control treatment during the July assessment.

As was the case with clipping weights, there were significant treatment differences ($P < .0001$) in the frequency of occurrence (%) of fescue in the sample plots. At the June evaluation, only treatment 5 had significantly altered the species composition (Figure 7). By July, treatment 5 showed a frequency of occurrence of 1.2% and by August this had dropped to 0.8%. This means that treatment 5 had almost completely eliminated the fescue. Given the need to maintain a turf and protect the site from erosion and vehicle traffic, treatments 5 and 6 do not offer an operational approach to turf management.

Although the "need to mow" was not specifically measured, estimates of when mowing would have been first required during the season were made by Jerry Bolen, orchard manager for James River.

In conclusion, treatments 3 and 4 seem to offer promise of reducing the growth of the fescue turf without destroying the fescue and the protection it affords the orchard site. However, it remains to be seen if the more promising treatments can be re-applied to the same treatment plots during the next growing season without eliminating the fescue.

Stability of Wood Specific Gravity in Open-pollinated Families of Loblolly Pine

The wide movement of selected sources and specific families of loblolly pine across the southeastern United States has resulted in a great deal of interest in both the growth rates and wood properties of this material. In the mid-1970's, the Cooperative established seedlots from Livingston Parish, LA, Marion County, FL, Gulf Hammock (Levy County), FL and the Eastern Shore of Maryland and Virginia together with over 40 open-pollinated families of loblolly pine in field trials throughout the southeastern U. S. to evaluate genotype x environment interactions.

A study recently completed utilized seven 12-year-old plantings of the above material to determine the stability of wood specific gravity of families when planted over a wide geographic area. These plantings spanned the southeastern U. S. from Virginia to Arkansas (Fig. 8). Wood samples were taken from each of the 4 seed sources and from 18 open-pollinated families common to the seven plantings. These plantings were chosen to sample both a north/south and east/west transect. Twenty trees per seed source and open-pollinated family were sampled at each location.

Results indicated that no important genotype x environment interaction existed. A high specific gravity family at one location tended to have high specific gravity at all locations. With the exception

Treatment	Mowing Requirement
1	Operational mowing beginning in late April and continuing every 2.5 to 3 weeks until mid-September.
2	Mowing required by mid-July and continuing until mid-September.
3	Mowing required by August and continuing until mid-September.
4	One mowing the entire growing season right before cone harvest.
5	Turf killed.
6	Turf essentially killed.

of one study location, family performance from site to site was highly correlated.

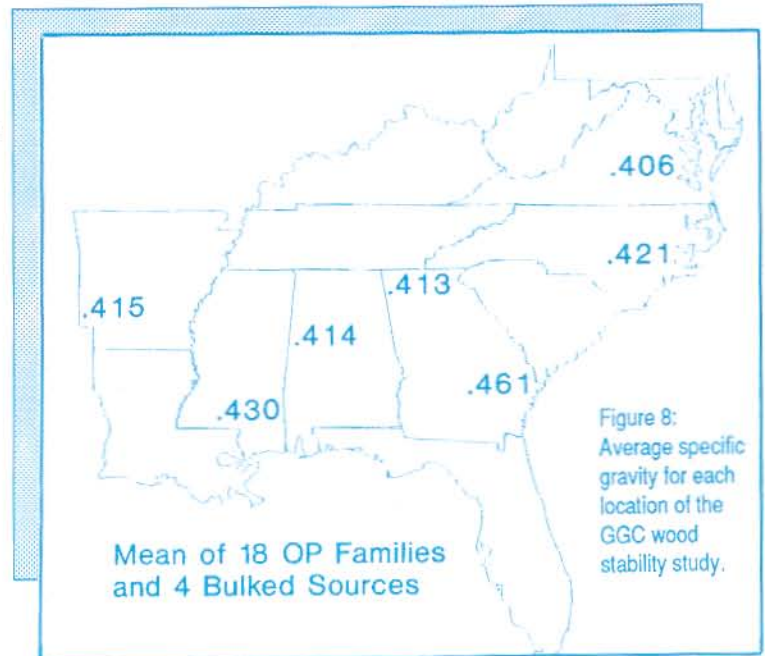
The seed sources from Florida and Livingston Parish consistently had the lowest wood specific gravity. Overall specific gravity across all families and sites was 0.422 while the average specific gravity of the two Florida sources was 0.399 and Livingston Parish was 0.414. These specific gravity values translate into a dry weight of 26.33 lbs/cu. ft. for the 18 O.P. families, 24.89 lbs/cu. ft. for the Florida sources, and 25.83 lbs/cu.ft for the Livingston Parish source. When evaluating these differences, it should be remembered that the families represented in this study are drawn from across the southeastern United States and consequently represent a wide range in specific gravity potential.

The mean specific gravity at each location increased going from the north to the south along the Atlantic coastal plain (Fig. 8). The mean specific gravity in Virginia was 0.406 in contrast to 0.461 for the Georgia site. This trend has been previously well established by numerous studies in natural stands.

These preliminary results indicate that family stability for wood specific gravity is generally good. However, on a provenance level there are differences in wood specific gravity that could prove to be important.

Verification Trial for Early Selection of Loblolly Pine

This study was initiated in the spring of 1989 by the Cooperative staff in conjunction with Great Southern Paper Company and International Paper Co. The use of stem elongation in first and second-year loblolly pine seedlings to predict 8-12 year field performance has been promising in a few studies. While other young traits have been evaluated, stem elongation appears to be the most reliable and most



easily assessed trait to use for early selection.

Each of these trials has been conducted with Coastal North Carolina and South Carolina provenances. The utility of using stem elongation has not been demonstrated for other provenances. It is possible that other provenances will not behave the same. For example, in the Western Gulf Cooperative, total stem dry weight at 4-6 months is the best prediction of field performance, but it has not been a good predictor in the study of the eastern North Carolina provenance.

As the Cooperative prepares to enter the third generation of breeding and testing, the ability to identify selections which could be dropped from the breeding population before expensive controlled crossing began would be very valuable. A reliable and repeatable early selection system for the Cooperative's third generation breeding and testing program will be needed in the mid 1990's.

The objective of this study is to evaluate the use of stem elongation for early selection among families from 5 provenances of loblolly pine. Open-

pollinated seed of 15 families from the following provenances were used:

South Atlantic Coastal Plain
Marion County, FL
Gulf Hammock, FL
Lower Gulf
Middle and Upper Gulf

The use of the different provenances will allow for reliable determination of variation among provenances for early selection.

The trees were planted in mid-March, 1989, and will remain in the field for two full growing seasons until fall 1990. Family means from older field studies will be correlated with family means for 1) total height after one and two growing seasons, and 2) stem elongation during the first and second growing seasons.

Plantation Selection Seed

Source Study

Breeding for the Plantation Selection Seed Source Study was initiated in the spring of 1988. Results from this study will be used to evaluate the patterns of variation among and within sources of the plantation selection population. Seven pollen mixes were made, each comprised of 40 plantation selections from each testing region. The 40 parents were chosen to represent the age and geographic distribution among plantation selections in each region. Each of the pollen mixes is being used to breed 20 females in their respective region.

Overall, good progress in the pollination work for this study has been made by cooperators. In 1988, breeding was initiated on 119 female clones; 45 females were finished. For each of the seven geographic regions in the study, 20 female clones are needed (140 total), but five to eight extra clones per region are being bred. If an unusually early flower crop and some late frosts do not cause too much damage, most breeding should be complete this year, but some breeding will continue at least thru 1990.

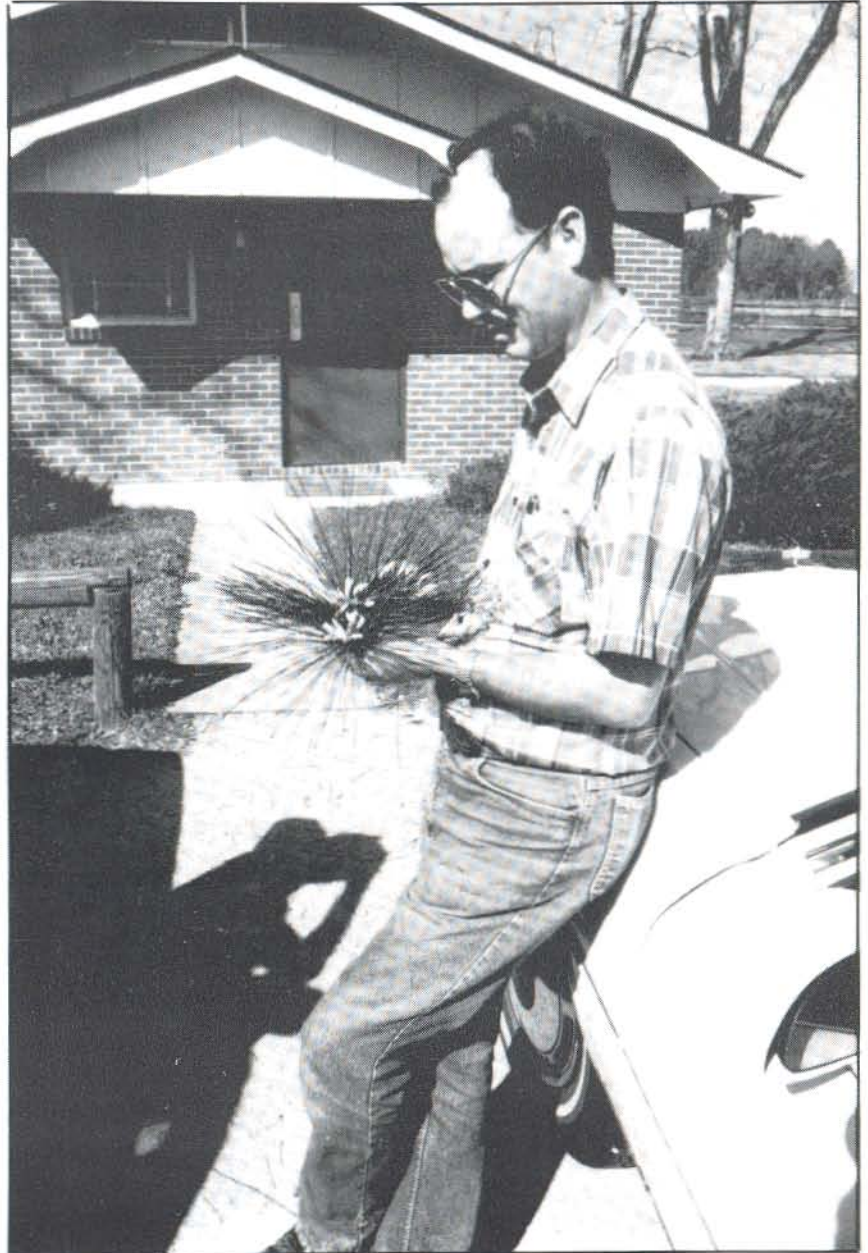


The Early Selection Verification Study was planted in March on this superb experimental site of Great Southern Paper Co. The companion planting was on an equally good site contributed by International Paper Company.

SELECTION, BREEDING AND TESTING

Breeding and testing 3300 plantation and 720 second-generation selections continued to be a major focus of the Cooperative in 1988. Presently, about 69% of the crossing for the plantation selections and 60% of the crossing for second-generation selections are completed. Test establishment is 12% complete for the plantation diallels and 18% for the second-generation (Fig. 9). Although the 1988 pollination season was a great disappointment due to meager flower crops for a majority of Cooperators, progress continued to be made in the test establishment phase. Another 61 tests were established in the 1988 season, including 13 second-generation tests and 48 plantation tests. This brings the total to 157 tests; already 20 have been established for the 1989 season with anticipation for another 30 this year. Figure 10 shows cumulative test totals since the first advanced generation tests were established in 1982. With about 67% of all crossing complete, the number of tests established in the next 4-5 years will increase dramatically. As it appears now, the bulk of the tests will be planted from 1991-1995.

With so many diallels nearing completion at once, a very real problem becomes the distribution of test workload. For example, at least one cooperator has 10 diallels ready for planting now which equates to 20 tests. To plant so many in one year is beyond reason for some organizations. On the other hand, other Cooperators have few, if any, diallels ready. To more evenly distribute the test load over time, some cooperators are trading tests.



*What is a tree breeder to do?
February 15th and the loblolly pollen is
flying. My goodness what weird
weather!!*

An organization with many tests ready for field planting may "give" some to a neighbor with few. Then, in the future, the receiving cooperator will send some tests back to the donor. Cooperation among members manifests itself in other areas, too. In some cases, a member may have completed one diallel of the two needed for test establishment. Rather than wait until another is ready, it can be paired with a diallel from a nearby cooperator, and the test establishment split. In other cases, a cooperator may have 3-4 selections left after breeding all diallels; by pairing up with another cooperator in the same situation, a full diallel can be put together by shipping the necessary pollens. In the second-generation work, two cooperators recently paired completed diallels which were planted by a 3rd member organization.

Cooperation to the ultimate degree was epitomized when Continental Forest Investments' (CFI) participation in the Cooperative ended in 1988. Approximately 220 plantation selections could have been lost from the breeding population, but cooperative members rallied to save the day. James Hodges (Georgia-Pacific) grafted the plantation selections from Coastal Georgia. Marvin Zoerb (Union Camp) and Dave Gerwig (Westvaco) grafted plantation selections from Virginia. Guy San Fratello (S. C. Comm. of For.) provided rootstock for members grafting the selections. Georgia-Pacific grew the

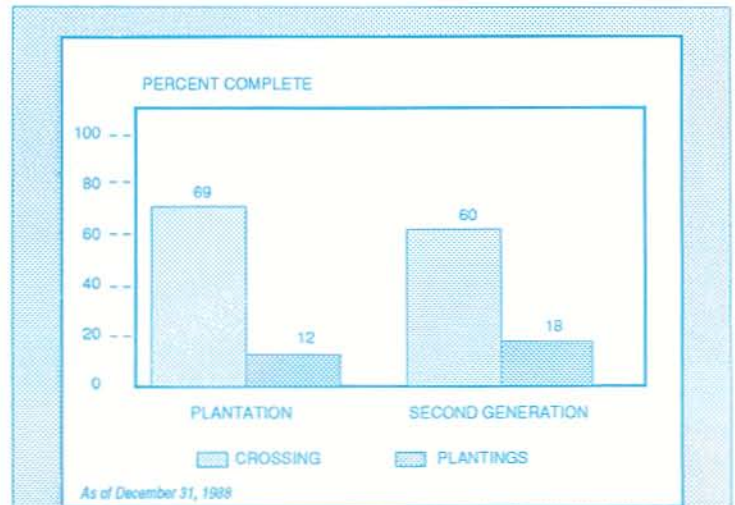


Figure 9: Overall status of the plantation and second generation breeding and testing program.

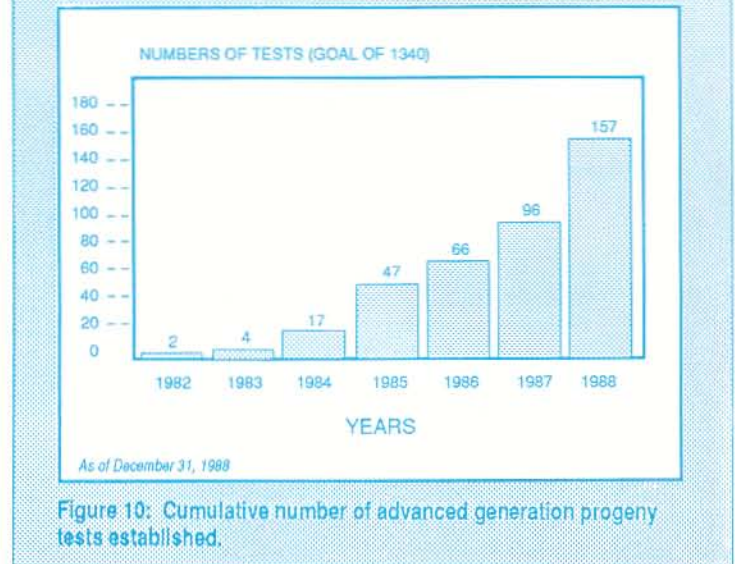


Figure 10: Cumulative number of advanced generation progeny tests established.



Virginia Department of Forestry's progeny test growing in the greenhouse. Substantial differences among families for germination speed are evident.

seedlings for two Georgia Piedmont second-generation diallels completed by CFI and Russell Pohl of the Georgia Forestry Commission established the tests. Westvaco volunteered to grow the seedlings and establish tests for some of the Virginia diallels that CFI completed. Cones from the control crosses made by CFI in the spring of 1987 were collected and processed last fall by Peter Ranalet of International Forest Seed Co. Georgia Forestry Commission will complete the testing of the Georgia Piedmont selections and Georgia Pacific will complete the breeding and testing of the Georgia Coastal selections. Westvaco, Union Camp, Chesapeake and the Virginia Department of Forestry will work to assimilate the Virginia selections into the Area 1 breeding population. A total of nine members have cooperated in this major effort to save CFI's selection work. This spirit of cooperation is highly commended.

In summary, much has been accomplished to date but beware! The big crunch is upon us!

Good General Combiner

Control-Crossed Tests

Several years ago, the Cooperative decided to systematically make crosses among the best first generation clones. The basic pattern was to do 4 crosses per clone; 3 were made with crosses within the same region and 1 was made with a clone outside the region. The tests were out-planted from 1980 to 1982.

Most of the parents of these crosses are well represented by progenies in seed orchards and in second-generation diallels. However, these Good General Combiner (GGC) crosses are new combinations of the best parents and offer an opportunity for more genetic gain. These selections have potential use for the following:

1. New blocks of second-generation seed orchards. Although only a few cooperators are currently expanding orchards, these selections could be

used. Again, new combinations of the best parents could mean increased gains from increased selection intensity.

2. Development of an elite breeding population for accelerating gains in the short term. Selections from these parents could be combined with other outstanding clones to form the base for an intensively selected breeding population. Several cooperators have expressed interest in this type of approach in addition to the mainline breeding program. In fact, IFSCO is already breeding a select population in this manner for rust resistance.

Though we had no firm plans for selections from these tests, we were concerned that we might lose this genetic resource if we did not act quickly. Therefore, this past year cooperators and staff made 74 selections from these tests.

The breakdown of selections made for each region follows:

Va and Northern NC (and other cold regions)
- 22 selections

Atlantic Coastal Plain and Lower Gulf
- 25 selections

Piedmont and Upper Gulf
- 27 selections

SEED ORCHARD PRODUCTION

Cone and Seed Yields

On the heels of the largest cone crop ever (1987), the 1988 crop was a disappointment as it was the lowest since 1982 (Table 2). Approximately 56,822 bushels of loblolly were collected, yielding 42.7 tons of genetically improved seed. The 42.7 tons represents a 54% decline over the 1987 record of 93.3 tons. Fortunately, most Cooperative members have good seed inventories due to several successive years of outstanding production. The cumulative production profile in Figure 11 illustrates how the Cooperative program members continue to impact the forests of the South and the nation in a major way through planting genetically improved loblolly pine. In Table 3 a detailed breakdown by species compares the 1988 and 1987 cone crops. The decreased production was not only for loblolly but for all other species as well. Total seed yields for all species combined was approximately 58% lower than in 1987 (96,603 vs 231,300 lbs.) In the minor species, Fraser fir seed yields decreased 92%, as did longleaf. Slash pine was down 70% and Virginia pine, 56%. However, with some of these species, it is difficult to know how much of the decrease is due to reduced seed yields and how much is due to the member deciding not to collect the cones.

The second generation orchard yields were a little better than average at 49% of the previous year. The total pounds of second generation seed produced was the lowest since 1985 (Figure 12). In 1988, second generation seed represented 11.7% of the total as compared to 11% in 1987. This percentage should rapidly increase in the next 3-5 years. Figure 12 shows the amount of second generation seed produced over the past 7 years which equates to approximately 730,000 acres of highly productive plantations.

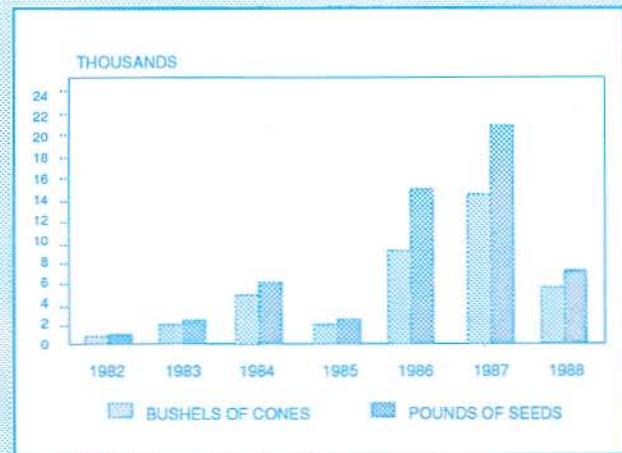


Figure 12. Second generation seed orchard yields for past 7 years.

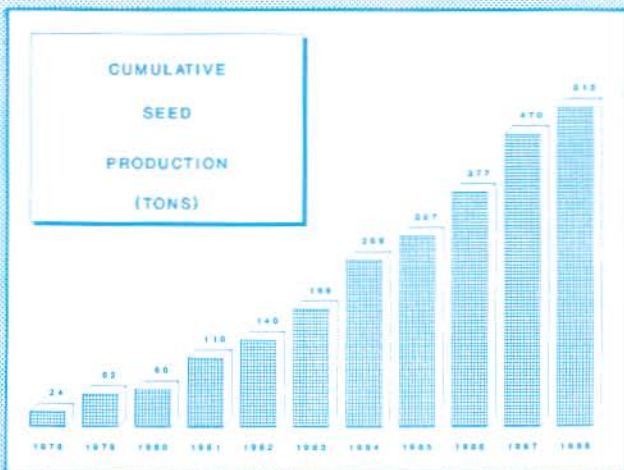
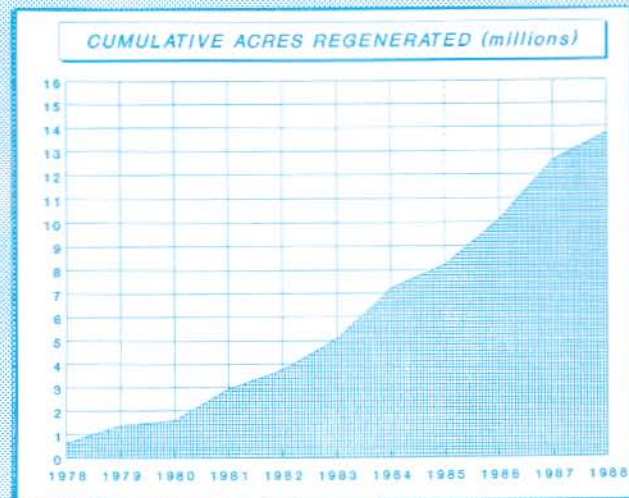


Figure 11. Cumulative Seed Production and Regeneration Profile for the Cooperative.



In light of the poor cone crop of 1988, what is even more disturbing is that the 1988 flower crop seemed to be down significantly; hence, another off-year cone crop for 1989 seems to be pending. The lesson to be learned from Mother Nature's fickleness is the need to take advantage of good seed years and reserve excess for the lean years. Complacency resulting from the outstanding crops in recent years could lead to problems. Let this past year be a reminder: we need to keep our seed inventories at a safe level!

10-YEAR SEED YIELDS FROM LOBLOLLY ORCHARDS

Table 2. Production of cones, seeds and seedlings from Cooperative members' loblolly pine seed orchards over the last 10 years, including an estimate of acres that could be regenerated with improved seedlings if all seed were used.

Harvest Year	Bushels Of Cones	Tons of Seeds	Millions Of Seedlings	Millions Of Acres Regenerated
1979	38,693	27.7	443	0.74
1980	15,296	7.9	126	0.21
1981	64,811	50.5	808	1.35
1982	44,761	30.5	488	0.82
1983	68,447	49.0	784	1.31
1984	105,239	80.1	1,282	2.14
1985	52,155	37.8	605	1.01
1986	84,953	70.1	1,122	1.87
1987	112,822	93.3	1,493	2.49
1988	56,822	42.7	683	1.14
Total	643,999	490	7,833	13.08

Table 3. Cone and Seed Yield Comparisons for 1988 to 1987

Species	Bushels of Cones		Pounds of Seed		Pounds of Seed per Bushel of Cones	
	1988	1987	1988	1987	1988	1987
Loblolly pine:						
Coastal 1.0 gen.	31,699	66,601	45,954	111,633	1.45	1.68
Piedmont 1.0	17,928	32,139	29,376	54,599	1.64	1.70
Coastal 2.0	4,584	10,010	6,303	14,781	1.38	1.48
Piedmont 2.0	2,611	4,072	3,702	5,687	1.42	1.40
Slash Pine	9,191	24,689	10,412	34,390	1.13	1.39
Longleaf	750	8,288	706	9,210	0.94	1.11
Virginia	97	200	93	210	0.96	1.05
White Pine	0	300	0	50	0	0.17
Fraser Fir	28.5	260	57	740	2.00	2.85
Totals	66,889	146,559	96,603	231,300		

Production Leaders

Every year it is a real pleasure to recognize the programs and orchard managers who set standards of excellence in seed production. As would be expected in an "off" year, the number of members appearing on the Two Pound Honor Roll was down in 1988 (7 orchards as compared to 9 in 1987). The orchards exceeding the two pound production efficiency level and their yield statistics are listed in Table 4.

The best yield in the Cooperative for 1988 was the Weyerhaeuser, NC Piedmont loblolly orchard which yielded 2.41 lbs/bushel. This is the second year in a row this orchard has made the honor roll and surprisingly, on a pound per bushel basis, yields were higher in 1988 than in 1987 (2.41 lbs/bushel vs 2.37 lbs/bushel). In 1988, this 11-acre,

26-year old, first generation orchard produced 555 bushels, from which 1339 lbs. of seed were extracted. In second place was Great Southern Paper Company's Coastal seed orchard at 2.18 lbs./bushel. Close on the heels of Great Southern in third place was the "Champion" from last year: Champion's NC Coastal seed orchard realized 2.12 lbs./bushel. North Carolina Forest Service appeared again this year but with a different orchard; their Piedmont orchard produced 2.08 lbs./bushel. Next in line was Weyerhaeuser, NC again with their South Coastal orchard at 2.03 lbs./bushel. This is the third straight year Weyerhaeuser has placed two orchards on the two pound Honor Roll.

Finally, rounding out the top seven was Catawba Timber Co.'s 1.5 Piedmont orchard at 2.02 lbs./bushel and S. C. State Commission's Coastal orchard at 2.00 lbs./bushel.

Table 4. Seed orchards exceeding two pounds of seed per bushel of cones harvested in 1988.

Cooperator	Orchard Type	Generation	Acres	Age	Lbs/Bu.
Weyerhaeuser Company	Piedmont	1.0	11	26	2.41
Great Southern	Coastal	1.5	40	11	2.18
Champion (NC)	Coastal	1.0	21	26	2.12
N. C. Forest Service	Piedmont	1.0	10	20	2.08
Weyerhaeuser Company	S. Coastal	1.0	31	28	2.03
Catawba	Piedmont	1.5	30	14	2.02
S. C. Commiss. Forestry	Coastal	1.0	36	18	2.00

Congratulations go to Gary Oppenheimer (Weyerhaeuser), Gary Cannon (Great Southern), Marc Davison (Champion), Gene Turner (NCFS), Jake Clark (Catawba Timber Co.) and Booth Chilcutt (S. C. State Commission) for setting such standards of excellence, especially in an off-year for loblolly seed production.

ASSOCIATED ACTIVITIES

Graduate Student Research and Education

The education of graduate students and the research they conduct as part of their degree programs continues to be an important activity of the Cooperative. During the past year, 14 graduate student programs have been conducted in association with the Tree Improvement Cooperative. Ten were directed toward Masters degrees and four were involved in Ph.D. programs. Of special note is the completion of degree programs by two students in 1988-1989: Jim Richmond and Roger Arnold.

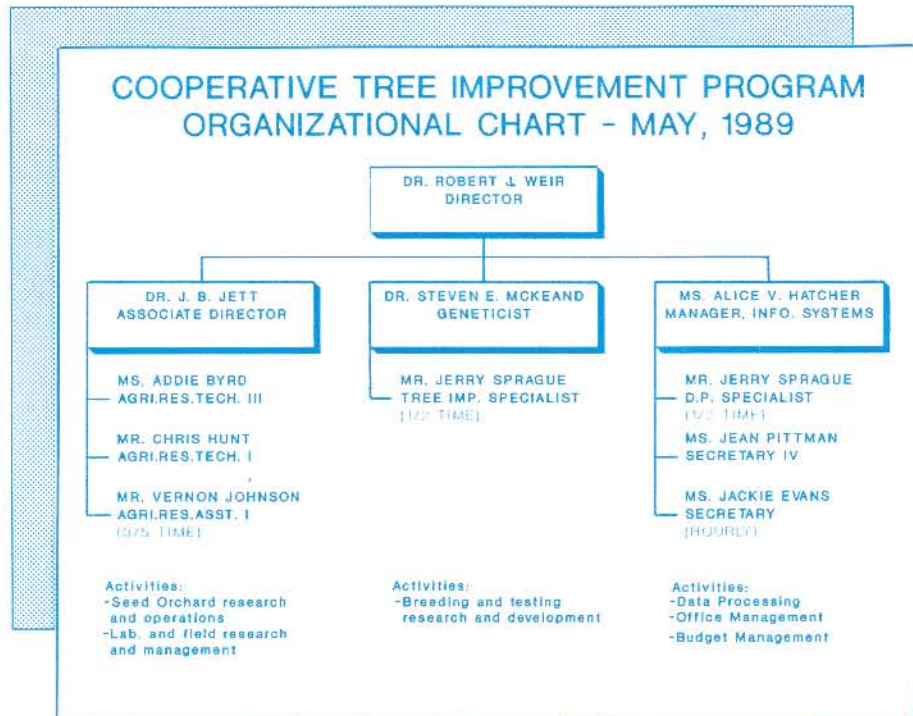
The graduate students working in association with the Cooperative, the degree to which each aspires and the subject of their research project are listed on the following page. Student research projects encompass a wide range of subject matter related to tree improvement. Financial support for students comes from a variety of sources — the Tree Improvement Cooperative, the College of Forest Resources - Department of Forestry, the North Carolina State University Agricultural Research Service, The U. S. Forest Service, Industry, various fellowship programs, and foreign governments.

Very qualified students continue to apply for admission to graduate studies in tree improvement. During the last year we received applications from 4 students, admitted 2 and we anticipate one will actually enroll during the fall. A substantial number of applicants to our graduate studies program are from foreign nations. This results from two primary factors: 1) the employment opportunities for domestic graduate students are few, and 2) the strong international recognition of our tree breeding research and development success.

Student/Degree	Research Project
Roger Arnold, Masters	<i>An evaluation of the DRIS approach for identifying mineral nutrient limitations on flowering and cone production in Fraser fir (completed)</i>
Claudio Balocchi, Ph.D.	<i>Modeling clonal propagation systems of Monterey pine in Chile</i>
Ann Margaret Hughes, Masters	<i>Seed quality studies in Fraser fir</i>
Keith Jayawickrama, Masters	<i>Nutrient and carbohydrate variation among loblolly pine clones grafted on different rootstock families</i>
Bailian Li, Ph.D.	<i>Genetic variation of nitrogen use in loblolly pine</i>
Yecai Liu, Masters	<i>Inbreeding in loblolly pine</i>
Mary Frances Mahalovich, Ph.D.	<i>Modeling the genetic consequences of positive assortative mating</i>
Luis Osorio, Masters	<i>Vegetative propagation of <u>Pinus maximinoi</u> and <u>P. tecunumanii</u></i>
David Porterfield, Masters	<i>An evaluation of interspecific hybrids of <u>P. clausa</u> x <u>P. virginiana</u> and <u>P. rigida</u> x <u>P. clausa</u></i>
Jim Richmond, Ph.D.	<i>Genetic variation among populations of pine cone worms (completed)</i>
Daniel Uribe, Masters	<i>Undecided (new student)</i>
Jerry Windham, Masters	<i>Undecided (new student)</i>
Youhau Zhang, Masters	<i>Genetic differences in seasonal development patterns associated with fusiform rust resistance in loblolly pine</i>
Lan Zheng, M.F.	<i>No thesis</i>

Program Staff

During the past year, there were a few fairly significant changes in the program staff. On June 30, 1988, Vernon Johnson officially retired from state services; however, he continues to work for us part-time. Addie Clark was promoted and is now in charge of our laboratory operations. Chris Hunt was hired as a field technician to replace Charles Echerd who left for another campus position.



MEMBERSHIP OF THE TREE IMPROVEMENT COOPERATIVE

Alabama Forestry Commission
Bowaters
Champion International Corp.
Chesapeake Corp. of Virginia
Container Corp. of America
Evergreen Corp.
Federal Paper Board Company
Georgia Forestry Commission
Georgia-Pacific Corp.
Great Southern Paper Company
International Forest Seed Company
International Paper Company
James River Corporation
Kimberly Clark Corporation

Leaf River Forest Products
MacMillan Bloedel Inc.
Mead Coated Board
N. C. Division of Forest Service
Packaging Corporation of America
Proctor and Gamble
Rayonier, Inc.
Scott Paper Company
S. C. State Commission of Forestry
Union Camp Corporation
Virginia Dept. of Forestry
Westvaco Corporation
Weyerhaeuser Company

Cooperative membership was relatively stable over the past year, though there were a couple of changes. Boise Cascade's Tree Improvement Program was bought by Evergreen Corporation, and fortunately, Evergreen plans to maintain the membership. Also Georgia Pacific bought Brunswick and plans to continue the Brunswick tree improvement program. This brings the total membership of the Tree Improvement Cooperative to 27 organizations which operate 27 base units and 11 supplemental units for a total of 38 active tree improvement programs.

PUBLICATIONS OF SPECIAL INTEREST TO MEMBERS OF THE COOPERATIVE

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- McKeand, S. E. 1988. Optimum age for family selection for growth in genetic tests of loblolly pine. For. Sci. 34:400-411.
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