PROCEEDINGS

23rd SOUTHERN FOREST TREE IMPROVEMENT CONFERENCE

June 20-22, 1995
Asheville, North Carolina

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THE SOUTHERN FOREST TREE IMPROVEMENT COMMITTEE

HOSTED BY

N.C. STATE UNIVERSITY-INDUSTRY COOPERATIVE TREE IMPROVEMENT PROGRAM

Proceedings of the 23rd Southern Forest Tree Improvement Conference
Compiled by Robert J. Weir and Alice V. Hatcher

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Sponsored Publication No. 45 of the

Southern Forest Tree Improvement Committee

FOREWARD

The 23rd Southern Forest Tree Improvement Conference was held at the Holiday Inn SunSpree Resort in Asheville, North Carolina. The Conference was sponsored by the Southern Forest Tree Improvement Committee and hosted by the N. C. State University-Industry Cooperative Tree Improvement Program.

A total of 37 presentations, three invited and 34 voluntary, were given. The voluntary papers were evaluated by the Southern Forest Tree Improvement Committee for the $200 Tony Squillace Award (best oral presentation/written paper). The paper General and Specific Combining Ability for Fusiform Rust Infection in Slash Pine by T. D. Byram and W. J. Lowe was selected to receive the Squillace Award. Congratulations to Tom and Bill for an outstanding contribution.

Ten posters were exhibited during the conference. The Baruch Foundation award of $100 for best poster was presented to H. V. Amerson, P. L. Wilcox, D. O'Malley, R. R. Sederoff, E. G. Kuhlman for their poster entitled: Role of Major Genes for Resistance in the Loblolly Pine Fusiform Rust Forest Pathosystem. Congratulations to Henry, Phil, Dave, Ron and George.

Conference attendees participated in a tour of Biltmore Estate, the birthplace of forestry in the United States. Preceding the tour, Bill Alexander, Landscape Curator at Biltmore Estate, presented an overview of forestry at Biltmore entitled "Breaking New Ground": 100 Years of Forestry at Biltmore Estate.

The staff of the N. C. State University-Industry Cooperative Tree Improvement Program expresses their appreciation to all conference participants for their contribution to a successful meeting.

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THE TIMBER SUPPLY SITUATION IN THE SOUTHEAST: IMPLICATIONS FOR INTENSIVE MANAGEMENT.

Robert C. Abt, Frederick W. Cubbage, Gerardo Pacheco[1]

Abstract.--For as long as we have been collecting inventory information, the southern timber inventory has been increasing. In the last decade, however, softwood removals in the South have exceeded growth. If current trends continue, hardwood removals will exceed growth in about a decade. If availability and operability constraints are considered, the supply situation looks even more serious. These structural changes in the supply situation, coupled with increasing demand on the resource have led to dramatic price increases. This paper analyzes past trends and assesses the future supply and price situation for the South. The potential effect of intensive management on both regional supply and wood cost will also be examined.

Keywords: timber supply, markets, prices, inventory.

INTRODUCTION

Timber supply issues have been a focus of forestry research and policy since the days of Pinchot. While our assessments and measures of resource scarcity have become more sophisticated, the central question of resource availability and cost competitiveness remains (Cubbage et al. 1995). The purpose of this paper is to examine the current timber supply situation in the South. This is best understood by considering the social, historical, regional, and economic context in which southern timber markets operate.

The southern timber market is one important component of an integrated U.S. and global fiber market. The South is the dominant supplier of both hardwood and softwood fiber in the U.S. Private investment in forestry will be a key determining factor in the future of the resource. For the softwood resource, this means the productivity of pine plantations is a key variable. Addressing the complexities of recycling markets, international fiber sources, and end-use markets is beyond the scope of this paper. Most analyses of these issues, however, conclude that the demand for fiber in the South will increase. The focus here is on the historical and economic context of the timber supply. An understanding of some fundamental changes in past trends provides a basis for examining implications for the future of the resource in the face of increasing demand, and the possible role of intensive management in influencing that future.

[1] Associate Professor, Professor & Department Head, and Research Assistant, Department of Forestry, North Carolina State University, Raleigh, NC 27695

TRENDS

The paper begins with a focus on historical inventory. The inventory of standing timber is not the same as the economic supply. Supply refers to that inventory which will be available for harvest at different prices. Regulations, landowner objectives, and accessibility are among the factors that create differences between inventory and supply. Inventory trends do provide insight into potential opportunities and problems.

Figure 1 shows the trends for softwood and hardwood growing stock levels in North Carolina. They indicate that, like the South as a whole, inventory has been steadily increasing over the last half century. In the last decade increases in inventory are smaller and for the South as a whole, softwood inventory have decreased slightly. Figures 2 and 3 show that this can be attributed to both an increase in harvest and reduced growth.

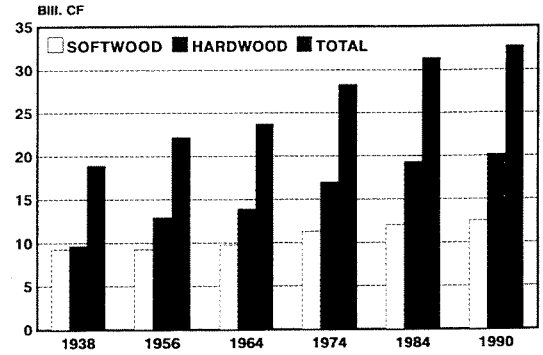

Figure 1. Growing stock volumes, NC

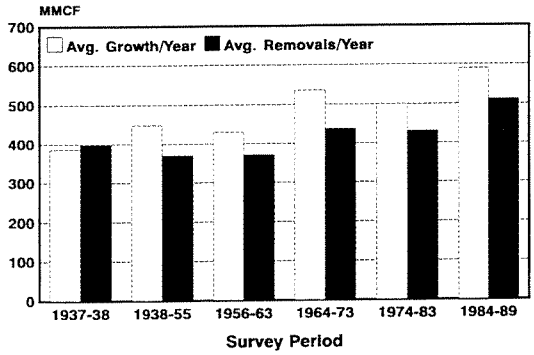

Figure 2. Softwoods net annual growth and removals, NC

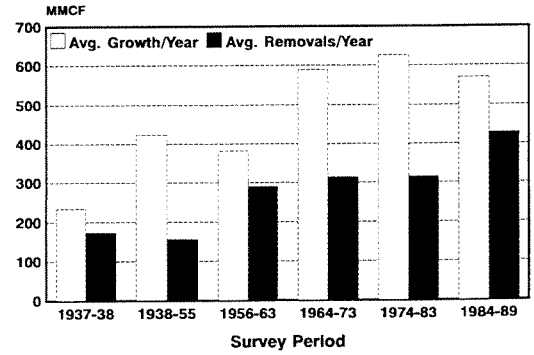

Figure 3. Hardwoods net annual growth and removals, NC

Harvest increasing faster than inventory also has economic implications as is shown in the stumpage prices for the Southeast in Figures 4 and 5. For the period since the mid 1980's softwood prices have doubled their rate of increase while hardwood prices have quadrupled as reported by Timber Mart South, Inc. Similar trends hold for sawtimber, though the prices don't start to increase until the early 1990's. Note that the price levels differ between states but the trends are similar. This is one result of a competitive market. As prices diverge between regions, harvest shifts to take advantage of lower prices until trends converge.

MODELING

The assumption of competitive markets for the resource is the basis for the projections to be discussed below. The SouthEastern Regional Timber Supply (SERTS) model simulates supply as a function of stumpage price and inventory (Abt et al. 1993). Empirical estimates indicate that timber supply is price inelastic, i.e. it takes a relatively large price increase to

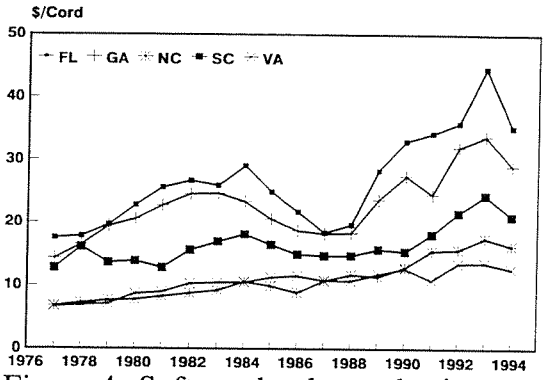

Figure 4. Softwood pulpwood prices

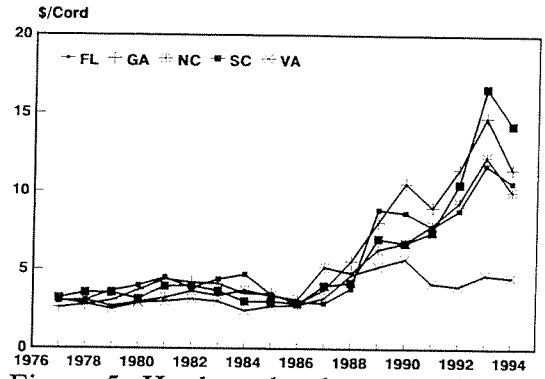

Figure 5. Hardwood pulpwood prices

yield an increase in harvest. These studies also indicate that inventory seems to have a proportionate affect on harvest, i.e. at a given price an increase in inventory leads to a proportionate increase in harvest. The range of elasticity estimates is large depending on the products, regions, and time periods being studied.

Other than the market elasticity estimates the model requires an assumption about future harvest levels. Based on this harvest, the model calculates the aggregate regional price trend and also the shift in harvest between regions. The harvest projection is based on the 1993 Draft RPA trend for the South. Growth is calculated in the model based on the latest FIA survey information for each state. The configuration discussed here shows results from running the 21 survey units of the Southeast region as one market. This model also does not differentiate between products so the price and inventory trends relate to the aggregate growing stock.

PROJECTIONS

Figures 6 and 7 show the inventory projections for softwood and hardwood growing stock respectively in the Southeast. As Figure 6 shows, softwood harvest currently exceeds growth and if harvest continues to increase as expected, inventory will continue to decline. For hardwoods, current growth exceeds harvest by a significant amount, but within the next decade the removals could exceed growth.

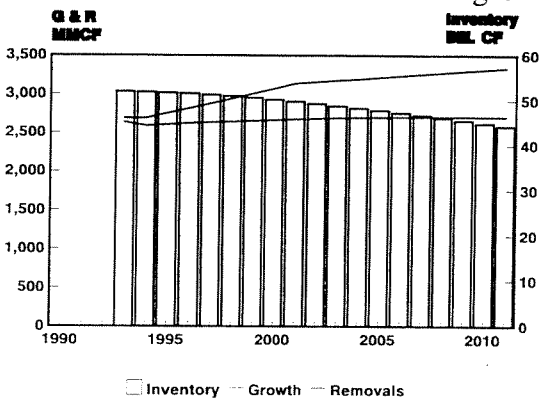

Figure 6. Softwood projections, Southeast

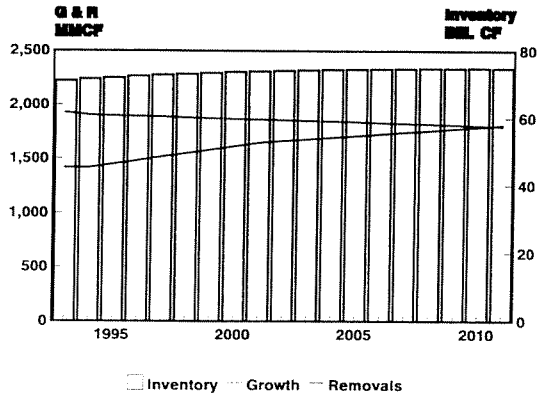

Figure 7. Hardwood projections, Southeast

Implicit in this forecast is a continuation of conversion pine plantations as shown in Figure 8. Productivity gains that might come from intensive management in these areas are expected to reduce the projected price trends, as explained below. These area trends by management type are based on those reported by the Forest Service South's Fourth Forest Report (U.S.D.A. Forest Service 1988).

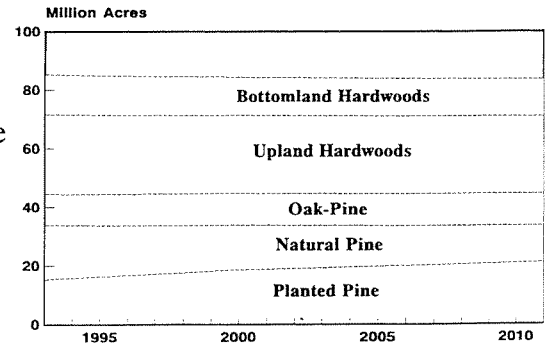

Figure 8. Southeast acreage trends

Over the projection period softwood harvest tends to shift to greater dependence on pine plantations in the coastal plain and the Atlantic Coast region as shown in Figures 9 through 11.

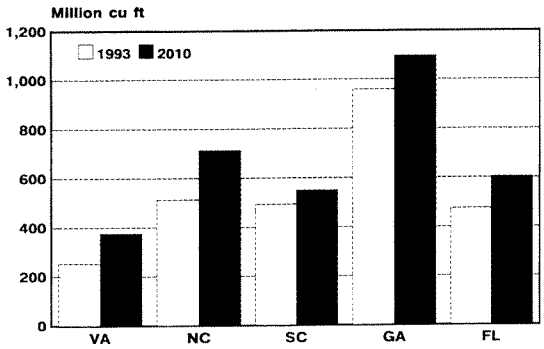

Figure 9. Projected distribution of softwood harvest

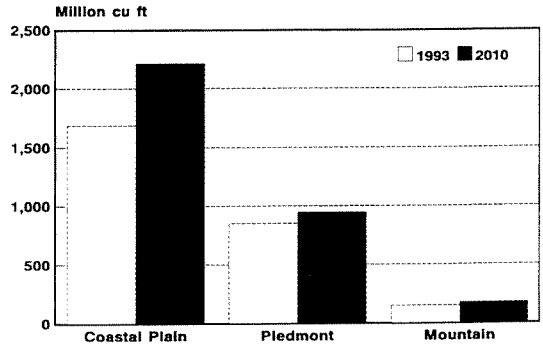

Figure 10. Projected softwood harvest by physiographic region

State projected harvest shifts appear to be larger in the states of Virginia and North Carolina. Our base projection suggests that the coastal plain region in the South might become a major source of future softwood harvest in the next fifteen years. A potentially significant implication of this projection is the major shift of harvest into pine plantations. If intensive management increases the productivity of these areas the economic consequences will be substantial, as explained below.

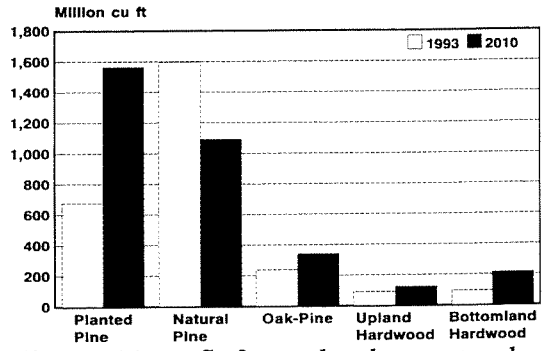

Figure 11. Softwood harvest by management type

For the region as a whole, increasing harvest with declining inventory implies large price increases over the next 15 years as shown in Figure 12. The effect of increasing growth rates on pine plantations is also shown. Though real prices increase in all scenarios, the potential impact of increased growth is significant. Estimates of productivity gains from intensive management (genetically improved stock, etc.) range widely. Our projection includes a base case that represents no gain, and incremental gains up to a 40% increase in growth. We also assumed that all of the pine plantations had the same growth boost. The results show a projected base softwood price over 220% higher than the projected price

increase with a 40% boosted growth. The significance of the economic consequences of intensive management depends on the realized productivity gains.

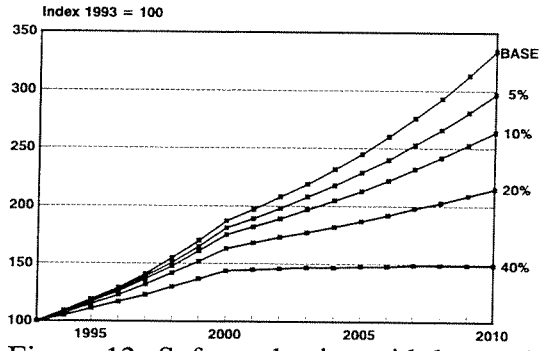

Figure 12. Softwood price with boosted growth in pine plantations.

In all of the above scenarios the total inventory was used to shift supply. A recent study by the North Carolina Forestry Association indicate that up to one fourth of the softwood inventory may not be available due to water, slope, or a variety of other accessibility problems (North Carolina Forestry Association 1993). Figure 13 shows inventory projections for North Carolina for the base case described above, the inventory decrease when acres are screened out (medium availability case), and the ameliorating impact of increasing growth 20 percent above current FIA levels in pine plantations. The lower line in the graph represents the impact on inventory from reduced availability screening. The projection shows that adding a 20% productivity gain to the reduced availability scenario reduces NC softwood inventory about half as much as the reduced availability screen and no productivity gain.

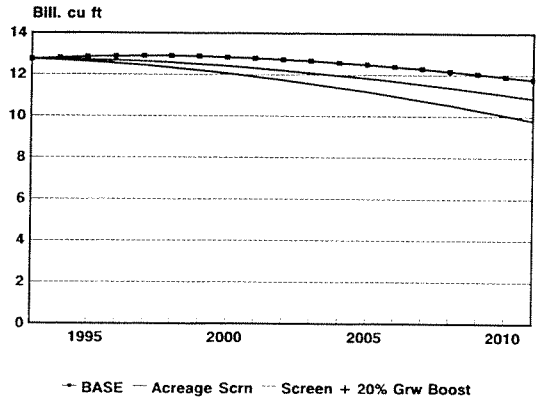

Figure 13. NC softwood inventory, screened acres and plantation growth boost.

While the above projections imply that wood costs will continue to increase, higher prices especially on higher faster growing trees make investment in intensive management profitable. Figure 14 shows the effect on the internal rate of return from various levels of volume and price increases.

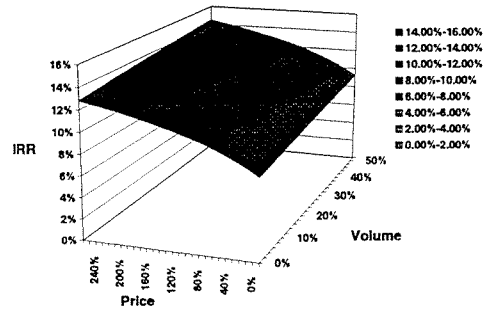

Figure 14. Relationship between price, volume, and the IRR. Cost=150, volume=40, price=25

In summary, these projections show that we may be entering a period of significant structural change in timber markets and investment. Increasing demand, less accessible supply, and the resulting higher prices imply a renewed interest in forest investment.

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The Impact and Value of Tree Improvement in the South

By David Todd[1], John Pait[2] & James Hodges[3]

Abstract. -- Anytime a review of impacts are done, one must summarize or account for activities of the past that have led us to the present -- How did we get to now? The impacts of tree improvement are significant. However, we are now just beginning to reap the increased wood and economic returns that have taken nearly 40 years to develop, implement and produce tree crops. And what a time it is for these benefits to be realized as available fiber resources are being pushed to the limit.

Impacts of tree improvement in the South can be classified as direct and indirect. Direct impacts are those which provide direct economic value, either cash or present value to affect owner equity. These direct impacts are associated with increase wood supply or quality and their net present value. An example is the planting of over 1 billion genetically improved seedlings each year in the South. This has a significant direct impact on owner equity.

Indirect impacts are associated activities that eventually will result in direct impacts, but in themselves do not have direct impacts. Examples would be research and developmental work. Such impacts have played a large role in attracting research dollars, furthering knowledge/understanding, and educating new generations of tree breeders. The value/benefit of such impacts is much harder to delineate and calculate than direct impacts.

Each type of impact is absolutely necessary for the long-term payoff of tree improvement. The interplay of these activities has been a catalyst to produce more and better quality trees and served as a model for other aspects of forestry. The tree improvement university/industry cooperative model has lead to a myriad of other cooperatives that have focused resources on forest productivity. This has placed the South at the forefront as a major wood fiber supplier.

Keywords: Tree improvement, economics, regeneration

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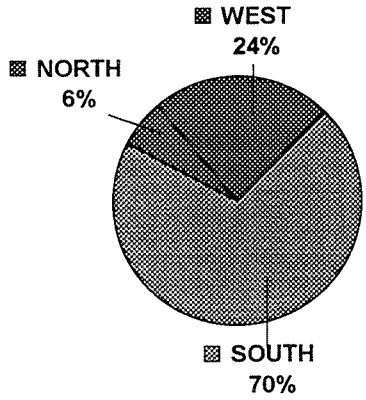
INTRODUCTION

Tree improvement has had a tremendous impact on the southern forest industry. Much of that impact we are now just beginning to realize. These impacts have come about through cooperative effort by industry, universities, and government agencies in a unique partnership.

Today, just like 40-45 years ago, the forest industry is keenly concerned with sustainable timber and fiber supply for the future. Then, as now, tree improvement is being investigated as being a key component of the long-term wood supply solution.

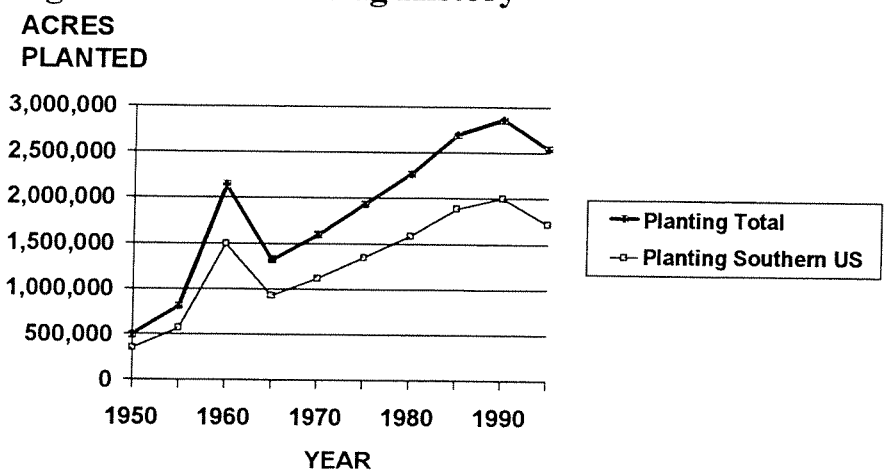
To provide some orientation, in the U.S., approximately 70% of all planting occurs in the South (Figure 1)(Moulton et. al.1993).

Figure 1. Planting by Region



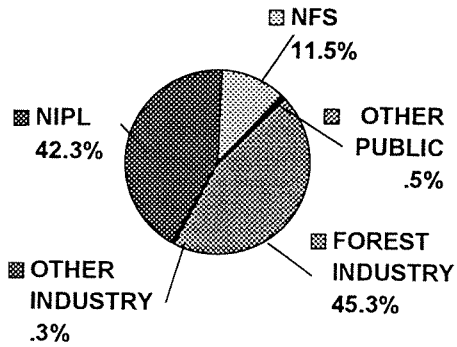
Historically, since the 1950's, tree planting has been on the rise. In 1950, about 500,000 acres were planted in the South. By 1990 the number of acres had risen to nearly 2.8 million (Figure 2)(Moulton et.al. 1993). Overwhelmingly this increase was due to the regeneration demand and commitment in the South. This increase also coincides with the development of tree improvement technology.

Figure 2. U.S. Planting History



Most of the tree planting is accomplished by forest industry (45%) and the non-industrial private landowner (NIPL)(43%) (Figure 3)(Moulton et. al. 1993) However, less than half of all lands harvested are regenerated. Most of the acres not regenerated occurs on NIPL property (Lantz 1994).

Figure 3. PLANTING BY OWNERSHIP



IMPACTS

Impacts from tree improvement can be classified as either direct or indirect. Direct impacts are:

a) **Those that increase wood supply or**
b) **Result in better wood quality**

Direct Impacts

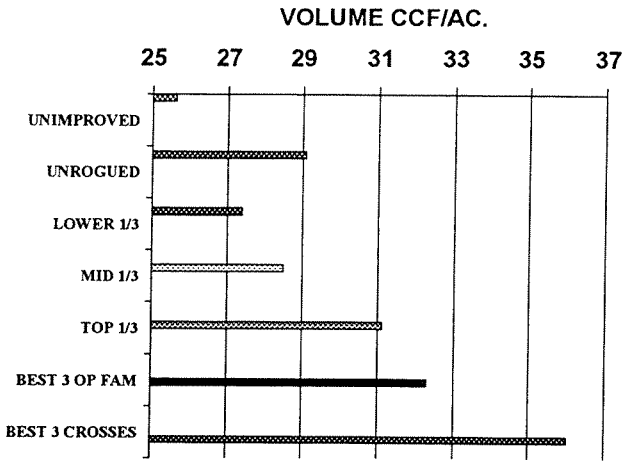
Species -- Although we do not think that much about species selection today, one of the great impacts of tree improvement has been on the choice of species to plant. In the 1950's nearly 80% of all planting was slash pine (*Pinus elliottii* Engelm. *var. elliottii*) and about 20% loblolly (*Pinus taeda* L.). The early fast growth of slash was deceptive in yield at rotation compared to loblolly on most sites. With the advances in tree improvement, we recognize loblolly will out perform slash on non-slash sites in volume yield at rotation. We learned a great lesson. Today, nearly 80% of planting is with loblolly and 20% with slash pine.

Potential Volume Gain -- Yes we can produce trees that grow bigger! Figure 4 is an example of the ways potential volume gain has been captured. The key to additional gain is information. At first, only seed from unrogued orchards was available and provided good potential to increase wood supplies. Then as more information became available orchards were segregated into bulk lots; the best, middle and lowest performers. With the planting of the best bulked families, potential gains increased. Then individual open-pollinated family lots were developed. And again, potential gain increased. Today controlled-pollinated family lots are being made and soon may be the standard for regeneration in the South. The reason -- increased potential volume gain.

Fusiform Rust Resistance -- Fusiform rust (*Cronartium quercuum* (Berk.) f. sp. *fusiform*) is the major disease of slash and lobllolly pine. It causes great mortality in slash pine stands. However, markedly less mortality and damage occurs in lobllolly stands. Another reason loblolly is being planted more than slash.

There has been great progress in improving rust resistance in both slash and loblolly pine. Specialty orchard have been used or resistant families have been identified and deployed in high rust hazard sites. Recent progress in the understanding and identification of the genetic mechanism for resistance will likely mean great progress in controlling this disease.

Figure 4. EXAMPLE OF METHODS USED TO CAPTURE GENETIC GAIN IN VOLUME



Straightness -- Straightness is generally considered a wood quality characteristic because it has great impact on the value of both solid wood and pulpwood products. Although straightness value gains are obvious for solid wood products, this is acute in the chipping of pulpwood. Today with tree length processing, very crooked stem will not physically fit in the throat of the chipper. One generation of selecting for straightness has resulted in significant gains in the straightness of loblolly pine.

Wood Quality -- Wood quality by what characteristic you wish to choose; specific gravity, tracheid length, etc, are traits that we do not quit know what to do with. Some traits for solid wood products have been specifically determine and are incorporated into product specification.

We know theoretically, for example, that specific gravity should make a difference in yield and quality in pulp and paper products. However we cannot measure the economic value in the mills. If we did change specific gravity, how would we determine the value?

Economic Value -- We are not going to go through any detailed economic evaluation. There are enough economist here to criticize our economic misgivings. But a simple valuation of tree improvement will serve to make the point of the magnitude of the revenue that is being generated in the South from tree improvement. A simple net present value of one year's regeneration in the South should illustrate this adequately.

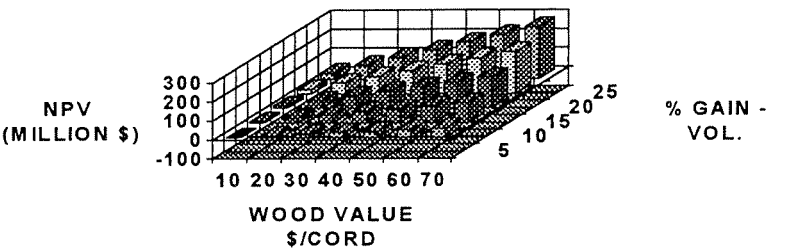
Simplifying Assumptions:

a) Annual planting of 1.5 million acres (1.7 million acres planted in the South in 1992 with 90% being planted with genetically improved stock.)
b) Rotation age of 25 years
c) Tree improvement cost of $7.50/acre
d) Discount rate of 4%

Net present values were calculated for a matrix of wood values and potential genetic gain in volume growth (Figure 5).

Since everyone has their own idea of gain and wood value, we will let you choose the one you like the most. However, the point is that we have a tremendous economic impact. Using reasonable gain and wood value assumptions, tree improvement has an impact in the hundreds of millions of dollars annually. At worst, genetic gains have to be less than 5% and wood value of $10 to break even. It is very difficult to make tree improvement not pay for itself.

Figure 5. NET PRESENT VALUE OF PLANTING GENETICALLY IMPROVED SEEDLINGS IN THE SOUTH



		wood value ($/cord)						
		10	20	30	40	50	60	70
	5	0.9	7.2	15.4	23.5	31.6	36.8	47.9
volume	10	7.2	23.5	39.8	56.0	72.3	88.6	104.8
gain (%)	15	15.4	39.8	64.2	88.6	112.9	137.4	161.8
	20	23.5	56.0	88.6	121.1	153.6	186.2	218.7
	25	31.6	72.3	112.9	153.6	194.3	234.9	275.7

Indirect Impacts

Cooperatives -- Cooperatives between universities, government agencies and industry are the backbone of tree improvement in the South. Enough cannot be said about these cooperatives. These organization have succeeded beyond anyone's expectation for over 40 years. Most are based solely on the word and commitment of the members. The secret to their success seems to be the genuine cooperation of the financial as well as the physical work. Industry's in-kind commitment is estimated to be 10 times the annual dues of any given tree improvement cooperative. Additionally, these cooperative formed the model for almost all successive cooperatives in the South. Southern tree improvement and related cooperatives include the:

Western Gulf Tree Improvement Cooperative
NCSU/Industry Cooperative Tree Improvement Program (Pine and Hardwood)
Cooperative Forest Genetics Research Cooperative - University of Florida

Southern Forest Tree Improvement Conference
NCSU Biotechnology Consortium
Institute of Paper Science & Technology

Silviculture -- Forest regeneration in the South leads any other forest region in the world in acres planted. This scale and commitment to afforestation and reforestation in the South, has intricately incorporated tree improvement with regeneration silviculture.

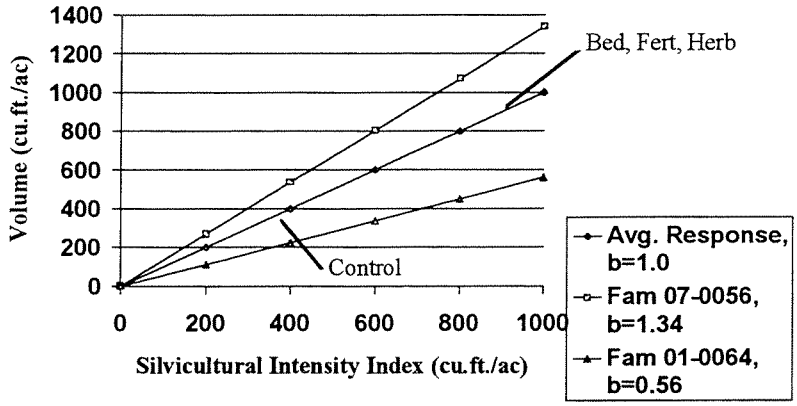
To begin the regeneration process, seed orchard are intensively managed to provide not only the best genetically improved seed available, but also to produce abundant, high quality seed. This has allowed the use of the best family lots on many more acres.

Seed processing has reach a very high level. Improvements in extraction and cleaning have led to higher seed yields and seed quality. The U.S. Forest Service's efforts in this area have been are extremely valuable. Additionally, information from the seed processor can help greatly in orchard management for higher quality.

Nurseries have also played a big role in tree improvement. Intensive management of seedling crops has made available large quantities of high morphologically quality seedlings that help allow the genetic expression of the traits desired. Today the growing of large numbers of family and specialty lots is challenging nursery operations.

Intensive site preparation and site specific management is being employed to insure maximum growth and take advantage of the improved stock. Research results generally show that tree improvement for growth and silvicultural intensity are at least additive. Figure 5 illustrates the relationship between family performance and silviculture intensity. Basically, most families interact with intensity of silviculture in a positive

Figure 5. AVERAGE RESPONSE TO VARIOUS TREATMENTS AND PREDICTED RESPONSE OF TWO FAMILIES



manner. That is, an increase in intensity has a corresponding increase family performance. Some individual families, however, perform much better than the average of all families. Family 07-0056 is such an example. This family performs on average 34% better when planted on better sites or when planted in conjunction with more intense silviculture (McKeand 1992).

Research -- Research has truly been a commitment on everyone's part in the South. The universities have undoubtedly been the leaders and major players in this area. For the most part, research results have been freely shared to increase the total wood supply. Research results have been aggressively pursued and implemented. Today, such efforts are in even greater demand. Everyone involved in tree improvement research should pat themselves on the back for a job well done. However, the best on most important research is yet to come.

Government -- State and federal agencies have played a tremendous role in the success of tree improvement in the South. Remember that the NIPL's plant almost as much land and forest industry in the South. The state agencies by being members of cooperatives or managing their own tree improvement programs have been the major source of improved stock for the NIPL.

The U.S. Forest Service has provided so much help in research & practical development. From orchard and seed technology to quantitative genetics and biotechnology, This group has been a tremendous resource for landowners and the industry. One area that first comes to mind is the work by the Forest Service to help control seed insects. Without this

work, tree improvement in the South would be much different, or at least much more difficult.

Southern Culture -- There is something unique about tree improvement in the South. Yes, it could be just the shear scale of the regeneration program. But it is different. The level of cooperation and commitment collectively and individually is different from any other part of the world. No where else has the commitment been so consistent for such a long period of time. Even more surprising is the fact that most of this effort has been sustained without formal contracts. It exists primarily on the will of those involved. Even those that move to the South become inoculated with the culture.

In summary, we have worked for over 40 years at developing improved trees. Today, we are just beginning to reap the rewards of these efforts. The first stands of genetically improved trees are just now starting to be harvested. With the current concern for sustainable wood supply, the timing of such harvest could not be better. These efforts should provide good evidence to the value of tree improvement and its value to sustainable forestry in the South and the world.

Today we are at the dawn of a new era in tree improvement. The era of "can we do it?" has past, the new era places higher expectations on tree improvement for higher productivity and to do it faster!

ACKNOWLEDGEMENTS

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GROWTH MODELS FOR SLASH PINE FAMILIES

D.L. Rockwood[1], B. Yang[1], and H. Gresham[2]

Abstract:--Height, DBH, and volume growth models in response to density, age, site index, interfamily competition, and genetics were developed for 29 15/16-year-old slash pine families in six progeny tests in Florida. Families significantly influenced the shape, asymptote, and rate parameters of height-age and site index curves. Separate base-age invariant height-age models were developed for each family to account for polymorphism associated with the shape and rate parameters. In Nelder design tests, tree height was not affected by density, but DBH and stand volume-index were negatively related to density as early as age four years. Growth dynamics, based on density, age, site index, competition level, and families, were fit to these data. There were significant differences for the estimated parameters of the equations: -0.0739 to 0.0616 (equivalent to 13% of average total height), -0.0513 to 0.1385 (20% of average DBH) and -0.1598 to 0.3331 (54% of average volume-index) for the poorest to best growing families. Interfamily competition coefficients ranged from -0.0026 to 0.0169 (2% of average height), 0.0206 to 0.0169 (10% of average DBH) and 0.0394 to 0.3758 (42% of average volume-index), and differences between mixed and pure plantings were not significant.

Keywords: Growth models, *Pinus elliottii*, Nelder Design, Row plots, Block plots.

INTRODUCTION

Growth models and stand simulators directly relate to management decisions about value and rotation length, and they are alternatives to traditional selection and genetic gain prediction systems. Stand growth models can project family differences from young ages to estimate selection differential and percent gain at rotation age (Knowe and Foster 1989). Selection on growth models promises greater flexibility and better maintenance of growth rates than pointwise selections (Magnussen and Kremer 1993). Buford (1986) and Buford and Burkhart (1987) concluded that stand-level gains could be estimated by determining the apparent increase in site index due to genetically improved loblolly pine. Knowe and Foster (1989), Buford and Burkhart (1987), and Nance and Wells (1981) discussed some of the problems related to modeling growth of genetically improved stock. Progeny tests are generally not suitable for developing necessary growth functions. Family plots of less than 10 trees efficiently rank families but provide poor estimates of stand parameters. With larger plots sufficient to construct individual growth trajectories, a functional analysis of growth as a stochastic process would furnish the necessary parameters for construction of a selection index and estimation of genetic progress. Growth model analysis offers the advantage of a succinct presentation of growth results.

This study presents total height (TH), diameter at breast height (DBH), and stand volume-index (SVI) growth models and their relationship to genetic parameters of 15- or 16-year-old trees in 29 slash pine (*Pinus elliottii* var. *elliottii* Engelm.) families. The growth patterns associated with families are examined. This study also addresses progeny tests with row plots and operational densities.

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MATERIALS AND METHODS

In 1978 and 1979, St. Regis Paper Company established 29 families in six tests near Cantonment, Florida (Table 1, Rockwood 1983). Two 1978 plantings had nine families in 1) a split plot design involving two competition levels (pure = all trees of the same progeny, and maximum = each measurement tree surrounded by trees of the other eight families and 2) Nelder plots (families assigned to spokes) with eight densities. The 1979 plantings included as many as 23 families in pure vs. maximum competition block plots, Nelder's plots, and 10-tree row plots.

TH, DBH, survival, and rust incidence were measured at age 4, 6, 8, 11, and 15 or 16 years. SVI was calculated as DBH^2 x TH x survival. In addition, the absolute growth increment and relative growth rate for each of the growth traits from age 4 to 6, 6 to 8, 8 to 11, and 11 to 15 or 16 were calculated.

Height and Site Index. After comparing various models, the analysis used the more flexible Richards' function (Balocchi et al. 1993) that permits each tree to have its own unique growth function. The height-age relationship of individual families was described by using the growth model:

$$H = A(1 - \exp(-b*age))^c + \sigma \qquad (1)$$

where

H = average height
A = asymptotic or maximum height
b = rate coefficient
c = shape coefficient
σ = random error

Equation (1) was fit to the dominant/codominant trees at each age for each family x replication x design combination. A full model expanded in the asymptote, rate and shape coefficients (A, b, and c) compared to the model (1) used for all 29 families was changed to:

$$H_d = b_1(1 - \exp(-b_2 age))^{b_3} + \sigma \qquad (2)$$

where

$b_1 = a_1 + \Sigma a_{1i}F_i$ $\quad a_1$ = ave. maximum height $\quad F_i$ = 1 for the ith family
$b_2 = a_2 + \Sigma a_{2i}F_i$ $\quad a_2$ = ave. rate coefficient $\quad$ = 0 otherwise
$b_3 = a_3 + \Sigma a_{3i}F_i$ $\quad a_3$ = ave. shape coefficient $\quad H_d$ = average dominant-codominant height

Table 1. Description of slash pine progeny tests established at Cantonment, FL, in 1978 and 1979.

Test ID (Co-Year-No.)	Design
8-78-1	Randomized complete block design (RCB), 2 reps; main plots of 454 and 907 trees/acre; subplots of 9 families; sub-sub-plots of pure and maximum competition block plots.
8-78-2	RCB, 6 reps; 9 families; 8 densities: 191, 250, 327, 428, 559, 731, 956, 1250 trees/A.
8-79-3	RCB, 4 reps; 9 families in main plots; sub-plots of pure and maximum competition block plots plus row plots; 545 trees/A.
8-79-4	RCB, 4 reps; 9 families in main plots; sub-plots of pure and maximum competition block plots; 545 trees/A.
8-79-5	RCB,6 reps; 23 families; 8 densities: 191, 250, 327, 428, 559, 731, 956, 1250 trees/A.
8-79-6	RCB, 6 reps; 18 families in 10-tree row plots; 545 trees/A.

The algebraic difference form of equation (2) (Borders et al. 1984) was selected for the final height-age equation:

$$H_2 = H_1((1 - \exp(-\beta_1 age_2))/(1 - \exp(-\beta_1 age_1)))^{\beta 2} + \sigma \qquad (3)$$

where

H_1 = dominant/co-dominant height at age 1 $\qquad$ β_1 = rate coefficient

H_2 = dominant/co-dominant height at age 2 $\qquad$ β_2 = shape coefficient

Replacing H_2 with site index (SI) and age_2 with base age (A_b = 15/16) produced an equation for predicting site index:

$$SI = H_d((1 - \exp(-\beta_1 age))/(1 - \exp(-\beta_1 A_b)))^{\beta}{}_2 \qquad (4)$$

Density and Family Effects. Means of densities and families for TH, DBH, and SVI were compared using the Nelder design tests. The phenotypic coefficient of variation was calculated for each density. For TH, DBH and SVI at age 15/16 years, the relationships with density were modelled by the simple linear model:

$$Y(TH, DBH, \text{ or } SVI) = \exp(c_1 + c_2{*}\ln(TPA) + c_3{*}(\ln(TPA))^2 \qquad (5)$$

where

TPA = trees/acre,

c_1, c_2, and c_3 are the intercept of the log transformed trait value at one density, the slope of the linear relationship between the log transformed trait value, and the curvature of this relationship, respectively. c_2 is the initial relative growth rate, and c_3 is half the rate at which the relative growth rate declines with density.

Growth and Yield Dynamics. Using data for individual trees at ages 4, 6, 8, 11, and 15/16 years, the growth model included site index, density, family, age, and competition level for TH, DBH, and SVI:

$$Y(TH, DBH, \text{ or } SVI) = \exp(d + d_1{*}\ln(TPA) + d_2{*}\ln(age) + d_3{*}\ln(SI) + \Sigma d_i{*}F_i + \Sigma d_j{*}C_j) \quad (6)$$

where

Age = measurement ages

d, d_1, d_2 and d_3 = intercept, Density, Age, and SI coefficients

d_i = parameter estimate for families $F_1 \ldots F_i$

F_i = 1 if family i,
0 otherwise;

d_j = parameter estimate for competition levels $C_1 .. C_j$

C_j = 1 if competition j,
0 otherwise;

Model (6) was used to predict growth for different families in various designs.

Linear Model Effects. All traits in each test were analyzed by the following linear model:

$$Y_{ijshl} = u + R_i + F_j + RF_{ij} + D_s + RFD_{ijs} + W_{ijsl} \qquad (7)$$

where

Y_{ijsl} is the value of the l_{th} tree in the i_{th} replicate in j_{th} family and s_{th} density, u is the overall mean, R_i is the effect of the i_{th} replicate, F_j is the effect of the j_{th} family, RF_{ij} is the interaction of families with replicates, D_s is the effect of s_{th} density, RFD_{ijs} is the interaction of replicates, families, and densities, and W_{ijsl} is the residual error. All terms in Model (7) were considered to be random effects with variances $\sigma^2 r$, $\sigma^2 f$, $\sigma^2 rf$, $\sigma^2 d$, $\sigma^2 rfd$, and $\sigma^2 w$ (within plot), respectively.

RESULTS AND DISCUSSION

Height and Site Index. The comparisons of the parameter estimates obtained for Model (1) fit to individual families (Table 2) suggested that polymorphism existed among families in addition to differences in level. The estimate for maximum height A was between 75 to 83' for most families but about 67' for 71-57 and 94' for 89-57 and 95-58. The estimate for b was about 0.12-0.13 for most families but only 0.10-0.11 for 89-57 and 95-58, and about 0.15 for 71-57. In addition, the estimate for shape (c) of 89-57 and 95-58 was conspicuously lower than the other families.

Estimates of the coefficients of model (3) and (4) for different families are given in Table 3. Family parameters ranged from 0.0927 to 0.1263 for β_1 and from 1.2529 to 1.6021 for β_2. Site index (SI) at a base age of 15 years can be calculated for different families using parameters β_1 and β_2.

Density and Family Effects. Density means for tree TH were not significantly different, but DBH and SVI were negatively related to density. Means of DBH versus density by ages indicated that DBH growth differences for densities as early as the fourth year became definite by years of 6, 8, 11, and 15/16. The SVI response was very similar to the DBH response, as would be expected. Applying model (5) to the age 15/16 data gave the overall equations:

$$TH = \exp(1.7403 + 0.5276*\ln(TPA) - 0.04296*\ln^2(TPA)) \qquad R^2=0.554$$

$$DBH = \exp(1.5962 + 0.1598*\ln(TPA) - 0.02795*\ln^2(TPA)) \qquad R^2=0.985$$

$$SVI = \exp(7.9945 + 0.0694*\ln(TPA) - 0.042*\ln^2(TPA)) \qquad R^2=0.985$$

For DBH and SVI between ages 4 and 15 years, the overall R^2 increased significantly, but for TH only increased slightly.

For individual families, the effectiveness of model (5) changed with age, and there were noticeable differences among the families. By 15 years, all families had responded to density for SVI (all $R^2>0.8$) except 62-57 and 74-57 which still had $R^2 < 0.6$. From 4 to 15 years, density had less effect on TH growth than on other growth traits (all $R^2 < 0.7$). TH usually has higher heritability than other growth traits and is also a good predictor of stand volume at rotation.

Table 2. Comparison of parameter estimates of height-age curves ($H=A((1-\exp(-b(age))^c)$ for 29 slash pine families in all six progeny tests.

	Parameter				Parameter				Parameter		
Family	A	b	c	Family	A	b	c	Family	A	b	c
132-56	80.4	0.1268	1.6289	76-57	76.3	0.1454	1.5984	99-58	71.1	0.1425	1.7721
330-56	72.5	0.1325	1.6972	78-57	86.3	0.1301	1.5220	56-59	85.6	0.1211	1.5218
45-57	77.3	0.1386	1.5953	89-57	94.4	0.1073	1.4324	27-59	71.3	0.1413	1.7198
49-57	80.3	0.1253	1.5572	102-57	81.3	0.1262	1.5642	86-63	83.1	0.1269	1.5502
51-57	71.4	0.1388	1.7213	70-58	70.4	0.1442	1.7197	95-63	77.4	0.1352	1.5971
62-57	87.4	0.1232	1.5023	79-58	76.3	0.1329	1.6520	105-63	77.3	0.1367	1.6129
67-57	78.4	0.1355	1.6526	84-58	84.4	0.1228	1.5129	106-53	76.9	0.1338	1.6409
71-57	67.6	0.1489	1.7578	85-58	89.6	0.1118	1.5043	128-63	85.6	0.1267	1.5117
72-57	78.1	0.1289	1.6062	91-58	76.5	0.1338	1.6563	86-65	82.5	0.1237	1.5732
74-57	85.4	0.1247	1.5176	95-58	94.2	0.1107	1.4331	Mean	80.0	0.1302	1.5976

Table 3. Estimates of the coefficients (β_1, β_2) for models (3) and (4).

Family	Parameters β_1	Parameters β_2	Family	Parameters β_1	Parameters β_2	Family	Parameters β_1	Parameters β_2
132-56	0.1045	1.4489	76-57	0.1224	1.4184	99-58	0.1225	1.6021
330-56	0.1134	1.5272	78-57	0.1101	1.3520	27-59	0.1231	1.5411
45-57	0.1196	1.4163	89-57	0.0965	1.2531	56-59	0.1011	1.3417
49-57	0.1062	1.3786	102-57	0.1059	1.4040	86-63	0.1057	1.3712
51-57	0.1168	1.5413	70-58	0.1239	1.5397	95-63	0.1148	1.4169
62-57	0.1016	1.3223	79-58	0.1106	1.4729	105-63	0.1157	1.4331
67-57	0.1137	1.4726	84-58	0.1031	1.3332	106-63	0.1148	1.4612
71-57	0.1263	1.5743	85-58	0.0927	1.3244	128-63	0.1071	1.3321
72-57	0.1076	1.4260	91-58	0.1145	1.4759	86-65	0.1041	1.3928
74-57	0.1023	1.3376	95-58	0.0983	1.2529	Mean	0.1103	1.4194

Growth and Yield Dynamics. Between ages 4 and 15/16 years, the average growth in TH, DBH, and SVI was from 13.9' to 54.9', 2.4" to 9.0", and 84 to 4445, respectively. The Model (6) coefficients for density, age, site index, competition level, and individual family terms for TH, DBH, and SVI are provided in Table 4. If a particular coefficient is negative, the associated term in the model reduces the trait value; if the coefficient is positive, the model term increases the trait value. If a family or competition level is included in the model, then its contribution is equal to the value of the coefficient; otherwise it contributes 0.

Family coefficients ranged from -0.0739 to 0.0616 for TH, -0.0513 to 0.1373 for DBH, and -0.1598 to 0.3331 for SVI (Table 4). A negative coefficient indicates a family with below average growth, and a positive value denotes a better than average family. The differences in family coefficients correspond to 13% of the average total height, 20% of average DBH, and 54% of average SVI from the poorest to the best growing families. Family 95-58, for example, was the most vigorous for TH and SVI, and the low growth families were 71-57 and 27-59. Overall, however, the relative performances of these 29 families, as reflected by their coefficients, were not correlated with their evaluations in traditional progeny tests, but the same lack of correlation existed at ages 4 (Rockwood 1983) and 6 (Rieghard et al. 1985).

The parameters for competition levels ranged from -0.0026 (Mix) to 0.0169 (Row) for TH, 0.0206 (Row) to 0.1122 (mix) for DBH, and 0.0394 (Row) 0.3758 (Mix) for SVI. These differences in interfamily competition coefficients represented 2% of average height, 10% of average DBH, and 42% of average SVI. Differences between mixed and pure plantings were not significant.

Model (6) and the coefficients in Table 4 may be used to estimate the growth of these 29 families under conditions similar to these six progeny tests. To predict TH, DBH, and SVI of family 330-56 in row plot competition at age 10 years on a SI 60 site (base age 15 years) with 400 TPA, for example, the models:

$$TH = \exp(0.9021+0.0072*\ln(TPA)+1.108*\ln(age)+0.0032*\ln(SI)-0.0123*F(330\text{-}56)+0.0169*row)$$

$$DBH=\exp(0.3348-0.1555*\ln(TPA)+0.9664*\ln(age)+0.0015*\ln(SI)+0.0548*F(330\text{-}56)+0.0206*row)$$

$$SVI = \exp(1.5718-0.3037*\ln(TPA)+3.0413*\ln(age)+0.0045*\ln(SI)+0.0972*F(330\text{-}56)+0.0394*row)$$

would estimate TH at 33.6', DBH at 5.5", and SVI at 1002.

Table 4. Parameters of Model (6) for density, age, site index, competition level, and families for Height, DBH, and Volume Index.

Variable	Height	DBH	SVI	Variable	Height	DBH	SVI
d (=Intercept)	0.9021	0.3348	1.5718	76-57	-0.0024	0.0254	0.0484
d_1 (=Density)	0.0072	-0.1555	-0.3037	78-57	-0.0059	-0.0081	-0.0220
d_2 (=Age)	1.1084	0.9664	3.0413	89-57	0.0501	0.1373	0.3247
d_3 (=SI)	0.0032	0.0015	0.0045	102-57	0.0184	0.1152	0.2642
d_j (=Competition)				70-58	-0.0573	0.0142	0.0197
Mix.	-0.0026	0.1122	0.3746	79-58	0.0148	0.0929	0.2006
Pure	-0.0015	0.1043	0.3758	84-58	0.0146	0.1328	0.1419
Row	0.0169	0.0206	0.0394	85-58	0.0606	0.1018	0.2643
d_i (=Family)				91-58	0.0067	0.0248	0.0563
132-56	0.0007	-0.0098	-0.0189	95-58	0.0616	0.1357	0.3331
330-56	-0.0123	0.0548	0.0972	99-58	-0.0554	-0.0253	-0.1061
45-57	-0.0195	-0.0129	-0.0453	27-59	-0.0571	-0.0513	-0.1598
49-57	0.0267	0.1153	0.2572	56-59	0.0222	0.0982	0.2186
51-57	-0.0389	0.0088	-0.0213	86-63	0.0194	0.0129	0.0702
62-57	0.0458	0.0532	0.1523	95-63	-0.0187	0.0502	0.0371
67-57	-0.0259	-0.0001	-0.0260	105-63	0.0057	0.0037	0.0129
71-57	-0.0739	-0.0022	-0.0784	106-63	0.0068	-0.0038	-0.0009
72-57	-0.0584	-0.0189	-0.0962	128-63	0.0227	0.0267	0.0117
74-57	0.0312	0.0732	0.1676	86-65	0.0121	0.0137	0.0732
				R^2	.88	.83	.79

Linear Model Effects. Age trends in the contribution of variance components to total TH, DBH, and SVI variances are provided in Figure 1. A linear increase in the total TH variance with age was evident through age 15. Most of the TH variance (1/2) was due to trees within plots. Family, density, replication, and competition contributed comparable amounts of variance (8 - 17%) to the total. As with TH, total variation in DBH increased with age. Density accounted for most of the variation in DBH, especially from age 8 years (Figure 1). Replication was the second largest source of variation at about 20%. Family and competition variances each contributed about 10%. Variation in SVI also increased substantially with age. The largest variance component for SVI after 11 years was due to density (about 1/3). Some 20% was typically due to families. Competition contributed about 15%.

These results have ramifications concerning progeny test design, deployment of genetically improved trees, differential growth patterns, and genetic selection in slash pine. No growth differences in TH or DBH were noted at age 6 due to intergenotypic competition (Rieghard et al. 1985). Based on the overall similarity of the Model (6) coefficients for mixed (i. e., maximum intergenotypic competition) and pure family blocks through 15/16 years, family evaluation and overall yields are not influenced by these extremes of test design. The relatively small influence of competition variance further supports this conclusion. However, only three of the 29 families in these six tests are as genetically elite as what is now typical of advanced generation trees. The observed difference between growth in row plots compared tc block plots must also be considered preliminary because the two types of plots did not occur in the same test and Test 8-79-6 was located slightly apart from the other 1979 tests.

Longer-term field evaluation appears necessary to assess volume related traits such as DBH and SVI. High densities affected DBH as early as four years (Rockwood 1983), and some density x family interactions were detected for TH at 6 years of age (Rieghard 1985). Density became increasingly

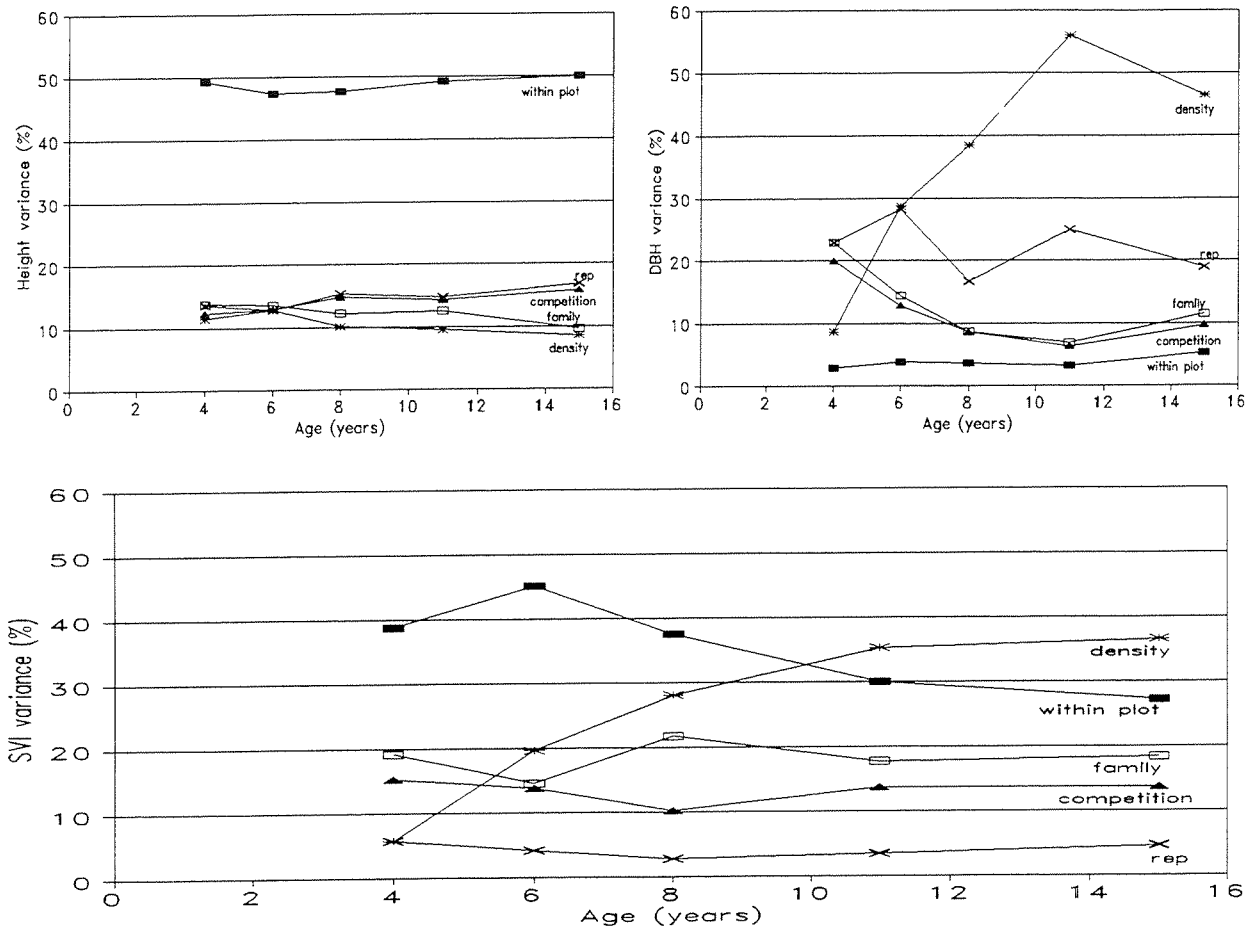

Figure 1. Age trends in contribution of replication, family, density, competition, and within-plot variance components to total variation in height (top left), DBH (top right), and volume index (bottom).

important on DBH after six years. Family variation in these tests, however, seems to remain steady for DBH and SVI after eight years. For these traits, under the moderate levels of fusiform rust incurred in these tests, evaluation periods of up to 10 years may be needed.

Models (4) and (6) are practical ways to incorporate genetic variation into growth and yield projections. Family specific coefficients may account for polymorphic height growth due to genetic differences. SI estimates reflecting these differences, i. e., applying coefficients such as in Table 3 to Model (4), can adjust for family differences. In turn, these SI estimates can be input into DBH and SVI models to accommodate genetic ranges in stand growth.

Model (6), however, is a whole stand growth and yield model and does not offer the potential of diameter distribution models or individual tree models (Burkhart and Matney 1981). Model (6) can only provide mean TH, mean DBH, and, assuming application of a tree content equation in place of the tree volume-index used here, a stand volume or weight. More useful would be a diameter distribution model that predicts the number of trees by DBH class, so that any genetic influences on the diameter distribution and the number of trees by DBH class in the stand can be depicted.

As shown by Spirek et al. (1981), genetic variation in slash pine may be expected to lead to varying diameter distributions and hence timber values. Improved families, for example, tended to have Weibull

DBH distributions with a higher shape coefficient. These positively skewed distributions consequently would have a larger proportion of large DBH trees than unimproved slash pine, and a stand of improved trees would potentially have more value.

Other aspects of stand development may be influenced by the use of improved families. As outlined by Burkhart and Matney (1981), any genetic influences on slash pine disease resistance, stem form, and wood density need to be represented in an operational growth and yield model for improved slash pine. The preliminary results reported here will be extended to other slash pine tests specifically designed for growth and yield analysis.

CONCLUSIONS

The shape, asymptote, and rate parameters of height-age models differed with families, and site index curves were developed for each family using parameters β_1 and β_2. These results indicate that, if the site index is appropriately specified for individual families and the appropriate height-age curve is used, the TH, DBH and SVI models can directly use them at the family level. Tree TH was not significantly affected by density, but DBH and SVI were negatively related to density as early as age four, becoming definite by years of 6, 8, 11, and 15/16. At the family level, DBH-Density or SVI-Density curves were related to age, but the levels of the DBH or SVI-density curves were influenced by individual families. For growth models based on the age, site index, density, and competition levels (row and block), 29 families had significant differences for the estimated parameters of the equations, on the order of -0.0739 to 0.0616 (TH), -0.0513 to 0.1385 (DBH), and -0.1598 to 0.3331 (SVI), by the poorest and best growing families. For the Mix, Pure, and Row design plots, corresponding coefficients were -0.0026, -0.0015 and 0.0169 for TH, 0.1122, 0.1043, and 0.0206 for DBH, and 0.3746, 0.3758, and 0.0394 for SVI. Growth differences between mixed and pure plantings were not significant.

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INFLUENCE OF FAMILY AND SPACING ON JUVENILE LOG QUALITY OF LOBLOLLY PINE

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Abstract.--Eight selected North Carolina families (based on growth rate and crown size from prior progeny tests) and one local unselected seed source were tested for juvenile log quality in a 10-year-old progeny test at three spacings in northeast Mississippi. The traits used to distinguish log quality were: sweep, number of limbs, average limb diameter, average size of largest branch, height to base of live crown, and form class. The North Carolina families exhibited significantly higher quality characteristics for all traits when compared to the local seed source. Differences were found among the eight selected families for all of the log quality traits except number of limbs. Spacing-by-family interactions were observed for average limb diameter. Interactions were associated with (a) smaller family differences at the closer spacing and (b) some large family rank changes between spacings. It was concluded that selection for fast-grown, small-crown families in North Carolina was effective in improving juvenile log quality in Mississippi, especially in plantations grown at wide spacings (8 x 8 ft. or greater).

Keywords: *Pinus taeda* L., sweep, stem form, limb traits, GxE interactions.

INTRODUCTION

Log quality and the resulting lumber quality of loblolly pine (*Pinus taeda* L.) are determined by number, size and soundness of limb knots, by stem straightness, and by stem taper. These traits can be influenced by spacing and genotypes among trees. Since the juvenile stem represents the core of the future log, measurements of limb and stem traits at an early age provide an indirect measure of the effects of spacing and family on the quality of that log and the resulting lumber quality. Campbell (1962) has stated that log imperfections are a result of the defects of the underlying wood.

The most important grading defects found in southern yellow pine are number and size of limbs (Campbell, 1962). Campbell (1962) defines a limb as a branch that is one-half inch or larger in diameter. This includes knots, stubs, holes, and overgrowths. The length and shape of the crown also influence the size of the limbs (Sprinz and Burkhart 1989). Another important stem quality characteristic is straightness. Williams and Lambeth (1989) concluded that a direct measure of stem curvature or sweep from a straight eight-foot pole was an easily obtained quantitative measurement of stem straightness in the first log of young trees, and that it was more repeatable than straightness scores of the entire stem that were determined subjectively.

Conflicting results have been reported on the relationship between stem characteristics and subsequent quality and yield of the resulting lumber. Gaby (1972) showed a positive correlation between knots and direction of warp.

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However, Shelley *et al.* (1979) found no relationship, and Beard *et al.* (1993) reported that only nine percent of the variation in lumber warp was related to growth traits. The fact that the number of lumber grades used in the latter studies was restricted is the most likely explanation for the difference in the results. It was noted that log quality can affect the relative percentages of lumber in the different grades.

Genetic variations in stem traits that might affect log quality have been reported for many species. Douglass *et al.* (1993) found significant genetic variation in sweep among five loblolly pine seed sources. These seed sources ranged from coastal North Carolina to Arkansas and Oklahoma. The North Carolina Piedmont source had the least sweep. A significant genotype-by-site interaction for sweep was present at the seed source level, but the interaction was much smaller for families within sources. The coastal North Carolina source and a central Mississippi-Alabama source had the greatest variability in sweep. In studies with other tree species, heritability has been higher for stem straightness and volume growth than for crown characteristics (Ferguson *et al.* 1977; Otegbeye 1988).

It is generally reported that spacing among trees influences straightness, frequency and size of knots, and diameter/height growth patterns (Daniel *et al.* 1979). Loblolly pines at wider spacings tend to have larger diameters, greater sweep and sweep length, larger knot size, and increased taper.[2] Wiley and Zeide (1988) also observed that wider spacings produced trees with larger d.b.h. and crown ratio. Closer spacings induce smaller diameter, less sweep, smaller limbs, and a base of the live crown that is higher than less dense spacings (Smith 1986).

The purposes of this study were to determine how family and spacing affect juvenile log quality and to learn if family-by-spacing interactions exist for juvenile log quality in a ten-year-old loblolly pine plantation.

METHODS

Plant Material

Open-pollinated progenies from eight selected trees in eastern North Carolina, as well as a commercial seed source (used as a check) from east-central Mississippi and west-central Alabama, were provided by Weyerhaeuser Company. The eight families were chosen based on performance in 12-year-old progeny tests in North Carolina and represented combinations of fast and slow growth rates with large and small crowns (Table 1). "Fast", "slow", "large", and "small" are relative terms referring to extreme combinations in a set of families represented in the progeny tests at 6 - 15 sites. Crown size was actually a performance level based on crown score, which was determined from crown length, crown width, and limb diameter.

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[2]Land, S. B., and J. D. Hodges. 1992. Influence of initial plantation spacing on defects and log grades in 33-year-old loblolly pine stands. Funded contract between International Paper Company and the Department of Forestry, Mississippi State University, Mississippi State, MS.

Table 1. Characteristics of families used in study.

Parent Tree Identity	Characteristics	
	Growth Rate	Crown Size
1-A	Fast	Small
1-B	Fast	Small
2-A	Fast	Large
2-B	Fast	Large
3-A	Slow	Small
3-B	Slow	Small
4-A	Slow	Large
4-B	Slow	Large
5	Commercial Check	

Field Planting

Seeds were collected in November 1984 and grown in leach tube containers for five months by Weyerhaeuser Company. The container seedlings were planted in early May 1985 at two sites on the Mississippi State University school forest in Winston County, Mississippi (Secs. 5 and 6, T16N, R14E). One site was an old field, and the other was a clearcut-and-site-prepared area that had previously been an old pine stand.

Three spacings were represented at each site: 5 x 5 feet, 8 x 8 feet, and 10 x 10 feet. Each spacing contained the eight families in "mixed-family" and "pure-family" plots. The unselected commercial check was included as a "pure-family" plot in each spacing to provide a local comparison for the North Carolina families.

Sample Size and Measurements

Sample size was determined by conducting a preliminary study and then basing calculations on the standard error of a key variable (number of limbs) being measured using the non-central f-distribution. The number of samples calculated yielded a power of 0.80 at an alpha level of 0.05 for a minimal detectable mean difference of 1.1 times the standard error (Kirk 1968). This indicated that a sample size of four trees per plot (replication by family by spacing) would be required. The following measurements were taken from November 1994 through February 1995 at the end of the tenth growing season:

1. diameter at breast height (dbh)
2. diameter outside bark at stump height (1 ft)
3. diameter outside bark at 17.0 feet
4. height to live crown (ft)
5. total height (ft)
6. crown class (dominant or codominant)
7. crown width in two directions (along rows) (ft)
8. diameter of largest limb (between 1-ft and 17-ft height on stem)

9. limb talley into four 1/2-in.diameter classes from 0.5 to 2.5 in.:
   (a) by first and second 8-foot logs (above 1-foot stump)
   (b) by status (live, recent dead, or old dead [where "old dead" represents punky, partly shed limbs]) (gives 24 variables for limb tallies)
10. height to lowest live and dead limbs (above 1-foot stump)
11. straightness score for the entire tree
   (1 = straightest ... 4 = most crooked)
12. sweep (to the nearest tenth inch), as measured in the first 16-foot log by the maximum deviation from a 12-foot straight pole)

DBH, crown width, and total height of tree are only reported as study means in this paper.

Statistical Analysis

Analysis of variance was for a split-plot design arranged in randomized complete blocks and repeated at two locations. The main unit treatment factor was spacing, and the subunit treatment factor was family. Four sample trees were randomly selected within each "pure-family" plot for each spacing and location, with the stipulation that these sample trees must be dominant or codominant in crown class, free of any disease, and not forked. In total, four trees within each of 216 plots (8 replications, by nine "families" by three spacings) were measured.

RESULTS AND DISCUSSION

The average tree was 38 feet tall, six-inches in diameter at breast height, and 11-feet in crown diameter.

Spacing

Spacing significantly affected average limb diameter, number of limbs, form class, height to live crown, and average size of largest branch (Table 2). The 5 x 5 ft. spacing had the smallest limbs, the fewest limbs per first 16-ft. log, the highest form class, and the smallest diameter of largest branch. However, no significant differences were found among spacings for sweep. Closer spacings did not improve straightness of the stem.

Family

The North Carolina families ranked higher in log quality than the commercial check for nearly all traits (Table 3). The only exception was that they had more limbs per 16-ft. log than the local source (Table 3). This indicates that selection for improved juvenile log quality in eastern North Carolina is beneficial when trees are planted in northern Mississippi.

For the most part, the faster growing families had better juvenile log quality than the slow-growing families (Table 4). Families representing fast growth rate had smaller limbs, better form class, and taller height-to-live crown than the slow growth families. Height-to-live crown was an indicator of how much of the stem was free of live limbs at age 10. Thus, having a greater height-to-live crown reflected no further increase in knot size, quicker

shedding of limbs, and faster overgrowth of knots. The slow-growing families did have less sweep than fast growing families, however (p-value=0.0002).

Table 2. Means and P-values for measured traits based on three different spacings.

Trait	Spacing*			P-value
	5x5 ft.	8x8 ft.	10x10 ft.	
Average limb diameter per tree (in)	0.8 A	0.9 B	1.0 C	0.0066
Number of limbs per 1st 16-ft. log	21 A	27 B	28 C	0.0372
Form class (17-ft. diameter/1-ft. diameter) (%)	74 A	73 B	70 C	0.0582
Height to the base of live crown (ft)	21.8 A	17.7 B	14.7 C	0.0080
Diameter of largest branch (in)	0.95 A	1.3 B	1.6 C	0.0055
Sweep (in)	1.2 A	1.2 A	1.2 A	0.8386

* Spacing means for the same trait are not significantly different at alpha = 0.05 if they are followed by the same letter.

Among the fast growing families, considerable differences existed between the two crown types. Comparisons within the fast-growth families showed that the small crown families had fewer number of limbs, smaller limbs, and less sweep than the large-crown families. However, differences between the two families within a growth-by-crown-size group were usually not significant. An exception was form class in the fast-growth, small-crown group, where family 1-B had a higher form than 1-A. Among the large crown families 2-B had smaller average limb diameter, smaller largest branch, and a much more crooked stem than 2-A. Family 2-B had the highest degree of sweep of any of the selected North Carolina families.

The differences found among slow-growing families were less than those among the fast-growing families. No significant differences were found for the important quality characteristics, of sweep, diameter of largest branch, or average limb diameter. The slow-growth families only showed significant differences for number of limbs per tree and height to live crown. Among the

small-crown families, 3-B had the least number of limbs and least sweep (family 3-B was the only family with a sweep less than one inch [0.95 in.]). Family 3-A had the highest height to live crown. Family 4-A had a larger average limb diameter, fewer limbs per tree, and a higher form class than family 4-B (p-values = .0449, .011, .013 respectively).

Selections made in eastern North Carolina for growth rate and crown size held true when planted in northern Mississippi. The family classes showed differences in juvenile log quality at all spacings. However, the differences among families are greater at the wider spacings. This is important, since the wide spacings are more often used today.

Table 3. Means and P-values for North Carolina families and the commercial check.

	Ave. limb diam. (in)	# limbs per tree (in)	Form class (%)	Ht. to live crown (ft)	Diam. largest branch (in)	Sweep (in)
North Carolina Selected Material	0.85	25	73	18.3	1.2	1.1
Commercial Check	0.96	26	69	16.3	1.5	1.6
P-value	0.0001	0.37	0.0002	0.0021	0.0126	0.0043

A significant spacing-by-family interaction occurred only for average limb diameter (p-value = 0.008). This interaction could be attributed to large family rank changes between spacings and smaller differences among the families at the 5 x 5 ft. spacing than in the wider spacings (Table 5). The only significant difference for the 5 x 5 ft. spacing was for the commercial check. The 8 x 8 ft spacing and the 10 x 10 ft. spacing provided significant differences among selected families as well as the commercial check. The wider spacings gave a much greater range in average limb diameter than the 5 x 5 ft. spacing. For all spacings the commercial check had the largest average limb diameter. The smallest limbs found at the 5 x 5 ft. spacing were in families 3-A and 4-B. At the 8 x 8 ft. spacing families 1-B and 2-B had the smallest average limb diameter. And at the 10 x 10 ft. spacing 1-A and 1-B showed the smallest limbs (1-A was the only family at this spacing that had an average limb diameter under 0.9 inches).

SUMMARY AND CONCLUSIONS

1. The selected North Carolina families proved to be superior to the local seed source for juvenile log quality when planted in northeast Mississippi.

Table 4. Means and P-values for comparisons among North Carolina families.

Preselected Characteristic	Ave. limb diam. (in)	# limbs per tree	Form class (%)	Ht. live crown (ft)	Diam. largest branch (in)	Sweep (in)
Fast Growth	0.85	25	73	18.8	1.2	1.2
Slow Growth	0.86	25	72	17.8	1.2	1.0
P-value	.0222	.7124	.0132	.0001	.1697	.0002
Fast Growth, Small Crown	0.84	24	74	18.4	1.2	1.1
Fast Growth, Large Crown	0.85	26	73	19.2	1.2	1.3
P-value	.0494	.0009	.0584	.0003	.1479	.0004
Slow Growth, Small Crown	0.86	26	73	18.1	1.2	1.1
Slow Growth, Small Crown	0.87	25	72	17.5	1.2	1.1
P-value	.2719	.0129	.0739	.0059	.8192	.9461

Table 5. Average limb diameter rankings of all families and their means for each spacing.

5 x 5 ft.		8 x 8 ft.		10 x 10 ft.	
Family	Mean*	Family	Mean*	Family	Mean*
3-A	0.76 A	1-B	0.82 A	1-A	0.89 A
4-B	0.76 A	2-B	0.82 A	1-B	0.91 AB
4-A	0.77 A	3-A	0.84 AB	2-B	0.91 AB
1-A	0.77 AB	1-A	0.84 AB	3-A	0.94 ABC
2-B	0.77 AB	4-B	0.85 AB	4-B	0.94 ABC
2-A	0.77 AB	3-B	0.85 AB	3-B	0.96 ABC
3-B	0.78 AB	4-A	0.87 AB	2-A	0.97 BC
1-B	0.79 AB	2-A	0.88 B	4-A	1.00 C
5	0.80 B	5	0.97 C	5	1.10 D

* Means followed by the same letters are not significantly different at alpha = 0.01.

2. Wider spacings gave greater average limb diameter, more limbs, lower form class, less height to base of live crown, and greater diameter of largest branch than narrow spacings. However, sweep was not significantly different between spacings.

3. Generally, family characterization based on prior tests in North Carolina held true in this progeny test in Mississippi.

4. Family 1-A was the best overall family in the study for juvenile log quality, because it usually ranked high in each category.

5. In conclusion, selection for fast growth and small crowns will be effective in improving juvenile log quality, particularly at the wide spacings.

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ECONOMIC EVALUATION OF FUSIFORM RUST RESEARCH AND PROTECTION

John Pye, John Wagner, Thomas Holmes, and Fred Cubbage

Fusiform rust causes the most commercial damage to southern pines of all diseases. Selection for genetic resistance to fusiform rust has yielded substantial improvements in resistance to the disease in loblolly and slash pine trees. We performed an economic evaluation of the research, development, and implementation of fusiform rust resistance in southern pines. Information on past and prospective gains in rust resistance was obtained from a survey of seedling producers in the South. Information on incidence and hazard of rust infection was obtained from several cycles of Forest Inventory and Analysis (FIA) data for the South. Stands level analyses using growth and yield models were used to compute financial gains for fusiform rust protection by site class and region throughout the South. These stand level analyses were aggregated to estimate past and future gains from fusiform rust protection, and the potential gains that could be achieved by greater levels of protection or elimination of fusiform rust. These gains were compared with estimated research expenditures to estimate total returns for fusiform rust protection research.

Three levels of targeting of rust resistant seedlings and four levels of utilization were considered. For random distribution of seedlings, the present value of research benefits for fusiform rust research ranged from $108 million for full utilization of all trees, regardless of rust, to $282 million for poor utilization that assumed all rust trees were culled. For optimal targeting of seedlings--placing all resistant seedlings in the highest risk sites--returns ranged from $261 million to $999 million. The potential benefits that could occur if rust were totally eliminated could range from $614 million to $4.6 billion. The present value of prior research costs was $49 million. Comparing benefits with costs yielded very positive returns to fusiform rust research, ranging from a low of 2.2:1 under a random seedling distribution and full utilization to a high of 20:1 if all seedlings were targeted to the highest risk areas and utilization was poor. The most likely targeting/utilization scenarios had benefit:cost ratios of about 2.3:1 to 6.9:1. Overall, the investments in selecting and breeding fusiform rust resistant southern pines had very good returns, and opportunity still exists for further gains.

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PHENOLOGICAL VARIATION IN HEIGHT AND DIAMETER GROWTH IN PROVENANCES AND FAMILIES OF LOBLOLLY PINE

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Abstract. We present results on the phenology of eight open-pollinated families from each of four different provenances in a trial (on two locations) in southwest Georgia. The provenances are: Atlantic Coastal Plain, Gulf Hammock (FL), Lower Gulf Coast and Upper Gulf Coast. The trees were measured from summer to fall in 1993 and 1994 when the trees were in their fifth and sixth growing seasons.

There was little difference between provenances as to when height growth started in spring, but there were very significant differences for the date of cessation of growth in fall. The fast growing Gulf Hammock provenance grew the longest (till the end of August) while the slowest growing Upper Gulf source was first to stop growing (early August). Provenances were also different for the date of cessation of diameter growth, and the order of cessation was the same as for height. Families within provenances were different for date of cessation of both height and diameter growth.

Keywords: Pinus taeda L., height growth phenology, diameter growth phenology.

INTRODUCTION

There is evidence that phenology of height growth in conifers is under strong genetic control (Hanover 1963, Li and Adams 1993, Mergen et. al. 1964). Diameter growth phenology of Douglas-fir is also moderately heritable (Li and Adams 1994). With regards to loblolly pine, southern sources appeared to have a longer growing season than northern sources (Perry et al. 1966). Height and diameter growth phenology appear to affect cold-hardiness, growth rate (Bridgwater 1990) and possibly wood properties. This paper presents phenology data from a study established to determine how wood properties are affected by the timing of the initiation and cessation of height and diameter growth of juvenile loblolly pine.

MATERIALS AND METHODS

Study Trees

The Early Selection Verification Study of the NCSU-Industry Cooperative Tree Improvement Program, planted in 1989, was used for this research. Details of the study are given in McKeand and Bridgwater (1993) and McKeand and Jett (1993). In the spring of 1991 and the summer of 1992, the two plantings in southwest Georgia (at Cedar Springs, by Georgia Pacific

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Corporation or G-P; and Bainbridge, by International Paper Company or IPCo) were thinned as follows:

- From each of the four provenances (Atlantic Coastal - ACP, Gulf Hammock - GH, Lower Gulf - LG, and Upper Gulf - UG), the tallest 4 families and the shortest 4 families were left, giving 32 families in all.
- About 10 trees were left per family per location as evenly spaced as possible.
- This left about 320 trees per location, 640 total.

Growth Phenology

Total height was measured in February 1993 (the beginning of the growing season). In 1993, total height was measured every two weeks from mid-June till growth stopped in late October. In the spring of 1994, flushing of the trees was observed. Following budbreak, height was measured in May, June and then every three weeks from late July to early October. To accurately measure height, a height pole was placed near each tree and the tip observed from a bucket truck with the aid of 15- to 20-X binoculars. The height pole was placed on a stake driven in the ground next to each tree, minimizing variation in placing the pole.

Dendrometer bands were installed on each tree, in the middle of the first flush originating in the trees' second growing season. In 1993, diameter growth was measured at the same time as height, and also once in November and once in December. In 1994, in addition to occasions when height was measured, weekly measurements were taken from July to the end of September, and also once in October, November and December.

Cessation of growth for a given tree (for both height and diameter) was defined as the Julian day when the tree completed 95% of growth for the season. Analysis of variance was conducted using the GLM procedure in SAS (SAS Institute Inc. 1990). Approximate F-tests were constructed for certain terms (Satterthwaite 1946).

RESULTS AND DISCUSSION

Budbreak and flushing for all the trees took place within a very short period (about a week) in the spring of 1994. Thus it was not possible to observe differences between provenances and families for the date of initiation of height growth. This contrasts with results for Douglas-fir where there were significant differences in date of bud-break (Li and Adams 1993). Our results may perhaps be attributable to the mild climatic conditions prevalent at the two sites.

Provenances were significantly different for all three traits analyzed (Table 1). Averaged over both years, the Gulf Hammock (north Florida) provenance finished 95% of the season's height growth 20 days later than the Upper Gulf Coast provenance. The difference was about the same for diameter growth in 1994. Total height is plotted for provenance means, by site, for 1994 (Figure 1) showing GH to continue growing longer in the fall and UG slowing down relatively early. Tests planted by the NCSU-ICTIP have shown the GH source to be consistently fast in growth. Provenance variation in height growth phenology has been reported previously (Hanover 1963, Perry et al. 1966).

Families within provenances were significantly different for the length of growing season. For height growth averaged over both years, the range was from 247.9 days (family 22-29, GH) to 211.8 (8-503, UG) (Table 2). The longest season for diameter growth also was from GH (22-27, 302.6 days) and the shortest from UG (8-526, 276 days). Annual height increment was

significant at the 10 % level (p=0.0516).

Thus provenances and families which continued height growth longer tended to continue diameter growth longer as well. Diameter growth continued about two months longer than height growth, which implies that if latewood begins to be formed after cessation of height growth (eg. Larson 1969), all four provenances have about the same period for latewood formation. Families which grew most in height tended to have longer growing seasons, and the family which grew least in height was the first to reach 95% of the season's diameter growth.

With respect to differences between **years**, height growth seemed to slow down earlier in 1994 than in 1993 (about 10 days) although on average the trees grew about 40 cm. more in 1994. 1994 was a very wet summer while there was a pronounced dry spell in July-August 1993. The fact that the trees were one year older may have contributed to the earlier transition although it was probably not the only factor. Most of the second and third order interactions were not significant.

CONCLUSIONS

Differences in the length of the growing season explained part of the difference in growth rate among provenances of loblolly pine, with faster-growing provenances tending to grow longer in the fall. Cessation of diameter growth took place in the same order as for height growth, and occurred about two months later. In continuation we are evaluating wood samples from this study to see how variation in height and diameter growth phenology affect wood formation, especially the proportion of latewood.

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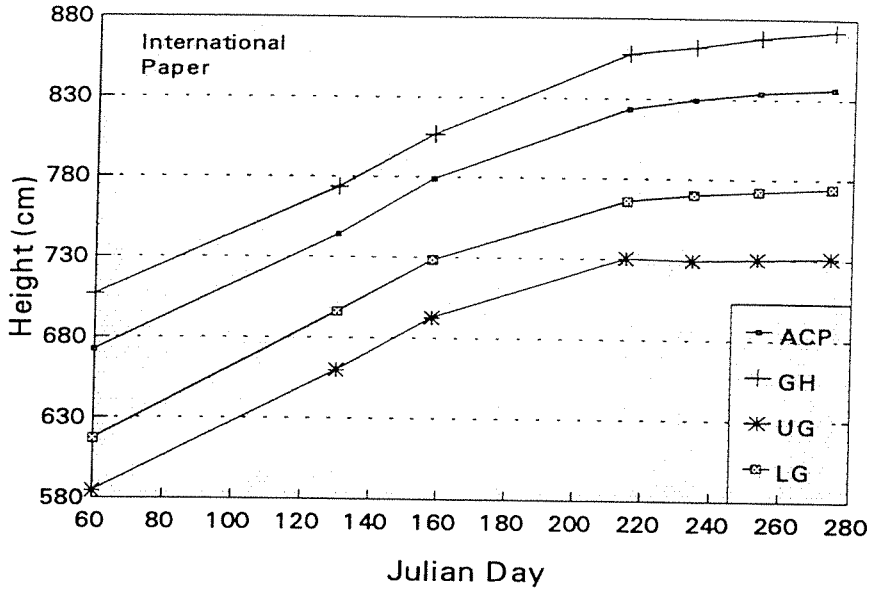
Table 1. Analysis of variance for three traits: Height increment and Julian days to complete 95% of season's height growth (for 1993 and 1994) and Julian days to complete 95% of season's diameter growth (for 1994)

		Annual Height increment (cm)	Julian Days to complete 95% of season's height growth
Source	DF	Prob>F	
Location	1	0.3632	0.2884
Block(location)	32	**0.0001**	0.0571
Year	1	0.1729	**0.0126**
Provenance	3	**0.0137**	**<0.0001**
Family(Provenance)	28	0.0516	**0.0005**
Year*Provenance	3	0.2797	0.8071
Year*Location	1	**0.0018**	0.4665
Year*Family(provenance)	28	0.2552	0.6456
Location*Provenance	3	0.2902	0.4546
Location*Family(Prov.)	28	0.5407	0.4682
Location*Year*Prov.	3	0.6856	0.5332
Location*Year* Family(Provenance)	28	**0.0174**	0.7370
Error	1144		

		Julian days to complete 95% of season's diameter growth
Source	DF	Prob>F
Location	1	.1238
Block(location)	32	**.0001**
Provenance	3	**.0072**
Family(Provenance)	28	**.0003**
Location*Provenance	3	**.0001**
Location*Family(Prov.)	28	.6248
Error	626	

Table 2. Least squares means for 1. Annual height increment in cm. 2. Number of Julian days to complete 95% of season's height growth and 3. No. of Julian days to complete 95% of season's diameter growth. Means for year, location, provenance and family(provenance). Traits 1 and 2 for 1993 and 1994, trait 3 for 1994 only

		Height Increment	Days (Height)	Days (Diameter)
Location				
GS		125.01	228.18	291.33
IP		143.22	229.9	286.88
Year				
93		113.34	234.21	
94		154.89	223.87	
Provenance				
ACP		142.84	233.07	290.89
GH		138.52	240.1	299.42
IC		126.34	218.68	279.14
LG		128.76	224.32	286.98
Family	**Proven.**			
5063	ACP	148.01	226.98	289.13
5065	ACP	135.63	232.51	288.04
7002	ACP	152.19	236.58	292.71
7004	ACP	138.27	226.78	293.49
7034	ACP	143.04	229.38	292.55
7056	ACP	149.8	236.04	297.25
7058	ACP	142.22	236.25	286.35
11009	ACP	133.57	240.04	287.56
22001	GH	135.19	241.59	302.15
22004	GH	134.81	240	299
22027	GH	137.96	241.83	302.57
22029	GH	149.4	247.87	299.42
22030	GH	140.27	244.56	300.7
22031	GH	137.09	232.23	295.91
22032	GH	135.87	237.99	296.24
22034	GH	137.55	234.74	299.36
8503	UG	131.11	211.79	277.79
8509	UG	124.8	221.94	281.56
8526	UG	123.87	212.01	276.02
12008	UG	141.53	227.99	283.19
17016	UG	126.94	215.38	283.92
17044	UG	134.12	229.89	283.53
19002	UG	111.66	214.83	270.6
19016	UG	116.71	215.58	276.47
17004	LG	123.35	218.2	277.95
17019	LG	114.56	229.58	286.27
23026	LG	129.22	219.9	284.08
23028	LG	133.41	221.4	287.2
24001	LG	137.54	223.69	291.85
24002	LG	135.08	228.67	291.05
24004	LG	127.04	220.06	289.49
24009	LG	129.89	233.08	287.98



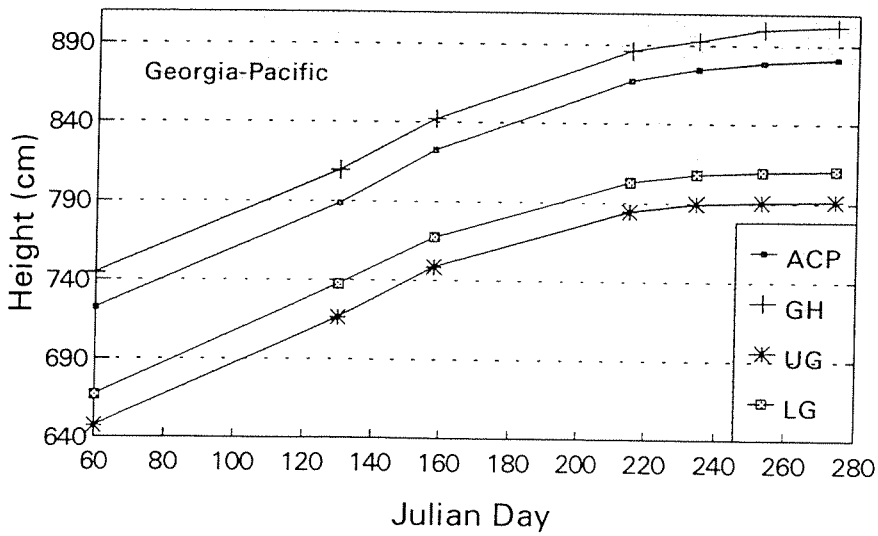


Figure 1. Total height by provenance, by site, during the 1994 growth season. (ACP = Atlantic Coastal, GH = Gulf Hammock, UG = Upper Gulf, LG = Lower Gulf).

ON THE GENOTYPE-BY-TIME INTERACTION: GROWTH INCREMENTS AND THEIR EFFECT ON GENETIC GAIN

F. Zamudio

Abstract.- A method useful to quantify the effect of genotype-by-time interaction (GxTime) for the genetic gain of cumulative traits is presented. Results indicate that the response to selection for cumulative traits can be partitioned into two components which reflect the contribution from the additive effect of growth increments and the contribution from the interfamily stability over time. A selection index which combines information for the intra- and interfamily variation over time is also developed. Data from four progeny tests of *Pinus tecunumanii* established in South America, as part of the international program conducted by the CAMCORE cooperative, were used to assess the method. The estimation of selection efficiency can be used to diagnose the effect of time in response to selection in a breeding program, to compare the chances for early selection in different locations, and to select individual trees for greater and more steady growth over time. As expected, the predicted response from the index selection exceeded the predicted response to individual selection for cumulative height at every test site.

Keywords: Early selection, *Pinus tecunumanii*, provenance/progeny tests, selection index.

INTRODUCTION

As more information from genetic tests become available, tree breeders increasingly turn their attention to developing optimum procedures for selection within these tests. One of these procedures encompass the use of correlated trait selection. Its most important application in forestry is to estimate mature tree performance by assessing progenies when they are young. The economic advantages of being able to observe traits in young seedlings and possibly shorten the generation interval are often great enough that juvenile selection becomes highly desirable. The success of early selection relies on the assumption that the genes and growth processes involved in early and late stages of ontogeny are the same and hence juvenile expression is correlated with mature tree performance. However, this is rarely the case because both the physiological system and gene expressions can change with the accumulation of size and through ontogeny (Namkoong et al., 1988).

Even though juvenile growth processes might not exactly be the same as those in adult trees, some growth processes may be identical, and some traits may foretell what later behavior will be even if it is not identical (Namkoong and Kang, 1989). The search for these traits motivated Nanson (1970) to develop a theory for selection at young ages in forest trees. Baradat (1975) later proposed and provided computational procedures for including juvenile-mature

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genetic covariances in combined and multi-trait indexes. Nevertheless, it has been recognized that estimates of breeding values from young sibs may be unstable and subject to rank changes over time. Maternal effects, unique characteristics of juvenile physiology, or genotype by year interaction have been mentioned as some of the possible causes for such instability (Stonecypher and Arbez, 1976).

The main objectives of the paper is to present a method useful to quantify the effect of GxTime interaction on the genetic gain of cumulative traits. The method partitions the genetic gain for cumulative traits into different components which reflect the effect of growth increments, and develops a selection index which combines information from the intra- and interfamily variation over time. Data from four progeny tests of *Pinus tecunumanii* established in South America (Dvorak and Donahue, 1992) were used to assess consequences of GxTime interaction in genetic gain and index selection.

METHOD

Rationale.- Zamudio (1995) showed that the heritability estimate for a cumulative trait measured at age **C** can be expressed as a function of the genetic control at different growth periods adjusted by the phenotypic variance for the cumulative growth at age C, plus a function of the genetic association among growth increments:

$$\mathbf{h^2(C)} = \Sigma^{c}_{t}\mathbf{h^2}_{t}\sigma^2_{Pt}/\mathbf{VP(C)} + (1/\phi_{xy})\ \Sigma^{c-1}_{t}\Sigma^{c}_{t<t'}\sigma_{ftft'}/\mathbf{VP(C)} \qquad (1)$$

where ϕ_{xy} is the coefficient of coancestry; h^2_t, σ^2_{ft}, and σ^2_{Pt} are the heritability, family variance component, and phenotypic variance for the growth increment at period t-th, respectively; and $\sigma_{ftft'}$ is the family covariance components between growth periods t- and t'-th. The first element in expression (1) can be considered as a "relative cumulative genetic control" (RCGC) and the second element can be defined as a "time stability factor" (TSF). Its value is unique to a set of families established at a particular site and summarizes the effect of the interfamily stability component of the GxTime interaction (Zamudio, 1995). A high positive value for $\sigma_{ftft'}$ means that family growth reflects a positive pattern during intervals t and t'; a value of $\sigma_{ftft'}$ close to zero suggests that families did not show any relation in their growth increment between periods t and t'; and a negative value of $\sigma_{ftft'}$ implies that families had a negative growth pattern from periods t to t'. As trees age, the inclusion of new family covariances as part of TSF can have a positive, neutral, or negative effect in the expression of genetic control at successive ages. Thus, it could be hypothesized that the higher the value of TSF for a particular site the better the chance for early selection to succeed. Zamudio (1995) also developed this idea by partitioning the genetic gain due to direct individual selection into two components:

$$\blacktriangle\mathbf{G(C)} = \Sigma^{c}_{t}\blacktriangle\mathbf{G}_{t}\ (\sigma_{Pt}/\sqrt{\mathbf{VP(C)}}) + 2\ \Sigma^{c-1}_{t}\Sigma^{c}_{t<t'}\blacktriangle\mathbf{G(A}_{t'}\mathbf{,P}_{t})\ (\sigma_{Pt}/\sqrt{\mathbf{VP(C)}}) \qquad (2)$$

The first component of (2) is the contribution to the total genetic gain from the additive effect of growth increments, and it encompasses the sum of genetic responses for each increment ($\blacktriangle\mathbf{G}_t$) adjusted by the ratio of their phenotypic variance with respect to the phenotypic variance for the cumulative trait at age **C**. The second component is the contribution to the total genetic

gain due to the interfamily stability over time or TSF for a particular site. It corresponds to the sum of correlated responses due to selection at earlier growth periods [$\blacktriangle$ $\mathbf{G(A_t,P_t)}$], and it is also adjusted by the ratio of phenotypic variance at earlier growth increments with respect to the phenotypic variance at age **C**. It is clear that the higher the family contribution to the interfamily stability, the higher the TSF for a test will be, and the better the chance to increase the genetic gain for cumulative growth.

Numerical Example.- The data used in this example are from four provenance/progeny tests comprising open pollinated half-sibs families of *Pinus tecunumanii*, collected from mother trees in the Mountain Pine Ridge, Belize, and established during 1982 in four different locations in South America as part of the international program conducted by the CAMCORE cooperative and its members (Dvorak and Donahue, 1992). The tests included in this paper are recognized as ARACRUZ 1 and 2 (established by Aracruz Florestal in Brazil), PROFORCA (established by Productos Forestales del Oriente C. A. in Venezuela), and JARI (established on lands of JARI Florestal also in Brazil). Trials were planted following a randomized complete block design, where each family was planted at 3x3 m (10x10 feet) spacing in six-tree row plots. More details about the trial establishment can be found in Jurado-Blanco (1989).

Measurements for total height (m) were obtained at three, five, and eight years of age after planting. Growth increments for individual trees were obtained by subtracting the cumulative growth at a particular age from the cumulative growth at the age immediately following. Considering H_t as the height increment at the t-th growth period after planting, there were three growth increments: H_1= growth during ages 0 to 3; H_2= growth during ages 3 to 5; and H_3= growth during ages 5 to 8.

PROC MIXED (SAS Institute, Inc. 1992) was used to obtain restricted maximum likelihood (REML) estimators for the different variance components for each growth period and covariance components between different growth periods. The value of $2\phi_{xy}$ (twice the coefficient of coancestry among individuals from the same family) was assumed to be 0.33, and used to estimate the additive genetic variances and heritability for each growth increment and additive genetic covariances between paired growth increments. Progenies established in the field tests originated from mother-trees occurring in natural stands, where there was a good chance for self-pollination and mating among related neighboring trees, which implied that families may present some degree of inbreeding (Squillace, 1974).

The genetic response to direct individual selection was calculated using expression (2) and the methodology presented by Zamudio (1995). The proportion selected (10%) was maintained constant through the comparison of responses (selection intensity = 1.76). Comparisons of the response to selection due to additive effects of increments and interfamily stability over time were made within each test and between tests to determine the effect of stability in the genetic response for cumulative growth. A selection index which maximizes the selection response for cumulative height at age 8 was developed. The index included the information about inter- and intrafamily variation for the three growth increments and was expressed as

$$\mathbf{I} = (\mathbf{b}_1 f_1 + \mathbf{w}_1 \mathbf{e}_1) + (\mathbf{b}_2 f_2 + \mathbf{w}_2 \mathbf{e}_2) + (\mathbf{b}_3 f_3 + \mathbf{w}_3 \mathbf{e}_3),$$

where f_t and e_t are the family and residual effects for an individual at the t-th growth period, respectively; b_t and w_t are the partial regression coefficients of the breeding value at age 8 on f_t and e_t, respectively. The genetic response in the index was compared to the response to direct selection for cumulative height at age 8.

RESULTS AND DISCUSSION

Phenotypic correlations among growth increments and cumulative growth are given in table 1. The largest positive phenotypic correlations between H_1 and H_2 were almost zero but the largest negative value was -0.477 at PROFORCA. The largest positive correlation between H_1 and H_3 was 0.185 at ARACRUZ 1, and three out of four correlations between H_2 and H_3 were also negative. Results from this study clearly show that GxTime interaction is present in the four CAMCORE tests sites analyzed, as reflected by the low and/or negative phenotypic correlations among increments.

TABLE 1.- Phenotypic correlations between growth increments and between cumulative growth for height

	PHENOTYPIC CORRELATIONS							
	GROWTH INCREMENTS					CUMULATIVE GROWTH		
	TESTS	H_2	H_3		TESTS	H5	H8
H_1	ARACRUZ 1	0.008 ns	0.185 ns	H3	ARACRUZ 1	0.781 **	0.669 **
	ARACRUZ 2	-0.191 ns	0.129 ns		ARACRUZ 2	0.724 **	0.685 **
	PROFORCA	-0.477 *	0.017 ns		PROFORCA	0.186 ns	0.220 ns
	JARI	0.068 ns	-0.089 ns		JARI	0.679 **	0.606 **
H_2	ARACRUZ 1		0.012 ns	H5	ARACRUZ 1		0.800 **
	ARACRUZ 2		-0.157 ns		ARACRUZ 2		0.840 **
	PROFORCA		-0.259 ns		PROFORCA		0.716 **
	JARI		-0.342 *		JARI		0.775 **

Not surprisingly, the estimators of correlations for cumulative growth were all positive. ARACRUZ 1 showed the largest correlation between H3 and H5, and PROFORCA the lowest value (despite having the largest phenotypic variance for H3, 1.2499). With the exception of PROFORCA, correlations between H3 and H8 decreased and the largest correlations were recorded between H5 and H8. These values ranged from 0.716 at PROFORCA to 0.84 at ARACRUZ 2.

Estimates of different genetic parameters are presented in table 2. Heritabilities for increments showed a tendency to diminish over the time. The PROFORCA and JARI test sites showed negative additive genetic covariances for height growth among periods 1 vs 2 and 2 vs 3. As a result, TSF was negative at PROFORCA and also low at JARI. The reasons for these negative covariances could be the results of adverse environmental conditions that predominate at both sites (Zamudio, 1992).

Predicted responses due to the effect of each increment and correlated responses due to interfamily stability among growth periods (TSF) are given in table 3. The largest contribution from the additive effect of increments to the total genetic response was recorded at PROFORCA (0.793/0.598=133 %). However, the correlated response due to interfamily stability was negative at this test site, which had the effect of subtracting gain from the response due to

the additive effects and reducing the total genetic response predicted at age 8. Nevertheless, the largest contribution of the correlated response due to stability to the total gain was recorded at ARACRUZ 1 (0.309/0.779=40 %), followed by ARACRUZ 2 (0.38/0.971=39 %), which implies that these sites present the best chances for early selection on height.

TABLE 2.- Genetic parameters for height. Subscript numbers represent the different growth periods. VP(8) and $h^2(8)$ are the phenotypic variance and heritability at age 8 respectively; h^2_t is the heritability for t-th growth period; $C(A_t,A_{t'})$ is the additive genetic covariance between growth increments t- and t'-th; RCGC is the relative cumulative genetic control; and TSF is the time stability factor.

	GENETIC PARAMETERS FOR HEIGHT INCREMENTS									
TEST SITE	$\sqrt{VP(8)}$	h^2_1	h^2_2	h^2_3	COVARIANCES $C(A_1,A_2)$	$C(A_1,A_3)$	$C(A_2,A_3)$	RCGC	TSF	$h^2(8)$
ARACRUZ 1	1.855	0.322	0.149	0.071	0.048	0.075	0.040	0.144	0.095	0.239
ARACRUZ 2	1.317	0.337	0.109	0.143	0.032	0.028	0.082	0.255	0.163	0.418
PROFORCA	1.693	0.068	0.195	0.059	0.223	-0.087	-0.230	0.266	-0.065	0.201
JARI	1.348	0.293	0.120	0.049	0.106	-0.052	-0.040	0.195	0.016	0.211

TABLE 3.- Genetic response to direct selection in cumulative height at age 8. Subscript numbers represent the different growth periods. ΔG_t is the genetic response for the t-th growth increment; $\Delta G(A_{t'},P_t)$ is the correlated response for growth increment t'-th after applying indirect selection at growth period t-th; and $\Delta G(8)$ is the total genetic response at age 8 and is the sum of responses due to additive affects for increments and the correlated response due to stability over time.

	RESPONSE TO INDIVIDUAL SELECTION FOR CUMULATIVE HEIGHT AT AGE 8								
TEST SITE	ΔG_1 (m)	ΔG_2 (m)	ΔG_3 (m)	DUE TO ADDITIVE EFFECTS	$\Delta G(A_2,P_1)$ (m)	$\Delta G(A_3,P_1)$ (m)	$\Delta G(A_3,P_2)$ (m)	DUE TO INTERFAMILY STABILITY	TOTAL RESPONSE $\Delta G(8)$ (m)
ARACRUZ 1	0.550	0.217	0.141	0.470	0.087	0.136	0.085	0.309	0.779
ARACRUZ 2	0.563	0.149	0.179	0.591	0.059	0.052	0.186	0.380	0.971
PROFORCA	0.134	0.596	0.128	0.793	0.351	-0.137	-0.233	-0.195	0.598
JARI	0.428	0.205	0.077	0.462	0.225	-0.110	-0.072	0.038	0.500

A better way to compare results is by dividing the total genetic response by the test means at age 8. The largest predicted response was at ARACRUZ 2 (0.971/12.5=7.8 %), followed by ARACRUZ 1 (0.779/11.6=6.7%), PROFORCA (0.598/14.4=4.2%), and JARI (0.5/14.4=3.5%). These results demonstrate the effect of interfamily instability. ARACRUZ 2 presented a low relative contribution of response due to additive effects of increments (0.591/0.971=61 %) than PROFORCA (0.793/0.598=133 %), but families established at PROFORCA were more unstable over the time than families at ARACRUZ 2. This triggered a lower total response to selection in PROFORCA than in ARACRUZ 2. This type of analysis warns breeders to be cautious when comparing genetic response at different sites. A negative contribution from the interfamily stability implies that the progeny of some trees with unstable growth could produce progenies that may perform well during the first five years but may change enough to decrease their growth in the next period(s). This would result in losing potential gain over time in the next breeding generation.

Values for the regression coefficients and response to selection in the index are given in table 4. The percentage of genetic response with respect to the test mean for cumulative growth at age 8 is also given for each test site. The ARACRUZ 1 test site ranked first for the predicted response. Conversely, JARI had the lowest predicted response.

TABLE 4.- Regression coefficients and response to selection in the index for height. The percentage of genetic response with respect to the test mean for cumulative height at age 8 is also given for each test. The efficiency was calculated as the ratio between the genetic response in the index and the genetic response due to direct individual selection for the cumulative growth at age 8.

TEST SITE	REGRESSION PARAMETERS FOR TOTAL HEIGHT AT AGE 8						GENETIC RESPONSE IN THE INDEX (m) ▲G(AI)	TEST MEAN AGE EIGHT (m)	PERCENTAGE OF GAIN (%) (RANKING)	EFFICIENCY OF RESPONSE IN THE INDEX OVER THE INDIVIDUAL SELECTION
	b_1	w_1	b_2	w_2	b_3	w_3				
ARACRUZ 1	1.050	0.729	1.044	0.376	1.054	0.124	1.60	11.6	13.8 (1st)	205
ARACRUZ 2	1.053	0.806	1.054	0.707	1.036	0.577	1.71	12.5	13.7 (2nd)	176
PROFORCA	-7.865	0.875	0.417	0.571	-9.323	-0.044	1.67	14.4	11.6 (3rd)	276
JARI	1.058	0.584	1.008	0.180	1.030	0.018	1.10	14.4	7.4 (4th)	129

Implications to Breeding. Results in this study indicate that the simple observation of age-age phenotypic correlations for cumulative growth can be misleading. Because GxTime interaction is mainly the effect of two components, namely interfamily and intrafamily stability (Zamudio, 1995), and the cumulative growth is a function of successive increments, the gain from early selection should be tested by analyzing how the performance of different families can affect the covariances among family and residual effects over time for the different growth increments. There will be families whose contribution to the covariance among family effects over time will be positive, but their covariance for residual effects over time can be positive or negative which affects the estimation of genetic gain at cumulative ages. This happened at the PROFORCA test site. Thus, families should not only be classified by their cumulative growth but also by their contribution to the two GxTime interaction components. The next logical step is a further comparison with their growth rate to fully detect which individuals and/or families are good candidates for early positive response to selection.

The estimation of the effect of instability on response to individual selection at cumulative ages is function of the heritability for each growth increment and genetic covariances between increments at different periods. It assumes that the same individuals selected at age 8 are also selected for each period but, given the effect of instability over time (reflected as imperfect phenotypic correlations between paired growth increments), probably some individuals would have to be replaced by others at different periods. Thus the first component in expression (2) may be underestimating the potential response to direct selection. Nevertheless, the usefulness of partitioning the estimated genetic response into cumulative effects and instability contributions has to be regarded as a diagnostic tool for breeders interested in knowing how much gain is lost due to the effect of GxTime interaction.

Genetic covariances or correlations among age-specific trait values quantitatively describe the genetic link between expressions of the same trait at different points in ontogeny. These genetic links between age-specific trait values have been mentioned to be the result of pleiotropy and linkage disequilibrium (Cherevud et al, 1983). In this case, the effects of one gene on the

phenotype is expressed at more than one age. But the differences in results indicate that there is also a strong influence of non-genetic factors on the expression of the trait through ontogeny. The fact that the interfamily stability component of GxTime interaction changed in different environments reflect the presence of genotype-by-time-by-environment interaction. This higher order interaction should suggest that breeders carefully assess selection strategies by measuring the impact of genotype-by-environment interaction as a main criteria, and also by considering how progenies evolve through ontogeny within each population test.

Though the response in the index looks promising at PROFORCA, results should be critically reviewed. A careful observation of the regression parameters for the index at this test site show that the b_1 and b_3 coefficients were the largest negative value among the total number of parameters estimated for the trait. This implies that if a candidate family had a highly positive family effect (family mean deviation from the total test mean) for the first or third growth period, or both, its index value can be very low. Because of the negative genetic correlations among periods 1 vs 3 and 2 vs 3, the interfamily instability component of GxTime in PROFORCA had a negative impact on the genetic response to selection for cumulative height at age 8. The high efficiency in response from using the index (table 4) and the negative regression coefficients imply that the index can successfully increase the genetic response to selection in height, but it will tend to favor families which on the average will grow less during periods 1 and 3 (negative family effects). Negative parameters have also been reported elsewhere. For example, Namkoong and Matzinger (1975) also estimated a mixture of positive and negative regression parameters to various growth points when selecting *Nicotiana tabacum* based on eight periodic heights. They hypothesized that some physiological constraints prevented the simultaneous seasonal increase in height growth causing a midseasonal drop in the index coefficients. Magnussen and Kremer (1993) also recorded negative index regression coefficients derived for selection of height in maritime pine (*Pinus pinaster* Ait.). They indicated that phenotypic height between ages 5 and 15 were inefficient as indicators of overall potential good height growth.

CONCLUSIONS

A comparison of the efficiency of response to selection in the index v/s individual selection for the cumulative height can be used to diagnose the effect of time in a breeding program, to compare the chances for early selection in different tests, and to select individual trees for higher and steady growth over time. As expected from theory, the predicted response in the index always exceeded the predicted response to individual selection for cumulative height at every test site.

A selection strategy based on the index suggests that ARACRUZ 1 had trees with the largest and most stable growth. The presence of a strong GxTime interaction for cumulative height in PROFORCA indicates that early selection will be less successful there than at other locations.

The results indicate the presence of a genotype-by-time-by-environment interaction that should be further investigated. Changes in the response to individual selection for cumulative height from one test to another suggests that progenies from *P. tecunumanii* can strongly and simultaneously interact over the time and planting location.

It was shown that the genetic response to individual selection depends on covariances between increments. Consequently, an early selection procedure can be optimized by selecting families and/or individuals which maximize the response function due to interfamily stability over time and thus choosing the moment when the response function due to stability over time show a maxima. This hypothesis needs to be further supported by the assessment of data collected at later ages.

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GENETIC IMPROVEMENT OF CHRISTMAS TREES: PROGRESS AND POSSIBILITIES

C. R. McKinley[1] and S. E. McKeand[2]

Abstract:-- Each year, over 35 million Christmas trees are harvested and sold in the United States. The need for quality and the relatively high value per tree result in significant opportunities for genetic improvement in several species. Efforts in the southern United States have been primarily directed at Fraser fir (*Abies fraseri* [Pursh] Poir.) and Virginia pine (*Pinus virginiana* Mill.), with limited breeding and seed production programs having been initiated. However, to reach the amount of genetic gain potentially available, additional efforts are needed in the selection, breeding and testing of species and individuals with desirable Christmas tree traits. Vegetative propagation techniques have also been developed for several species, and results indicate that both plantlets and seedlings can be successfully utilized for plantation establishment. By combining traditional breeding methods and vegetative propagation, a significant increase in the number of salable trees/acre and market value can be achieved.

Keywords: Christmas trees, genetic improvement, *Abies fraseri*, *Pinus virginiana*

INTRODUCTION

The Christmas tree industry in the United States produces about 35 million trees annually and involves about 15,000 growers (National Christmas Tree Assn.). The National Christmas Tree Association (1994) also estimates that 1,000,000 acres of Christmas trees are currently in production in the United States, and over 90 percent of the annual harvest are plantation-grown trees. Production in the southern United States is about 25% of the national total.

Because of the economic impact of the industry and the large-scale plantation management, it is a rather straight-forward assumption that genetic improvement should be considered a part of the Christmas tree production system. The ever-increasing costs associated with production and the need for a high quality product in order to maintain market share also lead to emphasis on genetic improvement.

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Genetic improvement of Christmas trees is, in many respects, similar to genetic improvement in other tree species. Growth and physiological characteristics are species dependent and must be considered in that context. However, any attempt at genetic improvement must also consider the product to be marketed and Christmas trees are quite different from solid wood or pulp products. Significant differences in this regard include:

1. Value

Christmas trees may be considerably more valuable at a young age than trees used for wood products. For example, a 1-0 Virginia pine planted into the field and grown for 4 years may bring $40 if marketed on a 'choose and cut' basis. Likewise, a 3-2 Fraser fir transplant grown for 7 years may bring $50 on a retail lot, with about half that value paid to the producer.

2. Rotation Age

As implied by the size of the product, Christmas trees are grown on much shorter rotations than timber species. The length of time in the field ranges from 3 to 10 years depending upon species, market, quality etc.

3. Management

Christmas trees are managed much more intensively than other forest species. Fertilization, weed suppression, and insect control are routinely practiced in Christmas tree plantings. In addition, pruning to correct form and shearing to give the 'Christmas tree look' are management activities applied to all species.

4. Consumer Acceptance

Christmas trees are highly dependent upon consumer acceptance. If a tree is not suitable, it is not sold, and there is no alternative use that will cover the cost invested. This necessity to meet consumer needs places a premium on the quality of the product not generally encountered in other forest species.

Given the production and marketing considerations, there appear to be several distinct benefits from genetic improvement in Christmas tree production. These include: 1) reduction of rotation age (age to harvest), 2) reduction of the variability of the product, 3) increase in the percentage of trees sold, and 4) reduction of chemical and labor inputs. In themselves, these are not specific traits which can be improved, but rather are the result of improvement in such traits as: survival, growth rate, straightness, pest resistance, color, needle length and retention, response to shearing, grade and value.

STEPS IN GENETIC IMPROVEMENT OF CHRISTMAS TREES

As with other improvement programs, a series of directed, coordinated steps must be followed to provide maximum genetic gain with minimal costs. In Christmas trees, these steps have closely paralleled those followed for other tree species. However, specific emphases and priorities have varied greatly depending on species, location, markets, etc.

Specific steps leading to genetic improvement include:

1. Selection of Species

In Christmas trees, the species to be used is dependent on consumer preference, shipping qualities, adaptability to sites, insect and disease problems, seed source/availability, and length of rotation (Bell and White, 1966). Several studies have been reported which provide information in this regard (Giliam 1961; Walterscheidt and others 1991; Whitfield and Davidson 1965; Thor 1972; Thor 1976). In the southern United States, the most favorable species have proven to be Fraser fir, Virginia pine, eastern white pine (*Pinus strobus* L.), Scotch pine (*Pinus sylvestris* L.) and eastern redcedar (*Juniperus virginiana* L). In recent years, Leyland cypress (x *Cupressocyparis leylandii*) has also gained popularity among growers and consumers.

In many cases, particularly in the southern United States, the evaluation of various species for Christmas trees has led to the subsequent use of species which are exotic to the area in which they are being produced. For example, Virginia pine is planted throughout the southern states, while being native only to the upper coastal plain, piedmont and Appalachian mountain regions. Likewise, Fraser fir is generally planted at elevations much lower than where the species grows naturally.

2. Testing Selected Material

Tests designed to evaluate geographic (provenance) variation in Christmas tree traits have been reported for several southern species (Arnold and Jett 1995; Brown 1987; Jett and others 1993; Schoenike 1969; Schoenike 1974). Other studies have not focused directly on Christmas trees but provide additional information relative to the amount of genetic variation present in a given species. Examples include Robinson (1968), Kellison and Zobel (1974), Thor (1978), Haverbeke and Read (1976) and Wright (1970). Results forn each of these species suggest that selection of proper seed source would make a significant contribution to the total amount of genetic improvement which could be obtained.

Genetic tests to evaluate family performances have also been established for Christmas tree species. These tests have subsequently provided a great deal of information regarding heritabilities, phenotypic and genotypic correlations, potential gains from selection and other important genetic parameters (Arnold and others 1994; Brown and Foster 1991; Diebel and others 1992; Warlick and others 1985). Individual heritabilities range from .2 to .7 depending on the trait, species, and specific trial. These values coupled with the economic importance of the

traits studied suggest ample opportunity to develop cost-effective genetic improvement programs.

3. Seed/Seedling Production

For many Christmas tree species, the production of genetically improved planting material has not received the priority of other activities.

Seed production areas and/or seed orchards, critical components of traditional tree improvement programs, have been established by a minimal number of organizations. The U.S. Forest Service currently manages a Fraser fir seed production area in a native stand on Roan Mt. N.C., while the North Carolina Forest Service has established both seedling and grafted orchards for Fraser fir selected for Christmas tree traits. Several private nurseries advertise availability of Fraser fir seed orchard seed, but the source is most often that of unselected Roan Mt. material. Brown (1978) traced the development of a Virginia pine seed orchard in Alabama, while McKinley (1989) described a Virginia pine orchard established in Texas. Orchards providing seeds for these and other species exist, but the material in those orchards has often not been evaluated for use as Christmas trees.

As an alternative to seedling production, efforts have also been underway for the production of vegetatively propagated material for several species commonly used as Christmas trees (Blazich and Hinesley 1994; Box and Beech 1968; Brown and others 1991; Chang and others 1991; Cohen 1975; Saravitz and others 1991; Tsai and others 1985). While favorable results have been reported, several difficulties remain. For example, vegetatively propagated material may initially show slower growth (Aimers-Halliday and others 1991) and often has a tendency to retain a plagiotropic growth habit (Wise and others 1986).

4. Recurrent Breeding Programs

For the most part, breeding programs design to be maintained on a continuing basis have not been implemented for Christmas trees. Some efforts have been made to make controlled crosses followed by selection for advanced generation programs (McKinley 1989), but to date these have not become operational systems. Because of the economic values involved, degree of genetic variation and suitability inheritance patterns, the potential to develop long-range breeding and testing programs appear positive. In the south, the best candidates for such programs are Fraser fir and Virginia pine due to the widespread planting of these species.

POTENTIAL FOR FURTHER WORK IN CHRISTMAS TREE IMPROVEMENT

Whether genetic improvement can be applied on a wide-scale basis in Christmas trees is dependent on species, market, genetic parameters, costs etc. However, in reviewing the industry, several factors favor the implementation of genetic improvement. These include: 1)

the high value product, 2) a relatively stable market, 3) sufficient genetic variation and heritabilities, and 4) applicability of new techniques.

Opportunities for Christmas tree improvement which appear to be most promising are:

1. Continued testing of new species

While a large part of the consumer acceptance of Christmas trees depends on the use of traditional species, growth traits of several yet-untested species appear to be suitable. In particular, several species of *Abies* may meet Christmas tree criteria, as well as requiring less intensive management. The current interest in Leyland cypress also illustrates that new species can be readily accepted into the market.

2. Better utilization of geographic variation

A number of tests have demonstrated the importance of utilizing geographic variation in the improvement of Christmas trees. The widespread use of non-native species and the limited number of organizations with sufficient funds to consolidate breeding, testing and seed production facilities result in many of the potential gains being left untouched.

3. Selection of best families followed by individual selection

Genetic gains in Christmas trees, as with other tree species, are greatest when some form of combined selection is practiced. Studies to determine selection and breeding strategies suggest that current gains could be greatly enhanced through selecting at both the family and within-family levels for Christmas tree traits (Arnold and others 1994; Brown 1987).

4. Increased utilization of vegetative propagation

Vegetative propagation offers a number of advantages in the utilization of genetic material. As techniques are developed to overcome plagiotropic growth and other problems, the establishment of clonal Christmas tree plantations could have a significant economic impact on the industry.

5. Application of genetic engineering techniques

Genetic engineering is no longer a series of techniques which have 'potential' for use in tree improvement. Those techniques are now being used extensively in many forest species. The high values associated with Christmas trees suggest that such species are prime candidates with which to develop and deploy genetically engineered plants.

CONCLUSIONS

The production system and economic impact of Christmas trees suggest that genetic improvement be considered for several species. Previous studies have demonstrated that such consideration is warrented from the standpoint of geographic, family and individual variation, plant production capability and the applicability of tissue culture and genetic engineering techniques.

To fully capture the potential gains from tree improvement, additional efforts in several activities, particulaly as they relate to the selection and production of improved material, is needed.

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Northern Red Oak Flower to Acorn Survival Increases Following Monthly Applications of Asana® XL

L. R. Barber[1], D. T. Barrett[2], and C. K. Proffitt[3]

Abstract. Many insect pests attack northern red oak flowers, acornets, and acorns. Selected trees on the USDA Forest Service, Watauga Northern Red Oak Seed Orchard near Elizabethton, TN were treated monthly during the 1993 and 1994 growing season with Asana XL. At harvest in the fall of 1994, 34 percent of the 1993 flower crop survived to harvest on the Asana® XL treated trees, as compared to 18 percent on comparable unsprayed trees.

Keywords: Northern red oak, *Quercus rubra L.*; filbertworm, *Cydia latiferreana* (Walsingham); acorn weevil, *Curculio, Conotrachelus, Callirhytis* spp., treehoppers, *Platycotis vitata* (F.); seed orchard, Asana® XL, esfenvalerate.

Introduction

Genetically superior pine seed orchards routinely produce improved seed to assist the timber industry in reforesting harvested land. Without the array of first and second generation orchards, reforestation would be based upon seedlings from seed trees or wild seed collections resulting in low genetic quality. Foresters are aware of the need for high quality, fast growing hardwood seedlings for reforestation but in most cases the seedlings that are available are of unknown origin.

The Forest Service maintains and operates the 17 acre Watauga Northern Red Oak Seed Orchard near Elizabethton, TN. The orchard was planted in 1973 as a progeny test by the Tennessee Valley Authority. The study was thinned in 1987-8 to become a USDA Forest Service seed orchard on the Cherokee National Forest in 1984 and the first large seed crops were documented in 1989. In 1993, the orchard yielded enough acorns to supply the entire southern appalachian area with high quality northern red oak seedlings for reforestation.

Larry Barber, Entomologist, USDA FS (unpublished data) tagged and followed to maturity the 1989 and 1990 flower crops on selected trees in the Watauga Northern Red Oak Orchard. Only 3.8 percent of the 1989 flower crop remained healthy at harvest some 18 months later while the 1990 flower crop faired better with 27 percent healthy at harvest. No insecticides were applied to either of these flower crops.

Previous literature indicates that acorn weevils of the genus *Curculio* cause the most insect damage to oak acorns (Gibson 1982). These weevils and others in the genus

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Conotrachelus, as well as the filbertworm *Cydia latiferreana* (Walshingham), can destroy a majority of the acorn crop (Solomon et. al. 1987). Both the pip gall produced by *Callirhytis operator* (O.S.) and the stone gall *Callirhytis fructuosa* Weld kill the acorn by either causing the nut to fall prematurely (pip gall) or by the stone gall replacing the seed. Tree hoppers, *Platycotis vittata* (F.), are potentially capable of damaging red oak flowers (Bob Ceich personel communication).

Little work has been done to control insect pests of oak seed crops (Kearby et al. 1986). Previous work, using both trunk implants and granular systemic insecticides showed a reduction in infestation levels but the insecticides may have also caused an increase in the percentage of desiccated acorns (Dorsey et al. 1962 and Dorsey 1967). Trunk implantation of the systemic insecticides phorate and Bidrin® yielded more sound acorns than granular applications of disulfoton and phosphamidon applied to the soil. In 1993, a study was initiated to investigate the effects of insect control measures on acorn production.

Material and Methods

In March 1993, 20 pairs of trees representing 17 families were selected for the study. A family consisted of half-sibling trees upon which acorn production with and without control spraying was assessed. One half of the trees received an insecticide treatment of esfenvalerate, Asana® XL, while the other half remained untreated. The treated trees were sprayed monthly throughout the summers of 1993 and 1994, with applications beginning in March. The Asana® XL solution was mixed at a rate of 9.6 fluid ounces in 100 gallons water. The application rate per tree varied during the season from approximately 2 gallons per tree in the early spring to nearly 10 gallons in the summer. The variation in application rates between spring and summer was because in the spring it took less spray solution to achieve proper coverage of the foliage and branches than in the summer when leaf production was at its peak. All applications were with an FMC DM020 high volume hydraulic sprayer set to apply the spray solution at 350 psi. The trees ranged in height from approximately 30 to 40 feet.

In May 1993, 20 branches on each tree were selected and tagged. Healthy pistillate flower structures were counted and recorded on data sheets at the first inventory. The branches and their developing acorns were revisited seven more times before harvest in November 1994, and the condition of their health was recorded.

In late August 1994, the final inventory was conducted, each tag was visited and the health of each acorn determined from visual observation. All acorns that were determined to be healthy were painted with one drop of fingernail polish and left attached to the tree. Nets were placed under each tree to catch the acorns when they dropped. Acorn collection began the first week of September and continued until November 2, 1995. During this acorn collection period, the nets were visited and the acorns collected three times each week. The painted acorns were separated from the rest of the acorns and placed into plastic bags and put into cold storage for later observation and dissection.

RESULTS AND DISCUSSION

Analysis of the 1993 flower crop survival from May 1993 to August 1994 indicated a significant difference at the one percent level in acorn production between treated and untreated trees. There was a significant family x treatment interaction, indicating that healthy acorn production in some families was not predicated upon the control measure. Thirty four percent of the flower crop survived to August 1994 on trees treated with Asana XL as compared to 18 percent on untreated trees. Both treated and untreated trees showed a dramatic decrease in healthy flowers at the second inventory (Figure 1). After this time, little difference or change in the percent healthy spread between treated and untreated trees was detected until the emergence and attack of acorn weevils in early August 1994.

Identifiable insect damage was observed only in the second year of acorn development and this was due primarily to pip gall, filbertworm, and acorn weevil attacks. Applying the results of the acorn dissections to the crop remaining in late August, we estimate that 28 percent of the original flower crop on treated trees would produce healthy acorns as compared to 7 percent on unsprayed trees (Figure 1).

Dr. Gerome Grant (personel communication) reported that several species of thrips were identified from the orchard as potential damaging agents to the newly formed flower and acornets. If thrips and treehoppers cause damage to oak flowers in the first year of the flower

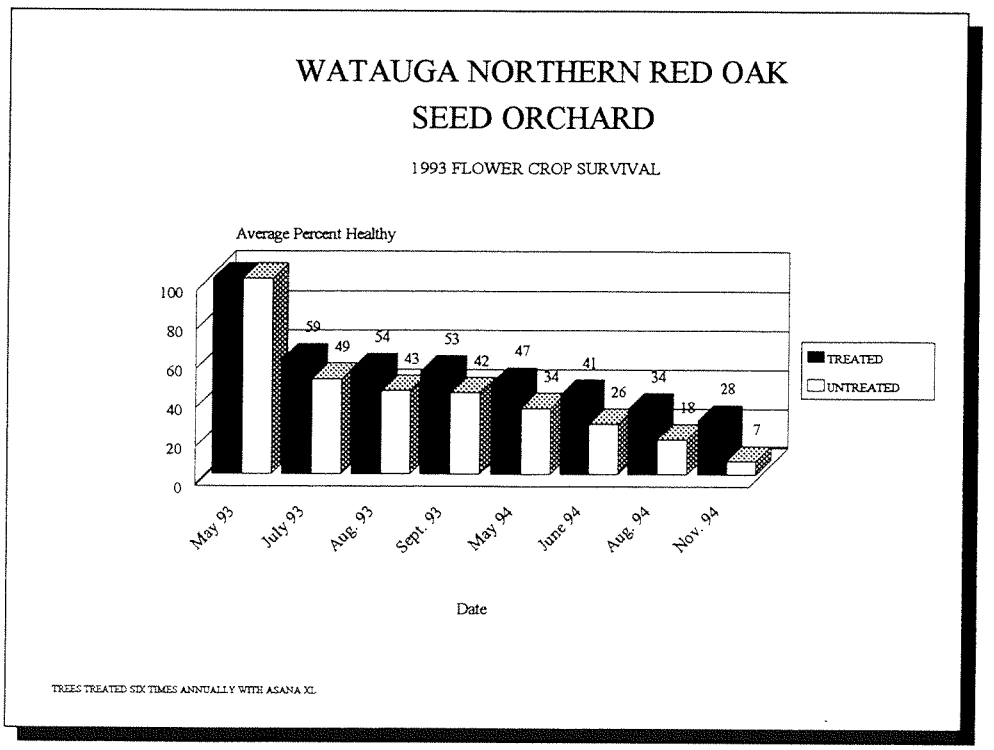

Figure 1. 1993 Watauga northern red oak seed orchard flower crop survival.

development cycle their control would explain the differences between treated and untreated flowers. Asana® XL is a broad spectrum insecticide and capable of controlling both pest groups. Neither potential pest was observed on the inventory trees during this evaluation.

In some families, more than two trees were used. There were six trees from family 915 (Appendix 1) and they responded similarly throughout the study and on average only 1 percent of the nuts were believed to be healthy at final harvest on untreated trees as compared to 29 percent on treated trees. In family 735, four trees were used and the untreated trees had more healthy acorns during the majority of the year, however, significant damage was detected at dissection. These dissections determined that many of the acorns were not healthy on the untreated trees and thus in the over-all rating for this family there were more healthy acorns on treated trees (Appendix 1). Family 323 was also represented by four trees and generally more healthy acorns were found on treated trees. In the remaining families, there were only one treated and one untreated tree. Generally for each pair of trees, more healthy acorns were present after harvest and dissection on the treated trees as compared to the untreated trees (Appendix 2). Only in family 565 were there more apparently healthy acorns at harvest in the untreated tree than on the treated tree. This may indicate genetic differences amoung the families in resistance to insect attacks. Family 526 showed the greatest treatment effect. The treated tree had 47 percent healthy acorns as compared to 2 percent on an untreated tree (Appendix 2). Comparative trees in families 526, 550, 903, and 913 also showed large differences in percent of healthy acorns between treated and untreated trees.

Wildlife such as deer, turkey, groundhogs, and squirrels are often observed in the fall and predation is usually not a problem. However, in 1994 deer were often observed leaving the orchard in the early morning and were assumed to be responsible for partially consumed acorns observed on the nets. Recovery of painted acorns was a problem in some trees. The percent recovery of painted inventory acorns ranged from 95 to 11 on the inventory trees (Figure 2).

At harvest, acorn weevils accounted for 37 percent damage on untreated trees as compared to 0.2 percent on treated trees. Filbertworm damage was less than one percent throughout the season including harvested acorns. Both pip gall and stone gall damage was observed at harvest. Losses from pip gall did not show up in our inventory data during August 1994 but were observed in the field. Losses from these two pests were less than one percent.

When dissected, some acorns appeared to be discolored and were categorized as being damaged by an unknown agent. This unknown damage category amounted to 25 percent of the acorns in the untreated trees and 16 percent in treated trees. This damage is characterized as having the appearance of potato rot and could be attributed to insects, such as acorn weevils, feeding in the tissue and introducing fungi or bacteria. In the untreated trees, this damage was found most frequently in acorns with insect damage. On the treated trees, however, it was found in acorns with no evidence of insect activity.

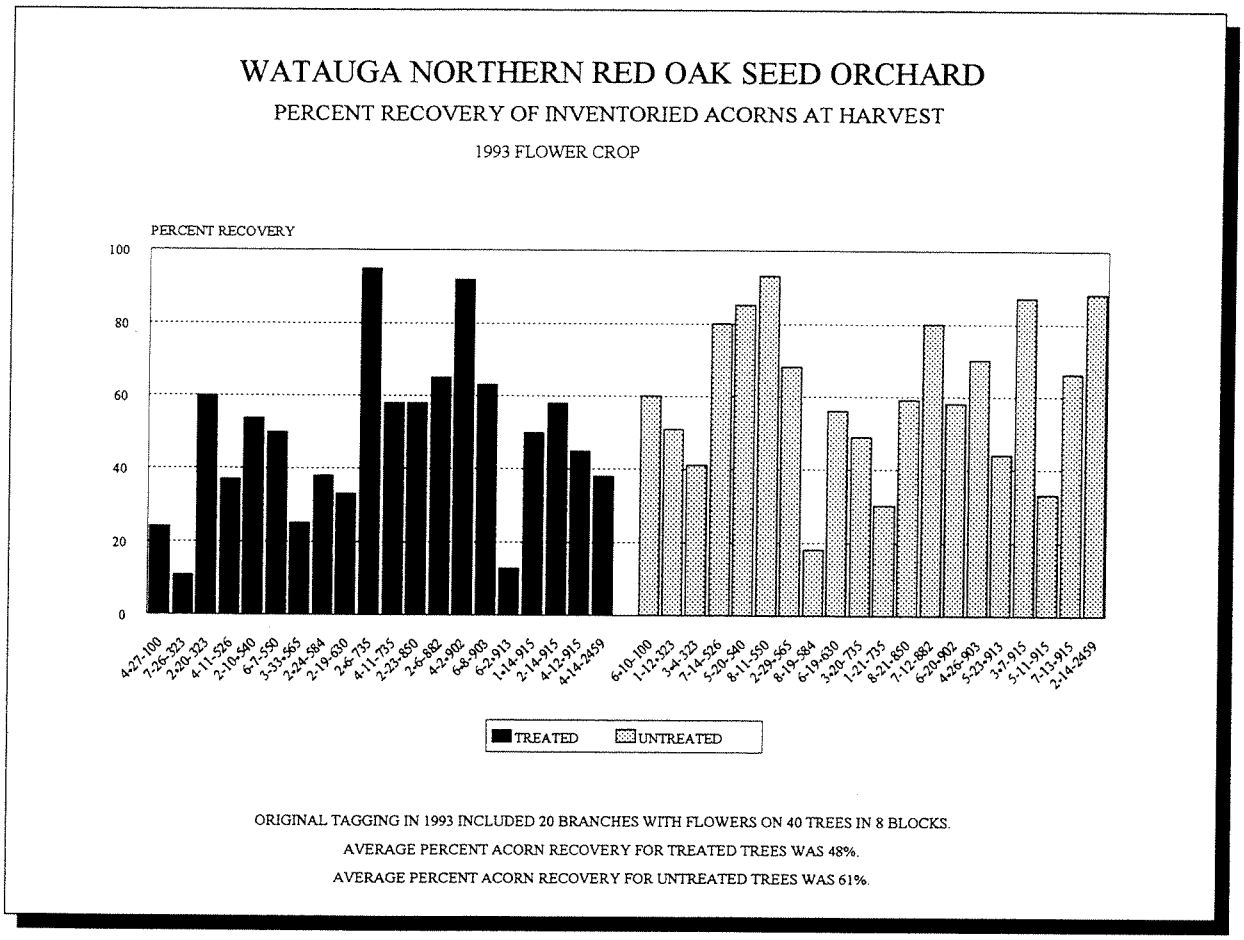

Figure 2. Percent recovery of inventoried acorns at harvest during the fall of 1994.

CONCLUSIONS

Monthly applications of Asana® XL, a synthetic pyrethroid insecticide, increased flower to acorn survival and produced more apparently healthy acorns on treated trees than on similar untreated trees. There appear to be two distinct time periods in the development of red oak acorns when insecticides are especially beneficial in increasing flower to acorn survival. These periods are in the early spring of the first year and in the late summer or fall of the second year.

Disclaimer

The use of trade, firm, or corporation names is for the information and convenience of the reader. Such use does not constitute an official evaluation, conclusion, recommendation, endorsement, or approval of any product or service to the exclusion of others which may be suitable.

Caution: Pesticides can be injurious to humans, domestic animals, desirable plants, and fish or other wildlife if they are not handled or applied properly. Use all pesticides selectively and carefully. Follow recommended practices for the disposal of surplus pesticides and pesticide containers.

ACKNOWLEDGMENTS

The authors sincerely thank Dr. Scott Schlarbaum, of the University of Tennessee for his assistance and guidance in the statistical analysis contained in this paper. Mark Remaley, graduate research assistant at the University of Tennessee is also thanked for his assistance in the statistical analysis.

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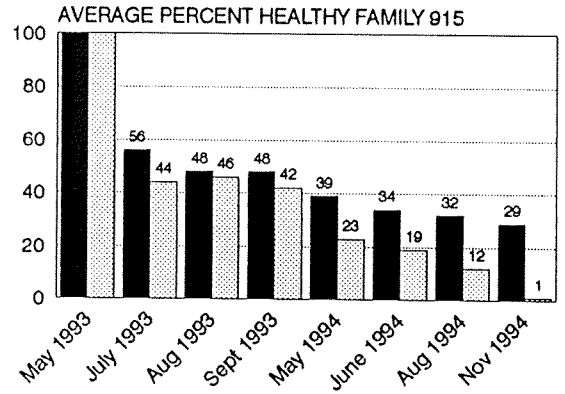
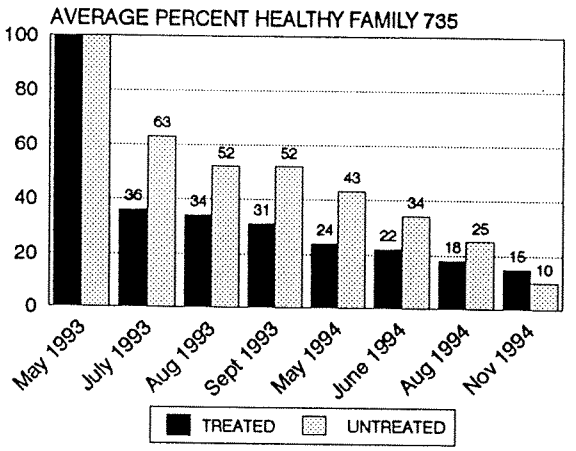
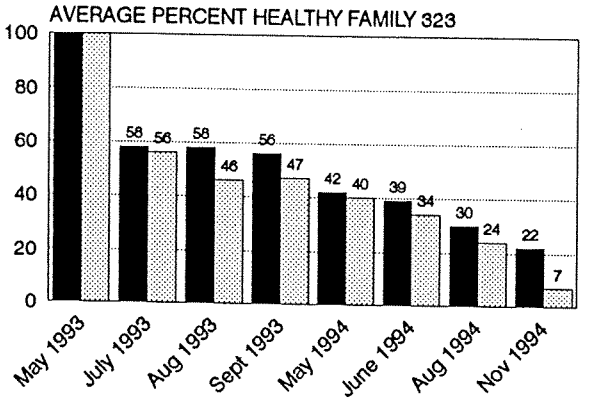
WATAUGA NORTHERN RED OAK SEED ORCHARD

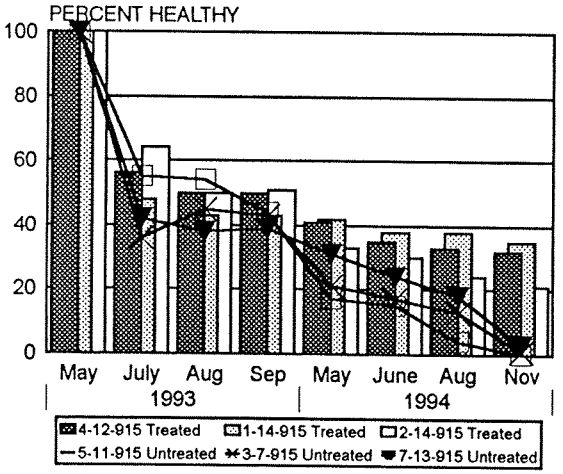
FAMILIES 915, 735, & 323 TREATED VS UNTREATED

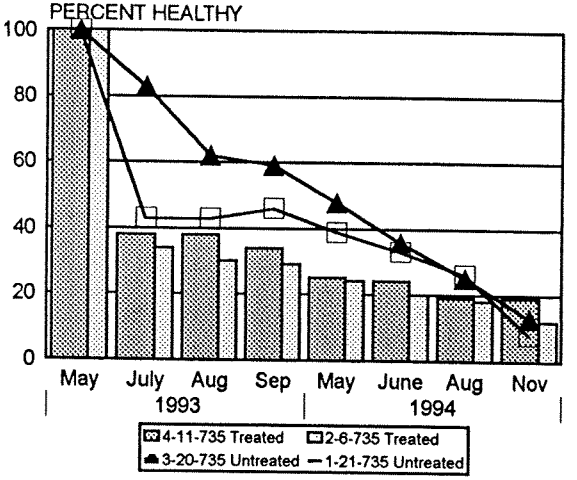
1993 FLOWER CROP SURVIVAL

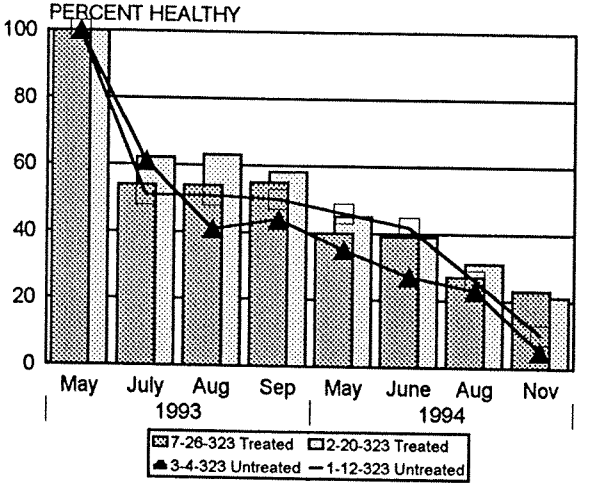










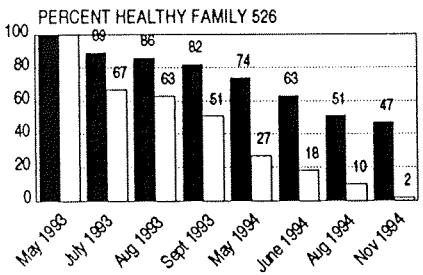


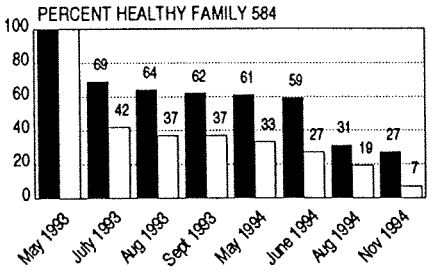
ORIGINAL TAGGING IN 1993 INCLUDED 20 BRANCHES WITH FLOWERS PER TREE.
THREE TREES TREATED MONTHLY WITH ASANA XL 1993 THRU 1994, THREE TREES UNTREATED
NOVEMBER 1994 ALL REMAINING TAGGED ACORNS WERE HARVESTED AND DISSECTED TO DETERMINE HEALTH

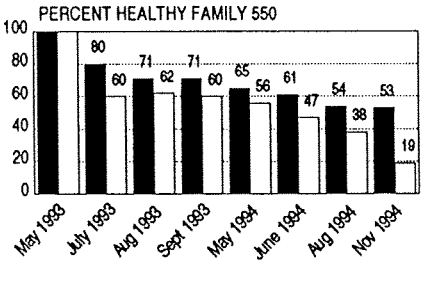
APPENDIX 1

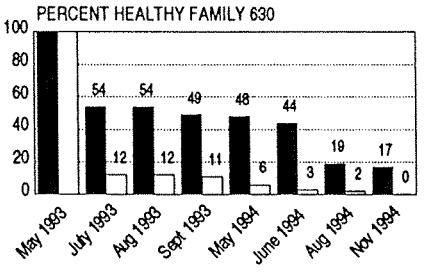
WATAUGA NORTHERN RED OAK SEED ORCHARD

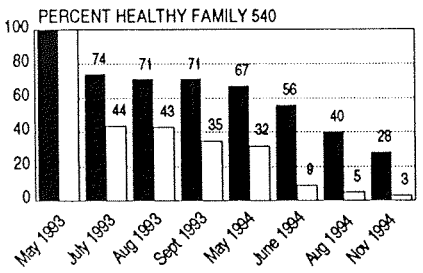
FAMILY COMPARISONS OF TREATED AND UNTREATED TREES

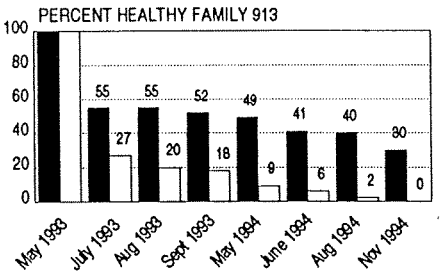


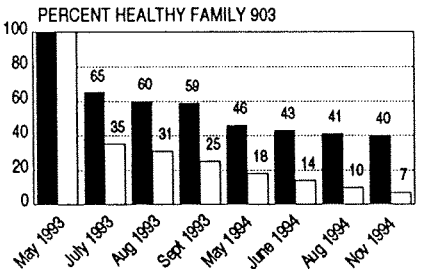


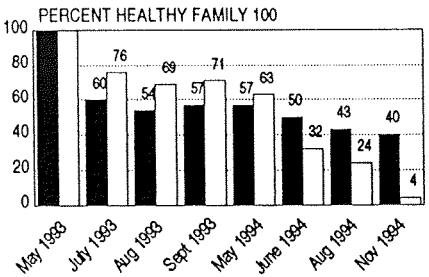


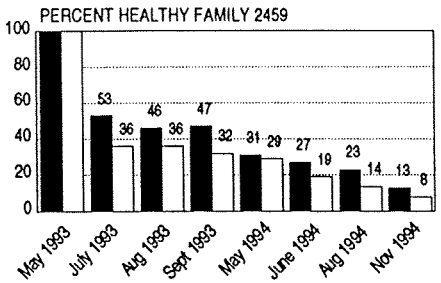


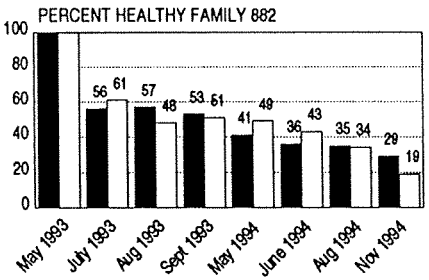


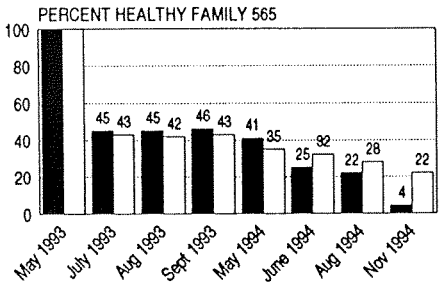


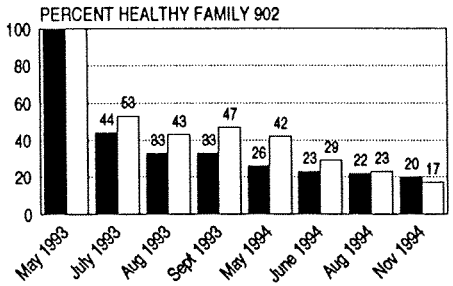


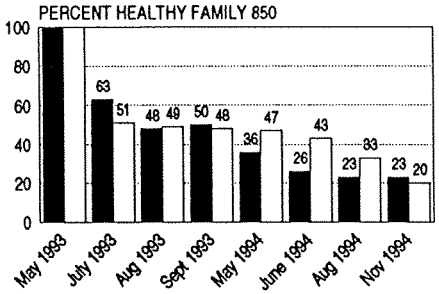












TREATED UNTREATED

TWENTY BRANCHES WITH FLOWERS WERE TAGGED IN MAY 1993.
TREATED TREES WERE TREATED MONTHLY WITH ASANA XL 1993 THRU 1994.
NOVEMBER 1994 ALL REMAINING TAGGED ACORNS WERE HARVESTED AND DISSECTED TO DETERMINE HEALTH

APPENDIX 2

SAMPLING VARIATION IN BLACK WALNUT (*JUGLANS NIGRA* L.) OUTCROSSING RATES AND FIXATION INDICES

Jinghua Zuo, F. H. Kung and George Rink[1]

Abstract: We studied black walnut mating parameters derived from electrophoretic analysis of open-pollinated embryos from local Jackson County, Illinois trees. Due to the periodicity of black walnut bearing, nuts could not be sampled from the same trees in all consecutive years. Thus we had nut collections from mother trees common to several or all years (1984, '87, '89, '91 and 1992) as well as nut collection from mother trees not common to all collection years. Also, there were sampling differences in allozyme patterns; in some years certain allozyme systems did not display variation and were not included in the data analysis. The objective of this study was to detect what effect the use of different open-pollinated families (i.e., mother trees), different years, and allozyme systems had on estimates of outcrossing rate and fixation indices. We applied analysis of variance to estimates of outcrossing rate and fixation indices developed from different subsets of data. The overall average outcrossing rate based on all five collection years was 0.97 (st. dev. = 0.17); the average inbreeding coefficient was -0.09 (st. dev. = 0.26). The implication of these parameters is that black walnut is predominantly an outcrossing species. However, results of the analysis of variance indicated that seed collection years had significant effects on outcrossing rates. Apparently black walnut outcrossing rates fluctuate somewhat from year to year. Outcrossing rates developed from use of seed collections with common mother trees were not significantly different from those developed using collections from trees uncommon to all years. Differences in allozyme systems used from year to year also had no significant effect on outcrossing rates.

Keywords: *Juglans nigra* L., isozymes, outcrossing.

INTRODUCTION

Black walnut (*Juglans nigra* L.) is an economically important species native to most of the eastern and central parts of the United States and southern Ontario, Canada. Black walnut is a monoecious, heterodichogamous, wind-pollinated tree species. It often occurs as a scattered, isolated tree or in small groups in the forest (Funk 1970). Pure natural stands of walnut are rare and usually small. Chief associates include yellow-poplar, white ash, black cherry and oaks (Schlesinger and Funk 1976). Low frequency of occurrence may result in inbreeding depression leading to poor tree quality in future generations. Intense harvesting may aggravate this by removing the best genotypes thus reducing the gene pool (Beineke 1989). Therefore, black

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walnut genetic resources need to be analyzed and conserved.

Population mating systems determine how individuals within a population mate and how the genetic information is transmitted between generations. Species with high outcrossing rates often maintain high genetic diversity with small differences among populations and high within population variation (Adams and Birkes 1991). If the mating system is mixed or if selfing is a part of the mating system, heterozygosity is typically lower. As a result, differentiation among such populations may increase (Loveless and Hamrick 1984; Hamrick and Godt 1990; Schoen and Brown 1991).

The earliest studies of mating systems often relied upon morphological markers. Such research was based on the behavior of pollinators and controlled crossing experiments. The results were often limited to the genotypes tested, the environments experienced, and the mode of measurement. For example, controlled pollination experiments were conducted to study the effects of inbreeding in black walnut (Beineke et al. 1976; 1977). Fortunately, the discovery of abundant and frequently codominant allozyme polymorphisms provided a valuable tool for estimating mating systems in tree species more effieiently. Electrophoresis was used to study allozyme variants (alleles) associated with specific genes to provide direct estimates of genetic variation at the DNA level. Such estimates are relatively free of environmental effects (Brown and Moran 1979). However, sampling variation among experimental units can affect mating parameter estimate. Brown (1990) found that using a larger number of families helped estimate the maternal fixation index accurately and stabilized variation of the inbreeding coefficients among loci. Shaw and Allard (1981) also found that use of different loci provided different results in Douglas-fir (*Pseudotsuga menziesii*). It was suggested that sampling more families and more polymorphic loci per family can increase precision in estimating mating system parameters (Shaw and Allard 1982). However, if alleles of marker loci or genotypes are associated with different flowering time, then heterogeneity in allele frequencies for these loci may result in temporal variation of estimates of mating system parameters (Sampson et al. 1990). In addition, micro geographic differentiation can also lead to inflated estimates of self-fertilization (Brown and Moran 1979).

Due to the periodicity of black walnut nut bearing, nuts could not be collected from the same trees in consecutive years. Thus sampling different families in different seed collection year could potentially affect estimation of mating system parameters. In addition, different enzyme systems were assayed in some years in attempting to find the best suitable isozyme markers for discriminating among black walnut genotypes. In this study we test the effect of different sampling schemes on the estimating mating system parameters.

MATERIALS AND METHODS

In the fall of 1984, 1987, 1989, 1991 and 1992, about 900 nuts per year were collected from the ground under the crowns of 24 to 37 trees that seemed representative of naturally growing walnut trees. No other selection criteria were imposed on the trees from which collections were made. Trees included are located in Jackson County, Illinois. To reduce the possibility of sampling closely related individuals, the distance between sampled trees was set at a minimum of 10 m, but most trees were between 0.5 and 1 km apart. Nuts collected from

these trees were dehusked and stratified at 2-5° C in the refrigerator. Embryos were removed and frozen at -80° C until used.

Before electrophoresis, embryos were individually ground and homogenized with an extraction buffer. The extraction buffer chosen was Marty et al. (1984). After homogenization, filter paper wicks were used to soak up the resulting liquid. A 2 mm x 15 mm wide wick per embryo was used to load samples into the gel with 30 samples per gel. The gels were placed into a refrigerator and 50 milliamperes/gel maximum electric current was applied for about 4.5 hours. After the first 20 minutes, the sampling wicks were removed from the gels to improve quality of enzyme migration.

When the indicator dye marker arrived at the anodal end of the gel (4.5 hours), the electric power was turned off and gels removed from the refrigerator. Each gel was marked in the upper right-hand corner of the gel to indicate its identity. Plexiglass guides (20mm x 240mm x 1mm) and nylon sewing thread were used to slice the gel. Each gel was sliced into 8 to 10 horizontal slices with the top slice being discarded.

The gel slices were stained and then rinsed with distilled water three to four times and placed onto the light table to score the allozyme bands. Eight enzymes with enzyme activity were analyzed: aconitase (ACO); alcohol dehydrogenase (ADH); aspartate aminotransferase (AAT); 6-phosphogluconic dehydrogenase (6PG); phosphoglucose isomerase (PGI); fluorescent esterase (FEST); acid phosphatase (ACP); and phosphoglucomutase (PGM). These eight enzymes gave eleven isozyme loci detectable variation: ACO-1, ACO-2, ADH, AAT-1, 6PG-2, PGI-2, FEST, ACP-2, PGM-1, PGM-2, PGM-3.

We partitioned the complete data set according to four control variables: (1) two levels of family identity (common between years as yes and no); (2) four levels of repeated seed collection (repeated 2, 3, 4, or 5 times); (3) five levels of sampling year (sampled in 84, 87, 89, 91, or 92); and (4) three levels of enzyme identity (alike loci, different loci, or all loci).

Each data subset was processed by the Multilocus Estimation Program (Ritland and Jain 1981) to obtain one estimate each for multilocus outcrossing rate (MT) single locus outcrossing rate (ST) and fixation indices (f). We had a total 179 subsets of data to estimate the mating parameters.

The three mating parameters (MT, ST, and f) were used as dependent variables and the four factors (CYON, NYR, YR and EM) were used as classification variables in a general linear model to test the null hypothesis that there are no differences among main effects and no differences among two-factor interactions. The F-values for testing each effect were obtained from PROC GLM (SAS 1988).

RESULTS AND DISCUSSION

The linear models for the three mating parameters are all significant. More than 60% of the total variance in outcrossing rate and 37% in inbreeding coefficient were explained by the linear model. The population mean outcrossing rate in black walnut was high in comparison with

its root mean square error (RMSE). Results for the multilocus estimate are similar to that for the single locus estimate (mean MT = 0.969 and mean ST = .965, RMSE MT = 0.128 and RMSE ST = .146). The implication of high outcrossing rate and low RMSE indicate that we can accept the hypothesis MT=ST=1 and reject the hypothesis MT=ST=0. Furthermore, the population inbreeding coefficient was not significantly different from zero (mean =-0.094, RMSE = 0.234). Thus, black walnut is predominantly an outcrossing species.

None of the main factors were significant for the fixation index. Outcrossing rates and fixation indexes developed from use of seed collections with common mother trees were not significantly different from those developed using collections from trees not represented in all years. This is in agreement with random sampling theory where sampling with replacement, sampling with partial replacement, and sampling without replacement all result in essentially the same estimate of population mean (Cochran 1977). Also, there was no significant effect of using various numbers of years in repeated seed collection. Among the four main factors, the effect of collection year was significant on multilocus and single locus outcrossing rates (Table 1). Apparently black walnut outcrossing rates fluctuate somewhat from year to year. Outcrossing rates were lower in the early years (1984 and 87) than that in the later years (1989, '91 and '92) The use of the same or different enzyme systems did not seem to have an effect on estimating the multilocus outcrossing rate, but did affect the single locus outcrossing rate. Using noncommon loci data resulted in lower values for the single locus outcrossing rates than when only common loci were used.

Table 1. F-Value and level of Significance for the four main effects and for the six interactions. The dependent variables in the general linear model were multilocus (MT) and single (ST) locus outcrossing rate, and inbreeding coefficient (f).

Source	DF	F-Value for		
		MT	ST	f
CYON	1	2.23	0.14	3.82
NYR	3	0.13	0.03	1.12
YR	4	18.29***	24.92***	1.92
EM	2	1.32	5.79***	0.40
CYON*NYR	3	0.65	0.46	0.74
CYON*YR	4	5.03***	3.92**	4.69**
CYON*EM	2	2.36	1.52	0.41
NYR*YR	12	0.61	1.04	1.09
NYR*EM	6	1.27	0.33	0.24
YR*EM	8	6.68***	10.30***	3.39**

*** Significant at the 0.001 level.
** Significant at the 0.01 level.

The interaction between family replacement and collection year was significant (Table 1). Outcrossing rates peaked at 1987 for common families, but for the noncommon families the

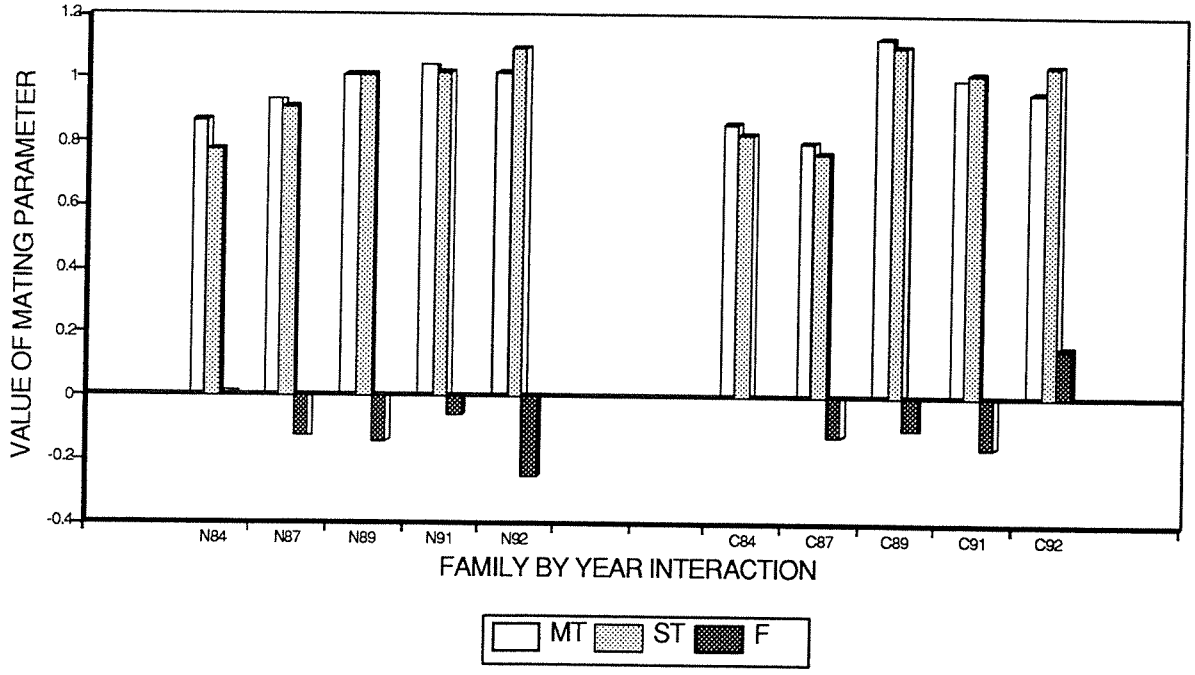

Figure 1. Multilocus (MT), single locus (ST) outcrossing rate and fixation index (F) between common (C) and noncommon (N) families in five years.

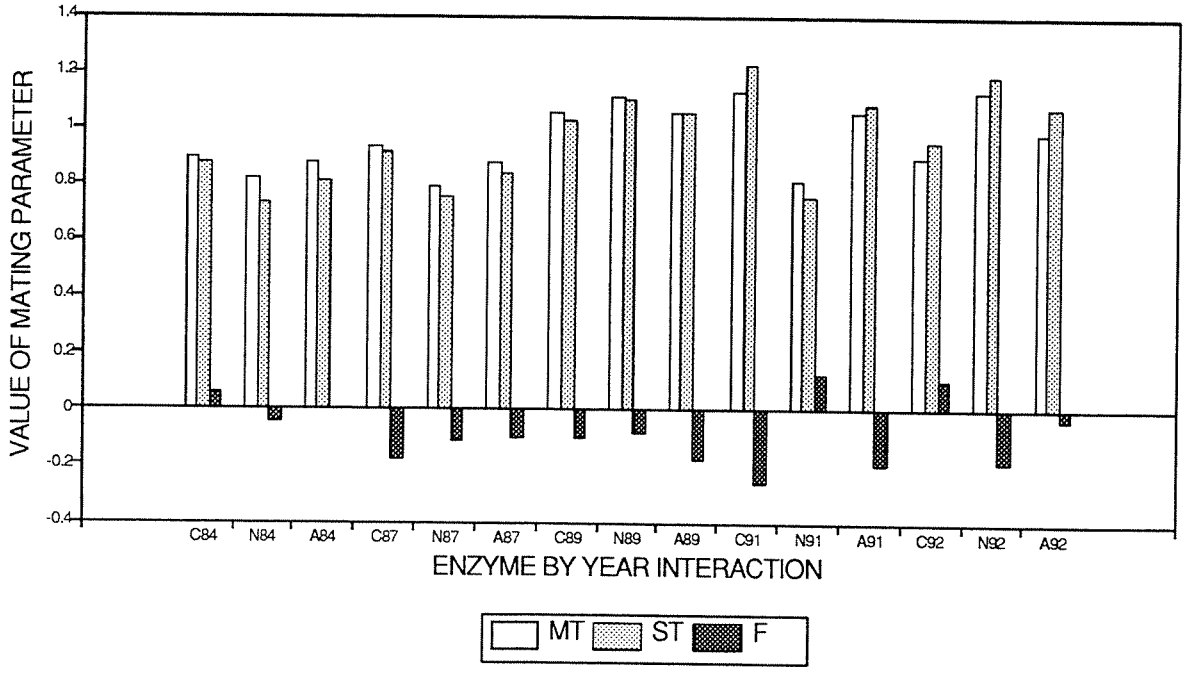

Figure 2. Multilocus (MT), single locus (ST) outcrossing rate and fixation index (F) among alike (C), different (N) and all (A) loci in five years.

outcrossing rate remained average. The trend was different between common and noncommon families; both multilocus and single locus outcrossing rates increased from 1984 to 1987 when data from the noncommon families were used, but with common families, outcrossing rates decreased (Figure 1). The fixation indeces from '87 to '92 were all negative for the noncommon families, but for common families the fixation index became positive in 1992.

Another significant interaction was observed with enzyme and year effects (Figure 2). In 1989 and in '92, the use of different loci provided higher estimates for outcrossing rates than the use of identical loci. However, in 1987 and in '91, outcrossing rates were lower if different loci were used. The trend was dissimilar between alike loci and different loci. The fixation index in 1991 was negative and in 1992 was positive for using alike loci, while using different loci the estimate in 1991 was positive and in 1992 was negative (Figure 2)

CONCLUSION

The choice of using alike or different loci will affect the estimate of single locus outcrossing rate appears to have a lessen impact on the multilocus outcrossing rate. The error variance for the multilocus outcrossing rate is smaller than that for the single locus outcrossing rate. Thus, using multilocus outcrossing rate for estimating mating parameters is preferred.

Mating parameters estimated from single year data may not be representative of the population mean. The choice of loci and the choice of families will affect the single year estimates. However, the choice of loci and the choice of families may have no effect on the mating parameters if more samples were collected in many years.

For the study of outcrossing rates, it is recommended that seeds should be collected for several years. When the same mother trees were not producing adequate number of nuts during the lean years, additional nuts may be collected from additional different mother trees. It may not be necessary to use the identical allozyme systems throughout the course of study.

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GENETIC ANALYSIS OF PUTATIVELY APOMICTIC SEED FROM AMERICAN SYCAMORE

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Abstract.--While conducting controlled pollinations of sycamore (*Platanus occidentalis* L.), it was observed that all six seed trees produced viable seed from the unpollinated flowers used as pollination controls. If the seeds proved to be of apomictic origin, exclusion of pollen would be an efficient means of cloning mature sycamore trees. We identified heterozygous loci in the five seed trees by screening for random amplified polymorphic DNA (RAPDs) markers that segregated in a 3:1 (band present:band absent) ratio in selfed progeny. Any individual seedling, or cohort, of apomictic origin should be band present for all heterozygous loci in the mother tree. We found no evidence for any of the five families of putative apomicts being of only asexual origin. Only five individuals out of 115 putative apomicts had the same RAPD banding patterns as the mother trees. Based on estimated gene frequencies, these five individuals are possibly of asexual origin and warrant further research. On average, these five individuals represent only 0.076% of all seeds (viable and nonviable), and only 4.3% of the viable seeds, from the unpollinated cohorts. The very low percentages of possible apomicts indicate that pollen exclusion is unlikely to be an efficient means of cloning mature sycamore trees.

Keywords: *Platanus occidentalis* L., random amplified polymorphic DNAs, RAPDs, arbitrarily primed PCR, apomixis, clone.

INTRODUCTION

The American sycamore is a common bottomland hardwood widely distributed throughout the southeastern United States, and has good potential for use in biomass production (Tuskan and De-la-Cruz 1982). Genetic improvement programs for sycamore are in progress in the southeastern United States, using methods for controlled crosses (Land 1991) and vegetative propagation (Land and Cunningham 1994). The use of vegetative propagules is of interest because they capture non-additive genetic variation in addition to

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additive genetic variation (Zobel and Talbert 1984). As Biondi and Thorpe (1982) have reviewed, techniques for vegetative propagation in forest trees (including advanced layering, rooted cuttings, grafting and budding, and tissue culture) are becoming practical reforestation methods. However, most methods of vegetative propagation are highly sensitive to the effects of maturation of the donor tree (Bonga 1982). Additionally, when compared with seed propagation, poor survival and growth of some vegetative propagules, coupled with the high cost per propagule, have offset the genetic gain advantage for many forest trees (Aimers-Halliday et al. 1991). Therefore, an efficient clonal propagation method that is able to supply large numbers of clones, is still needed.

As part of an on-going sycamore breeding program, controlled pollinations were conducted at Mississippi State University in the spring of 1988. It was observed that bagged flowers serving as pollination controls (i.e., no pollen was applied) averaged 2.3 percent sound seeds per bagged, globular head of pistillate flowers. These seeds could have resulted from pollen contamination, selfing, or apomixis (Bashaw 1980). If the seed proved to be of apomictic origin, the exclusion of pollen might be an efficient means for cloning mature trees.

Random amplified polymorphic DNAs (RAPDs)(Welsh and McClelland 1990, Williams et al. 1990) are a type of DNA marker based on the polymerase chain reaction (PCR). RAPDs overcome some limitations of traditional PCR (Caetano-Anolles et al. 1992) and have gained widespread acceptance and use. The RAPD technique can be used to detect genetic variation among individuals within a species (Williams et al. 1990), and has proven to be a useful genetic fingerprinting technique for parentage determination (Welsh et al. 1991), kinship relationship analysis (Hadrys et al. 1992) and pathotype identification (Goodwin and Annis, 1991). RAPDs were useful in assessing the validity of controlled crosses in hardwood species (Roy et al. 1992) and should be useful for determining clonal identity (Smith et al. 1992).

The objectives of this study were to identify RAPD markers in the seed trees used for contolled pollinations, and then to use these markers to determine the origin of the seed in the unpollinated seedlots.

MATERIALS AND METHODS

Leaf tissue was collected from the five parent trees and 15 to 30 progeny from self-pollinated and unpollinated treatments from each tree. All samples were stored at -85°C prior to use. DNA from ten grams of frozen leaf tissue from each sample was extracted by modified procedures of Murray and Thompson (1980). Two µg of DNA from each extract was further purified using the BioRad Prep-A-Gene™ DNA Purification Kit (BioRad). The RAPD reaction was performed in a modification of Williams et al. (1990) protocol consisting of the following in a 25 µl reaction volume: 2.0 ng of template DNA, 2.5 µl of 10x buffer (500 mM KCl, 100 mM Tris-HCl, pH 8.4), 2.0 µl of $MgCl_2$ (25 mM of $MgCl_2$), 2.0 µl of dNTPs (1.25 mM of each dNTP), 1.0 µl of Operon® Primer (5 µM), 1 unit of *Taq* polymerase, and sterile distilled water. The mixture was loaded in 96 well Falcon® 3911 MicroTest III™ Flexible Assay Plate (Becton Dickinson), overlaid with 50 µl of sterile mineral oil, covered with Saran Wrap® and placed in a programmable temperature cycler.

Nelson et al. (1993). Amplified products were separated in a 1.4% agarose gel in 1x TAE buffer.

Seed trees, and 15 to 30 selfed progeny from each family, were screened with primers to identify heterozygous loci in seed trees. At least ten heterozygous loci which were band present in a seed tree and segregated (both present and absent) in its selfed progeny, were identified in each family. Chi-square analysis was conducted to determine if a locus was inherited in a 3:1 (band present:band absent) ratio as expected for a dominant marker in the selfed progeny. Next, the unpollinated progeny were screened using the primers that identified heterozygous loci in the seed tree. If the putative apomicts were of somatic origin, the apomicts would be band present at all loci that are heterozygous in the seed trees. If one, or more than one, of the bands was absent in an individual, this individual was scored as not being of apomictic origin.

If an individual was band present for all identified loci, the probability that this putative apomict was of asexual origin was estimated with the following formula: for a particular locus i, the frequency of the band present allele from the female heterozygote of locus i was assumed to be 0.5. If p_i is used to represent the frequency of the band present allele of the ith locus in the pollen pool, then for the particular locus i, the probability that an individual resulting from pollen contamination has a particular band present is expected to be:

$$P_i = 0.5+p_i/2.$$

Then, p_i can be estimated as:

$$p_i = 2P_i - 1.$$

Assuming that loci in each family were independent, then the probability of an individual resulting from pollen contamination (P_C) being band present for all of the loci can be estimated to be:

$$P_C = \prod_{i=1}^{n} (0.5 + P_i/2)$$

RESULTS

Ten or eleven heterozygous loci in each of the five maternal trees were identified and are listed in Table 1. A chi-square (χ^2) test was performed to verify that the polymorphisms were segregating as a single genetic locus in the selfed progeny (3:1 Mendelian ratio was expected), and 15 of the 53 loci were rejected (α=0.05). In addition, inheritance of some loci in each family could not be distinguished from a 1:1 ratio. After ten or eleven heterozygous RAPD loci were identified in each seed tree, these loci were scored in the unpollinated individuals. Only five individuals, out of 115 unpollinated progeny, were band present for all identified heterozygous loci (Table 2).

Table 1. Identified heterozygous loci for each family.

Marker	Operon Primer/Fragment Size(kb.)				
	B209-08	O110-09	K110-19	H205-55	S210-19
1	A17/2.4	C8/0.72	A17/2.3	A7/0.8	A7/1.15
2	C4/0.9	X1/1.75	A17/2.0	D8/1.55	A7/0.8
3	C8/0.72	X1/1.5	A17/1.0	W2/0.9	A17/1.0
4	E2/0.95	X1/0.75	A20/1.4	W4/0.35	A17/0.75
5	W11/1.2	X18/1.15	C8/1.2	W6/0.5	X2/1.5
6	W11/0.85	Y1/0.7	C8/0.72	W11/1.1	X2/0.8
7	X4/1.25	Y1/0.62	C8/0.62	X4/0.7	X4/1.45
8	Y3/0.9	Y3/1.55	X1/1.75	X4/0.5	X9/1.5
9	Y5/1.8	Y3/1.0	Y9/0.65	Y1/0.8	Y20/1.5
10	Y13/0.6	Y3/0.9	Y13/1.4	Y1/0.7	Y20/1.4
11		Y13/0.6	Y13/0.6	Y13/1.4	

Table 2. Summary of heterozygous loci in the mother trees that are found in the putatively apomictic progeny.

Parent	Number of Heterozygous Loci in the Seed Trees	Putative Apomictic Progeny		
		Number of Progeny	Number of Loci Scored Band Present[1]	# of Individuals Band Present at All Loci[2]
B209-08	10	22	4~10	2
O110-09	11	15	7~11	2
K110-19	11	22	3~9	0
H205-55	11	27	4~10	0
S210-19	10	29	5~10	1

[1]Range in the number of loci present per individual in each cohort.
[2]Individuals that are band present for all loci segregating in the mother trees.

In order to estimate the likelihood of an asexual origin of the five individuals with all loci fixed, the frequencies of band present alleles (p_i) in the pollen pool at all the 53 loci were estimated. The result indicated a 3% probability that individuals 12-110 and 12-112 from family B209-08 are from pollen contamination; individuals 22-201 and 22-305 from family O110-09 have a 5% probability of being from pollen contamination. Additionally, individual 62-220 from S210-19 has about a 1% chance being from pollen contamination.

DISCUSSION

There are three plausible explanations for the existence of full seed obtained from unpollinated (control) flowers in this study. One possible explanation for seed production without the addition of external pollen would be the existence of hermaphroditic flower structures in sycamore. We found no published literature on the existence of hermaphrodism in sycamore, and no evidence of hermaphrodism has been noted for any of the trees used in this study. A second possible origin of the seed is pollen contamination from surrounding trees. Since the pollen exclusion bags were not placed over the buds until they began to open, and vegetative buds could be differentiated from flower buds, there was the possibility of pollen contamination. A third possible origin of the seed, and one which could simplify the clonal propagation of superior genotypes, would be the existence of apomixis.

Parthenogenic origin of the seeds in the unpollinated progeny was impossible for two reasons. First, when comparing mother trees with their unpollinated progeny, we found extra bands in the unpollinated progeny, which would be inconsistent with parthenogenic origin. Second, parthenogenic seeds should show a low germination rate and poor growth (Richards 1986). The trees resulting from the unpollinated control seeds are growing as well as open pollinated sycamore trees.

Based on the models of dominant inheritance of RAPD markers, progeny from selfed pollinations will produce banding patterns that represent a subset of the parental patterns (with heterozygous loci in the parent segregating in a 3:1 ratio in the progeny). However, we found that the selfed progeny, in comparison to the parent tree, had some non-parental bands. In fact, these additional bands were commonly found in each family. Limitations of RAPD markers, such as the possibility of co-migration, non-specific amplification or even artifact caused by mismatch at primer sites (Williams et al. 1990) may account for some of the variants we observed. Therefore, certain markers may be amplified unreliably, and may not represent useful genetic variation (Riedy et al. 1992). However, it has been confirmed that most of the RAPD markers are inherited in a Mendelian fashion and therefore will make them reliable and valuable tools (Williams et al. 1990). The reproducibility of most RAPD markers in this study excludes non-specific priming and other artifact as causes for the non-parental bands (Hadrys et al. 1992). To minimize artifacts of RAPD markers and exclude most of the unreliable RAPD polymorphisms, we selected only specific, reproducible heterozygous loci in mother trees to estimate levels of pollen contamination and apomictic production in sycamore.

A dominant marker (e.g. a RAPD marker) will segregate in a 3:1 ratio in the selfed progeny of a heterozygous individual. However, there are several reasons for the segregation of alleles to depart from a 3:1 ratio. One possibility, which would affect the segregation ratio, is that of self-incompatibility (Richards 1986). Furthermore, gametophyte selection or linked deleterious mutations could bias transmission or survival of those chromosomal regions bearing the RAPD markers (Echt et al. 1992). Additionally, amplification of non-nuclear DNA, such as mitochondrial DNA or chloroplast DNA, would follow the inheritance pattern of the organelles (Hadrys et al. 1992). The possibility of co-migration of fragments of the same size from non-homologous loci still exists, and cannot be ruled out without extra

analysis by Southern blotting (Hadrys et al. 1992). Due to the small sample size of selfed progeny, segregation of alleles at a locus can deviate from a 3:1 Mendelian ratio just by chance. Therefore, the loci in which bands were present in a mother tree and segregated in selfed progeny were selected to identify apomicts in unpollinated progeny if they segregated between 1:1 and a 1:0 ratio (band present:band absent). However, in family S210-19 seven of the ten loci were inherited in a 1:1 or a 1:0 ratio, and not a 3:1 ratio. This unusual departure from a 3:1 ratio was most likely the result of high levels of pollen contamination. These results also were consistent with the much higher percentage of sound seeds obtained from unpollinated flowers of S210-19; therefore, the identification of apomicts in this family is probably unreliable.

Another limitation of identifying heterozygous loci with this approach was that expected ratios in the progeny were based on the frequency of band present alleles in the pollen pool. If allele frequencies in the pollen pool approached the extreme, {i.e. a>>A or A>>a (A=band present allele, a=band absent allele)}, then the expected segregation ratio would approach 1:1 (band present: band absent) or 1:0 (all band present) in open pollinated progeny. If allele frequencies were equal in a given pollen pool, the expected ratio would be 3:1 (band present:band absent), similar to selfed progeny. Seed that resulted from open pollinated flowers would have band frequencies between 1:1 (band present:band absent) and all band present, depending upon the frequency of band present alleles in the pollen pool. Since heterozygotes could also occur from fertilization events such as open pollination described above, the presence of a maternal genotype at only one locus does not imply that the embryo is of apomictic origin. Therefore, when discussing the probability of apomixis, we could only establish a maximum value. The actual frequency of apomixis might be lower (Aly et al. 1992). By increasing the numbers of identified DNA markers, it would be possible to increase our confidence of identifying seed of apomictic origin.

Our conclusions are limited by small sample sizes; results from five families cannot represent all sycamore trees in all years. Also, the number of selfed progeny used for identifying heterozygous loci ranged from 18 to 30, and because of this small sample size, it is difficult to differentiate a 3:1 segregation ratios from 1:1 or 1:0. Furthermore, only five individuals out of 115 unpollinated progeny (approximately 4%) were identified as being possible apomicts. The small number of unpollinated seeds makes it difficult to accurately estimate the rate of apomixis. Additionally, all progeny from each seed tree came from one year's controlled pollinations, which were done in the same place and at the same time. Therefore, replication of this work would strengthen our conclusions.

CONCLUSIONS

Most of the putative apomicts resulted from pollen contamination. The greatly different frequencies of the band present alleles shown among different loci within a family and among the same loci from different families might be due to different pollen parents for each seed tree. The probability of an individual being produced by the apomictic process was estimated under the assumed condition of independence among the loci within a family. Only

0.076% of all seeds (both viable and nonviable seeds) collected from the unpollinated cohort might be apomicts and are unlikely to supply sufficient number of seeds for efficient cloning purposes.

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SEED TRANSFER AND GENECOLOGY IN LONGLEAF PINE

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Abstract.--Twenty seed sources of longleaf pine (*Pinus palustris* Mill.) were grown in seven locations in Georgia and Florida for 25 years. The plantings and seed sources approximated a north-south transect of the entire species range through Georgia and Florida, with plantings and seed sources representing all physiographic provinces. Tree heights were related to latitude and climatic variables with polynomial regression models. The most important climatic variable associated with north-south variation was average annual minimum temperature at the seed source. Results of different plantings were combined by expressing growth as a percent deviation from the local source and by expressing temperature at the source as a deviation from that of the planting site. The combined analysis using minimum temperature difference between the seed source and the planting site and the square of this value accounted for 58.9% of the total variation. The regression equation predicts that moving seed sources northward from areas with minimum temperatures 3° F warmer (approximately 100 km in central Georgia) than the planting site results in the maximum gain in height over local sources. Moving seed sources northward more than 6° F results in less growth than that of the local source. Ecotypic differentiation did not appear to be an important factor in geographic variation.

Keywords: *Pinus palustris*, provenance, geographic variation

INTRODUCTION

The area of longleaf pine (*Pinus palustris* Mill.) in the Southern United States has declined from 12.2 to 3.8 million acres over the past 30 years (Kelly and Bechtold 1990). In many ways, longleaf is the most valued of the southern pines (Croker 1990), and there is now a concerted effort to restore longleaf to its historical and ecological prominence.

Restoration of longleaf pine will necessarily require a great deal of planting (or perhaps direct seeding). Choosing the proper seed source will be essential to ensure long-term success. It is necessary to define geographic variation in longleaf pine precisely to identify suitable seed sources for restoration planting.

The most effective way to measure the range of genetic variation and limits to germplasm movement is to establish seed source studies or provenance tests. Ideally, long-term experiments are established using seed collected from natural stands, sampling from the entire natural distribution of the species and planted in common gardens in many locations representing the

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full range of climatic and edaphic variation. Growth of the trees is measured over a period of time, preferably to rotation age, since stress increases with age. Such provenance tests have been used for more than 200 years to examine geographic variation and genecology in forest tree species (Langlet 1971).

A major factor in the performance of a seed source in a particular location is the difference in climate between the planting site and the seed source. Seeds moved a modest distance northward often out-perform seeds from the local source (Wells and Wakeley 1966). If moved too far to the north, however, they suffer cold damage and do not perform as well as the local source. If moved to the south, they also do not perform as well as the local source. These results suggest a curvilinear relationship between growth and climatic differences between seed source and the planting location.

Differences in temperature are certainly important, and yearly average minimum temperature at the seed source was the best variable found to predict effects of seed transfer in loblolly pine (*Pinus taeda* L.) (Schmidtling 1994). In the present study, results of a provenance test established in Georgia and Florida were used to explore the genecology and to predict the effects of seed movement on the growth of longleaf pine.

MATERIALS AND METHODS

The study is unique in that intensive sampling was conducted on the entire north-south distribution of longleaf pine in Georgia and Florida (Fig. 1), as well as all the physiographic provinces where the species occurs. This test was established in 1970.

Plots representing single seed sources contained five rows of five trees at 2.5-m by 2.5-m spacing. These plots were replicated six times. Trees from 20 sources were planted at each site. Kraus and Sluder (1990) completely described the study. Climatic and other location data, in addition to those in Wells and Wakeley (1970), were obtained from USDA Forest Service (1969), USDA Agricultural Research Service (1990), and NOAA (1991).

In an initial examination, 15-year data from each of the seven plantings was reanalyzed separately. Mean heights and survival of the provenances were used as dependent variables. Independent variables included each seed source's latitude, mean temperature, yearly average minimum temperature, frost-free period, and the squares of these. The variables were included in step-wise multiple regressions to determine the most important variables. Overall, mean temperature and minimum temperature are probably the most useful, apparently because they integrate the effects of latitude, elevation, and maritime effects into a single variable. It also is important, however, to know what other variables are affecting growth.

In the spring of 1995, height and DBH were measured in four of the plantings (111 through 114), and DBH only was measured in the southernmost planting, number 115 (Figure 1). Plot volumes or basal areas were analyzed in the same manner as the 15-year data.

In the regression analysis of combined data, the percent survival and the percent deviation in height or volume from the local source were the dependent variables. Independent variables

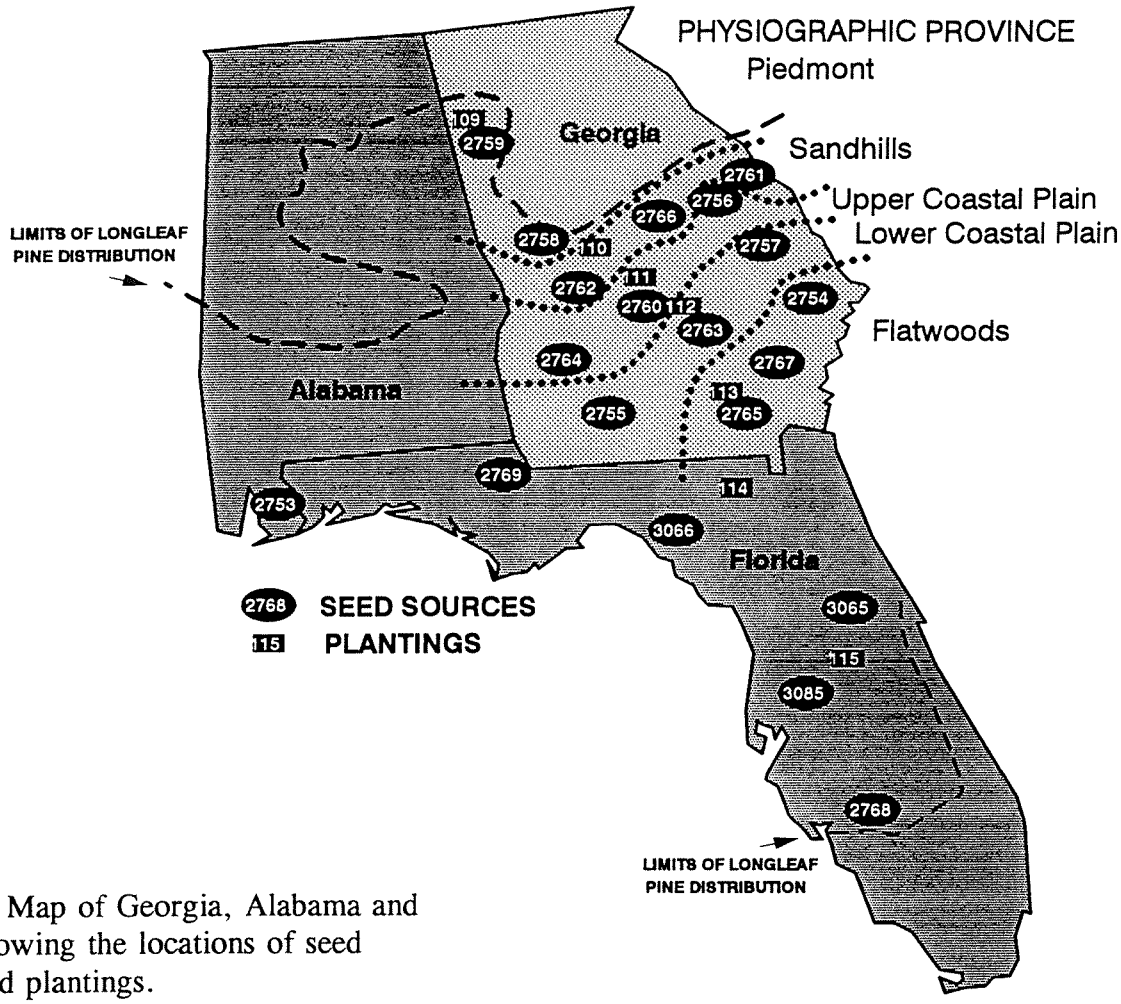


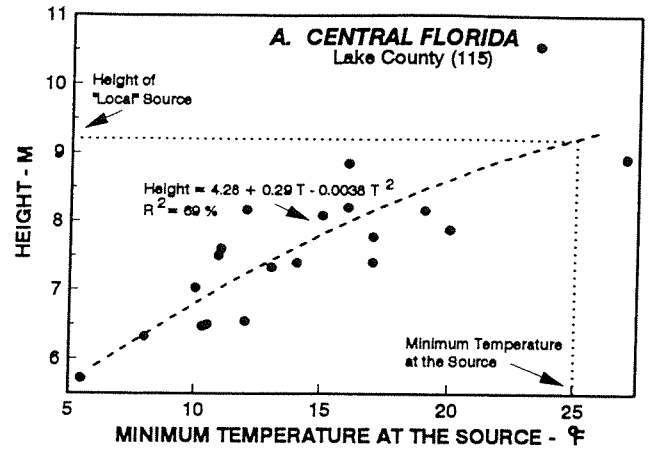
Figure 1. Map of Georgia, Alabama and Florida showing the locations of seed sources and plantings.

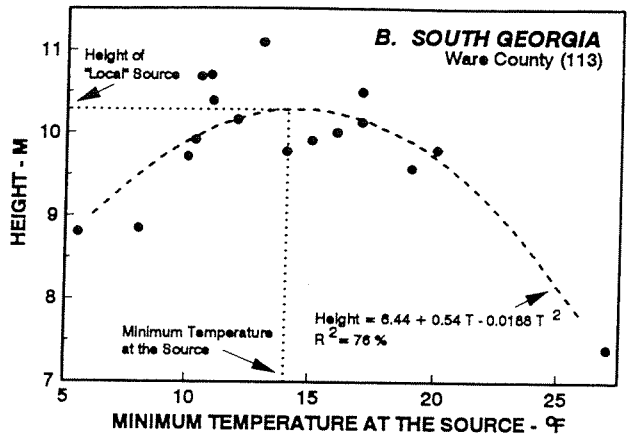
were the differences between the location and the seed source for latitude, minimum temperature, mean temperature, frost-free period and rainfall, and the squares and cross-products of these variables. Differences among physiographic provinces (Figure 1) were examined by plotting residuals from the regression models.

RESULTS AND DISCUSSION

In the original analysis of the study, there was a strong interaction between planting location and seed source (Kraus and Sluder 1990). Their analysis showed that the relative performance of the seed sources depended on the planting location. This result is common, and even expected, in seed source studies, and is evident when comparing height growth with temperature in Figures 2a-c.

Consistently, the best single predictor for height growth was average annual minimum temperatures at the seed source. In only one of seven plantings, planting 111 (Figure 1), was no relationship found between height and any independent variable. In the other six





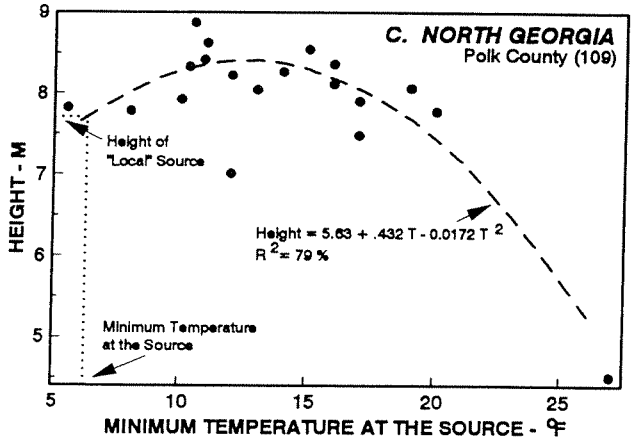


Figure 2. Height at age 15 versus yearly average minimum temperature at the seed source for three selected plantings representing southern (A), central (B), and northern (C) responses.

plantings, from 51 to 79 percent of the variation in height at age 15 years was explained by a quadratic relationship with minimum temperature at the seed source. Latitude, mean temperature, frost-free season, and the squares of these variables also were significantly related to height in individual analyses.

The relationship between height and minimum temperature at the source was nearly linear in the most southern planting (Figure 2a). The sources from areas with the highest minimum temperature, those from the southernmost collection points, were the tallest, up to 10.6 m, and those from area of the lowest minimum temperature averaged less than 6 m. The climate in this southern planting was not cold enough to adversely affect the growth of any source.

A curvilinear relationship between minimum temperature at the source and growth is apparent in a mid-latitude planting (Figure 2b). The linear regression with minimum temperature explained 36 percent of the variation in height; adding the square of minimum temperature improved the fit to 76 percent. The sources with the tallest trees at age 15 were those that were collected from areas with minimum temperatures somewhat above that of the planting location--those from south of the planting location. Seed sources from climates colder than the planting location, as well as those from climates much warmer than the planting location, did not grow as well as the local stock.

The curvilinear relationship also can be seen in a plot of height versus minimum temperatures in one of the northern plantings (Figure 2c). A linear fit with minimum

temperature at the seed source explained 36 percent of the variation; addition of minimum temperature squared improved the fit to 79 percent. In this planting, the poor performance of sources from far south of the planting site is more evident than in the mid-latitude planting (Figure 2b).

Differences in site index present difficult problems in combining data from different locations (Mátyás and Yeatman 1992). The approach used in the present study was to first express growth as a percent deviation from the local source, and then combining the data from the different plantings.

The definition of the "local source" is often problematical, and in this study, exact local sources do not exist for many of the plantings. There may be one or more sources from nearby areas that could be used. In this analysis, the height of the "local" source was determined by regression (Figure 2a-c). As Mátyás and Yeatman (1992) have pointed out, the height of the local source is not known without error. Using a regression model to determine this height may result in less error than using any one particular source.

When combining studies from different locations, the differences in latitude, temperature, precipitation, etc., between the planting location and the seed source are probably more important than the absolute values of these variables. Giertych (1977) used "latitude displacement" to combine seed source data from several nurseries. In developing their seed transfer model in jack pine (*Pinus banksiana* Lamb.), Mátyás and Yeatman (1992) used the difference in latitude and the difference in heat sums between the planting location and the seed source to define "ecological distance."

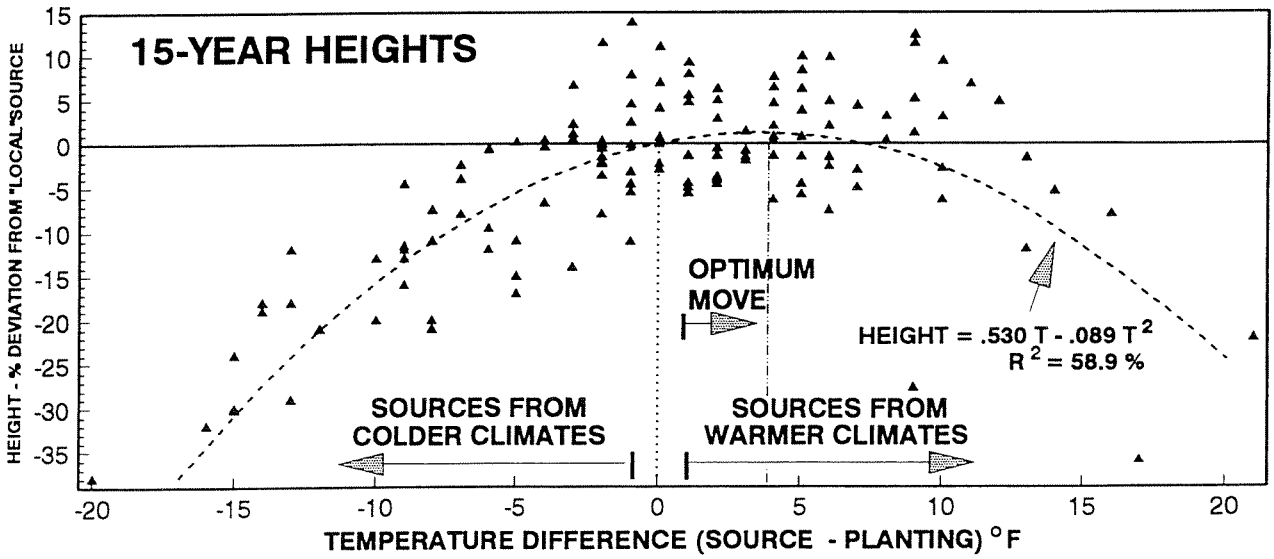


Figure 3. Height at age 15 versus minimum temperature for the combined data. On the vertical axis, heights are expressed as deviations from the local source. On the horizontal axis yearly average minimum temperatures are expressed as differences between the seed source minimum temperature and the planting location minimum temperature.

Minimum temperature difference and minimum temperature difference squared were the most important variables when the data from the seven plantings were combined (Figure 3). This combination accounted for 58 percent of the variation in height deviation from the local source. No other independent variables were significantly related to height deviation in the multiple regressions in this study, after effects of temperature were accounted for.

The analyses that are summarized in Figure 3 show that moving seed sources northward from areas with minimum temperatures of 3 °F warmer than the planting site result in the maximum gain over local sources. Moving seed sources southward or northward more than 6 °F results in less growth than that of the local source.

Survival at age 15 years varied significantly by seed source, but the seed source by planting interaction was minimal (Kraus and Sluder 1990). There was very little additional mortality in any of the measured plantings at age 25. In general, the sources from the coldest climates survived better. The sources from colder climates are adapted to colder winter temperatures and shorter growing seasons. Dormancy is longer and deeper, allowing them to survive adverse conditions. As a result of the shorter active growing season, they generally grow slower than sources from warmer climates. However, there is a tendency for lower than expected survival when northern sources are moved very far south. If survival is analyzed in the same manner as height, the combined data predicts that survival will be reduced if seed sources are moved from a warmer to a colder climate, and enhanced if seed sources are moved in the opposite direction (Fig. 4). For survival, the optimum movement is farther, and in the opposite direction as for height (Fig. 3).

Both height and survival are important components of plot volume (quantity of wood produced on a given area of land). Plot volume is often more variable than height because of large variation in early survival (Wells 1983). In regressions using the deviation in plot volume from the local source as the dependent variable, only 24 percent of the variation in plot volume was explained by minimum temperature and its square (Figure 5). Plot volumes for the "local" sources were determined by regression in a manner identical to those used for heights in Figure 2 and combined as in Figure 3.

As with heights, the most important independent variables in the step-wise regressions were mean annual minimum temperature deviation and its square. No other independent variables were significantly related to volume. The regression formula predicts a decrease in volume for any movement away from a mean annual minimum temperature different than that of the source (Figure 5). The volume data is undoubtedly biased by differences in early survival. With time, lower survival is compensated for by greater diameter growth of the surviving trees on poorly-stocked plots, and self-thinning due to competition on the better-stocked plots. Little, if any, self thinning has occurred in this study, and the relationship between volume and seed movement shown in Figure 5 may change with time. Analysis of 25-year volumes from the Southwide Southern Pine Seed Source Study (Schmidtling 1994) showed a relationship similar to that found for height in Figure 3, that is, some gain in volume could be expected for a movement of seed sources from a warmer to a colder climate.

In the analysis of the 15-year data, effect of physiographic province of the seed source was not statistically significant (Kraus and Sluder 1990). Examination of residuals from the minimum-temperature model also failed to show any consistent seed source differences among physiographic provinces. There also were no consistent interactions between physiographic province of the seed source and planting site, e.g., the seed sources from the Piedmont did not grow or survive in the Piedmont significantly better than sources from the Sandhills, or vice-versa.

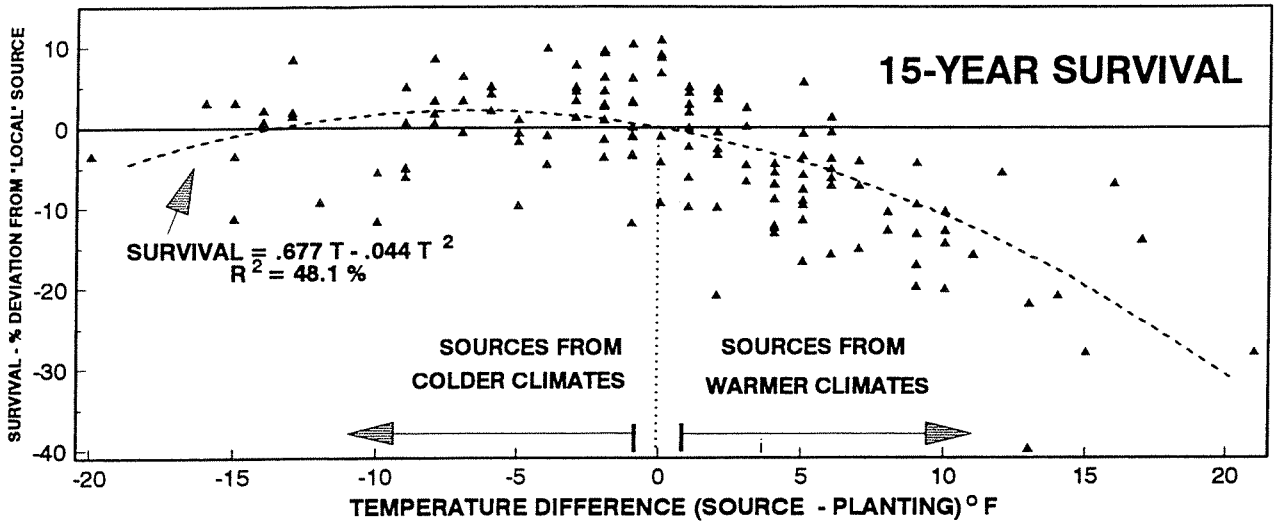


Figure 4. Survival at age 15 versus minimum temperature for all seven plantings combined. On the vertical axis, survival is expressed as deviations from the local source. On the horizontal axis, yearly average minimum temperatures are expressed as differences between the seed source minimum temperature and the planting location minimum temperature.

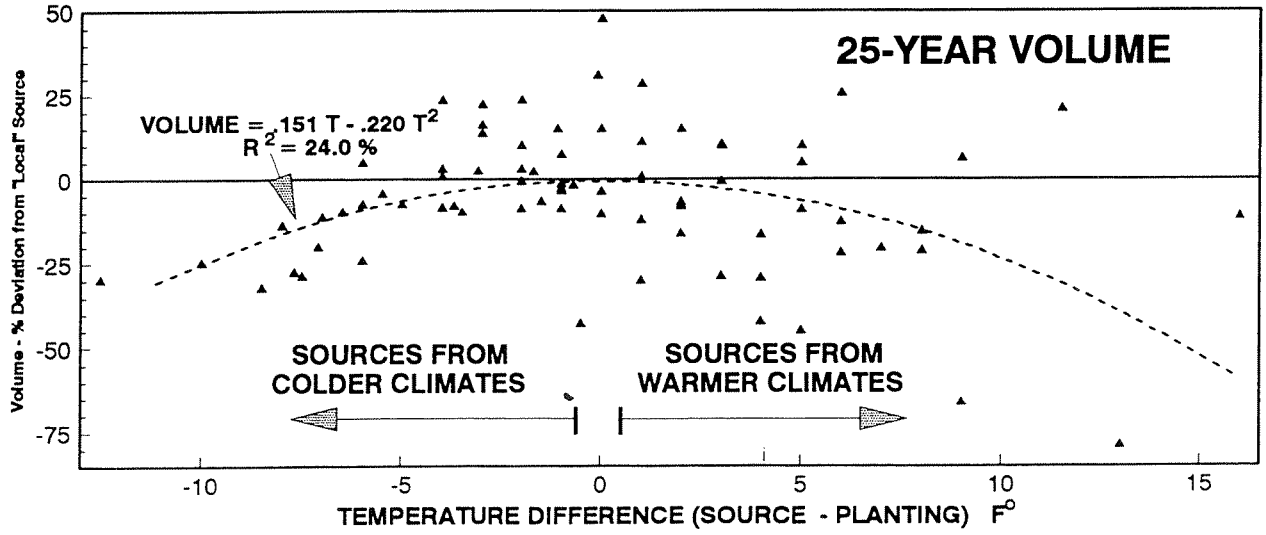


Figure 5. Volume at age 25 versus minimum temperature for plantings 111 through 114 combined. On the vertical axis, volumes is expressed as a deviation from the local source. On the horizontal axis yearly average minimum temperature is expressed as a difference between the seed source minimum temperature and the planting location minimum temperature.

CONCLUSIONS

Provenance tests are often analyzed planting by planting, to determine which seed source is best at a given planting location. That approach is a simple way to deal with the strong and complex interactions between seed source and planting site. The approach described here provides an overall picture. Growth variables are related to climatic factors at the seed source by regression. Performance in different plantings is combined by expressing growth as a percent deviation from the local source. Temperature or other climatic factors at the source are expressed as deviations from conditions of the planting site. The result is a general picture of the effects of seed transfer.

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HARDWOOD COVER CROPS: CAN THEY ENHANCE LOBLOLLY PINE SEEDLING PRODUCTION

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Abstract--It has been extremely difficult to obtain more than two loblolly pine (*Pinus taeda* L.) crops following even effective soil fumigation with methyl bromide in southern forest tree nurseries. The traditional agronomic cover crops such as sorghum and sudex, unless followed by fumigation, do not normally produce satisfactory loblolly pine seedling crops. Various species of hardwoods appear to stimulate the following pine crop even in the absence of fumigation. In the present study, we fumigated immediately before the hardwood and sudex cover crop sequences because no effective herbicide was available to control weeds in the hardwood nursery beds. Heights and root collar diameters (RCD) of loblolly pine seedlings from all cover types were comparable. Stem weights were generally greater for seedlings in the hardwood-pine rotation. Also, the needles were longer and thicker in pine seedlings grown after hardwoods as compared to those followed the sudex cover crop.

Keywords: methyl bromide, *Pinus taeda*, cover crops, hardwoods

INTRODUCTION

Loblolly pine is the most widely planted southern pine and is indispensable to the forest economy of the southern United States. Its importance increased following World War II, and artificial regeneration became the principle method of establishment with the development of the various tree improvement programs throughout the region. Accompanying the increased planting of loblolly pine was a rapid expansion of forest nurseries to provide seedling to meet the planting needs on public, private, and industrial lands. For many years, the demand for pine seedling exceeded the capacities of the established nurseries to produce them, and intervals between cover crops and seedling productions were altered. The compression between cover crop sequences probably became feasible because of effective fumigation with methyl bromide. Although other soil fumigants are available, maintaining loblolly pine seedling production has relied heavily upon continued use of methyl bromide (Chapman 1992). Before an array of

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fungicides and herbicides began to come on line, this compound appeared to be a panacea for nursery production. It proved to be effective as a preemergent herbicide, as well as a most effective treatment for controlling potentially destructive soil borne organisms (Cordell 1982; James et al. 1993). However, methyl bromide may not be available for use in seedling production after the year 2000 (James et al. 1993).

During many periods of rapid expansion of planting loblolly pine, the numbers of seedlings produced were at least as important as their quality. However, it soon became evident that, essentially, only two acceptable successive pine crops were possible following even effective fumigation (May 1985a). Although most rotation sequences stress buildup of organic matter during the cover crop sequence as being important to maintaining valuable soil properties and fertility relationships (May 1985a; Rose 1993), significantly increasing soil fertility levels have had little effect on undesirable seedlings produced in the third or fourth successive seedling crop. The scientific community has not yet addressed the obvious question of why 3 or more successive southern pine seedling crops are difficult to produce. This is even more unusual when one realizes that different annual crops have been grown for centuries without the benefit of soil fumigation and successive tree rotations have continued for centuries without fumigation. Unfortunately, methyl bromide's effectiveness masked the need for researching the biology behind growth decline associated with successive crops of loblolly pine seedlings. Research is needed to determine why successive pine seedling crops, even in absence of potentially destructive soil borne organism, results in depressed seedling development.

In our early studies on the heritability of first-order lateral roots (FOLR) on loblolly pine at the Institute of Tree Root Biology (ITRB), Athens, Georgia, we would alternate between sweetgum and loblolly pine studies in our experimental nursery beds (Kormanik et al. 1986; Kormanik et al. 1990; Kormanik et al. 1991). Fumigation would follow each sweetgum seedling crop to facilitate introduction of specific ectomycorrhizal fungi into the pine nursery beds. The emphasis of our researches soon focused on the morphology, physiology, and biochemistry of seedling development rather than mycorrhizal relationships. Fumigation to maintain specific ectomycorrhizal fungi was eliminated. After five or six successful sequences at alternating pine and hardwood crops in the same nursery beds without fumigation, the value of hardwoods as a cover crop became evident but was not considered as a practical alternative in commercial nurseries.

Few nurseries were growing many hardwoods in the early 1980s and those that did were using completely different soil fertility regimes for producing pines and hardwoods. These nurseries did not normally precede pine crops with hardwoods because of soil fertility considerations. However, the Georgia Forestry Commission and the U.S. Forest Service ITRB began to develop nursery fertility protocols that maintained the traditional crop rotation but would readily permit alteration of crops between hardwoods and southern pines (Kormanik et al. 1992). Following two pine seedling crops, we found that any number of either ectomycorrhizal or endomycorrhizal hardwood host species developed very well without fumigation. The major problem encountered has been herbaceous weed competition during the hardwood sequence

since so few effective herbicides are available to use over them. Thus, fumigation was still recommended before planting the hardwood cover crop as a preemergent herbicide even though presence of potentially destructive soil borne organisms was not evident.

Over a seven year period in our Whitehall Experiment Nursery, Athens, Ga, several 2:1:1 rotations of pine:hardwood:pine proved to be effective without fumigation. We hand weeded during the hardwood sequences and used herbicides to control herbaceous competition during the pine sequence. It was observed in several cases, but essentially ignored, that following the hardwood sequence the pine seedlings were somewhat larger and had darker green foliage than the pine seedlings produced either with or without fumigation in the sudex cover crop sequence. Parallel observations in two of the Georgia Forestry Commission's nurseries indicated a similar situation. Initially no attempt at using hardwoods in a cover crop sequence was considered until we had developed a nursery soil fertility protocol similar to that reported earlier for loblolly pine (Kormanik et al. 1994). After the hardwood protocol was developed, we considered whether hardwood crops could reverse whatever undefined soil effects or microbial problems were induced by successive pine cropping. The short and long term objectives of this research are: (1) to determine response of loblolly pine seedlings following normal fumigation schedules but including various commercially important hardwood species in the cover crop sequence; and (2) to determine the effect of hardwood cover crops on subsequent pine rotations when hardwood cover crops are not preceded by soil fumigation.

METHOD

In 1990, as a normal procedure at the Georgia Forestry Commission's Flint River Nursery, several fields were fumigated and sown with sudex in their cover crop sequence. In the 1991 and 1992 growing seasons, loblolly pine seedlings were produced according to the soil fertility protocol reported by Kormanik et al. (1992). In 1993, one of the fields was fumigated and sown with a sudex cover crop again. This field served as the control. An adjacent field was fumigated and sown with one of 19 hardwood species as the cover crop sequence. The hardwoods were all planted at 65 per m^2 and were grown according to the soil fertility protocol by Kormanik et al. (1994). The field containing the hardwood beds was carefully mapped so that each specie's location could be re-established after the seedlings were lifted. The 19 hardwood species used were: *Quercus acutissima, Q. alba, Q. nigra, Q. prinus, Q. virginiana, Q. michauxii, Q. rubra, Malus anqustifolia, Diospyros virginiana, Liriodendron tulipifera, Lagerstroemia indica, Liquidambar styraciflua, Plantanus occidentalis, Catalpa bignonioides, Cercis canadensis, Nyssa sylvatica* var *biflora, N. aquatica, Fraxinus pennsylvanica,* and *Carya aqualica*. After lifting the hardwood seedlings, a composite soil sample from each of the 19 hardwood beds and a single composite sample from the field containing the sudex cover crop were collected and sent to the A&L Laboratory (Memphis, Tennessee) for soil analysis.

In March 1994, before sowing in April, both fields had their soil fertility adjusted to the standard baseline used at the Flint River Nursery. Both fields were sown with a precision seed sower to the same mixed loblolly pine seedlot at a planned density of 284 per m^2. Ten randomly located positions were established in beds from which each of the hardwood species had been growing and were monitored at two week intervals for pine seedling height growth. Standardized curves had already been established for the normal sudex cover crop sequences and after mid-July their development was not monitored, except on required intervals.

The nursery protocol requirement is that the seedlings be between 15-20 cm tall by mid-July when the final growing season application of nitrogen is applied. Depending on the actual height in mid-July, additional nitrogen and irrigation can then be applied or held back to obtain final seedling height of 25-35 cm. Unless adjustments are needed, based primarily on environmental conditions, the final nitrogen application is applied in early to mid-September after terminal buds have set and dry weight growth is then being allocated primarily to the root systems (Sung et al. 1993; Sung et al. 1994). With this protocol, seedlings can be lifted for outplanting in early November.

In early November of 1994, seedlings from five of the ten permanent 0.93 m^2 sampling plots were lifted from each of the beds previously grown hardwood species. Root collar diameter (RCD) and height were measured and FOLR were counted. Fresh weights were obtained from two of the five lifted plots to obtain top:root (T/R) ratios. Approximately a total of 6 million seedlings were produced in each of the two fields used in this experiment. Loblolly pine seedlings from the hardwood cover crop beds were the first to be lifted and were all shipped out by mid-December. In early February, only control seedlings from the sudex cover crop beds remained and 5 plots were resampled to follow seedling development during the lifting season. The soils were resampled and both fields were again planted to the same seedlot for their second successive crop during the 1995 growing season for continued study. The current study design precluded statistical analysis of the effects among the 19 hardwood species. No replications among fields were available. This is not an unusual situation for large applied nursery studies such as this one.

RESULTS AND DISCUSSION

The unusually heavy rains during early July of 1994 that caused massive flooding in south Georgia had a significant impact on the entire nursery, including the fields used in this experiment. For extended periods through July and early August, water stood in the allies half way up the raised beds. As is characteristic of any nursery, specific portions of any field may be affected to different degrees by excess water. Thus, while the floods may not have affected the general outcome, they may have affected seedling development in specific portions of the fields. In general, by early August pine seedlings grown after the hardwood cover crop were noticeably greener and had larger needles than those seedlings which followed the sudex cover crop.

Because of their increased vigor, seedlings were lifted beginning the first week of November, 1994. The effects of different cover crops were examined in several fashions.

Seedling Survival

Typically, the Commission allows for a 20% mortality factor during the growing season and increases sowing rate accordingly. The nursery protocol used requires constant monitoring; thus mortality has been less than experienced earlier. This 20% mortality factor is currently being reduced. Overall, in both fields the number of seedlings per m^2 was 299, about 10% more than we planned to have. This should be acceptable even though within some of the sampled plots for any given hardwood cover crop a 15-20% variation from the norm was encountered. The 10% overall increase in seedling survival was attributed to improvements in soil fertility and moisture regimes, and not to cover crop effects. However, the pattern of water logging could easily have affected seedling survival, since the bed space for given species of cover crops often extended 100 meters or more, and some of the species covered four beds. The low survival in some plots was definitely related to long term standing waters.

Seedling Growth

In year to year operations, seedling growth is monitored several times since the normal growth curve for the nursery had been developed with a sudex cover crop sequence for the Flint River Nursery. Mid-July is critically important, for the seedlings should be between 15-20 cm tall in order to reach the 25-35 cm target height at lifting. It is when the seedlings reach the 15-20 cm height that the secondary needles begin to elongate and mature. Stem growth continued for the next two months until terminal bud formation which signals a shift of photosynthates to root growth. This 15-20 cm height can be easily reached in early to mid-June with excessive nitrogen applications. If this occurs, it becomes very difficult to control seedlings growth without root pruning, top clipping, or significant reduction in irrigation (Kormanik et al. 1992; Sung et al. 1994).

Average seedling size with both cover crop sequences were within 1.5-2.0 cm of the desired of the mid-July height (Figure 1). Figure 1 showed the pine seedling growth curves for five of the hardwood cover crop species commonly produced in large numbers in the Commission's nurseries. If the loblolly seedlings reach 15-20 cm much earlier than mid July, it is difficult to slow their height growth down to achieve the specific desirable sizes. However, one can see that seedlings were closer to 15 cm in mid-July and were on the lower side of the desired heights when lifting started. These smaller sizes can readily be attributed to the slow mid-season growth due to early July floodings. We did not want to deviate too much from our normal procedures in the two fields in which this experiment was carried out and thus followed the established fertility protocol in spite of growth reductions the flooding may have caused.

The seedling did not shrink between October 17 and November 7 (Figure 1). Seedlings from the same area was used for height monitoring throughout the summer and for final lifting.

Because of flooded conditions, footing was less than ideal and succulent tips were easily damaged. Therefore, seedlings were periodically measured to the tips of the small terminal needles through October. After the seedlings were hardened and lifted, they were measured to the base of the terminal bud, thus causing the apparent reduction in final harvested size.

In Table I, the pertinent growth data for the seedlings from the different cover crops were collected on November 7, 1994 when lifting was initiated. Even on the earliest lifting date, the mean heights and RCD for seedlings for all cover crop types, except for green ash and swamp chestnut oak, were within protocol limits. However, as in mid-July, the seedlings were on the lower end of the desirable limits. The average number of FOLR for seedlings from all cover crops were within acceptable limits (Kormanik et al. 1990; Kormanik et al. 1991). Only two cover crops, swamp chestnut and green ash, produced less top weights than control seedlings from the sudex cover crop sequence. This extra weight resulted from the longer, thicker needles of the seedlings grown after the hardwood crops. The difference among foliage characteristics usually becomes quite evident between the hardwoods and sudex cover crops by mid-August. Although we have not attempted detailed soil investigations at this time, the effect almost appears similar to several short interval applications of a foliar fertilizer. It may well be that it takes several months for the residual hardwood roots to decompose. The more succulent sudex roots may be breaking down before the pine root systems have developed sufficiently to benefit from cover crop root decomposition and resulted release of nutrients into the soil. In this case, hardwood cover crops proved to be noticeably beneficial to loblolly pine seedling development.

It has been reported that T/R ratio of 1:1 or 2:1 are most desirable for loblolly pine (May 1985b). Anything approaching these ratios are attainable only if mechanical top clipping is undertaken with just about any nursery protocol being used in Southern pine seedling nurseries. We have found that top:root ratios of 7:1 and 8:1 are characteristic during the early part of the lifting season and has not affected survival or growth of outplanted seedlings. The biology of loblolly pine seedling seasonal root development readily accounts for these ratios and is apparent in Control 2 (Table I) and has been reported (Sung et al. 1993). In Table I, from early November to mid-February, tops of pine seedlings after the sudex cover crops increased by only about 18% and the root weights increased by over 100%. Thus T/R ratio was reduced from 6:1 to 3:1. This latter ratio is the approximately T/R recently reported for mature loblolly pine (Van Lear and Kapeluck 1995) and is characteristic for 3-9 year old loblolly pine in plantations (Kormanik, unpublished data). Interestingly, seedlings grown at 130 per m^2 at the Flint River Nursery in 1994 with the heavier applications of nitrogen recommended by others for nursery production of loblolly pine had T/R ratios as large or larger than those observed in this study. For example, in November, 1994 root pruned seedlings were 38 cm tall with 5.5 cm RCD and 8:1 T/R ratio. The unpruned ones were 45 cm tall with 6.0 cm RCD and 9:1 T/R ratio when lifted in early November. Thus, while hardwood cover crops appear to benefit the loblolly pine seedling development, it is difficult to say if, or how, this affects T/R ratio, since tops and roots respond as a unit rather than separate entities.

Whether the benefits of hardwood cover crops are due to the heavier fertilization program utilized in their production and/or changes in the soil microbial relationships is currently open to speculation. We don't know whether one large scale hardwood cover crop can be effective without being preceded by soil fumigation. Certainly, the many beneficial effects of using methyl bromide, even in absence of known destructive soil borne organisms, cannot be discounted. However, the price that was paid scientifically for chauvinistic reliance on methyl bromide may turn out to be high since it seriously reduced research in specific areas such as soil born organisms and soil mediated processes in nursery soils. Nevertheless, this research clearly demonstrates that under the proper soil fertility programs, hardwood cover crop rotations may have a significant advantage over the traditional ones .

CONCLUSIONS

In many early trials, hardwood cover crops proved beneficial to succeeding loblolly pine crops. Even with uncharacteristic flooding affecting this study, the beneficial effects of the hardwood cover crop was quite obvious. How a second pine crop develops after a hardwood crop is currently being followed. The question that must be clarified is whether hardwood cover crops, in the absence of known root pathogens, can eliminate the depressive effects of repetitive loblolly pine crops without the benefit of soil fumigation. This is a critical question that must be examined before methyl bromide and other effective soil fumigants are banned from forest nursery practices. Their demise at this time would be a serious blow to economic production in many Forest Tree Nursery programs.

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Figure1. Cumulative height growth of first year loblolly pine seedlings during1994 at the Georgia Forestry Commision Flint River Nursery following different hardwood species used as cover crops in 1993.

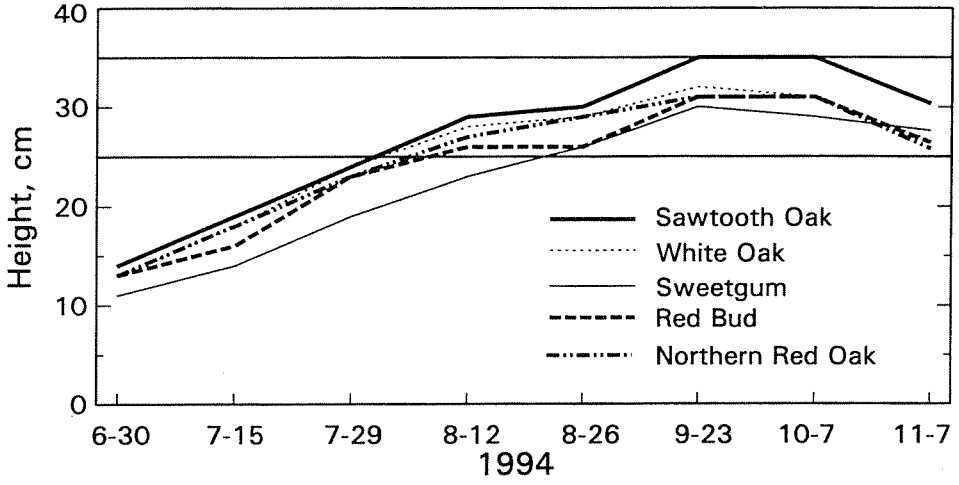

Table I. Loblolly pine 1-0 seedling morphological and growth data following different cover crops. Control cover crop is sudex. Seedlings from all treatments were lifted on November 7, 1994.

Cover crop	FOLR #	Ht cm	RCD mm	Top FW g	Root FW g	T/R Ratio
Sawtooth oak	5	30.3	4.1	11.2	1.5	8 : 1
White oak	4	26.7	4.0	10.0	1.4	7 : 1
Water oak	5	26.6	4.0	8.3	1.1	8 : 1
Chestnut oak	4	26.7	3.8	8.3	1.1	8 : 1
Live oak	5	28.8	4.0	10.7	1.4	8 : 1
Swamp chestnut oak	3	24.3	3.4	5.6	0.9	6 : 1
Nothern red oak	4	25.8	3.6	7.7	1.2	7 : 1
Crabapple	5	26.1	4.0	8.3	1.2	7 : 1
Persimmon	5	28.9	4.0	9.4	1.1	8 : 1
Yellow poplar	3	28.8	3.7	8.7	1.2	8 : 1
Crepe mrytle	4	27.5	3.7	8.6	1.1	8 : 1
Sweetgum	4	25.9	3.6	8.0	1.3	6 : 1
Sycamore	4	25.1	3.7	7.5	1.2	6 : 1
Catalpa	4	25.6	3.7	7.6	1.1	7 : 1
Redbud	4	26.4	3.8	8.3	1.2	7 : 1
Swamp tupelo	5	28.3	4.2	10.3	1.8	6 : 1
Water tupelo	6	26.8	4.2	8.6	1.7	5 : 1
Green ash	4	22.8	3.4	6.3	1.1	6 : 1
Water hickory	5	26.2	3.8	8.9	1.7	5 : 1
Control 11/7/94	4	25.4	3.7	7.0	1.1	6 : 1
Control 2/15/95	6	26.2	4.4	8.3	2.5	3 : 1

PROPORTION OF SPECIES' GENOMES AFFECTS SURVIVAL AND GROWTH OF SHORTLEAF X LOBLOLLY PINE HYBRIDS

E. R. Sluder[1]

Abstract. To study the performance of shortleaf x loblolly pine hybrid progenies with differing proportions of the two genomes, families with 25, 50, or 75 % of each genome and the corresponding 75, 50, or 25 % of the other were produced and outplanted along with pure families of each species. In February 1986, 39 seed lots grouped into five genome proportions and eight progeny types were planted in four randomized blocks in each of two areas. At age five years, variation among seed lots without regard to genome proportion or progeny type was significant ($P < 0.01$) for survival, height, and within-plot coefficient of variation in height in both plantations. Progeny groups (either genome proportion or progeny type) differed in height in both plantations and in survival in one ($P < 0.01$). Generally, the greater the percentage of the loblolly genome, the better the survival and growth. Hybrids with 75 % of the loblolly genome performed almost the same as pure loblolly in height and survival; those with 75 % of the shortleaf genome, almost the same as pure shortleaf. Infection by fusiform rust was too low for evaluation of rust resistance.

Keywords: *Pinus taeda* L., *P. echinata* Mill., *Cronartium quercuum* (Berk.) Miyabe ex Shirai f. sp. *fusiforme*, selection, breeding

INTRODUCTION

Interspecific hybridization of forest trees may produce planting stock that will outperform either parent species under unfavorable conditions, such as poor site, severe climate, or high disease and insect incidence (Duffield and Snyder 1958). The southern fusiform-rust fungus (*Cronartium quercuum* (Berk.) Miyabe ex Shirai f. sp. *fusiforme*) is a serious pest on loblolly pine (*Pinus taeda* L.) throughout most of its range. In contrast, shortleaf pine (*P. echinata* Mill.), a closely related species, is rarely affected by the fungus. Because the two species overlap in range and have relatively weak reproductive barriers, hybridization might be used to transfer genes for rust resistance from shortleaf to the faster-growing loblolly pine.

Shortleaf and loblolly pine apparently hybridize naturally (Mergen et al. 1965, Zobel 1953). The first control-pollinated hybrids of these two species were produced in 1933 in Placerville, CA (Duffield and Righter 1953). The hybrid has been botanically described (Little and Righter 1965), with distinguishing characteristics noted by Mergen et al. (1965). F_2 hybrid progenies produced in Placerville have grown well and demonstrated fusiform rust resistance in progeny tests in Louisiana (Henry and Bercaw 1956) and Georgia (Sluder 1970).

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Based on the promising results from these tests, the USDA Forest Service, Southern Research Station, has continued the research with hybrids of these two species in Macon, GA. Shortleaf and loblolly pine clones from Georgia Forestry Commission's seed orchards and the largest, best-formed trees from an F_2 hybrid progeny produced at Placerville and grown in Georgia were used as parents. Results indicate that resistance to fusiform rust is inherited from the shortleaf parent (LaFarge and Kraus 1975, 1977, 1980; Kraus 1985, 1986).

The most recent study assesses the relative performance of the two species and a series of their hybrids containing three different proportions of the genomes of each species. Fifth-year results from the two experimental plantations in this study are reported.

METHODS

Hybrid families with 25, 50, or 75 percent of the loblolly genome and, respectively, 75, 50, or 25 percent of the shortleaf genome were produced. The 50:50 loblolly:shortleaf group had three types of progenies and the 75:25 group had two types. Each of the other three genome proportion groups (0:100, 25:75, and 100:0 loblolly:shortleaf) had one type of progeny, bringing the total to eight types. Five families from each of the six hybrid types plus five pure loblolly and four pure shortleaf families or bulks comprise the 39 seed lots in the study (Table 1).

The 50:50 hybrids (types 3-5) were F_1 and F_3 progenies. Backcrosses (types 6-8) were made with F_1 and F_2 hybrids. With the exception of progeny types 5 and 6, the hybrid progenies were produced by controlled pollinations. Progeny type 5 was assumed to be an F_3 from wind pollinations among F_2 parents in an older study plantation. Progeny type 6 was assumed to be a backcross to surrounding loblolly pines from wind pollinations on the F_1 female parents growing in a young hybrid seedling seed orchard (Table 1).

The seedlings were planted in two plantations in Georgia during February 1986. The field design for each plantation consists of four randomized blocks, 16-tree square plots, and 2.5 m x 2.5 m spacing. In the plantation located in the Hitchiti Experimental Forest in Jones County, all four blocks are contiguous (plantation 150). In the second plantation, seed lots 3573, 3577, 3601, and 3604 are excluded. This plantation is located on two noncontiguous sites in Meriwether and Putnam Counties, with two blocks in each county (plantation 152). All sites were cut-over. The Jones County site was prepared by discing; the Meriwether and Putnam County sites by windrowing. All are in the Piedmont physiographic province, and site quality in each varied from medium to low.

Data recorded at age five years were survival, height, and infection by southern fusiform rust. Within-plot coefficients of variation (CV) in height were calculated. Survival, height, and CV in height data were subjected to analysis of variance (Table 2). Rust incidence was too low, even on pure loblolly, for meaningful analysis of rust data. Comparisons among group means (percentage loblolly, progeny type) were made with Bonferroni's method (Miller 1981), and eight contrasts between progeny types were tested with the F-statistic.

Table 1. Description of seedlots in the study.

Seed lot	Progeny type	Percentage Loblolly	Percentage Shortleaf	Parent[1] Female	Parent[1] Male
3566	1. Loblolly	100	0	520	W
3567				512	W
3568				541	W
3569				Bulk, seed orchard	
3570				Bulk, Piedmont	
3571	2. Shortleaf	0	100	2006	W
3572				2017	W
3573				2019	W
3574				Bulk, seed orchard	
3575	3. F_1 hybrid	50	50	2006	512
3576				2017	541
3577				2019	541
3578				2004	617
3579				2008	625
3580	4. F_3 hybrid	50	50	HH-5	HH-39
3581				HH-11	HH-26
3582				HH-19	CH-4
3583				HH-5	HH-3
3584				HH-10	HH-26
3585	5. F_3 hybrid[2]	50	50	HH-5	W
3586				HH-6	W
3587				HH-11	W
3588				HH-20	W
3589				HH-15	W
3590	6. Backcross,	75	25	2006 x 515	W
3591	F_1 to			2006 x 512	W
3592	loblolly[3]			2017 x 541	W
3593				2019 x 541	W
3594				Bulk lot	W
3595	7. Backcross,	75	25	515	HH-6
3596	F_2 to			624	HH-6
3597	loblolly			541	HH-11
3598				617	HH-39
3599				624	HH-26
3600	8. Backcross,	25	75	2003	HH-6
3601	F_2 to			2019	HH-6
3602	shortleaf			2011	HH-29
3603				2004	HH-39
3604				2008	HH-11

[1] Three- and four-digit numbers (520, 2006) are loblolly and shortleaf clones, respectively, in Georgia Forestry Commission seed orchards. HH-5, etc., are F_2 hybrid trees in the Hitchiti Experimental Forest. CH-4 is a F_2 at Callaway Gardens. W is wind-pollinated.

[2] Female F_2 trees in a plantation in the Hitchiti Experimental Forest, assumed crossed with other F_2 trees in the plantation.

[3] Female trees in a young seedling seed orchard in Baldwin County, assumed backcrossed to loblolly.

Table 2. Expected mean squares for the analysis of variance.

Source	Degrees of freedom Plant. 150	Degrees of freedom Plant. 152	Expected mean square[1]
Block	3	3	$V_e + V_b$
Seed lot	38	34	$V_e + bV_l$
Progeny group[2]	4 (7)	4 (7)	$V_e + bV_{l(g)} + Q_g$
Lot in group	34 (31)	30 (27)	$V_e + bV_{l(g)}$
Error	114	102	V_e
Total	155	139	

[1] V_e, error variance; $V_{l(g)}$, lot in group variance; Q_g, quadratic function of progeny group; V_l, seed lot variance; V_b, block variance.
[2] Genome proportion or progeny type (d.f. for progeny type in parentheses).

RESULTS

Seed Lots

Variation among seed lots without regard to genome proportion (Table 3) or progeny type (Table 5) was significant ($P < 0.01$) for all three variables in both plantations. Also, variation among seed lots within genome proportion and progeny type groups was significant ($P < 0.01$ or 0.05) for all traits except height in progeny type in plantation 150.

Genome Proportion

Variation due to genome proportion (percentage loblolly) was significant ($P < 0.01$) for survival in plantation 150 and for height in both plantations (Table 3). Percentages of the two genomes showed no significant effect on within-plot CV in height in the analyses of variance (Table 3), but Bonferroni's method of comparisons among treatment means ($P < 0.05$) showed pure shortleaf to be more variable in height within plot than 75 percent loblolly in plantation 150 and than pure loblolly in plantation 152 (Table 4). Generally, the greater the percentage of loblolly, the greater the survival and height and the less the within-plot CV in height (Table 4).

Progeny Type

The eight progeny types varied in survival in plantation 150 and in height in both plantations ($P < 0.01$) (Table 5). Tables 6 and 7 show progeny type means and multiple comparisons among them (Bonferroni's method). Table 8 shows F-statistic tests of eight of the more meaningful contrasts between progeny type means. While none of these contrasts were significant for plantation 152, seven were significant for plantation 150.

Table 3. Analyses of variance of fifth-year data grouped by percentage loblolly pine (genome proportion).

Source	Plantation 150			Plantation 152		
	Survival	Height	CV height	Survival	Height	CV height
	Mean square					
Block	753.56**	1.82**	0.0107	59.98	3.32**	0.0170**
Seed lot	512.35**	0.86**	0.0157**	386.20**	0.59**	0.0056**
Percent lob	2908.75**	5.15**	0.0278	253.22	2.55**	0.0119
Lot in pct.	230.42**	0.37*	0.0142**	403.93**	0.33**	0.0048**
Error	99.66	0.22	0.0080	112.26	0.12	0.0026

* P < 0.05
** P < 0.01

Table 4. Means data by percentage loblolly pine, age 5 years.[1]

Percent loblolly	Plantation 150			Plantation 152		
	Survival (%)	Height (m)	CV height (%)	Survival (%)	Height (m)	CV height (%)
0	30.1b	1.95c	34.7a	72.1a	2.40b	21.5a
25	38.7b	1.96bc	32.0ab	72.1a	2.44b	18.1ab
50	61.4a	2.34b	29.1ab	72.4a	2.41b	20.3a
75	69.7a	2.88a	25.7b	77.9a	2.86a	17.9ab
100	68.6a	2.88a	29.0ab	83.4a	3.09a	15.4b

[1] Within a column, means followed by a common letter do not differ at the 0.05 level, Bonferroni's method (Miller 1981).

Table 5. Analyses of variance of fifth-year data grouped by progeny type.

Source	Plantation 150			Plantation 152		
	Survival	Height	CV height	Survival	Height	CV height
	Mean square					
Block	753.56**	1.82**	0.0107	59.98	3.32**	0.0170**
Seed lot	512.35**	0.86**	0.0156**	386.20**	0.59**	0.0056**
Progeny type	1257.50**	3.18**	0.0270	177.36	1.64**	0.0069
Lot in type	231.19**	0.34	0.0131*	440.35**	0.32**	0.0053**
Error	99.66	0.23	0.0083	112.26	0.12	0 0026

* P < 0.05
** P < 0.01

Table 6. Means data by progeny type, age 5 years, plantation 150.[1]

Progeny type	Percent		Trait		
	Loblolly	Shortleaf	Survival (%)	Height (m)	CV height (%)
1. Loblolly	100	0	68.59a	2.88a	29.00ab
2. Shortleaf	0	100	30.08c	1.95c	34.07a
3. F_1, c.p.	50	50	64.06a	2.57abc	28.45ab
4. F_3, c.p.	50	50	64.73a	2.22bc	30.05ab
5. F_3, wind	50	50	55.31ab	2.24bc	28.72ab
6. B.C. to lob., wind	75	25	68.91a	2.84ab	30.07ab
7. B.C. to lob., c.p.	75	25	70.51a	2.93a	21.42b
8. B.C. to shtl., c.p.	25	75	38.75bc	1.96c	31.96ab

[1] Within a trait column, means followed by a common letter do not differ at the 0.05 level, Bonferroni's method (Miller 1981).

Table 7. Means data by progeny type, age 5 years, plantation 152.[1]

Progeny type	Percent		Trait		
	Loblolly	Shortleaf	Survival (%)	Height (m)	CV height (%)
1. Loblolly	100	0	83.44a	3.09a	15.42a
2. Shortleaf	0	100	72.12a	2.40bc	21.53a
3. F_1, c.p.	50	50	76.17a	2.61abc	19.91a
4. F_3, c.p.	50	50	71.67a	2.24c	20.01a
5. F_3, wind	50	50	70.00a	2.41bc	20.78a
6. B.C. to lob., wind	75	25	78.44a	2.85abc	17.77a
7. B.C. to lob., c.p.	75	25	77.46a	2.86ab	18.01a
8. B.C. to shtl., c.p.	25	75	72.12a	2.44bc	18.07a

[1] Within a trait column, means followed by a common letter do not differ at the 0.05 level, Bonferroni's method (Miller 1981).

Table 8. Eight contrasts between progeny type trait means at age 5 years.

Contrast	Plantation 150			Plantation 152		
	Survival	Height	CV height	Survival	Height	CV height
-Type-	F-value					
1 vs 7	0.17	0.08	4.38*	0.74	1.65	1.28
2 vs 8	1.50	0.00	0.51	0.00	0.03	1.37
3 vs 7	0.85	3.92	3.77	0.04	1.72	0.61
3 vs 8	11.02**	10.77**	0.93	0.33	0.68	0.44
7 vs 8	18.01**	27.68**	8.46**	0.17	4.29	0.00
6 vs 7	0.14	0.26	5.71*	0.01	0.00	0.01
4 vs 5	2.45	0.01	0.13	0.02	0.91	0.11
3 vs 4	0.09	3.59	0.19	0.30	3.96	0.00

* $P < 0.05$.
** $P < 0.01$.

Correlations

Correlation between the two plantations was good for data means for height, reasonably good for survival, and poor for CV in height. For seed lots, progeny types, and genome percentages, respectively, correlation coefficients were: 0.76 ($P < 0.001$), 0.88 ($P < 0.01$), and 0.90 ($P < 0.05$) for height; 0.41 ($P < 0.05$), 0.61 (ns.), and 0.72 (ns.) for survival; and 0.27 (ns.), 0.32 (ns.), and 0.56 (ns.) for CV in height.

DISCUSSION

If the assumptions about pollen source for the wind-pollinated progenies are not totally correct, type 5 (F_3) may be more than 50 percent and type 6 (backcross) less than 75 percent loblolly. Each of these two wind-pollinated progeny types can be compared with its corresponding F_3 (type 4) or backcross to loblolly (type 7) produced by controlled pollinations. Contrasts shown in Table 8 indicate no differences between the two types of F_3 progenies (type 4 vs. type 5), but do indicate a difference between the two types of backcross to loblolly progenies (type 6 vs. type 7) in plantation 150 for within-plot CV in height ($P < 0.05$). The wind-pollinated type 6 progenies were more variable in height than the type 7 progenies, even though they differed little in mean height or survival (Table 6). This difference may be caused, in part, by greater variation within half sib than within full sib progenies.

Based on fifth-year results, hybrids with 75 percent of the loblolly genome performed almost the same as pure loblolly in survival and growth. Conversely, hybrids with 75 percent of the shortleaf genome performed almost the same as pure shortleaf. The 50-50 hybrids' performance was between the two.

The trait for which hybridization is deemed most beneficial, resistance to fusiform rust, could not be evaluated at age five years because almost no infection had occurred. Cool, moist weather early in the growing season is necessary for infection. This type of weather did not occur during the first five years of this study. Perhaps by age 10 years weather conditions will promote more normal exposure to innoculum, and resistance to fusiform rust can be evaluated.

These and earlier study results indicate that the favorable traits of loblolly and shortleaf pines can be combined into a genetic stock with the fast growth rate of loblolly and the high fusiform rust resistance and straight stems of shortleaf. Only one or two generations of backcrossing from 50:50 hybrids to loblolly will be required to regain the fast growth rate of loblolly. Still uncertain is how many generations of selection and breeding after backcrossing to loblolly will be required for high, stable rust resistance. Confidence and progress would be greatly enhanced if the number, location, and mode of action of all available resistance genes in each species and their interaction with genetic variation in the fungus were known. Breeding research with these two species should continue until broadly-based genetic stocks of resistant loblolly pines are developed for high fusiform rust hazard areas of the Southeast.

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GENETIC MAPPING IN LOBLOLLY PINE

M. M. Sewell and D. B. Neale[1]

Abstract.--A consensus map for loblolly pine (*Pinus taeda* L.) was constructed using restriction fragment length polymorphisms (RFLPs) as genetic markers. This map is based on segregation data from two unrelated three-generation pedigrees (Devey et al., 1994; Groover et al., 1994) and was assembled using the linkage computer program JoinMap (Stam, 1993). The merger of individual genetic maps into a consensus map for loblolly pine allows for the integration of genetic information from independent sources onto a single map and facilitates the consolidation of linkage groups to represent the 12 chromosomes of loblolly pine. This consensus map contains many known and characterized genes, and serves as the foundation for present and future genetic studies in loblolly pine and for studies of genome organization and evolution in conifers.

Keywords: *Pinus taeda* L., genetic mapping, RFLP molecular markers, genome organization.

INTRODUCTION

An increasing number of genetic linkage maps are being constructed for forest tree species. These maps are commonly used for mapping Mendelian traits such as disease resistance and quantitative traits of agronomic importance. Therefore, multiple maps for a single species are often constructed from individual pedigrees that segregate for a specific trait of interest. Synthesis of these individual maps into a single consensus map to represent each species will be a valuable resource for breeders and evolutionary biologists alike. Breeders can ascertain the map positions of genes controlling the synthesis of important traits as well as the relative position of those genes to one another. Evolutionary biologist can determine the copy number of a gene found within the genome (and the relative map position of genes within these gene families), the frequency of these multigene families within the genome, etc. As more genetic information accumulates for a species and is incorporated into a consensus map, hypotheses regarding genome organization and evolution in conifers can be further explored.

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For loblolly pine (Pinus taeda L.), an independent linkage map, based primarily on restriction fragment length polymorphism (RFLP) markers, was previously constructed from each of two outbred three-generation pedigrees (Devey et al., 1994; Groover et al., 1994). Each map contains unique genetic information; Devey et al. (1994) mapped complementary DNA (cDNA) and isozyme loci, while Groover et al. (1994) mapped additional cDNAs and quantitative trait loci for wood specific gravity.

The objective of this study is to integrate these two loblolly pine maps, derived from genetically independent pedigrees, into a single consensus map. This objective will serve two purposes:

1) to synthesize the available genetic information into a single consensus map, which will serve as a foundation for genetic study in loblolly pine and for studies of genome organization and evolution in conifers, and

2) to consolidate linkage groups to represent each of the 12 chromosomes of loblolly pine.

Our strategy is to first saturate each independent linkage map with as many markers as are readily available, thereby consolidating linkage groups *within* each pedigree. Secondly, we will map loci that are common to each pedigree. These "common" loci then serve as anchor points to integrate the linkage data from each pedigree and will further consolidate linkage groups *among* pedigrees.

MATERIALS AND METHODS

Mapping Populations

Genetic linkage maps for each of two independent three-generation outbred pedigrees were recently completed. The first map (here-to-fore called the *base* map) was constructed from F_2 segregation data for 90 RFLP and six isozyme markers from 95 progeny (Devey et al., 1994). This pedigree was constructed and maintained by the North Carolina State University Cooperative Tree Improvement Program (NCSU Coop). The second map (called the *qtl* map) was constructed from F_2 segregation data for 142 RFLP markers from 175 progeny (Groover et al., 1994). This pedigree was constructed and maintained by the NCSU Coop and Weyerhaeuser Company and was selected based on extreme-high and -low values for wood specific gravity among grandparental pairs.

Source of Probes

We chose three sources of genetic markers for mapping: RFLPs (Devey et al., 1991), isozymes (Conkle, 1981) and

random amplified polymorphic DNA (RAPDs) (Kiehne and Neale, 1995). We use RFLP and isozyme markers because they are 1) codominant and multiallelic, 2) highly repeatable and syntenic across genetic backgrounds (Conkle, 1981; Ahuja et al., 1994) and 3) RFLPs are the most efficient way to map cDNAs. Kinlaw et al. (1995) has initiated "single-pass" sequencing of these cDNAs which are then compared to known genes in nucleotide sequence databases. Consequently, many of these markers are of known genes and they can also be used as orthologous markers among different species of pines. We use RAPDs because they are an efficient source of a high number of markers that are putatively found at random from throughout the genome.

Strategy for Map Integration

A. Integration of linkage data within a pedigree.

Since recombination occurs independently during the production of maternal and paternal gametes, the genetic segregation observed in the progeny represents both sources of recombination. Therefore, we arrange the segregation data into independent maternal and paternal datasets.

In an outbred pedigree, four informative mating types are possible for any given locus (see below). Each mating type reflects which parent is heterozygous and therefore produces alternate alleles that segregate among the progeny.

Mating Type	Cross
Maternally Informative (MI)	HxA
Paternally Informative (PI)	AxH
Fully Informative (FI)	$H_i x H_j$
Both-Informative (BI)	$H_i x H_i$

where, A = homozygote
H = heterozygote
(H_i, H_j are different heterozygotes)

For MI and PI mating types, only one parent is heterozygous. However, for FI mating types, each parent is heterozygous for a different pair of alleles. Therefore the segregation data for a locus of an FI mating type can be recoded once as MI and again as PI (i.e., $H_i x H_j$ is recoded as H_ixA and A$x H_j$). Parents of BI mating types are also heterozygous and can be treated in a similar manner. However, since each parent is heterozygous for the same pair of alleles, some segregation data is ambiguous (i.e., it is difficult to determine which parent contributed the alleles of the heterozygous progeny class).

The loci from FI and BI mating types, after being recoded and placed into the appropriate maternal or paternal

dataset, serve as common loci among these datasets. Utilizing these FI and BI loci as anchor-points, these independent datasets are integrated using the linkage computer program JoinMap (Stam, 1993), which uses a modified least squares procedure for estimating map distances from independent and weighted joint estimates of pairwise recombination frequencies.

B. Integration of linkage data among pedigrees.

The integration of linkage data among independent pedigrees is performed in a manner similar to the integration of maternal and paternal linkage data within a pedigree. Instead of uniting maternal and paternal linkage data via loci from FI and BI mating types, linkage data from each pedigree is integrated via loci that are "common" to each pedigree dataset.

To determine commonality of loci among pedigree datasets, comparison of molecular markers are first made by inspection of migration distances of RFLP bands on autoradiograms. Secondly, map distances between putatively common loci pairs are compared for collinearity. Loci that meet both of these criteria can then be used to integrate linkage data from independent pedigrees.

RESULTS AND DISCUSSION

Map Integration

Figure 1 illustrates the integration of independent linkage data from the *base* and *qtl* pedigrees into a single *consensus* linkage group. In this example, two BI and three FI loci (represented by dashed arrows) serve as anchor-points to unite the maternal and paternal linkage groups within the *qtl* map; one BI and four FI loci unite the maternal and paternal linkage groups within the *base* map. Six loci serve as "common" loci that unite linkage groups among pedigrees (represented by solid arrows). This example demonstrates that more than one linkage group from any given dataset can be united by this integration process (e.g., two maternal linkage groups from the *qtl* map are brought together via integration with the *base* map). Note that at least two anchor-points between linkage groups are necessary for unambiguous orientation of individual linkage groups.

In an attempt to saturate the linkage groups within each pedigree, we have added approximately 150 new genetic markers to the existing *base* and *qtl* maps. Forty-one markers from the *base* map and 49 markers from the *qtl* map serve as anchor-points to unite the maternal and paternal linkage groups within each pedigree and 42 "common" markers serve as anchor-points to unite linkage groups among each pedigree. Although

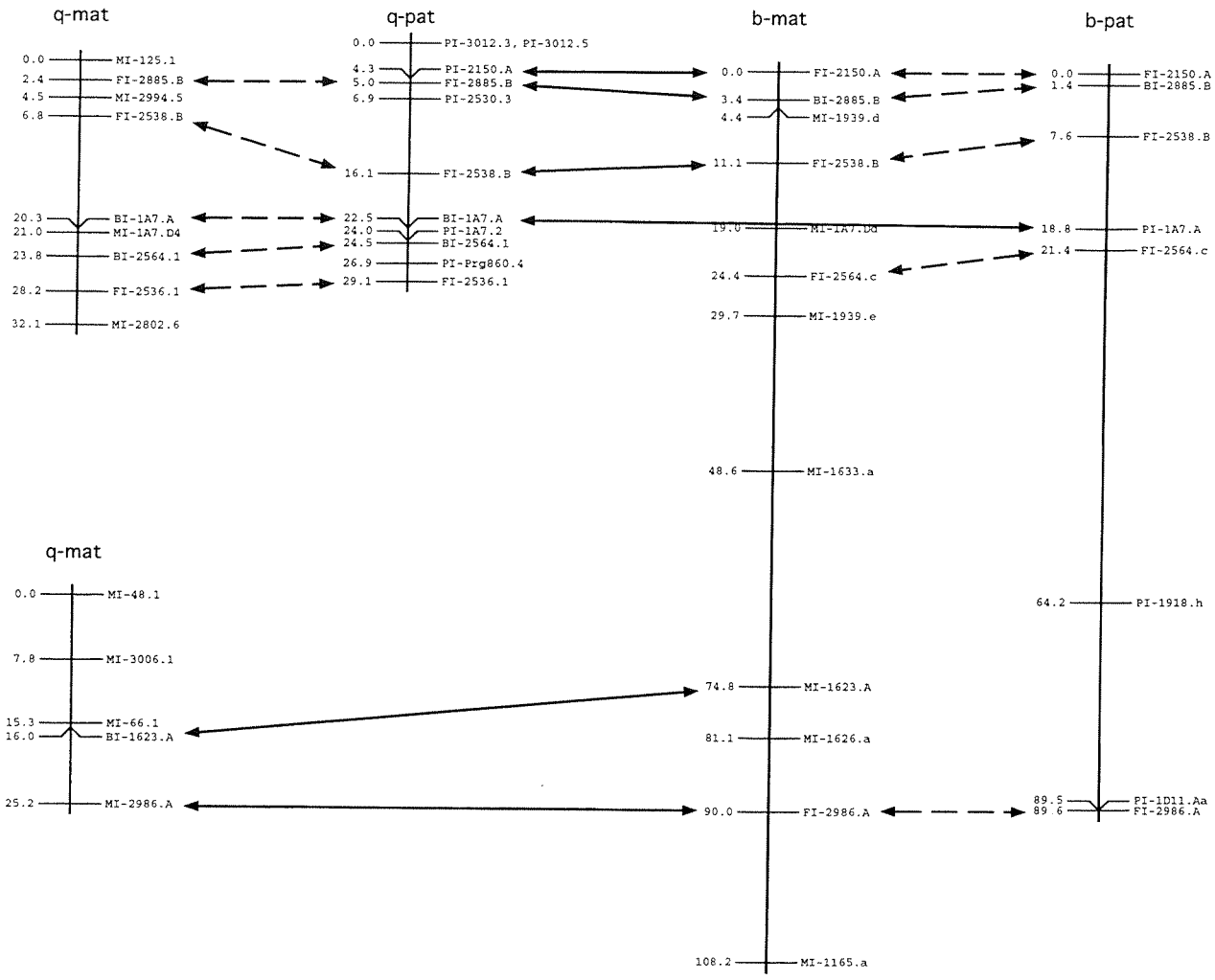

Figure 1. Integration of linkage data from within and among pedigrees. Using *consensus* linkage group 2 as an example, maternal (mat) and paternal (pat) linkage data from the *base* (b) and *qtl* (q) pedigrees are integrated into a single linkage group. Within a pedigree, FI and BI loci serve as anchor-points (dashed arrows); among pedigrees, "common" loci serve as anchor-points (solid arrows). See text for more details.

the number of markers and the degree of integration of linkage groups on the *consensus* map is reasonably high, we have yet to achieve our goal of 12 discrete linkage groups that represent the 12 chromosomes of loblolly pine (Table 1).

Table 1. Saturation of genetic markers on the *base*, *qtl* and *consensus* maps.

	base	*qtl*	*consensus*
Total unique loci	126	236	320
MI loci	45	96	--
PI loci	41	91	--
FI loci	33	36	--
BI loci	8	13	--
Common loci	--	--	42
No. linkage groups	9	17	18
Total distance (cM)	575	908	936
Average distance (cM)	6.7	4.4	3.5

Mapped Genes of Known Function

To identify functions for the molecular markers used in our genetic mapping project, Kinlaw et al. (1995) has determined partial DNA sequences for more than 200 loblolly pine cDNAs. Approximately 44% of these sequences have matched known genes based on database searches. Most sequence similarities are to genes from other plant species and include many enzymes involved in cellular metabolism and photosynthesis. This data is being used to study conifer genome organization and evolution. Sequencing efforts are being expanded to a variety of clones from specific conifer tissues. In addition, various collaborators have contributed cDNAs of characterized genes from loblolly pine (J. Cairney; D. Harry; C. Loopstra; D. O'Malley) and Scotts pine (P. sylvestris) (Jansson and Gustafsson; Karpinski et al.). Data for 18 isozyme loci is also included. Approximately 200 of these genes of known function are placed on the *consensus* map.

Future Directions

A logical extension from integrating independent maps from loblolly pine is comparative mapping among species from the genus Pinus. In collaboration with other laboratories, we have begun comparisons among loblolly, Monterey and slash pine. We will extend this effort to representative species from subsections of Pinus.

Comparative genetic mapping has become a powerful technique for investigating the mode and tempo of chromosomal evolution (Whitkus et al., 1992). However, for our goals of

mapping important traits in conifers, a more important aspect of comparative mapping is the potential to establish collinearity of linkage groups among pine genomes. If pine genomes are collinear, then it is likely that the location of a gene in one species can be used to predict the location of the homologous gene in another species (Jena et al., 1994).

The practical application of collinearity is that map locations for genes of important traits in one species will lead to a directed search for homologous genes in another species. For example, our success in mapping quantitative trait loci (QTLs) for wood specific gravity in loblolly pine (Groover et al., 1994) may help uncover QTLs for wood specific gravity in other pine species. Consequently, the emphasis of forest genetics research is not on a single species, but instead can focus on many species, each with regional importance and concerns. For this reason, establishing the extent of collinearity among pine genomes is fundamental to understanding genome organization and function in pines.

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SPATIAL PATTERNS OF MITOCHONDRIAL DNA VARIATION WITHIN JACK AND LODGEPOLE PINE POPULATIONS

T. Li[1], J. Dong[2], R. C. Hamelin[3], R. N. Patel[1] and D. B. Wagner[1]

Abstract.--We have studied maternally inherited mitochondrial variation in a sample of 1655 individuals from six natural populations of jack and lodgepole pines (*Pinus banksiana* Lamb., *Pinus contorta* Dougl.) in Alberta, Canada. Diversity was sufficient for analyses of within-population spatial distributions in one allopatric lodgepole pine population and three mixed-species populations. Surprisingly, no mitochondrial variants typical of jack pine were found in the three mixed populations. Spatial patterns were nonrandom in the three mixed populations, but random in the lodgepole pine population. These results provoke speculation that within-population mitochondrial spatial patterns may be restricted to hybridizing or introgressed populations. This conjecture is testable and has general implications for population genetic studies, as well as for germplasm improvement and conservation programs.

Keywords: *Pinus banksiana* Lamb., *Pinus contorta* Dougl., spatial autocorrelation, maternal inheritance

INTRODUCTION

Nonrandom spatial patterns of genetic variation within populations can result from the action of evolutionary forces. For example, effective gene flow tends to eliminate such patterns, while mating by proximity promotes their development (Epperson 1993). Similarly, natural selection can counteract dispersal and produce genotypic clusters within populations (Epperson and Allard 1989). However, interpretation of spatial patterns is complicated by many factors (Slatkin and Arter 1991).

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Regardless of the evolutionary cause(s) of spatial patterns, their presence or absence within populations is relevant to the exploration activities of germplasm improvement and conservation programs (*e.g.*, Epperson 1992). Also, such patterns affect population genetic statistics that are sensitive to pooling of genetically subdivided samples, such as gametic-phase disequilibria (*e.g.*, Prout 1973).

It is well established that organellar markers strengthen population genetic and systematic studies (*e.g.*, Avise *et al.* 1987). Pines (*Pinus* L.) have special advantages for such studies, due to their paternal chloroplast yet predominantly maternal mitochondrial inheritance (Schnabel and Asmussen 1989; Dong *et al.* 1992; Dong and Wagner 1993). Consequently, we have been including organellar markers in our studies of natural hybridization and introgression in jack and lodgepole pines.

Organellar markers are no different than nuclear markers in requiring us to understand spatial patterns prior to interpretation of population genetic data. In preliminary work, we found that chloroplast DNA (cpDNA) variants, despite their paternal inheritance, form patches within populations in a sympatric region of natural hybridization between jack and lodgepole pines (Wagner *et al.* 1991). Here we show that mitochondrial genotypes can also form patches in mixed-species populations.

METHODS

Based on the distributional ranges of the two species (Critchfield 1985), we sampled two allopatric lodgepole pine populations, two allopatric jack pine populations, and two populations in a sympatric region of natural hybridization between the two species, all located in Alberta (Table 1). The two sympatric populations are those in which cpDNA spatial pattern was detected previously (Wagner *et al.* 1991). We reused the sympatric DNA samples, but DNA's from the other four populations were extracted from new foliage collections.

Within the sampled area of each population, we sampled all cone-bearing trees. Distances and compass bearings between trees were recorded, in order to map the locations of all sampled individuals.

A mitochondrial *coxII* restriction fragment length polymorphism (RFLP) was assayed as described by Dong and Wagner (1993), except that a 1:1 mix of two cloned white spruce (*Picea glauca* (Moench) Voss) *coxII*-associated restriction fragments (Sutton *et al.* 1991) was used to probe pine *Sst*I fragments from the four allopatric populations (instead of a maize *coxII* probe; Fox and Leaver 1981). The maize *coxII* probe was used to probe DNA of the two sympatric populations. Comparative assays showed that the maize and white spruce probes identified the same RFLP (T. Li and D.B. Wagner, unpublished data).

We studied spatial patterns by spatial autocorrelation analysis of genotypes, the computational methods of which have been described in detail by Sokal and Oden (1978). Briefly, an analysis of each genotype in each population leads to a plot of

Table 1. Mitochondrial Variant Frequencies in Samples from Six Natural Populations

	Population Names and Initial Classifications of Population Type[a]					
	Allopatric Lodgepole Pine		Sympatric		Allopatric Jack Pine	
Variant[b]	Coleman	Edson	Carson Creek	Windfall	Wandering River[c]	Bellis
2.9-7.6 (jack pine)	.	.	.	.	.	288
3.1-10.2 (lodgepole pine)	137	37	41	31	95	.
5.2-10.2 (lodgepole pine)	2	202	232	422	161	.
4.4-10.2	.	.	1	.	.	.
6.8-10.2	.	.	1	.	.	.
8.1-10.2	.	.	.	.	5	.
Total	139	239	275	453	261	288

[a] Allopatric populations are named by nearest town. Additional information on sympatric populations available in Wagner *et al.* (1991); Bellis location shown in Dong and Wagner (1993). See also footnote "c" regarding classification of Wandering River population.

[b] Variants are named by sizes, in kilobase pairs, of *Sst*I fragments hybridizing with *coxII* probes (this nomenclature includes only variable fragments). Species origin indicated parenthetically for each variant if established by surveys (Dong and Wagner 1993; T. Li and D.B. Wagner, unpublished data).

[c] The Wandering River location was reclassified as a mixed-species population after analysis (see text for details).

standard normal deviates (SND's) as a function of distance (*i.e.* a correlogram, *e.g.*, Figure 1). Each SND was associated with a specific range of distances between trees and was based on observed and randomly expected numbers of pairs of trees in which the two trees of a pair both had the same genotype. One additional correlogram was constructed for each population, based on the total observed and expected numbers of pairs of all possible combinations of unlike genotypes (TU). SND's were computed by Pascal programs (Wagner *et al.* 1991).

Inspection of correlograms permits interpretation of spatial pattern (Figure 1). For example, organellar TU SND's are inversely related to gene identity probabilities (Epperson 1993). Thus, significant negative TU SND's in small distance classes, together with non-negative TU SND's in higher distance classes, imply the existence of genotypic patches.

We accepted an SND as a "valid" test of spatial pattern only if its expected number of pairs was greater than one (Cochran 1954). Because we computed many individual SND's, we used Šidák's probability ($p_S=1-(1-m)^k$) to evaluate overall statistical significance of mitochondrial spatial structure within each population (Oden 1984). Correlograms of different genotypes within a population are interdependent, because genotypic frequencies sum to one. Thus, for each population we conservatively took m as the minimum valid individual p value, and k as the total number of valid SND's, in all correlograms of the population.

RESULTS AND DISCUSSION

Population Subdivision and Hybridization/Introgression

Except for the Wandering River population, each allopatric population appeared monospecific, based on cone morphology, cpDNA, and mitochondrial DNA (mtDNA). However, the Wandering River (putatively jack pine) sample contained a mixture of jack and lodgepole pines, hybrids, and/or hybrid derivatives (T. Li and D.B. Wagner, unpublished data). Therefore, we treat Wandering River hereafter as a mixed-species population, rather than as a jack pine population.

Predictably (Petit *et al.* 1993), mitochondrial variant frequencies (Table 1) differed between the two allopatric lodgepole pine populations ($\chi^2=240.8$, d.f.$=1$, $p<0.001$). Although variant frequencies also differed statistically among the three mixed populations ($\chi^2=115.2$, d.f.$=4$, $p<0.001$), the magnitude of these frequency differences was less striking. Strong mitochondrial population subdivision has been reported previously in these pines (Dong and Wagner 1993).

A rangewide survey of jack and lodgepole pines (Dong and Wagner 1993) permitted us to ascertain the species origin of mtDNA variants (Table 1). We encountered only jack pine mtDNA in the Bellis population, but, surprisingly, we found no mtDNA variant typical of jack pine in any mixed-species population. In contrast, chloroplast genotypes and cone morphologies typical of both species were

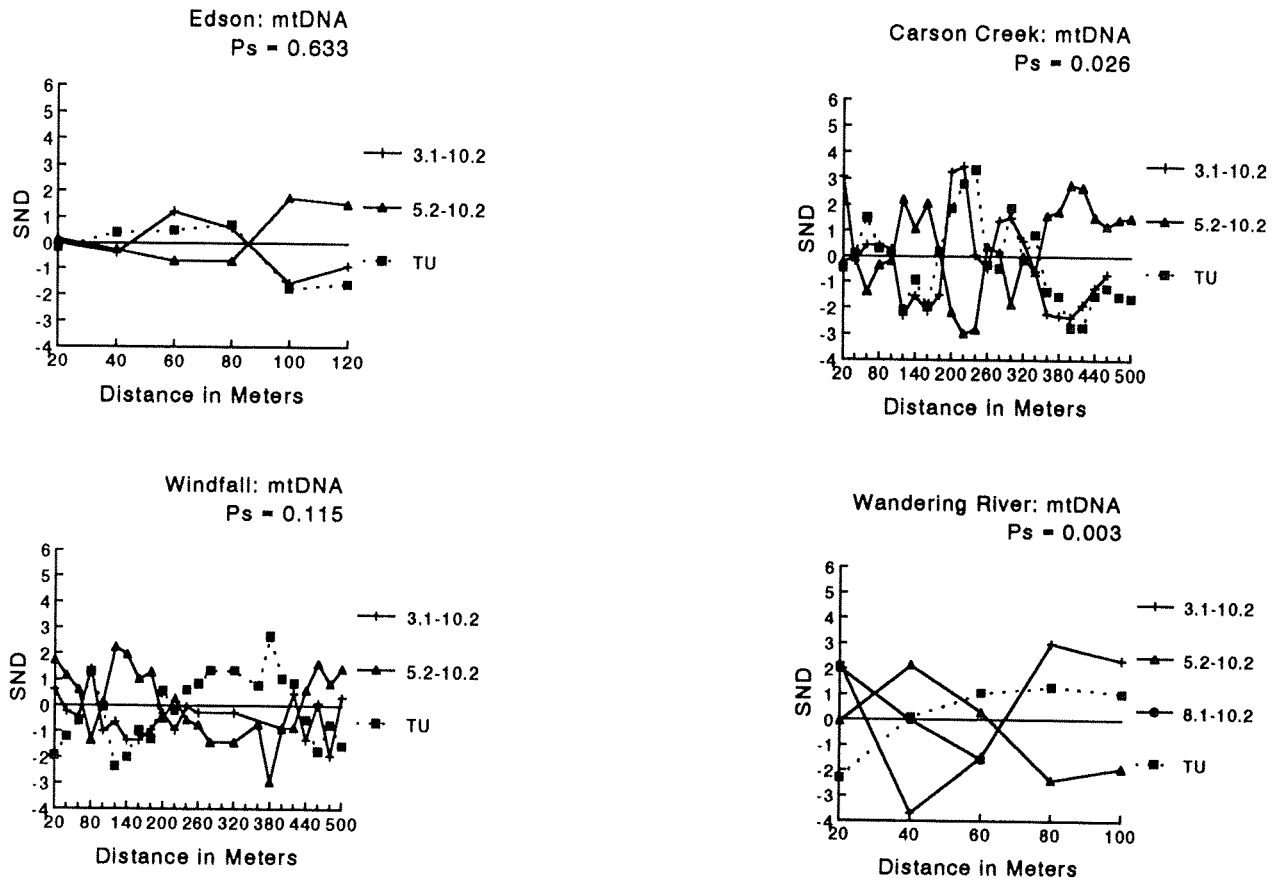

Figure 1. Mitochondrial correlograms, depicting only "valid" SND's (*i.e.*, those with expected numbers of pairs greater than 1). Edson is an allopatric lodgepole pine population; Carson Creek, Windfall, and Wandering River are mixed-species populations. Šidák's overall probability (p_S) is indicated for each population.

found in all three mixed populations (Wagner *et al.* 1991; T. Li and D.B. Wagner, unpublished data). Due to the mode of organellar inheritance in pines, these results indicate unidirectional hybridization in which lodgepole pine tends to serve as the female parent. The replacement of jack pine mtDNA by lodgepole pine mtDNA is compatible with phenological differences between the two species (Critchfield 1980) and has also occurred in a jack pine population located in an ancient sympatric region, hundreds of kilometers east of current areas of hybridization (Dong and Wagner 1993).

Spatial Autocorrelation

Two of the sampled populations were each fixed or nearly fixed for a single variant (Table 1). Thus, spatial analyses are restricted to the Edson lodgepole pine population and the three mixed populations (Figure 1).

At Edson, we detected no deviation of mtDNA variants from a random distribution (p_S=0.633). However, mitochondrial spatial patterns were significantly nonrandom in two of the mixed populations, Carson Creek and Wandering River (Figure 1). Although p_S=0.115 for the Windfall population, this probability is conservative (Oden 1984) and two features of this third mixed population's correlograms suggest spatial pattern. First, each variant's SND is positive in the lowest distance class (the 5.2-10.2 variant's SND's are, in fact, positive in the first three distance classes). Second, the TU SND is negative in its lowest three distance classes (significantly so in the 0-20 meter distance class).

Previous allopatric isoenzyme studies in these two species detected little spatial pattern, except for loci on chromosome segments that may be subject to selection (Epperson and Allard 1989; Xie and Knowles 1991). Studying mtDNA variants, we too failed to detect spatial pattern in an allopatric lodgepole pine population (Edson). Thus, the assumption that genotypes are usually randomized spatially within populations of conifers (and other wind-pollinated, outcrossing plants with efficient dispersal mechanisms) may not depend on the mode of inheritance. However, it would be premature indeed to advance this notion as more than conjecture, after study of mitochondrial diversity in only one allopatric population and in the face of prediction that spatial patterns could be strong for maternally inherited markers (Petit *et al.* 1993).

The nonrandom distributions of mtDNA variants found in all three mixed populations may represent an effect of natural hybridization. This effect could arise through any of several mechanisms, including reproductive and genomic incompatibilities between jack and lodgepole pines (*e.g.*, Critchfield 1980).

Note that the three mixed populations' mtDNA variants were mostly typical of lodgepole pine (Table 1). It is intriguing that the mitochondrial spatial pattern observed in these populations involved variants of only one of the two hybridizing species. We speculate that physiological effects of the lodgepole pine mitochondrial

genotypes may be variable and dependent on the genetic backgrounds in which they occur. Ongoing studies of chloroplast and nuclear genetic markers in these same populations may permit tests of hypotheses arising from this speculation.

CONCLUSIONS

1. Maternally inherited mitochondrial genotypes can form patches within sympatric populations of jack and lodgepole pines.

2. Mitochondrial spatial patterns are population specific; *a priori*, such patterns may not be predictable for a population of interest.

3. Limited seed dispersal may not be the most important factor responsible for mitochondrial spatial patterns; mechanisms associated with natural hybridization may be equally or more influential.

4. This report of cytoplasmic spatial patterns within populations is not isolated (van Damme 1986; Wagner *et al.* 1991); thus, failure to account for spatial structure may lead to serious artifacts (*e.g.*, Prout 1973).

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Detection of QTLs for economically important traits in Loblolly Pine

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Abstract. Understanding the genetic basis of economic traits in forest trees has been a goal of tree breeders and forest geneticists. Genomic mapping using molecular markers is a powerful new tool that extends conventional quantitative approaches to genetic analysis. Phenotypic variation in height, volume, disease resistance, lignin etc. can be associated with segregating genetic markers if these traits are under the control of one or a few major genes (quantitative trait loci, QTLs). Our studies are directed toward detection of QTLs inherited from the common seed parent in a large half-sib family. The effects of these QTLs are representative of average effects across the population and thus can be interpreted as components of breeding value.

Cyclic shoot elongation was evaluated in an open-pollinated family from clone 9-1020. Yearly height measurements on 1,000 trees were taken over 3 years and QTLs have been identified. The heritibility (additive genetic variation/phenotypic variation) for shoot elongation in young trees is approximately 1/2. The 2 QTLs found explain approximately 50% of the additive genetic variation expected to be transmitted by the seed parent in the half-sib family, which is about 14% of phenotypic variation. While these QTL effects are only a small portion of the total phenotypic variation in the family (3-4% each), they are the average effect of the alternative QTL alleles in the maternal with respect to a larger breeding population.

Yearly height measurements will continue to be taken to study QTLs over time. QTLs for volume, lignin content and rooting ability will also be evaluated.

Keywords: shoot elongation, QTLs, RAPDs, loblolly pine, half-sib family

A HEAT SUM MODEL FOR LOBLOLLY PINE POLLEN DEVELOPMENT

W. C. Woodbridge, F. E. Bridgwater[1], and D. L. Bramlett[2]

Abstract. Catkin development was recorded at 2 to 7-day intervals in February and March of 1988 to 1992 for 90 ramets from 31 families at the Weyerhaueser Company's loblolly pine seed orchard in Lyons, Georgia. Four to twelve clusters per ramet were observed each year and scored for development on a 6-point scale. The timing of catkin development varied widely over the study period. For example, the date when pollen release began (score = 4.0) varied from February 17 in 1990 to March 6 in 1988 for the earliest family and from March 7 in 1990 to March 26 in 1988 for the latest family. A heat sum model was developed to account for this annual variation using the first four years of data from 12 families measured all five years. The model which explained the most variation accumulated heat units above a threshold of 37 °F starting January 17. Different families required different heat sums to reach a given stage of catkin development. The model was validated using development data from 1992. The model predicted the time of 50 % pollen shedding (score = 5.5) within 1 day for 7 families, within 2 days for 3 more families, and within 4 and 6 days for the remaining two families.

Keywords: Loblolly pine, seed orchard, clonal, pollen, degree hour, heat sum, model.

INTRODUCTION

The phenology of pine pollen development is known to vary among years, among species, and among individuals within a species (Dorman and Barber, 1956; Blush, et al., 1993, and many others). In Loblolly pine seed orchards, between family variation of pollen shedding has been reported by Bramlett and Bridgwater (1989). The timing of clonal pollen release is useful to orchard managers for the scheduling of operations related to pollen collection, and the estimation of interclone crossing potentials and genetic composition of the seed crop. Wheeler and others (1993) believe that phenology is the most important influence on gene flow in forest seed orchards.

Clones tend to maintain their relative rank in timing from year to year (Blush, et al., 1993), and experienced workers can predict pollen release by monitoring catkin development and noting the relative timing of shedding of different clones for several years. However, the development of a heat sum model to predict different stages of pollen development could be useful for: 1) making predictions earlier in the season, 2) allowing for continuous adjustments to predictions based on updates in weather data, 3) quantifying clonal composition of the pollen cloud at any given time, and 4) reducing the expertise needed to make predictions. Such a model would not replace the need for field observations, but could reduce the work required by determining when field observations should begin.

A heat sum model predicts that a certain developmental event will occur when a defined heat unit total is reached. Heat units are accumulated during days or hours of the growing season during which the temperature is above a defined threshold value. The parameters of the model are: 1) the **start date** of the growing season, 2) the **threshold** temperature, and 3) the **critical heat sum** required for the target event to occur. Many assumptions and simplifications are made; the limitations of heat sum models have been well reviewed by Wang (1960). Nevertheless, they have had useful applications in agriculture (Wang, 1960) and

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forestry, including the description of bud development in Douglas-fir (Thomson and Moncrieff, 1982), red pine (Sucoff, 1971) and Loblolly pine (Boyer, 1970), and pollen shedding in southern pine (Boyer, 1978).

The objective of this study was to create a heat sum model that would account for the annual variation in timing of pollen development for different clones of Loblolly pine. The model would use a single threshold temperature and start date for all clones in the orchard, but each clone would have a different critical heat sum. The model would be used to predict the timing of pollen shedding based on weather data in future years.

METHODS

Pollen Development Classification System

A method for consistent assessment of catkin development and degree of pollen shedding was a prerequisite to model development. Bramlett and Bridgwater (1989) presented a Pollen Development Classification System (PDCS) that measured development on a six-point scale; pollen shedding begins at a score of 4.0 and becomes measurable at 5.0 (Table 1). An important feature of this scale is that in addition to identifying the stage of pollen release, the percentage of pollen shed is estimated.

TABLE 1. Pollen Development Classification System. (Abbreviated from Bramlett & Bridgwater, 1989).

PDCS stage	Description	Approximate date for Lyons, GA
1.0 -3.9	Various stages of catkin elongation.	Fall - Feb.
4.0	Pollen release begins, less than 10% of pollen released.	mid Feb. - Mar.
5.0	Pollen release increases, more than 10% released.	Mar.
5.2	20% released.	
:	:	
5.5	50% released.	
:	:	
5.9	90% released.	
6.0	All pollen released.	Apr.

These stages apply to individual catkins. When assigning a PDCS score to a cluster comprised of catkins in various developmental stages, the cluster was scored to reflect the average of the catkins.

Field measurements of pollen development

Observations of catkin development were made on 90 ramets from 31 families from 1988 to 1992 at the Weyerhaueser Company seed orchard in Lyons, Georgia. The families included in the study varied from year to year due to 1) roguing in the orchard, 2) reducing the overall sample size, and 3) missing the shedding season of early clones in 1989. Seven families were measured all five years and twelve were measured in all but 1989. The model described in this report is based on those 12 families. Ten to twelve clusters distributed throughout the crown of each ramet[1] were tagged and scored at two to seven-day intervals during the flowering season. Ramet scores were means of ramet clusters, weighted by the number of catkins in the clusters. Based on 1988 preliminary results, the number of families and the number of ramets per family were reduced, and scores of ramets of the same clone were averaged when clones were represented by more than one ramet.

Temperature data was recorded at hourly intervals by an onsite weather station. Missing observations were filled with data from the nearest National Weather Service stations.

[1] In 1989 only 4 clusters were measured and the beginning of shedding season was missed.

Choosing the heat sum model parameters

The first step was to choose the target developmental stage. We examined targets of 4.0, 5.0 and 5.5; the target used for example in this report is a PDCS stage of 5.5, the stage when half of a clone's pollen is shed. This date was determined for each clone by graphical analysis of the plot of average PDCS score over time. The heat sum at which stage 5.5 was reached depends on the threshold and start date. These two parameters of a heat sum model are correlated with each other, complicating the task of choosing best fit values (Boyer, 1973). The method used is an adaptation of Boyer's work with Longleaf pine. He modeled the timing of pollen shedding of a *single population*, choosing the start date and threshold that reduced the variation over years of the critical heat sum: the heat sum accumulated at the time of pollen shedding. Once these two parameters were determined, the critical heatsum required in a given year was related to and modified by the length of time required to reach that heat sum. In our study, we proceeded similarly for *each family*, choosing a different start date and threshold for each family. We then chose single values for these two parameters that were the best overall choice for *all* families. The model critical heat sum was calculated for each family as the average observed critical heatsum over years.

To determine the best start date and threshold for a single family, heat sums were calculated using all combinations of values of 30 to 60 degrees F. for the threshold and Julian day 1 to 42 (Jan 1 to Feb 11) for the start date. For each combination, the heat sum at which each clone reached a score of 5.5 was determined for each year. The standard deviation and coefficient of variation of these heat sums over years were determined for each combination. The best combination, *i.e.* the model that explained the most variation, was determined by examination of the effect of start date and threshold on standard deviation and coefficient of variation. This analysis is confounded because when the threshold is lowered, standard deviation will *increase* due to the size of the means while coefficient of variation will *decrease* because annual variation is dampened (Boyer, 1973). However, minima and slopes of these functions indicated the best threshold and start date parameter values.

Model Validation

The model predicts that PDCS stage 5.5 is reached when the heat sum reaches a certain value for a given clone. The model's accuracy can be checked by comparing the day that this was observed to occur to the day predicted to occur for each year. This was done for the years used in model formation, 1988 to 1991, and for 1992, a year of data independent from the model formation data.

RESULTS AND DISCUSSION

The variation in timing of all (31) clones in a single year (1988) was described by Bramlett and Bridgwater (1989). Their plot also showed the similarity in shape of the development curves for the different clones. For this study, plots of PDCS score against time for all years were made for each clone. Figure 1a is an example plot for a single clone, "19", and shows patterns that are typical of all the clones: 1) ramets of the same clone in a given year develop synchronously, 2) large differences exist in the timing of pollen development between years of a given clone. For this clone, there was a 4 or 5-day difference between the times the earliest and latest ramets reached stage 5.5 in a single year while there were over 20 days difference between the earliest and the latest year. These results gave us the confidence to average scores from ramets of the same clone for a given year. Note that in a plot of these averages (figure 1b), the development curves for 1990 and 1988 fluctuate. This is because data were averaged by date and ramets were not all measured on the same day.

Plots of the average development score for a family allowed determination of the dates that PDCS stage 5.5 (or other stage) was reached in the various years by the family. In figure 1b, the vertical arrows placed at the intersection of the curves and the target PDCS stage of 5.5 intersect the horizontal axis at these dates. Some adjustment is required to accommodate fluctuating lines, but we considered that this error was not important in estimating dates to the nearest day. Table 2 shows the Julian dates at which the 12 clones

used in the model attained PDCS scores of 4.0 and 5.5. Field measurements began late in 1989 and some clones had already finished shedding pollen before they were visited. The results in Table 2 show that the relative timing of clones remains stable over time, but the magnitude of the differences in timing and the amount of overlap of shedding is variable. The difference between when the earliest and latest clone reached stage 5.5 was 16 - 18 days for most years and there was no overlap in shedding (score 4.0 - 6.0) between the earliest and latest clones. In 1992, however, the difference is only four days, so most of the clones were shedding at the same time. This phenomenon may be explained by the high temperatures proceeding and during pollen

FIGURE 1. Pollen Development Classification Scores for Loblolly pine clone "19" plotted over day. Weyerhaueser company seed orchard, Lyons, GA, 1988 - 1991.

a) PDCS scores for individual ramets; three for 1990, one each for 1989 and 1991, and eight for 1988.

b) Date that average PDCS score (means of all ramets measured in a given year) reached 5.5, 50% of pollen shed, in 1988 - 1991. Fluctuating average in 1990 and 1988 occurs because different ramets were measured on different days.

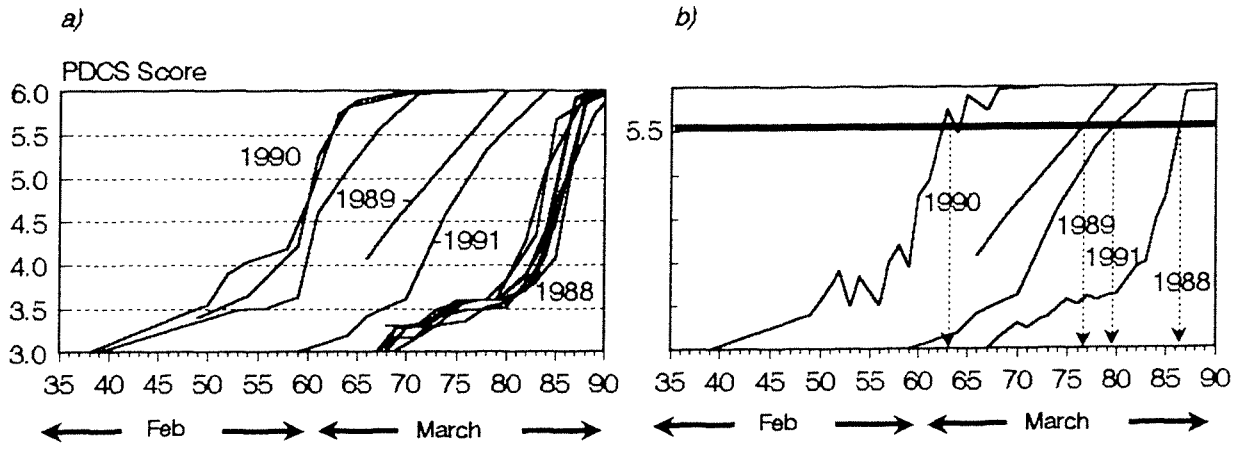


TABLE 2. Dates that PDCS score reached stages 4 and 5.5 in 1988 - 1992. Clones are ordered based on average development dates.

CLONE	*1988*		*1989*		*1990*		*1991*		*1992*	
	4	*5.5*	*4*	*5.5*	*4*	*5.5*	*4*	*5.5*	*4*	*5.5*
53	66	70	-	-	48	54	59	64	64	67
22	72	78	-	-	-	-	61	66	60	65
43	76	83	-	-	44	52	64	72	65	68
28	76	83	-	-	44	55	66	72	65	68
00	76	81	56	65	51	57	67	73	65	68
33	75	81	62	71	54	60	67	73	66	68
39	81	85	66	74	57	63	71	75	66	69
20	81	86	61	74	59	65	70	75	66	68
19	83	86	65	76	58	63	71	79	66	70
37	84	87	60	75	51	64	71	79	67	70
73	83	87	-	-	60	64	72	77	68	70
31	85	88	71	78	65	70	77	82	68	71

FIGURE 2. Effect of start date and temperature threshold on the heat sum development curve of Loblolly pine clone 19 in years 1988 - 1991. Year 1992 is included in *d)*.

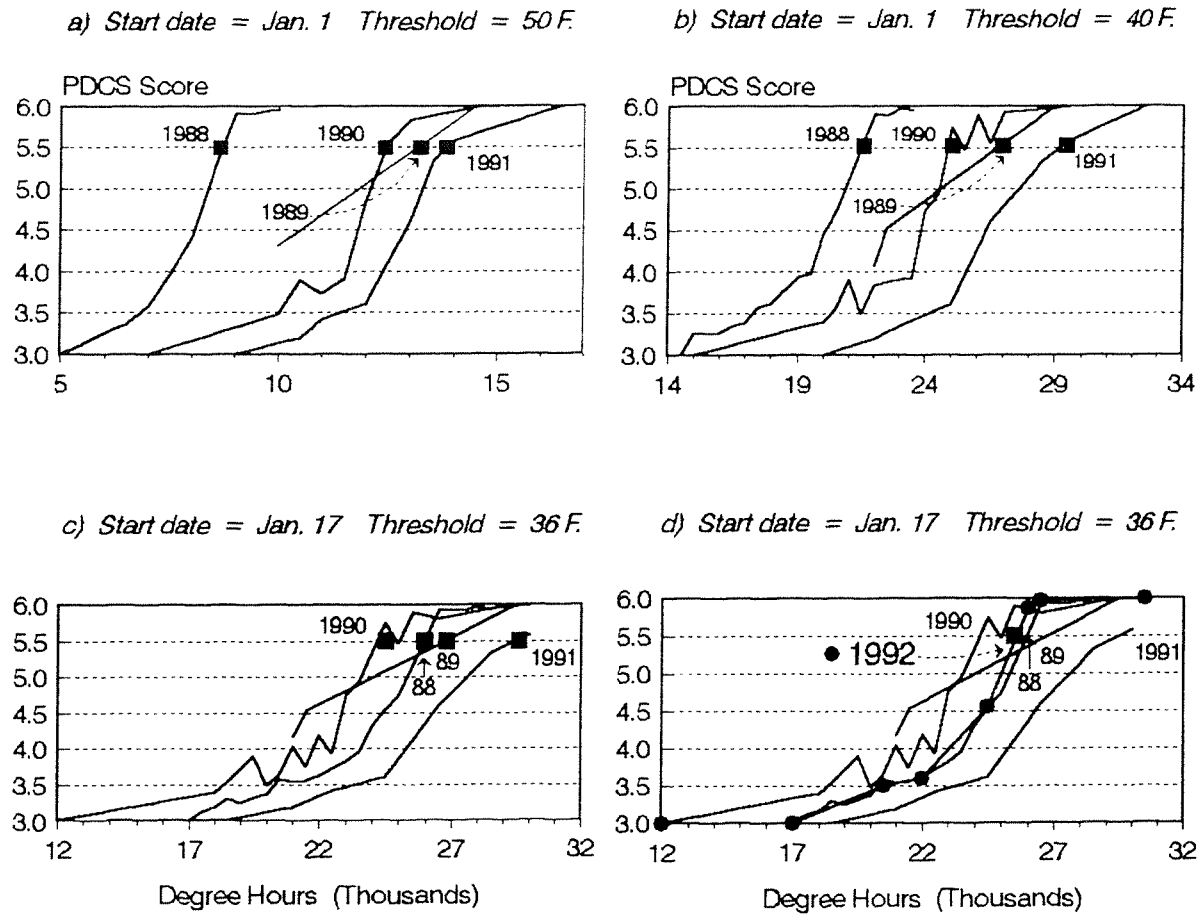
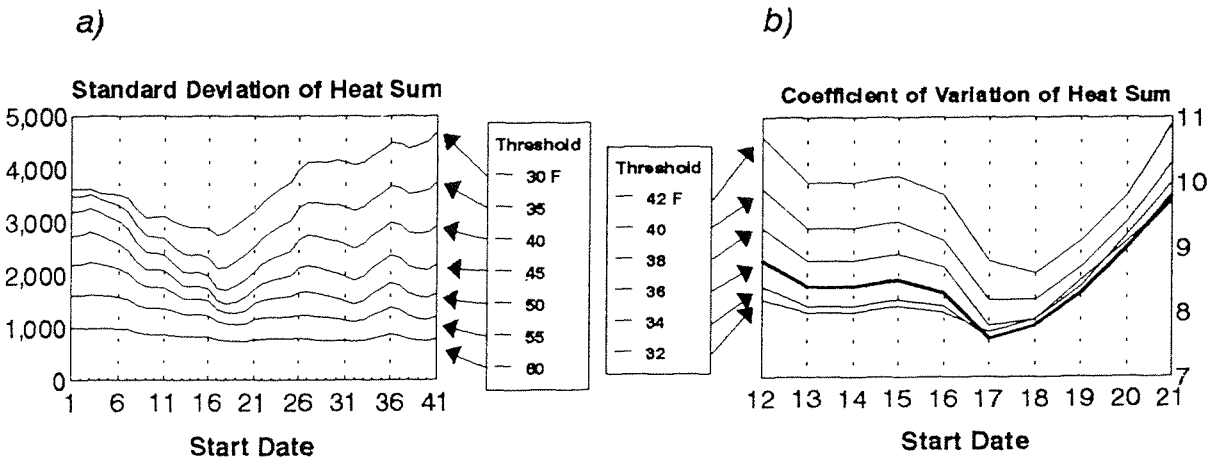


FIGURE 3. Effect of start date and temperature threshold on *a)* standard deviation and *b)* coefficient of variation of heat sum required for Loblolly pine clone 19 to reach PDCS stage 5.5 in years 1988 - 1991. Note that *b)* contains more narrow ranges in parameter values.



shedding in 1992 and is consistent with a heatsum model. The difference in timing of stage 5.5 between the coolest year (1988) and the warmest year (1990) for a given clone was greater than the between clone variation within years and ranged from 16 to 31 days with a mean of 23 days.

Once these target dates had been established, the heat sum at which PDCS stage 5.5 was reached was determined for a model with a given threshold and start date, and the standard deviation and coefficient of variation of the heat sums for the four years were calculated. This process was repeated for all combinations of threshold and start date. The effect of varying the parameters is shown in figure 2. Figure 2a shows PDCS scores for clone 19 plotted against heat sums based on a start date of January 1 and a threshold of 50 degrees F. With this model, score 5.5 was reached at a lower heatsum in 1988 even though this was the coldest, latest year. Boyer described a similar relationship, *i.e.* lower heat sums required when development times were longer, but this relationship could be modeled in our study by lowering the threshold temperature and assuming a constant critical heat sum over years. Figure 2b shows that a change of threshold from 50 to 40 degrees has "pulled in" the critical heat sum for 1988. This is because addition of heat units between 40 and 50 degrees occurs over a longer period of time in 1988.

The results of the parameter grid search for one clone are presented in figure 3. In figure 3a, the standard deviation increases with decreasing threshold because the heat sums are larger. However, for a given threshold, the minimum s.d. indicates the best start date. For clone 19, this best date is between day 17 and 20, depending on the threshold. No other minima were found outside the range shown in the graph so we are confident that the best start date occurs after January 1. Plots of s.d. for other clones showed similar patterns and optimal start dates. Figure 3b shows the coefficient of variation for the different models. An increase in the threshold results in higher a c.v. because differences in temperature become relatively more important. These curves are roughly parallel, but note the line for threshold = 36: for a start date after day 17, the c.v. is actually lower than for a threshold of 32 or 34. This is an indication that 36 is the best choice to reduce the variation in heat sum required to reach PDCS stage 5.5 for this clone. Some clones were fairly insensitive to changes in threshold or start date, but most showed a reduction in s.d. and c.v. with a threshold near 36 degrees Fahrenheit and a start date near day January 17, so these parameters were chosen to represent the entire orchard. Figure 3c shows the PDCS score curves using this model.

TABLE 3. Observed and predicted Julian dates and residual days that clones of Loblolly pine reached PDCS stage 5.5 using a heat sum model with a start date of Jan. 1 and a threshold of 36 F.

Clone	1988			1989			1990			1991			1992		
	Obs	Pred.	Res	Obs	Pred.	Res	Obs	Pred.	Res	Obs	Pred.	Res	Obs	Pred.	Res
53	70	77	-7	-	-	-	54	55	-1	64	64	0	67	63	4
22	78	80	-2	-	-	-	-	-	-	66	65	1	65	65	0
43	83	82	1	-	-	-	52	61	-9	72	67	5	68	66	2
28	83	83	0	-	-	-	55	61	-6	72	68	4	68	67	1
00	81	82	1	65	72	-7	57	60	-3	73	67	6	68	66	2
33	81	83	-2	71	73	-2	60	61	-1	73	68	5	68	67	1
39	85	85	0	74	75	-1	63	64	-1	75	72	3	69	69	0
20	86	86	0	74	75	-1	65	65	0	75	72	3	68	70	-2
19	86	87	-1	76	76	0	63	67	-4	79	74	5	70	71	-1
37	87	87	0	75	76	-1	64	67	-3	77	74	3	70	71	-1
73	87	87	0	-	-	-	64	67	-3	77	74	3	70	71	-1
31	88	90	-2	78	79	-1	70	70	0	82	77	5	71	77	-6
Average			-1			-2			-3			4			0

Table 3 shows the difference between observed and predicted dates of reaching stage 5.5 for twelve clones using this model. The residual between observed and predicted dates was only one or two days for most clones in most years. Exceptions seem to be early clones ("53" and "00") that flower much

earlier than predicted in some years. Average residual was three days early in 1990. This year was a warmer than the rest and our model did not completely account for the difference. In 1991, the residuals were four days "late". We believe that this is due to an unusual cold snap that occurred in mid February of that year. In its simple form, a heat sum model does not account for any developmental setback that may occur from cold weather. Residuals were low for 1992, the independent year, indicating that our model may be useful for predicting pollen development in future years. However , these accurate predictions are due in part to the fast speed at which pollen matured from stage 4.0 to 5.5 in 1992.

CONCLUSIONS

Our results indicate that the selected heat sum model should be useful for predicting pollen shedding for the study location. Ramets of the same clone develop at the same time and rate, so clones can be represented by a single ramet. Pollen develops faster in warm years, indicating a relation to temperature and development. A heat sum model with a threshold of 36 degrees F. and a start date of Jan 17 reduced the s.d and c.v. for most of the clones. The accuracy of the model is not highly sensitive to these parameters and may vary if a different data set is used. Predicting the timing of pollen shedding seems feasible, but less so for very early or late clones, and years with unusually cold or warm periods during pollen maturation may reduce the accuracy of the predictions.

Orchard managers need not make detailed observations of pollen development to use a heat sum model for predicting shedding. A record of the timing of a certain observable event (*e.g.* the beginning of pollen release for a single clone) along with temperature data over several years would be adequate data for rudimentary model develpment.

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EFFECTS OF ROOTSTOCK GENOTYPE AND SCION CHRONOLOGICAL AGE ON GROWTH AND FLOWERING OF SLASH PINE GRAFTS.

S. R. Parker, T. L. White, G. R. Hodge and G. L. Powell

Abstract.--In 1988 and 1989, the University of Florida Cooperative Forest Genetics Research Program (CFGRP) established clone banks throughout the Southeastern U. S. for breeding and scion multiplication for seed orchards. This provided an opportunity to study rootstock-scion interactions, screen for potential seed orchard rootstock families and study the effects of scion maturation on growth and reproduction of grafted slash pine clones across many sites and ages. This study included nine clone banks, seventy-six open-pollinated slash pine rootstock families, approximately 460 scion clones of different chronological ages and over 3600 ramets. Comparisons among rootstocks were made for height and diameter growth, disease resistance, female strobili production, male strobili production and survival. The scion clones had chronological ages (age from time of seed germination) of 5 to over 40 years. Comparisons of height growth, diameter growth and female and male strobili production were made between older and younger scion clones.

Keywords: *Pinus elliottii*, seed orchard, rootstock-scion interaction, chronological age, juvenility.

INTRODUCTION

Industrial forestry in the Southern United States is primarily based on the use of genetically-improved seed for plantation establishment, which has significantly increased both the volume and quality of wood throughout the region. As a result, clones in breeding programs and seed orchards are of immense economic value. Most existing slash pine (*Pinus elliottii* Engelm. var *elliottii*) seed orchards were grafted using unselected rootstock, and some orchards have experienced incompatibility, poor growth and inadequate or delayed flowering. Many methods of controlling these problems have been tried such as top-pruning, fertilization and irrigation timing and partial girdling (Schmidtling 1980, 1985). Some methods have been effective (fertilization and irrigation), while others seem to be of limited use (top-pruning) (Varnel 1969, Greenwood and Bramlett 1989). The use of genetically-selected rootstock is another possible means of providing cost-effective control of scion characteristics for the life of grafted trees, which has proved effective with horticultural perennials such as apples and peaches (Schmidtling 1980, Simons 1987).

As woody plants age, their development begins with the juvenile phase, which can last from a few days to as long as 30-40 years and is characterized by vegetative growth with little or no reproductive growth. The mature phase follows and is characterized by consistent reproductive growth (Hackett 1985, Hackett et al. 1990, Greenwood and Hutchison 1993). This process is commonly called phase change or maturation and has many synonymous names such as ontogeny, cyclophysis, ontogenetic ageing, meristem ageing, ageing and juvenility (Brink 1962, Oleson 1973, Hackett 1985). A great deal of research on phase change has been done in horticultural crops especially citrus, prunus and apple species (Visser 1964, 1965, Zimmerman 1972, Hackett 1985, Oliveira and Browning 1993, Snowball et al. 1994). However, less work on maturation has been done with conifers and almost none with slash pine (Hood and Libby 1978, Greenwood and Nussbaum 1981, Greenwood 1981, 1984, Bolstad and Libby 1982, Greenwood et al. 1989, Burris et al. 1991).

There are three main objectives to this study: (1) To ascertain whether some genotypes, when used as rootstock in grafted seed orchards; will confer desirable characteristics to the scion; (2) To screen a large number of open-pollinated families for their potential as rootstocks; and (3) To examine the effects of scion chronological age on the growth and reproduction of slash pine in clone banks and seed orchards. Characteristics that would be desirable to screen for are: early flowering (precocity), heavy flower production (fecundity), graft compatibility, rust resistance, pitch canker resistance and dwarfing.

METHODS

The nine clone bank locations are planted on a 15 by 30 foot (4.6 by 9.2 meters) spacing with from 5 to 12 blocks (1 block is 0.62 acres or 0.25 hectares), 40 to 120 scion clones and 8 to 10 open-pollinated (OP) rootstock families per clone bank with between 30 and 72 ramets grafted onto each rootstock family. The scion clones have chronological ages from 5 to 30 years among the forward selections, and greater than 40 years among the backward selections. Backward (parental) selections are original selections made in the 1950's which have been maintained in orchards. Forward (offspring) selections are offspring of the original selections and were selected from progeny tests. The rootstocks are placed in 5 groups with 2 OP rootstock families in each, selected based on their parents' performance in various progeny tests and research studies. Each rootstock group was intended to test one of 5 performance traits which were: (A) growth, (B) flowering, (C) graft compatibility, (D) fusiform rust resistance and (E) pitch canker resistance. Each group consists of a high and low performer for each trait (i.e., a fast and slow grower in group A in each clone bank).

An ideal clone bank contains 10 randomized complete blocks, 10 rootstock families and 100 scion selections, with 5 blocks established in 1988 and 5 in 1989. Each block consists of 20 row plots, of three trees each. A given rootstock group was assigned to 4 row plots (12 ramet positions per block), and two scion clones were assigned to each rootstock group. Each scion clone was grafted onto one row plot of each rootstock within a group (i.e., 6 positions per block). Thus, each group of 4 row plots can be viewed as a 2 X 2 factorial (2 rootstocks by 2 scion clones). The two scion clones are therefore nested in each block by group combination, and cross classified with rootstock families within the group. There are five rootstock groups (A through E) and thus five different contrasts going on within each block. The rootstock groups are considered whole plot factors, and the two rootstock families within each group are considered subplot factors. Single degree of freedom contrasts within the groups measure the difference between the two rootstocks in each group. Having scion clones nested within rootstock groups within blocks allows for the many scion clones needed in these operational clone banks.

Measurements were taken each year from 1988 until 1995 of: planting code, status (whether living, dead, fusiform rust infected, pitch canker infected and graft incompatible), total height, height to graft union, scion and rootstock diameter (near the graft union), number of lateral branches, female strobili (flower) counts and male catkin cluster counts. Low disease incidence, high graft compatibility and good survival in the clone banks precluded the analysis of the disease, graft incompatibility and survival variables. The seven key variables that could be analyzed were: rootstock diameter, scion diameter, ramet height (total height minus height to graft union), ratio of scion diameter to rootstock diameter, ratio of ramet height to scion diameter, number of flowers per tree and number of male catkin clusters per tree.

Analysis of the Rootstock and Scion Effects

The rootstock, scion and the rootstock-scion interaction effects were examined to determine their overall effects on growth and reproduction. The linear model for the analysis was:

$$y_{ijkl} = b_i + g_j + bg_{ij} + r_{k(j)} + br_{ik(j)} + s_{l(ij)} + rs_{kl(ij)}$$

where b_i = random effect of ith block,
g_j = fixed effect of jth rootstock group,
bg_{ij} = random effect of interaction of ith block and j^{th} rootstock group,
$r_{k(j)}$ = fixed effect of kth rootstock within j^{th} rootstock group,
$br_{ik(j)}$ = random effect of interaction of ith block with k^{th} rootstock,
$s_{l(ij)}$ = random effect of the ith scion within the ith block and j^{th} rootstock group,
$rs_{kl(ij)}$ = random effect of interaction of kth rootstock and l^{th} scion.

There was too little growth in year 1 to include it in the rootstock analysis. And there were too few flowers and catkin clusters before years 5 and 6 to test the effects of flowers per ramet and male catkin clusters per ramet. All analyses was done using procedure GLM in the SAS® programming language (SAS Institute 1989). Tests were considered significant if the F-test was significant at the α=0.10 level. To strengthen the test of the within-group contrasts the rootstock scion interaction ($rs_{kl(ij)}$) was pooled with the error. Only 33% of the F-tests for the interaction were significant at the α=0.25 level, with no consistency by trait or measurement year, and their mean significance was greater than α=0.25, which is an acceptable level for pooling (Bozivich et. al. 1956, Bancroft 1968). This reduced linear model was used to test the single degree of freedom contrasts for each rootstock grouping in each clone bank. Due to low disease incidence, high graft compatibility and high survival only contrasts within the flowering and growth groups were used (ie., rootstock groups A and B). Further, only contrasts in measurement years 5 and 6 were analyzed since persistent rootstock effects are the ones of most interest.

In order to estimate the relative effects of the rootstock and scion on growth and reproduction of ramets in the clone banks, a ratio of the rootstock family variance component to the sum of the rootstock and scion variance components was calculated. The variance component estimates were obtained through the VARCOMP procedure in SAS® (SAS Institute 1989). Since, due to common pollen parents, the intraclass correlation among rootstock OP families is probably closer to 0.30 than 0.25 the constant 3.3 ,instead of 4, was used to multiply the rootstock family variance to obtain an estimate of additive variance (Squillace 1974). The scion variance component is among scion clones and hence estimates total genetic variance. It was used in the denominator of the ration without amplification. Several methods of variance component estimation were tried and found to produce similar results, so the ANOVA Type I sums of squares method was used, since it is both simple and unbiased. The ratio gives the amount of rootstock variance compared to the total rootstock and scion variance. Thus, the closer the ratio is to 0, the less variance that is accounted for by the rootstock, and the closer to 1 the more variance accounted for by the rootstock.

Scion Chronological Age Analysis

To quantify the effects of scion chronological age (age from time of seed germination) on growth and flowering of clone bank ramets, four variables were analyzed at ramet ages 1 to 6 (age of a ramet since it was grafted). They were: scion diameter, ramet height (total height minus height to graft union), flowers per ramet and male catkin clusters per ramet. Since the only true replication of scion clones was due to the two different grafting years and the nine clone bank locations, the units of observation for this analysis were means by chronological age group (backward selections or forward selections) within year-grafted (1988 or 1989) within location (9 clone banks). For example, the mean of all forward selections grafted in 1988 in a given location is considered one observation. The linear model for the scion age analysis was:

$$y_{ijk} = l_i + y_j + ly_{ij} + g_k + lg_{ik} + yg_{jk} + lyg_{ijk}$$

where l_i = random effect of ith location (i=1,··· 9),
y_j = random effect of jth year (j=1988 or 1989),
ly_{ij} = random effect of interaction of ith location and j^{th} year,
g_k = fixed effect of kth age group (k= backward or forward),
lg_{ik} = random effect of interaction of ith location and k^{th} age group,
yg_{jk} = random effect of interaction of jth year and k^{th} age group.

The three way interaction was considered the error term to increase its power and the "sometimes pooling" technique was used to determine which two-way interactions could be pooled with it (Bozivich et. al. 1956, Bancroft 1968). The year-grafted by age group (yg_{jk}) and the location by age group (lg_{ik}) interactions had few significant F-tests with no consistency by trait or measurement year and were thus acceptable for pooling (Bozivich et. al. 1956, Bancroft 1968). Pooling led to the use of a reduced model in which the residual error consisted of the pooled lyg_{ijk} and yg_{jk} interactions for most F-tests and lyg_{ijk}, yg_{jk} and lg_{ik} interactions for the few remaining F-tests. This model was used to test the difference between backward and forward selections.

RESULTS AND DISCUSSION

Overall rootstock effects were only occasionally significant at the α=0.10 level for the 4 growth variables, and effectively nonsignificant for the other 3 variables. However, overall scion effects were almost always significant. The interaction between rootstock and scion had more significant tests than the rootstock effects alone. The rootstock effects when present were small compared to the scion effects (Table 1). Only 18% of 92 rootstock group contrasts showed significance at the α=0.10 level. At the same time 19% of all possible contrasts (the 7 variables by 5 rootstock group contrasts by 9 clone banks) were significant at the α=0.10 level. That is, the high flowering or growth rootstock, chosen on the basis of its progeny test performance, was almost never significantly different than the low flowering or growth rootstock family in the same group. If the groups were well-chosen they should have a higher percentage of significant contrasts than for all contrasts. Thus, the choices of rootstocks to contrast in the flowering and growth groups were not effective, which is not surprising given the small overall rootstock effects relative to overall scion effects (Table 1). Scion effects seem to be so large that they seem to overwhelm the rootstock effects.

Table 1. Number and percentage of tests of the overall rootstock, scion and rootstock by scion interaction effects that were significant, at α=0.10, level for 7 key variables. The N is the total number of tests for the variable, while the % value is the percentage of tests that were significant at the α=0.10 level.

	Model effects					
	Rootstock		Scion		Interaction	
Variables	N	%	N	%	N	%
Flowers / ramet	17	6	18	83	18	22
Height / scion dia. ratio	41	24	41	78	40	23
Catkin clusters / ramet	18	6	18	83	18	39
Ramet height	43	9	43	95	43	21
Scion dia. / rootstock dia. ratio	41	34	41	54	41	22
Rootstock diameter	41	17	41	73	41	27
Scion diameter	40	23	41	78	41	24

The ratio of the rootstock variance to the sum of the rootstock and scion variance components indicates that the relative contribution of rootstock to the genetic variance is much less than the scion contributions. All variables, except the scion diameter to rootstock diameter ratio, had ratios of about 0.1 to 0.2. Also, the ratio for the ramet height and scion diameter started high, but fell after year 2 implying the rootstock effect diminished quickly as the ramets aged (Table 2). Height to scion diameter ratio diminished slowly over the 5 years. The ratio of scion diameter to rootstock diameter remained constant and fairly high over all 5 measurement years. Rootstock effects on the 2 flowering variables may be increasing, but with only 2 year's flowering data there is too little information to call it a trend (Table 2).

Table 2. Ratio of the rootstock variance to the sum of the rootstock and scion variance components for the 7 key variables averaged across the 9 clone banks. Each measurement year's value is the mean of the values of all 9 clone banks. Insufficient flowers were present in years 2 through 4 to estimate the flower per ramet and catkin clusters per ramet ratios.

	Measurement Year					
Variables	2	3	4	5	6	Mean
Flowers / ramet				0.05	0.15	0.11
Height / scion dia. ratio	0.29	0.36	0.12	0.19	0.02	0.19
Catkin clusters / ramet				0.14	0.23	0.19
Scion dia. / rootstock dia.	0.29	0.38	0.33	0.40	0.38	0.36
Ramet height	0.31	0.08	0.12	0.05	0.06	0.12
Rootstock diameter	0.29	0.29	0.22	0.13	0.22	0.23
Scion diameter	0.24	0.02	0.11	0.08	0.10	0.11

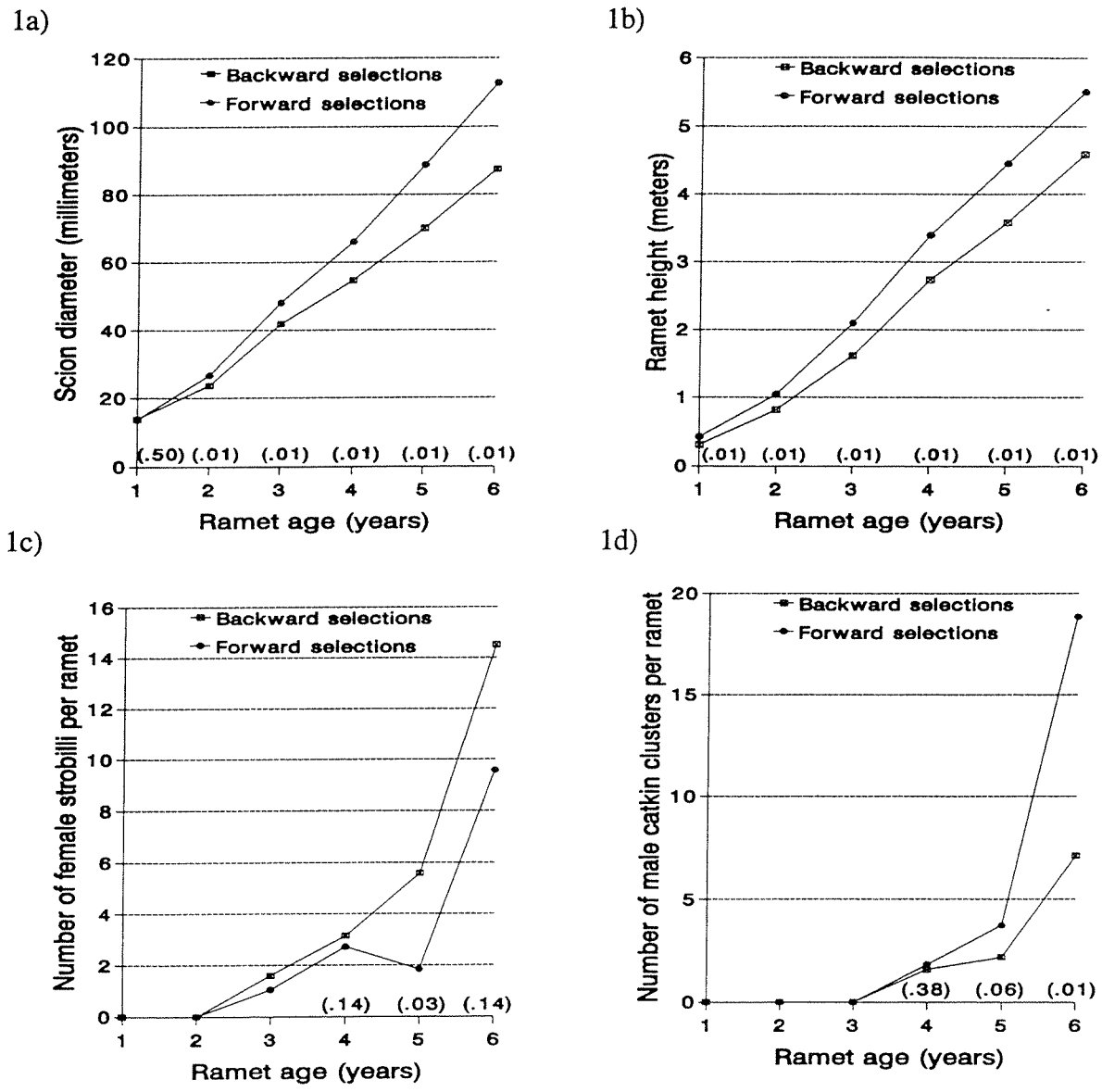
No ranking of the 76 different rootstock families was done for several reasons. First, there was too little disease incidence, graft incompatibility and too high survival to determine the rootstock effects on fusiform rust, pitch canker and graft compatibility. Second, the rootstock effects were too small relative to the scion effects to effectively rank the rootstocks for the remaining growth and flowering variables.

Scion Chronological Age Effects

For almost all dependent variables (across all 6 years) there was a statistically significant difference at the α=0.05 level between forward and backward selections. In all except the first measurement year the difference between scion diameters of backward and forward selections was increasing and significant at the α=0.01 level (Figure 1a). The lack of significance in the first year was probably because no appreciable growth had yet occurred. Differences between the ramet height of backward and forward selections were significant at the α=0.01 level in all 6 years (Figure 1b). Also, in all but the first year the forward selections grew more in diameter than backward selections. Therefore, there were significant differences in growth rates between the backward and forward selections.

In years 1 through 3 there were not enough female strobili present to test the difference between backward and forward selections (Figure 1c). In year 4, the difference in female strobili production between backward and forward selections was only significant at the α=0.14 level. But, by year 5 the backward selections produced significantly more female strobili at the α=0.05 level than the forward selections. However, in year 6 the difference became nonsignificant again, even though there was a greater number of flowers present on backwards selections despite them being smaller in diameter, shorter and having fewer branches. However, the absolute differences in number of female strobili per ramet was only about 3 flowers (Figure 1c).

Figure 1. Differences in growth and flowering between backward selections (chronologically older scion) and forward selections (chronologically younger scion) for 6 years after grafting: a) Total scion diameter growth; b) total ramet height growth; c) female strobili production; d) male strobili production; The values in the parenthesis are the α levels at which the contrasts between backward and forward selections are significant.



Forward selections produced significantly more catkin clusters per tree than the backward selections in years 5 and 6 (Figure 1d). This could be because most forward selections, though chronologically younger than the backward selections, were close to maturity. Of the forward selections 72% were 6 to 10-years-old. Also, in conifers most catkins are produced in the lower crown, which is chronologically younger than the upper crown where female strobili are formed. Thus, the forward selections may be at an excellent age to produce catkins, and the backward selections may be beyond the prime age to produce catkins.

CONCLUSIONS

This study leads to the following conclusions:

1. Rootstocks had a small but significant effect on the taper and diameter growth, and no noticeable effect on the height and flowering of clone bank ramets.

2. Scion had a large, highly significant effect on the height, diameter, taper and flowering of clone bank ramets. This effect was at least 5 to 10 times larger than the rootstock effect.

3. Interaction between the scion and rootstock was significant for all tested variables and larger than the rootstock effect.

4. Rootstock effects were too small relative to the scion effects to make it possible to effectively rank the rootstocks for the tested variables. The evidence here suggests that there is little reason to select for rootstocks in slash pine.

5. Scion chronological age effects were highly significant, even after six years of ramet growth. Chronologically older scion grew less in diameter and height than chronologically younger material. Chronologically younger scion grew more in both height and diameter and produced about 2.5 times as many male catkin clusters per tree as chronologically older material. Age effects on female flower production were small and generally not significant.

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SOUTHERN PINE SEED QUALITY: INFLUENCE OF SEED ORCHARD MANAGEMENT

J. P. Barnett[1]

Abstract.--Tree improvement programs have significantly influenced the quality of southern pine seeds produced when compared to collections from native stands. Studies show that seed orchard management practices such as fertilization increase seed size and reduce seed dormancy. These result in the need for less complex pregermination treatments. Repeated cone collections from the same clones facilitate collections according to ripening (cone specific gravity), which can improve seed germination and storage. However, cultural practices may result in seed properties that are more sensitive to damage during processing procedures and result in lower quality unless special care is provided during this stage of handling. The effect of orchard management practices on seed quality also varies by species, with loblolly pine being less affected than longleaf pine.

Keywords: Pinus spp., seed germination, seed dormancy, seed storage, cone maturity.

INTRODUCTION

Remarkable progress has been made in tree improvement of the southern pines (Pinus spp.) during the past four decades. Seed orchards produce seeds for the vast majority of the more than 1 billion pine seedlings produced annually in the southern United States. In fact, the more than 10,000 acres (4,000 hectares) of southern pine seed orchards now produce seeds of most species in quantities in excess to our current needs. During the development of these orchards and incorporation of improved seeds into nursery practice, there has been a shift in seed management practices to adjust to differences in seed properties caused by more intensive tree culture.

Much of the definitive research on physiology of southern pine seeds was conducted with seeds collected from native stands. Through the years, there has been a modification of practices to reflect the character of seeds from managed orchards. Since this has been a gradual process, there has been no critical evaluation of the impact of tree improvement on seed quality. However, there have been a number of changes in methodology that reflect impact of more intensive tree culture. The purpose of this paper is to review the influence of seed orchard management on seed quality. Since there are few studies designed to compare these effects, many of the influences are documented from personal observation, evaluation of data from related studies, and from changes in seed processing practices over time.

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ORCHARD MANAGEMENT

The goal in managing seed orchards is to produce high-quality seeds with a desirable genotype mix in quantities sufficient for regeneration needs in the shortest time (Pait et al. 1991). Rapid, juvenile growth is achieved by controlling weeds, insects, and water stress and ameliorating soil nutrient deficiences. Management activities shift as orchards begin to produce cones. Fertilization continues to maintain desirable levels of nutrients for overall tree vigor and cone production. Irrigation to reduce moisture stress, subsoiling to overcome compaction, and applications of insecticides to control insects are additional practices that may improve vigor and enhance cone and seed production of orchard trees.

CONE COLLECTION AND SEED MATURITY

Wakeley (1954) reported the germination of southern pine seeds is directly related to cone maturity at the time of extraction and cones are mature enough for collection when their specific gravity drops below 0.89. The critical determinant of maturity is cone moisture content, but measuring specific gravity by flotation is the quickest way to estimate cone moisture. Wakeley (1954) determined specific gravity by flotation in oil, but another simple method is described by Barnett (1979a). Typically, cones were collected over a 2- to 3-week period, and seed extraction was accomplished within a few weeks after collection. However, as the demand for southern pine seeds escalated and seed orchards came into production, this period was too short to allow collection of enough cones to provide an adequate seed supply. It became necessary, then, to extend the collection period by beginning a few weeks before the traditional specific gravity index.

An extended collection period is possible if slightly immature cones are stored or precured prior to kilning (Barnett 1976a, McLemore 1975). It became obvious that there were different responses in seed maturity that are related to cone collection and storage. Collections of loblolly (P. taeda L.) and slash (P. elliottii Engelm.) pine cones can begin 2 or 3 weeks before maturity if specific gravity is 1.0 or less, but seed yields from these immature cones may decrease even after cone storage. Acceptable yields and germination can be obtained when collections of loblolly pine cones begin about mid-September (specific gravity <1.0) and when collections of slash cones begin in late August (specific gravity 0.95). Generally, optimum yields and germination are possible only if the cones are mature when collected (Barnett 1976a). Early collections are advisable only if large quantities of seeds are needed immediately or if labor or the collectable crop is limited. Seeds from immature loblolly pine cones are apparently mature when extractable, and slash pine seeds continue ripening during cone storage. However, viability of longleaf pine (P. palustris Mill.) seeds from immature cones decreases during cone storage. Therefore, only mature longleaf cones should be collected. Once ripe, longleaf cones can be stored or precured for 3 to 5 weeks to increase seed yields without reducing viability, but the storage period for mature cones should not exceed 8 weeks (McLemore 1961).

The method of cone storage has varied from burlap bags to 20-bushel (7 hL) wire-bound boxes, outdoors or in unheated indoor facilities. Although several studies of the influence of the type of cone storage on seed quality have been conducted (Barnett 1979b, Bonner 1987), there has been no clear indication of the superiority of one method over the other when collections are restricted

to the major southern pines (loblolly, slash, longleaf, and shortleaf (P. echinata Mill.).

The greatest emphasis in southern pine seed orchard production has been on loblolly pine. It is fortunate this species is, of the southern pines, the least affected by seed orchard practices that require extension of cone collection and processing. Such extended periods are feasible without any major reduction in seed quality of loblolly pine. However, seed quality of slash and longleaf pines can be markedly reduced by changes in collecting and processing variables.

Orchard managers normally determine the relative sequence and approximate dates of ripening of all their clones. Once this clonal cone ripening sequence is determined, collections should be scheduled to start with those maturing early and follow the order of ripening. The time between ripening dates of the earliest and latest clones in one orchard was documented as 50 days (Zoerb 1969). The use of this collection strategy will reduce the adverse effects of early cone collections.

An alternative to collecting cones by hand is the net retrieval system that is used to collect loblolly, Virginia (P. virginiana Mill.), and eastern white (P. strobus L.) pine seeds (Pait et al. 1991, Wynens and Brooks 1979). Polypropylene netting is spread on the orchard ground before cone opening. Seeds fall to the netting naturally as the cones open (McConnell and Edwards 1985). When most of the seed crop is judged to be on the netting, it is rolled up and the seeds are recovered. High seed moisture contents are common and can be troublesome during retrival and processing. However, with proper care, good quality can be maintained in loblolly pine seeds for use immediately or after at least 1.5 years of storage (Bonner and Vozzo 1986).

CONE AND SEED PROCESSING

Extraction

Seeds are usually extracted from southern pine cones in forced-draft kilns. Temperature and duration of kilning are critical for southern pine cones, particularly longleaf: temperatures of 115°F (46°C) or more markedly reduce germination (Rietz 1941) and those under 90°F (32°C) may not result in effective cone opening (Bonner 1987). Optimal temperatures are 95°F (35°C) to 105°F (41°C). Increases in the length of treatment may also reduce viability. A general recommendation is that cones and seeds should be removed from kilns as soon as cones open sufficiently to release the seeds.

Cleaning

After seeds are extracted, they must be dewinged, cleaned, and dried. The wings on seeds of all southern pines, except longleaf, are completely removed by brushing and tumbling in mechanical dewingers. The structure of longleaf seeds makes dewinging difficult; the wings are mechanically reduced to stubs, so dewingers must be carefully regulated to prevent injury to these thin-coated seeds. Wing removal that is done carefully and does not damage the seed coat has no effect on seed storability (Barnett 1969, Belcher and King 1968), but dewinging is a common cause of seed injury and loss of viability. Orchard management practices generally result in larger seeds and thus increase seed sensitivity to damage during processing activities such as dewinging. The dewinging process for species other than longleaf is hastened

and improved by moistening dry seeds, but this moisture may need to be removed prior to seed storage.

Before seeds are used or stored, empty seeds should be removed from the seedlot. This is the easiest means of upgrading a seedlot. This can be accomplished by mechanical cleaning equipment, or, when lots are small, as in progeny tests, it is often convenient to use flotation in water or organic solvents to separate unfilled seeds. In the appropriate liquid, sound seeds sink, while unsound ones float and can easily be skimmed off. Examples of appropriate solvents for seed separations are: water for loblolly pine, n-pentane for longleaf pine, and 95-percent ethanol for slash, shortleaf, sand (P. clausa [Chapm. ex Engelm.] Vasey ex Sarg.), and spruce (P. glabra Walt.) pines (Barnett and McLemore 1970). To maintain seed quality, flotation in ethanol should be delayed until just prior to use, because if the ethanol is not thoroughly removed by drying, seeds so treated rapidly loose viability in storage (Barnett 1971).

Sizing

The reported effects of seed size on germination and early seedling growth are conflicting. The operational objective of sizing is to produce a uniform crop of seedlings. Medium to medium-large seeds have been reported to produce larger and more uniform seedlings than smaller seeds (Ghosh et al. 1976). Larson (1963) reported that although seed size can influence subsequent seedling development when seedlings are grown under uniform conditions as in greenhouses, seed size has a more pronounced effect on germination. Uniform speed of germination may, therefore, be the most important consideration in sizing. More recent tests under laboratory conditions of minimal environmental stress have shown that germinant size after 28 days of growth was strongly correlated with seed size (Dunlap and Barnett 1983). The faster germinating seeds in each size class produced larger germinants after 28 days of incubation (fig. 1). All seeds reached a maximum germination rate by the sixth day, but smaller seeds were slower to initiate germination (fig. 2).

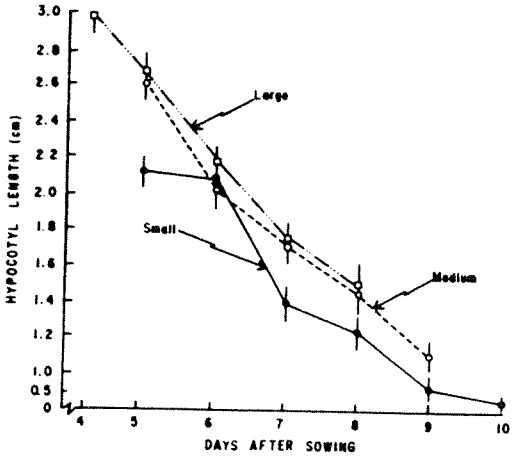

Figure 1. Mean hypocotyl length of loblolly pine germinants 28 days after germination (Dunlap and Barnett 1983).

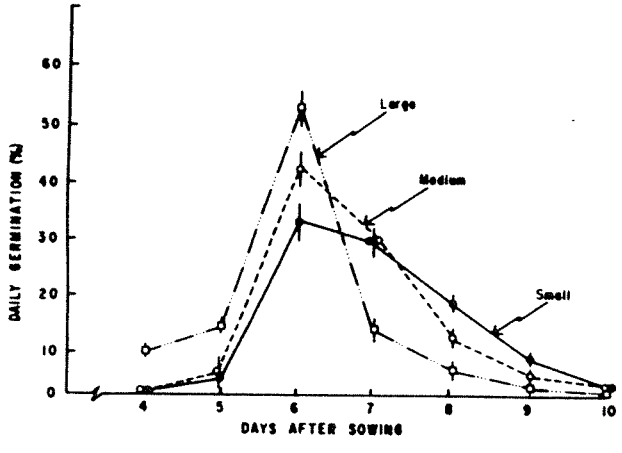

Figure 2. Mean daily germination of loblolly pine seeds from the large medium and small classes (Dunlap and Barnett 1983).

These results are in agreement with Venator's (1973) findings indicating faster growing Caribbean pine (P. caribaea var. hondurensis Barrett and Golfair) seedlings tend to develop from early germinating seeds. Consequently, seedling size and possibly uniformity of growth are primarily a function of germination patterns, which are partially determined by seed size.

It is generally recognized that seed size varies by genetic and geographic source, and fertilization practices commonly used in seed orchards increase seed size (McLemore 1975). When orchard collections are bulked into a single lot, sizing can result in the exclusion of certain families. Some seed managers may be tempted to discard the small-seed fraction of their lots; however, such a practice could eliminate certain families and narrow the genetic base of the planting stock (Silen and Osterhans 1979). All sizes of seeds should be used in seedling production.

Seed processing techniques have been developed specifically for loblolly pine since it is the species more often produced in southern pine seed orchards. Of the southern pines, loblolly seeds are the most dormant, hardseeded, and the least susceptible to damage during processing. It is not surprising then that application of the processing technology developed primarily for loblolly pine to a species like longleaf pine may result in damage that reduces seed quality and performance.

SEED STORAGE

Inconsistent cone crops require that seed managers store seeds to offset years of low production. Though good seed-orchard management has reduced the fluctuations in southern pine cone crops, seed supplies must be maintained in storage for unanticipated needs. Fortunately, seeds of southern pines are relatively easy to store for long periods. Viability of slash and shortleaf seeds stored for 50 years at above-freezing temperatures was 66 and 25 percent, respectively (Barnett and Vozzo 1985); vigor declined as expected, but no serious chromosomal damage was noted. Longleaf seeds, which are the most difficult of the southern pines to store, have been held for 20 years at low seed moisture and subfreezing temperatures without significant losses in viability (Barnett and Jones 1993).

Careful control of seed moisture content and storage temperatures is essential to maintain viability (Barnett and McLemore 1970, Barton 1961, Jones 1966). General recommendations for long-term storage are to dry seeds to 10 percent or less moisture content and store at subfreezing temperatures. Seeds that are damaged or are known to have low vigor can be preserved by drying to 8 to 10 percent and lowering storage temperatures to about 0°F (-18°C) (Kamra 1967). The genetic source of the seeds seems to have little effect on their storage capabilities (Barnett and McLemore 1970).

SEED DORMANCY AND PRETREATMENT

Seed dormancy in the southern pines seems determined by the magnitude of seed coat constraint. The ratio of the seed coat to total seed weight provides a means of rapidly estimating relative seed dormancy among closely related species (Barnett 1972, 1976b; Carpita et al. 1983). The theory is that thicker and heavier seed coats of the more dormant seeds restrict imbibition by preventing swelling of the megagametophyte and embryo and thus limit water absorption sufficient for germination. Loblolly pine seeds are

considered the most dormant, and longleaf pine seeds, the least dormant of the southern pines. Although not thoroughly documented, one of the more significant effects of seed orchard culture may be on seed dormancy. Orchard fertilization increases seed size. Dunlap and Barnett (1983) found larger seeds tend to germinate more quickly and produce larger germinants than smaller ones, although final germination is typically lower in the larger seeds. Therefore, seed orchard management results in seeds with less dormancy than those from native stands.

Maternal factors such as seed coat properties that influence the speed of germination can obscure the nature of genetic control of subsequent growth processes (Perry 1976). Seed dormancy varies by geographic location or ecotype (Barnett 1991). Less than 15 percent of the weight of a conifer seed is in the embryo, which is the only portion with a genetic component from the male plant. In nature, moist prechilling or stratification is usually optimized as a result of natural conditions, but in nursery production, the genetic component from the male parent may be obscured when managers do not optimize the prechilling needs of the seedlot. Prechilling needs should be determined under the stress conditions that relate to nursery bed conditions where seeds are to be sown.

Overcoming seed dormancy is one of the major steps to ensure prompt and uniform germination. Presowing treatments to speed germination are discussed in detail by several authors (Bonner et al. 1974, Tanaka 1984). Typically, prechilling is done after an 8 to 24 hour period of moisture imbibition. Fully imbibed seeds are placed in polyethylene bags and held at temperatures of 34° to 40°F (1° to 5°C). The length of treatment varies by the extent of dormancy present in the seeds.

In recent years, techniques other than prechilling have been investigated in an attempt to accelerate the dormancy-breakage process or obtain more desirable germination patterns for genetically improved seeds (Barnett 1989). However, none of these newer presowing treatments have been developed to the point where they are as consistently effective as conventional moist prechilling.

PREDICTING SEED PERFORMANCE

For decades, nursery managers and seed physiologists have sought techniques, generally with little success, that would more accurately predict seed performance in the nursery. In an evaluation of the problem, Barnett and McLemore (1984) found that laboratory germination tests performed on prechilled seedlots provide the best predictors of nursery-tree yield for southern pines. Germination tests are standardized by conducting them under optimum light and temperature conditions (AOSA 1980). However, tests conducted under optimum conditions do not reflect germination of dormant seeds on nursery beds were temperatures and photoperiods are often considerably less than optimal (table 1). Since orchard culture results in changes in seed properties, comparative tests of untreated and prechilled (various durations) seeds are needed to develop optimal recommendations. A technique to improve prediction of seed performance is to determine prechilling needs under stress conditions that relate to the nursery conditions where the seeds are to be sown (Barnett 1992). The prechilling period should be extended to minimize the effect of the less than optimal conditions on initial seedling development.

CONCLUSIONS

Tree improvement programs have significantly influenced the properties of southern pine seeds produced compared to seeds from native stand collections. The effects of orchard cultural practices include:

1. Varying responses among species to management practices. Fortunately, loblolly pine, which is the major southern species produced in tree improvement programs, is most tolerate to cultural manipulation. Longleaf pine seeds are the most sensitive to impacts of management.
2. Reductions in seed quality related to extended cone collections result from the processing of immature cones and seeds. Repeated collections (year after year) from the same clones allow orchard managers to collect by ripening sequence and thus overcome some of the problems of collecting large quantities of immature cones.
3. Increases in seed size, resulting from orchard fertilization, reduce dormancy levels in loblolly pine seeds, increases sensitivity of longleaf pine seeds to damage during processing, and changes the nature of presowing treatments in most species.
4. Insignificant effects on seed storability.
5. The reevaluation of prechilling needs based on comparative germination tests is required and germination should be optimized for nursery-bed conditions.

Table 1. Effect of length and method of stratification of a mixed loblolly pine seedlot in two testing environments (McLemore 1969).

Days stratification	Stratified in refrigerator at 34°F: Germination percent	Stratified in refrigerator at 34°F: Germination value[a]	Stratified outdoors: Germination percent	Stratified outdoors: Germination value
	Tested at 60°F with 11-hour photoperiod			
0	< 1	0.0	< 1	0.0
30	68	7.1	59	6.0
60	95	17.3	91	11.4
113	99	24.0	98	19.6
	Tested at 72°F with 16-hour photoperiod			
0	96	20.8	96	20.8
30	99	37.6	98	41.8
60	99	47.1	99	47.0
113	100	50.3	99	56.3

[a] Germination values represent the speed as well as completeness of germination (Czabator 1962).

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PRODUCING AND TESTING LARGE NUMBERS OF SELF-FERTILIZED LOBLOLLY PINE SEEDLINGS

F.E. Bridgwater[1] and D.L. Bramlett[2]

Abstract.--Self-fertilization combined with selection is the fastest way to fix favorable alleles in breeding populations. However, inbreeding depression dramatically reduces the average numbers of self-fertilized seeds . In fact, the numbers of viable seeds from self-fertilizations are often so few that it may not be practical to maintain a parental line in the breeding population. If self-fertilization is to be a part of a breeding strategy it is important to know how much effort will be required to produce adequate numbers of self-fertilized seedlings for subsequent selection and breeding. Forty, first-generation loblolly pine (*Pinus taeda* L.) parents were self pollinated in a seed orchard in an operational trial. The results of that breeding and testing trial through the first two years in field plantings are reported and the implications in planning a breeding strategy are discussed

Keywords: Pinus taeda, breeding strategy, self-fertilization, inbreeding depression

INTRODUCTION

Inbreeding offers a means of rapidly increasing homozygosity in breeding populations. Coupled with selection, the frequency of favorable alleles will theoretically increase as will the additive genetic variance. Self-fertilization (hereafter, selfing) is the most rapid form of inbreeding and has the advantage of a greater among-family selection intensity than for bi-parental crosses. With selfing, the parents with the best general combining abilities will be mated to themselves, and thus can be advanced to the next generation without carrying genes from poorer parents. Since selfing is perfect assortative mating,

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the additive genetic variance among lines will increase rapidly. Although selfing has the greatest potential to produce genetic gains rapidly it also has some critical disadvantages. Inbreeding depression in metrical traits and in reproductive capacity is well documented for many conifers (See Williams and Sovolainen , 1995 for a thorough review). Both of the largest loblolly pine breeding programs in the southern United States have adopted the use of sublines to avoid inbreeding depression in progenies used to establish plantations (Lowe and van Buijtenen 1989, McKeand and Bridgwater 1992). Thus, the critical question for breeders is how to maintain breeding populations composed of parents with reduced vigor and reproductive capacity. One of the major hurdles to implementing a breeding strategy incorporating self-pollinations is in the early generations. Models of embryonic lethal allele systems in conifers suggest that these can be purged in a few generations of selfing (Bishir and Namkoong 1987). However, the difficulty of producing sufficient numbers of S_1 progenies for testing and selection should not be minimized. Herein, we report an operational trial of self-pollination on 40, first-generation loblolly pine (*Pinus taeda* L.) parents. The results of breeding and testing through the first two years in field plantings are reported and the implications in planning a breeding strategy are discussed

METHODS

Fresh pollen, pollen that had been processed and stored in a freezer since 1985 and 1986 at 4 C°, and pollen that had been dried in a vacuum in a freeze desiccator and stored since 1982 and 1983 at 4 C° were used to accomplish the self-pollinations. Polymix crosses were made using one of two 5-parent mixes of pollen frozen in 1986.

Pollinations were made in the Weyerhaeuser Company's Lyons, Georgia Seed Orchard in the spring of 1987. Counts of surviving conelets were made in the fall of 1988 and cones were harvested in October of 1989. Seeds were extracted and numbers of total seeds were counted. The numbers of filled seeds were determined from radiographs. Seeds were sown in a greenhouse in Raleigh, North Carolina in April and May of 1992. Surviving self- and cross-pollinated sibs from 25 families were planted in a split plot experimental design near Lyons, Georgia on January 14-15, 1993. Main plots were pollination types. Heights to each whorl of branches and total heights were measured at the end of the first growing season in the greenhouse and again in June, 1993. Total heights were measured in January, 1993 and 1994.

RESULTS AND DISCUSSION

There were no significant differences among the pollen types in the total numbers of seeds per cone produced nor in the percentages of filled seeds per cone. In fact, fresh pollen produced approximately average numbers of total and filled seeds per cone. There three pollen types ranked as expected for total seeds, filled seeds and percentage filled seeds (Table 1). Lower numbers of total seeds were produced in both self and polymix crosses. The percentage of filled seeds was less for polymix than wind pollinations, probably because the polymix males were related as half-sibs to the

female parents in 11 of the matings.

Table 1. Average cone and seed statistics for selfed and outcrossed parents and three pollen types.

Pollen Parent	Numbers of Cones	Total Seeds	Filled Seeds	Empty Seeds	% Filled Seeds
WIND	198	132	99	33	72
POLYMIX	152	88	58	30	61
SELF	365	94	9	85	9

Conelet abortion during the first growing season after pollination was not great nor significantly different for wind and self pollinations (Table 2). Conelet abortion during the second year was important and significantly more self-pollinated conelets were lost for both polymix and selfed cones. Only about 1 in 5 wind-pollinated strobili and 1 in 7 self- or polymix-pollinated strobili survived until harvest. Conelets and cones which had been damaged by insects or disease were excluded from the counts.

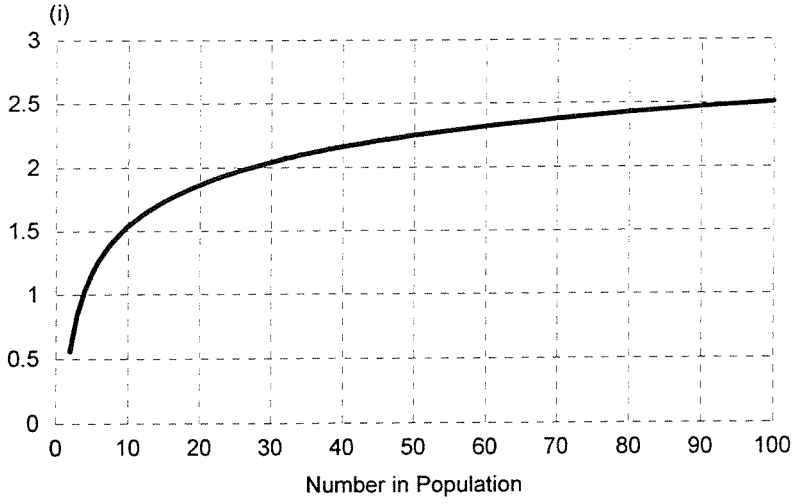
Table 2. Strobilus survival for self and outcrossed parents and three pollen types.

Pollen Parent	Numbers of Pollination Bags	Mean Number of Strobili Pollinated	Conelet per Strobilus Pollinated Year 1	Cone per Strobilus Pollinated Year 2
WIND	-	3.1	0.92	0.63
POLYMIX	99	3.6	0.98	0.55
SELF	252	3.6	0.97	0.50

Thus, producing large numbers of self-pollinated seeds requires pollinating large numbers of female strobili. Furthermore, the effort required is increased if there is no prior knowledge about which parents produce very low percentages of filled seeds when selfed.

When a breeding and testing plan are developed the question of how many S_1 progenies to plant arises. Examination of Figure 1 suggests that at least 20 individuals should be planted for efficiency. That is, the marginal increase in expected selection differential per individual added is greatest from two to twenty and decreases thereafter. It may in fact be desirable to plant more than twenty individuals since mortality, selection based on multiple traits and the need for insurance, all suggest planting more individuals.

Figure 1. Standardized selection intensity (i) for selecting one individual from varying population sizes less than 400. (After Becker, 1984).



The effort required to produce a desired number of S_1 seedlings is a direct function of : (1) the number of strobili pollinated, (2) the germination percentage for filled S_1 seeds, and (3) the numbers of filled seeds produced per strobilus pollinated. The number of strobili that can be pollinated is determined by the availability of pollen and female strobili and the investment that can be made in making controlled pollinations. The percentage of filled seeds that can be expected to germinate is, on average, 75% to 85% (Franklin, 1969 and McKeand, N.C. State University, Pers. Comm., respectively). The numbers of filled seeds expected per strobilus pollinated is more problematical. The assumptions made to produce the data in Table 3 were that 75% of filled, S_1 seeds would produce a seedling, and that there would be an average of 3.6 strobili per pollination bag (Table 2).

Thus, if 50 seedlings were desired for outplanting, and one was willing to accept that only half of the parents would reach that goal, the number of pollination bags that would have had to be used per parent could be calculated as ((1/1.27 filled seeds per strobilus pollinated)/3.6 strobili per bag) x (50 seedlings / 0.75 seedling per seed) = 15 bags. If no prior knowledge is available, the number of pollinations to be made on each parent must assume that each parent will produce the number of filled seeds per strobilus pollinated for which the plan is developed. Thus, all 40 parents in the seed orchard represented in Table 3 would have had to have 15 pollination bags installed and pollinated for a total of 600 bags. As the percentage of filled seeds per strobilus pollinated decreases, the numbers of strobili that must be bagged increases exponentially (Table 3).

Table 3. Estimated numbers of pollination bags to produce desired numbers of S_1 seedlings for outplanting. Assumptions were 75% germination of filled, S_1 seeds and 3.6 strobili per pollination bag.

% of 40 Parents Producing Desired # of Seeds	Filled Seeds per Strobilus Pollinated	# of Bags Per Parent to Produce n seeds			Total # of Bags to Produce n seeds		
		n=20	n=30	n=50	n=20	n=30	n=50
2.5	22.62	1	1	1	40	40	40
5	15.43	1	1	1	40	40	40
10	8.25	1	1	2	40	40	80
15	7.1	1	2	3	40	80	120
20	6.32	1	2	3	40	80	120
25	4.47	2	2	4	80	80	160
30	4.15	2	3	4	80	120	160
35	3.67	2	3	5	80	120	200
40	2.22	3	5	8	120	200	320
45	1.87	4	6	10	160	240	400
50	1.27	6	9	15	240	360	600
55	1.15	6	10	16	240	400	640
60	0.94	8	12	20	320	480	800
65	0.63	12	18	30	480	720	1200
70	0.52	14	21	36	560	840	1440
75	0.48	15	23	38	600	920	1520
80	0.44	17	25	42	680	1000	1680
85	0.33	22	33	56	880	1320	2240
90	0.24	31	46	77	1240	1840	3080
95	0.19	39	59	98	1560	2360	3920
100	0.11	67	100	167	2680	4000	6680

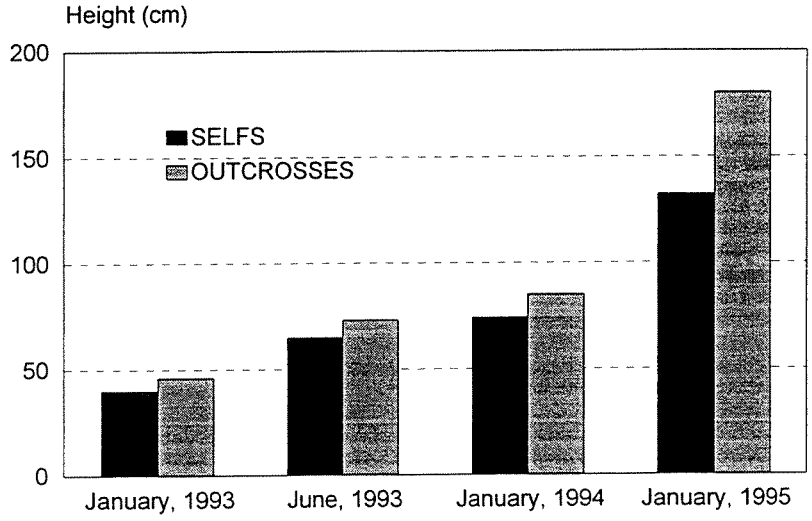
Once the population of S_1 progenies has been planted, survival until selection age becomes a concern. After one year in the field there was significantly more mortality in S_1 progenies than in their outcrossed siblings (Table 4). Most S_1 mortality occurred during the first year after planting (21% mortality) but continued through year 2 (7% additional mortality). Survival was determined for all trees including damaged trees and trees that had been used as border row trees.

Table 4. Survival of outcross and self progenies for 2 years after planting.

	Percentage Surviving			
Pollen Type	January 1993 (Planted)	June 1993 (5 months)	January 1994 (1 year)	January 1995 (2 years)
Outcross	100	100	97	97
Self	100	99	79	72

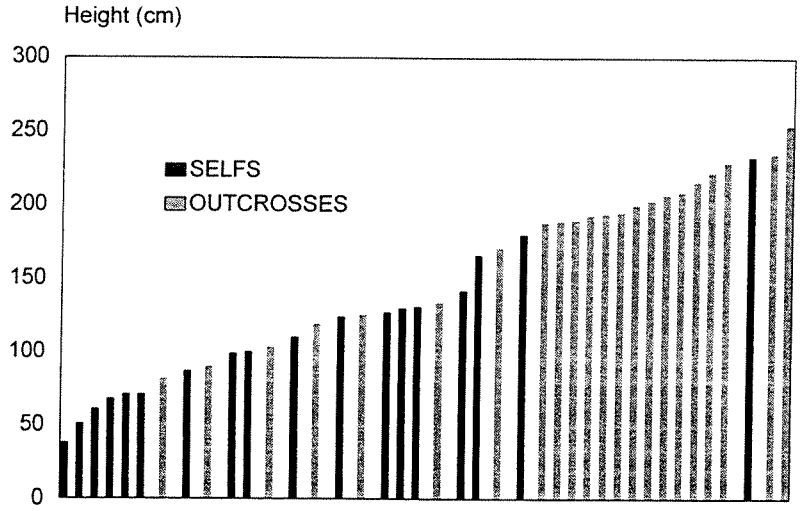
S_1 progenies also grew more slowly than outcross siblings (Figure 2). After 2 years in the field outcross siblings were 36 % taller than their S_1 siblings. However, there were S_1 individuals in most lines with growth that was greater than many of their outcrossed siblings (Figure 3, for example).

Figure 2. Average height growth of self and outcross progenies.



Thre are two important issues to consider when contemplating selection in trials of self-pollinated progenies. First, there is a real probability that the fastest growing S_1 progenies are the result of pollen contamination and are not selfs. Secondly, selecting the fastest growing S_1 progenies may not be the best way to increase general combining ability. If there is a substantial amount of non-additive variance contributing to the genetic variance, the correlation between self- and out-cross performance may not be high (Williams and Sovolainen , 1995).

Figure 3. Heights of self and outcross progenies from a single parent after 2 years in the field.



If Phenotypic selection is to be done in tests such as those reported here, it may be possible to make selections at fairly young ages. After two years in the field, there were 127 S_1 progenies representing 17 parental lines that survived and were undamaged. Six of these had trivial numbers of individuals upon which to compare height ranks over time (four lines had two individuals and two had only one). Of the remaining 11 lines, the tallest individual after two years was also tallest after one year for five lines. If the tallest individual from each of the 11 lines had been selected after five months in the field their average rank would be 5 after two years in the field. If the tallest individual from each of the 11 lines had been selected after one year in the field their average rank would be 2 after two years in the field. Further assortment among individuals within lines may occur as they grow older. However, examination of the heights of all individuals from a typical line over the period of the study suggests that much of the assortment may have occurred during the first two years in the field.

SUMMARY AND CONCLUSIONS

The basis for estimating the effort required to produce desired numbers of S_1 loblolly pine seedlings for testing is provided. This should be a useful tool for breeders who plan to make large numbers of self-pollinations of non-inbred loblolly pines. If phenotypic selections for height growth are to be made among S_1 progenies in field trials, it may be possible to do so after two years in field tests.

The large amount of effort required to produce large numbers of S_1 progenies, their subsequent lower germination and survival rates, and uncertainty about the efficacy of selecting good general combiners from selfed lines suggest that embarking upon such a program be done with caution.

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EXPRESSION AND FUNCTION OF ARABINOGALACTAN-PROTEINS IN XYLEM OF LOBLOLLY PINE

C.A. Loopstra[1], E.-G. No[2], and R.R. Sederoff[3]

Abstract. Genomic and cDNA clones of two genes encoding xylem-specific proteins were previously isolated and characterized. Transcripts of these genes are extremely abundant in differentiating xylem, much less abundant in needles, and are present at very low or non-detectable levels in embryos and megagametophytes. Both genes appear to encode arabinogalactan-proteins (AGPs). AGPs are highly glycosylated proteins thought to play important roles in plant development. AGPs have been found to be abundant in differentiating xylem of loblolly pine. We are attempting to produce genetically engineered loblolly pine plantlets with reduced amounts of these AGPs in order to examine their function in xylem development. Possible roles include cell-cell signalling or cellular interactions, transport of cell wall components, autolysis, or they may act as gums or humectants.

We have isolated both promoters and found them to be functional in bombarded pine tissues and in transgenic tobacco, poplar, and white spruce. We are currently searching for the elements responsible for xylem-specificity and for the high levels of expression observed. These elements will be of value in future attempts to genetically engineer wood properties.

Key words: *Pinus taeda*, xylem development, arabinogalactan-proteins, gene expression.

INTRODUCTION

In recent years, progress has been made towards understanding the molecular basis of xylem development in coniferous species. Several enzymes involved in lignin biosynthesis have been purified and cloned (Whetten and Sederoff, 1992; O'Malley et al., 1992; Bao et al., 1993), an extensin-like cell wall protein has been purified (Bao et al., 1992) and regulatory elements of genes expressed in xylem have been isolated. There is however, much we do not yet understand about the genes involved in the formation of wood. There are genes involved in xylem differentiation with unknown functions. We also have a very poor understanding of the regulation of gene expression in xylem. In this paper, we describe work in progress to characterize two genes preferentially expressed in differentiating xylem, to determine their function, and to examine their regulation.

Isolation and characterization of putative xylem-specific arabinogalactan-proteins

The isolation and characterization of two genes preferentially expressed in newly differentiating xylem of loblolly pine (PtX3H6 and PtX14A9) have previously

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been described (Loopstra and Sederoff, 1995) and are summarized here. A cDNA library constructed using RNA isolated from newly differentiating xylem was differentially screened to identify clones of genes with xylem-specific expression. Of the sixteen clones isolated, thirteen were found to be the same (PtX3H6) and three were the same (PtX14A9). From this, we conclude that although it is likely other genes with preferential expression in xylem exist, transcripts of PtX3H6 and PtX14A9 are the most abundant.

Northern blot analyses were used to look at transcript abundance in various tissues and at different stages of development. Transcripts of both PtX3H6 and PtX14A9 are much more abundant in xylem than in needles, embryos, or megagametophytes (Figure 1). Some hybridization to needle RNA was observed. This was not unexpected due to the presence of vascular tissues in needles. RNAs isolated from 6 week, 1 year, 2 year, and 10 year old trees as well as from earlywood and latewood were also examined. The only difference observed with tree age was a lower level of expression in the stems of 6 week old trees, possibly because pure xylem could not be isolated. No differences were detected between earlywood and latewood.

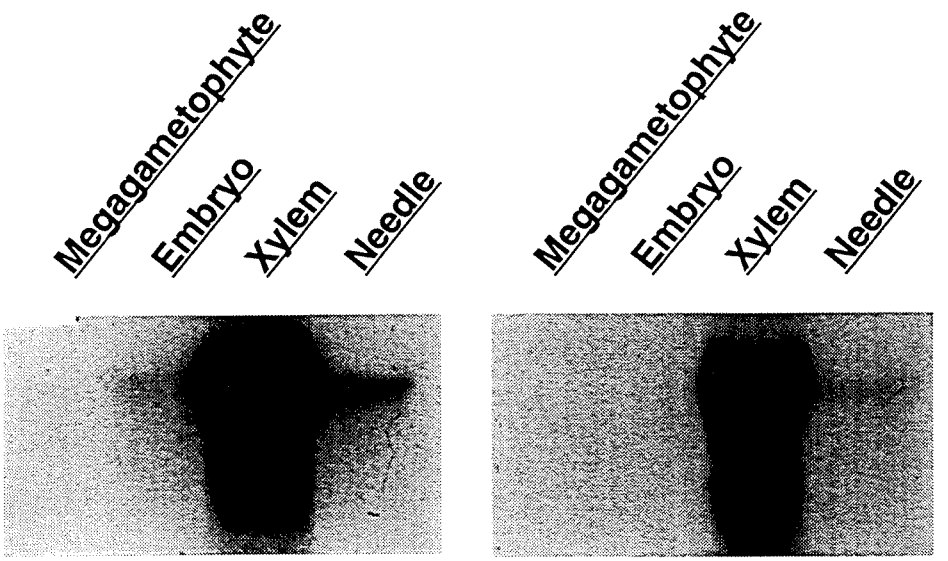

Figure 1. Northern blot hybridizations to examine abundance of the PtX3H6 and PtX14A9 transcripts in various tissues.

The relative abundances of PtX3H6, PtX14A9, phenylalanine ammonia lyase (PAL), and cinnamyl alcohol dehydrogenase (CAD) transcripts were compared by hybridizing a radioactive xylem cDNA probe to cDNA clones of the genes. PAL and CAD are both enzymes involved in lignin biosynthesis. Both enzymes have been purified from differentiating xylem of loblolly pine (Whetten and Sederoff, 1992; O'Malley et al., 1992). PtX3H6 transcripts were found to be more abundant than those of PtX14A9 and both were much more abundant than those of PAL and CAD.

The nucleotide sequences of PtX3H6 and PtX14A9 were determined and the amino acid sequences inferred. PtX3H6 is rich in prolines or hydroxyprolines and contains repeats similar to those found in proline-rich cell wall proteins (PRPs). The amino acid composition of PtX3H6 however, is much more similar to the arabinogalactan-proteins (AGPs). PtX3H6 and AGPs are rich in prolines or hydroxyprolines, alanine, threonine and serine and have few tyrosines, an amino acid usually found in cell wall proteins. The PtX14A9 sequence is not particularly similar to those previously published but does contain the sequence Ala-Pro-Ala-Pro-Ser-Pro-Ala-Ser near the amino terminus. This sequence has been found at the amino terminus of at least three AGPs. Both PtX3H6 and PtX14A9 appear to have signal peptides at the amino termini and hydrophyllic helixes at the carboxy termini. The hydrophobicity plots are very similar to that of a recently reported pear AGP (Chen et al., 1994). We are currently working under the assumption that both PtX3H6 and PtX14A9 encode AGPs.

Arabinogalactan-proteins

AGPs are a class of proteins recently receiving increased attention due to their utility in studies of angiosperm development and evolution and their potential roles in developmental processes. The properties of arabinogalactans and AGPs have been reviewed by Clarke et al. (1979) and Fincher et al. (1983). AGPs are a widely distributed class of proteoglycans and glycoproteins. Generally, only 2-10% of the weight of an AGP is made up of protein. Ninety percent or more of the molecule is carbohydrate, including galactose, arabinose, uronic acids, glucose, rhamnose, mannose, and glucosamine. The majority of the carbohydrates are attached to the protein backbone by hydroxyproline linkages and the remaining are likely to be attached to serines and threonines (van Holst and Klis, 1981). Tissue-specific expression of AGPs has been observed and more than one AGP can be found within a tissue. AGPs are found in the extracellular matrix, associated with the plasma membrane, inserted into the cell wall, or secreted into the medium of cell cultures. They have been found in almost all tissues of higher plants and have been detected in every taxonomic group tested, including in the wood of some angiosperms and gymnosperms (Showalter and Varner, 1989). A comparison of carbohydrate-protein complexes from cell walls and cytoplasm of Siberian larch xylem revealed AGPs to be associated with the primary cell walls (Antonova and Stasova, 1990). AGPs are also abundant in xylem of loblolly pine (approximately 0.1% of fresh weight) and are extractable (R. Whetten, personal communication). AGPs have also been extracted from Douglas-fir and loblolly pine tissues collected at different stages of development (Bobalek and Johnson, 1983).

The functions of AGPs are not known but many potential roles have been postulated. Antibodies have been used to show that AGP carbohydrate epitopes display developmentally regulated patterns of cell surface expression directly reflecting cell fate in both root and floral meristems of angiosperms (Knox et al., 1991; Pennell and Roberts, 1990; Pennell et al., 1991). It has been suggested that AGPs may not only be markers of development, but may have a role in cell-cell interactions or cellular signalling during morphogenetic processes. There is also a theory that AGPs may be involved in programmed cell death. We are interested in determining the roles of AGPs in xylogenesis and the development of wood and in using AGPs to gain a greater

understanding of the molecular events leading to the differentiation of xylem. Due to recent developments in pine transformation methodologies and the isolation of pine AGP clones, we are now in an excellent position to pursue these interests. Experiments in progress to examine AGP functions in xylem are described in the results and discussion section.

Promoter analyses

In order to obtain the promoter elements thought to be involved in controlling the expression of PtX3H6 and PtX14A9, a genomic library was constructed and screened. Genomic clones of each were isolated and characterized. Primer extension analyses were used to identify the transcription start sites. Approximately 953 bp of PtX3H6 5' flanking sequence and 750 bp of PtX14A9 5' flanking sequence were determined. Both promoters contain regions of high A/T content. The PtX3H6 promoter contains a 467 bp region containing 86.5% A/T. These promoter segments may contain scaffold attachment regions (SARs). The PtX3H6 promoer contains a pair of 63 bp direct repeats with four nucleotide differences and a pair of 36 bp repeats with two nucleotide differences and a one base insertion. Other smaller repeats are also found. Several sequences of 8 to 10 bp are found in both promoters. It is not known if this is due to chance or if they are conserved functional elements. The sequence CTGCATG is found in both promoters and in the promoters of two vascular-specific genes from bean (Keller and Baumgartner, 1991). In the GRP1.8 promoter, this sequence has been shown to be part of a negative regulatory element required for vascular-specific expression. It seems unlikely that this element would appear by chance in two vascular-specific bean promoters and two xylem-specific pine promoters. It is likely this element is involved in the xylem-specific expression observed but it is also likely that other elements are also involved. Experiments used to test the isolated promoters are described below.

RESULTS AND DISCUSSION

Determining the roles of AGPs in xylem development

It may be possible to gain insight into the function of AGPs in differentiating xylem by reducing their levels in genetically engineered pine plantlets and comparing the wood produced to that of normal control plants. Antisense constructs are being made by inserting the cDNA clones for PtX3H6 and PtX14A9 in an orientation opposite to normal relative to a promoter (Figure 2). We have developed two cassettes useful for the production of antisense constructs. Each contains a gene for kanamycin resistance (*npt* II) under the control of the nopaline synthase promoter (NOS) for selecting transformed cells and an antisense cassette containing the cauliflower mosaic virus 35S promoter with enhancer elements followed by the restriction sites Xho I, Kpn I, and SacI and a NOS terminator. The order of the restriction sites relative to the promoter allows any cDNA clone isolated from a Lambda Zap library (Stratagene) to be inserted in an antisense orientation. Both constructs also contain the 35S promoter driving a reporter gene. One contains the gene for β-glucuronidase (GUS) and the other contains a modified green fluorescent protein gene (mGFP) from jellyfish. The above pieces are located between *Agrobacterium tumefaciens* right and left borders in plasmids derived from pBin19.

We may also produce constructs containing the gene in a sense orientation. Introduction of extra copies of a gene has frequently been shown to reduce rather than enhance expression by way of cosuppression. The plasmids containing the constructs will be introduced into *Agrobacterium* strains known to be virulent in pines such as EHA105 (E. Hood) via electroporation. The *Agrobacterium* containing the plasmids will be used to inoculate loblolly pine apical shoot meristems using a technique developed by Jean Gould at Texas A & M University and transformed plants will be regenerated.

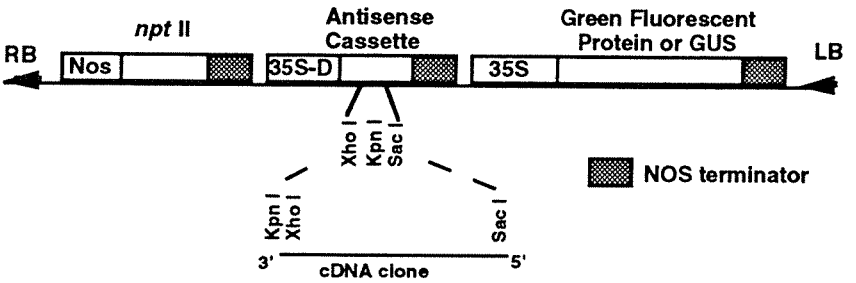

Figure 2. Diagram of the constructs being used to produce loblolly pine plantlets with reduced levels of xylem AGPs. The segment shown is within pBin19 derivatives.

We also plan to characterize the AGPs found in differentiating loblolly pine xylem. AGPs have been shown to be abundant in this tissue and are extractable (R. Whetten, personal communication). AGPs will be purified from differentiating xylem, deglycosylated, and the numbers, sizes, and tissue-specificities determined. AGPs that appear to be the sizes expected if encoded by PtX3H6 or PtX14A9 and are xylem-specific will be further purified and the amino acid compositions will be determined. Those that remain candidate proteins will be partially sequenced to determine if they are indeed encoded by PtX3H6 or PtX14A9.

Promoter analyses using microprojectile bombardments

In order to examine the regulation of PtX3H6 and PtX14A9, their promoters were fused to the gene for β-glucuronidase in the vector pRT99-gus (Topfer, 1988) and in Bluescript KS (Stratagene). PRT99-gus contains the *gus*A gene and a gene for kanamycin resistance (*npt*II), both under control of the CaMV35S promoter. The CaMV35S promoter preceding the *gus*A gene is flanked by restriction sites making it possible to remove the promoter and insert one of interest. An approximately 3.2 kb promoter fragment from genomic subclone G3H(5.7) and a 750 bp fragment from subclone G14A9(5.1) were cloned into PRT99-gus. A promoterless *gus*A construct was made in pRT99-gus by removing the CaMV35S promoter.

A method for examining transient gene expression in differentiating xylem of pine using microprojectile bombardment has been previously reported (Loopstra et al. 1992). In this study, loblolly pine xylem (stem sections), embryos, and megagametophytes were bombarded with constructs containing putative xylem-specific promoters to look for evidence of tissue-specific expression. Bombardments using the promoterless-*gus*A constructs failed to produce any staining cells after incubation with 5-bromo-4-chloro-3-indolyl-β-D-glucuronic acid (X-gluc). Previous

bombardments using pUC 18 plasmid DNA or a *gus*A fusion containing an inverted Em promoter from wheat (Marcotte et al. 1988) also resulted in no observable stained cells (Loopstra et al. 1992). Bombardments of xylem samples with the 35S, PtX3H6, and PtX14A9 constructs resulted in cells exhibiting expression of the *gus*A gene, demonstrating that the isolated promoters are functional. The number of staining foci resulting from bombardments with the CaMV35S and PtXH6 constructs were similar with averages of 66.5 and 55.1 staining foci per stem section respectively. Bombardments done with PtX14A9 constructs resulted in far fewer stained foci with an average of 1.0 per stem section.

Previous bombardments and hand sections of bombarded material had demonstrated expression in at least three cell types; tracheids, ray parenchyma, and axial parenchyma associated with resin canals. In xylem bombarded with the 35S and PtX3H6 constructs, 20.4% and 18.9% of the staining cells were tracheids, similar to the 20% previously reported (Loopstra et al., 1992). Only 36 stained foci have been observed following bombardment with the PtX14A9 construct and all have been tracheids. It is not known if this is due to the sequences found in the promoters or if expression from the PtX14A9 promoter is just so low it is only noticable in the larger tracheids.

To determine if the promoter-*gus*A fusions are expressed in a xylem-specific manner following bombardment, megagametophytes and embryos were also bombarded. The 35S, PtX3H6, and PtX14A9 constructs all resulted in staining foci when bombarded into megagametophytes and embryos. To determine if the expression observed was transient due to wounding, bombarded megagametophytes and embryos were maintained on non-selective media before staining. Blue staining was observed in tissues bombarded with all three constructs for at least 3 weeks after bombardment, arguing against a transient wound response.

Expression of pine promoters in transgenic tobacco

Transgenic tobacco plants were produced to determine if the PtX3H6 and PtX14A9 promoters would direct expression of the *gus*A gene in a non-woody angiosperm and to determine if any expression observed is xylem-specific. Constructs containing the pine promoters, a CaMV35S promoter, or no promoter along with the reporter gene *gus*A were made in pBin19. The plasmids were moved into *Agrobacterium tumefaciens* strain LBA4404 using triparental matings. Transgenic *Nicotiana tabacum* plants were produced using leaf disc transformation and plant regeneration methods. Stem and leaf samples were analyzed by histochemical staining with X-gluc and by fluorescence produced by homogenization and incubation with 4-methylumbelliferyl-β-D-glucuronide (MUG) in microtiter plate assays. Wells containing buffer and MUG only were included as well as samples from nontransformed plants.

The PtX3H6 promoter was found to function in at least 93% of the plants surviving selection on kanamycin. Expression was not however, restricted to xylem tissue. MUG assays using leaf tissue from plants transformed with the PtX3H6 construct resulted in lower levels of fluorescence in microtiter plates than assays of plants containing the 35S construct. However, the percent of plants giving a positive

MUG result were similar. The relative levels of fluorescence in stems vs leaves were similar in PtX3H6 and 35S plants. A positive MUG result was obtained in only three of the 54 plants assayed that were transformed with the PtX14A9 construct. In two of the cases, the fluorescence was low. In the third, it was quite high, possibly due to the position where the DNA was integrated. No fluorescence was seen in samples taken from non-transformed plants or when buffer alone was incubated with MUG. The results of the MUG assays on leaf tissue are given in Table 1.

Table 1. Analyses of loblolly pine xylem-specific promoters in transgenic tobacco.

Promoter	Plants harvested[a]	Plants assayed[b]	Positive plants
CaMV35S	206	38	35 (92%)
Promoterless	127	26	0 (0%)
14A9	229	54	3 (5.6%)
3H6	180	57	53 (93%)

[a] Number of plants harvested at end of experiment.
[b] Number of plants assayed for GUS activity in leaf tissue using a MUG assay.

Expression of pine promoters in transgenic poplar and spruce

The 3.2 kb PtX3H6 promoter, the 750 bp PtX14A9 promoter, an inverted PtX14A9 promoter, and the CaMV35S promoter were cloned in front of the *gus*A, *lux*, and *luc* genes in the *Agrobacterium* binary vectors ppCV812, ppCV813, and ppCV814. The plasmid was moved into *Agrobacterium* strain 8145 using electroporation. Poplar stem sections were co-cultivated with the *Agrobacterium* and plantlets regenerated in the laboratory of Dr. Olle Olsson at Swedish Agricultural University in Umea Sweden. The patterns of GUS expression driven by the promoters were determined by staining with X-gluc. In some regenerated plants, expression of the *gus*A gene was greatest in differentiating xylem. However, in various plants, many patterns of expression were observed.

Three lines of transgenic spruce were regenerated by Dr. David Ellis (U. of Wisc.) using the PtX3H6-*gus*A construct in pRT99 and microprojectile bombardment of white spruce somatic embryos as described by Ellis et al., (1993). All three lines appear to have very different patterns of blue staining.

Since we have not observed xylem-specific expression of the gusA gene under the control of the PtX3H6 or PtX14A9 promoters using four different systems, it is likely that our constructs are lacking an element required for xylem-specificity. It is possible that important elements are located further upstream or that they are located within the transcribed or 3' portions of the genes. Experiments are now underway to make and test constructs containing a reporter gene and various putative regulatory regions. Initial transformations are likely to be in tobacco or poplar due to the relative ease of transformation. Once isolated, the elements responsible for xylem-specificity and those responsible for the high levels of expression observed will be valuable for genetic engineering of wood properties.

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ISOLATION AND CHARACTERIZATION OF WATER DEFICIT STRESS INDUCIBLE cDNAS AND THEIR GENOMIC COUNTERPARTS FROM *Pinus taeda* (LOBLOLLY PINE)

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Abstract. Water deficit stress (WDS) is one of the most important factors affecting trees in forest stands. To survive the stress, plants activate certain genes of which the translated products are assumed to play a major role in tolerance. Previously we have cloned and characterized 3 genes. Here we describe another WDS induced gene.

The predicted translation product of *lp5* is rich in glycine (40%) and serine (20%), and appears to be a cell wall targeted protein with a possible function in cell wall reinforcement. In order to understand the function *in vivo, lp5* gene has been cloned behind a CaMV 35S promoter and used in transformation studies.

Also the genomic clones of *lp3* and *lp5* genes were isolated from a lambda library and sequence analysis was done. A 1.1 kb fragment of *lp3* and 2.3 kb piece of *lp5* upstream of the transcription start site have been cloned in front of *gus* genes and these constructs have been used in the transformation of tobacco. Preliminary data indicate that the *lp5* promoter is functional in cell suspension tissue. Analyses of sequence data and results of transformation studies are presented.

This study will enable us to understand the molecular mechanism involved in stress tolerance and gene regulation in forest trees.

Keywords: *Pinus taeda,* tater deficit stress, cDNA, promoter analyses, transformation.

INTRODUCTION

Pinus taeda is an important conifer in the reforestation program in the semi-arid regions of U. S. and especially in Texas. These trees are well known for their rapid growth, high biomass production and drought tolerance among conifers. Still many seedlings are lost in the field due to lack of water (Williston, 1972), and the losses can amount to several million

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dollars annually. Further studies have revealed WDS as the cause for up to 90% of the variation in annual ring-width of conifers in the humid temperate and arid climates (Zahner, 1968). Perry et al., (1994) reported that water deficit stress was responsible for more than half of the variation in stem volume index at the end of the first growing season in pine seedlings.

Plants cope with WDS by avoidance, postponement and/or tolerance (Kramer, 1983). Because of their immobility, plants that cope WDS by postponement or tolerance have to make some metabolic and/or structural adjustments to survive the stress (Ho and Sachs, 1989). Techniques of molecular biology now have opened new avenues to study these adjustments in detail at the gene level. An understanding of these alterations and their regulation facilitate breeding of new and improved plants through genetic engineering and formulation of better management strategies for drought tolerance to increase productivity of plants.

Although many advances have been made in the study of stress induced responses of plants, namely gene expression, little is known about these mechanisms in *P. taeda*. Thus, this study was undertaken to gain an insight into genes induced by water deficit stress in loblolly pine. We have cloned several WDS induced cDNAs isolated through differential screening of a WDS root cDNA library. These clones isolated from loblolly pine - named lp were sequenced and their expression patterns have been analyzed by Northern screening. Three of them were described previously. We report here two other clones not described before and the isolation and characterization of genomic clones of two of the above described WDS cDNA clones.

METHODS

Plant Growth and Water Status

Full-sib *P. taeda* L. seedlings grown to heights of 30 to 45 cm with an age of 8 to 13 months were used in the experiments. One week before WDS treatments began, all seedlings were acclimatized by daily irrigation with reverse-osmosis water. The seedlings were then separated into 5 different groups and water was withheld, in staggered fashion so that different groups were deprived for various periods. All plants were harvested predawn on the same day. Immediately before harvest, the water potential of a medium-aged needle fascicle from each seedling was measured using a Scholander pressure chamber. Then each seedling was separated into root, stem and needle portions and immersed in liquid nitrogen. These tissues were stored at -80° C for subsequent nucleic acid isolation from individual seedlings that had attained targeted water potentials.

Nucleic Acid Isolation

The RNA extraction was done according to protocol of Chang et al., (1993). The DNA was isolated using the procedure developed by Doyle and Doyle (1990).

Northern and Southern Analysis

The Northern and Southern analysis were performed according to Maniatis et al., (1982). Each sample in Northern analysis is represented by 10 μg of total RNA, and in Southern blots 15 μg of total DNA, digested with the given enzyme were run/lane in gel electrophoresis. All gels were photographed after ethidium bromide staining, for mobility measurements, and future references. The probes were made using a random primed labeling kit (USB).

Genomic Library Construction and Molecular Methods

A genomic library of loblolly pine was created in bacteriophage Lambda GEM 11 obtained from Promega (Madison, Wisconsin), with a titer of 8 X 10^5. The library was screened with the lp cDNA clones to isolate the genomic counterparts after plating at a density of 2000 pfu/plate on a *E. coli* KW251 lawn.

The positive plaques were isolated separately and were further screened by secondary and tertiary plaque lifts.

Sequencing of DNA and Data Analysis

The sequencing of cDNA and genomic clones were done using a combination of a manual and Dye-Deoxy automated sequencing method. Sequencing reactions were performed using a Sequitherm kit (Epicenter Technologies) or a Dye-Deoxy sequencing kit (Applied Biotechnologies Inc) in a thermocycler.

The sequences were analyzed by the MacVector program (Kodak). Computer searches of the NIH Genbank and Swiss Protein Databank were also performed.

RESULTS AND DISCUSSION

Isolation and Characterization of WDS Induced Gene *lp5*, and genomic counterparts of *lp3* and *lp5*

Previously, we have isolated 28 putative WDS induced clones by differential screening of a WDS root cDNA library. Out of these gene *lp5* was sequenced and characterized.

The cDNA clone of *lp5* consists of 981 bp (data not shown) and the Northern analysis (Fig 1) indicate the message to be about 1 kb. This gene is mainly expressed in roots and to a lesser extent in stems, with very little or no expression in needles. There is an open reading frame that corresponds to a translation product of 205 amino acids (Fig. 2) with a calculated molecular weight of 17,264, and a pI of 6.39. It is very rich in glycine (41%) and serine (20%) and has no proline, tryptophan and histidine residues. When aligned to sequences at the GenBank, the putative LP5 protein showed the greatest similarity to silk fibroin heavy chain protein of *Bombyx mori*, and glycine rich cell wall structural proteins from *Arabidopsis*, petunia and *Phaseolus*. Thus, LP5 appears to be a cell wall related structural protein.

There is evidence for this assumption to be correct in the predicted amino acid sequence. First, it has a very hydrophobic amino terminal that is 17 (5th - 21st) amino acids long which is characteristic of a signal peptide. The peptides with such a signal peptide are translocated to endomembranes during translation and are secreted out of the cell unless an endoplasmic retention signal is found. The second feature that indicates that LP5 is a cell wall protein is the 9 direct repeats of a GXGXGY sequence in the carboxy terminal. All of the above mentioned proteins to which LP5 is similar have been predicted to have an anti parallel, ß pleated sheet structure. According to this model, the residues alternating with glycine have all their bulky side chains projecting on the same side of the ß pleated sheet. The primary sequence of LP5 allows the formation of a structure similar to the above with all the tyrosine residues having their side chains on one side of the ß pleated structure (Fig. 3). Third, Lei and Wu (1991) who also isolated a cell wall targeted GRP, proposed the reinforcement of the cell wall as the function of their protein based on the exposed tyrosine residues in the direct repeats. It has been shown that the peroxidases can form isodityrosine bonds between two tyrosines in the same protein or in two different proteins (Liyama et al., 1994) forming a network that serves as a matrix for linkage of proteins with polysaccharides or polyphenols, thus reinforcing the cell wall (Epstein and Lamport, 1984). The possibility of crosslinking between LP5 and other cell wall proteins and/or lignin via tyrosine residues in the ß pleated sheet structure exists as tyrosine residues are placed in alignment to each other as described by de Oliveira et al.(1990).

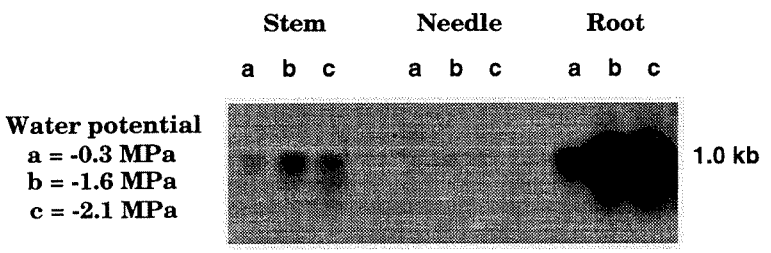

Figure 1. Northern Analysis of *lp5*.

MSKQKLLIFAAMAGLLFACAAVESRIARSDLGLDLGGGLGLGVGVGAGLGLGGGSASGSGSGSGS
GSGSGSGAGSAAGSGSGSGAGSGAGSYAGSGAGNGGGQGRGSGSGYGSGSGYGAGNGNGNGYGAG
SGYGAGNGNGNGYGAGSGSGSGSGSGSGRGYGSGSGTGSGYGSGSGSGYGNGSGSGSGYGAGDDG
SNEGASGGGY

Fig. 2. The predicted translation product of *lp5*. The putative signal peptide is given in bold letters. The tyrosine residues with potential to be involved in crosslinking are underlined.

```
                                                    Strand #
AA # 106          G
                    S-G-S-G-Y-G-S                       1
AA # 114                        G
                    N-G-A-G-Y-G-S                       2
AA # 122          G
                    N-G-N-G-Y-G-A                       3
AA # 130                        G
                    N-G-A-G-Y-G-S                       4
AA # 138          G
                    N-G-N-G-Y-G-A-G-S-G-S-G-S           5
AA # 152                                      G
                    S-G-S-G-Y-G-R-G-S-G-S-G-S           6
AA # 166          G
                    T-G-S-G-Y-G-S-G                     7
AA # 175                          S
                    S-G-N-G-Y-G-S-G                     8
AA # 184          G
                    S-G-S-G-Y-G-A-G    (AA # 192)       9
```

Figure 3. The proposed anti parallel ß pleated sheet structure of the LP5 at the direct repeats.

In order to confirm the function of LP5 in-vivo, the *lp5* cDNA clone was cloned behind a CaMV 35S promoter, and was inserted into pBI121 in between *nptII* and *gus* genes. This vector was then inserted into *Agrobacterium tumefaciens* LBA4404, and was used in transformation of tobacco leaf discs. More than twenty different, stable transformed, GUS positive tobacco plants were obtained after selection on kanamycin media. These were grown to maturity, and were allowed to seed. The seeds were collected and analyzed for both GUS activity and kanamycin resistance. Almost all GUS activity was limited to the phloem tissue in all transformed plants. This may be due to the 35S promoter which has the origin in a pathogenic virus which are adapted to live in the vascular system. Northern analysis indicated the presence of transcripts for both *gus* and *lp5* in the transformed leaves (Fig. 4). No differences were observed in the phenotype of the transformed plants compared to nontransformed. Southern analysis indicated the possible incorporation of two copies of T-DNA in one of the plants and the presence of *lp5* homologs in tobacco. Chi Square analysis of the number of seedlings resistant to kanamycin compared to kanamycin sensitive seedlings in the F1 generation of transformed plants confirm this observation. Furthermore, GUS activity was observed in root hairs of the seedlings in the F1 generation. These studies are continuing to determine the function of LP5 in pines.

In our quest to characterize the regulatory elements in the 5' upstream sequences of *lp3* and *lp5*, Southern analysis indicated that the *lp3* gene belongs to a multi-gene family and that *lp5* has

only two members in it's family in loblolly pine. During the screening for genomic clones, 2 clones representing *lp3* and one representing *lp5* were isolated.

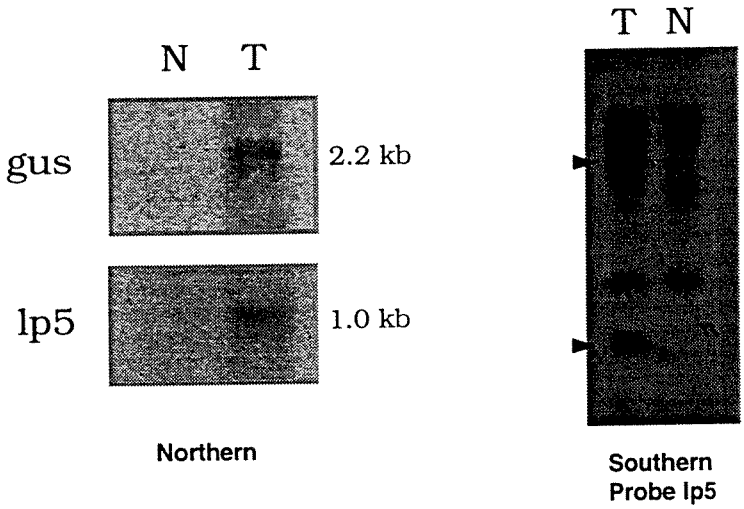

Figure 4. Northern and Southern analysis of transformed tobacco. Northern was done with leaf RNA and probed with *gus* and *lp5*. Southern was done on BamHI digested total DNA. Arrows indicate possible *lp5* inserts in the transformed genome. N = Nontransformed, T = Transformed.

The *lp3* genomic clones were members of that gene family and did not represent the exact cDNA clone. But the predicted translational products were very similar to each other. There is an intron in one clone and the other is being characterized. The *lp5* gene did not have any introns and matched base to base to the cDNA clone, indicating that it is the exact genomic counterpart of the *lp5* message.

The 5' transcription start sites of *lp3* and *lp5* genes were located by primer extension. It appears that the start site of the *lp5* transcript is flexible within 3 adjoining bases about 25 bases downstream from a putative TATAA box. A convenient EcoRI site is located just a base pair upstream of the predicted transcription start site. A 2.3 kb HindIII:EcoRI fragment containing the whole 5' upstream sequence was subcloned in front of the *gus* gene. Likewise, a 1.2 kb 5' upstream sequence of the *lp3* genomic clone was also cloned in front of *gus* in pBI221. Both these vectors were used in biolistic transformation of slash pine suspension cultures using DNA coated gold particles. Both *lp5* and *lp3* promoters were functional when tested, and gave similar number of blue spots (positive for GUS with X-Gluc) against a CaMV 35S promoter (Data not shown). When the bombarded cells were placed on media with mannitol concentration adjusted to have an osmotic potential of 1.1 MPa, there was a marked increase in the number of blue spots observed (Fig. 5). This increase was dramatic with the *lp3* promoter compared to the *lp5* 5' upstream sequence (Data not shown).

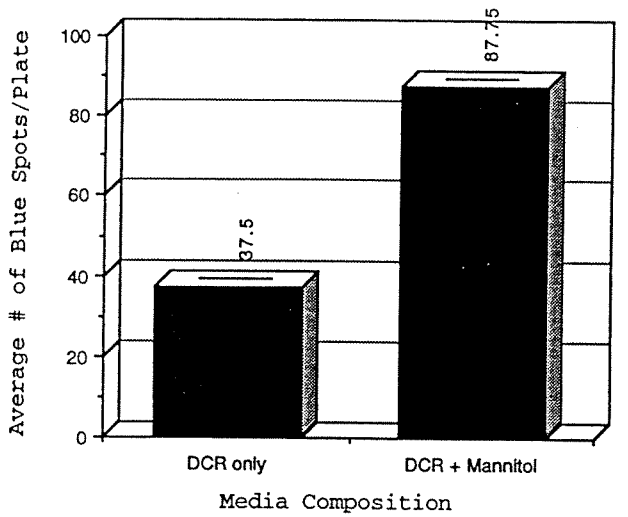

Figure 5. GUS activity of cells bombarded with *lp5* promoter:*gus* construct under water deficit stress.

Modern molecular techniques have proven successful in manipulating genomes to produce transgenic plants of a desired character. These approaches are now being applied to forest species. Plantlets of loblolly pine have been regenerated from tissue culture (Gupta and Durzan 1986), although the efficiency of plantlet regeneration is often low.

However, the ability to propagate plant cells and produce fertile plantlets in vitro has perhaps its greatest potential when applied to the techniques of gene cloning and transfer. Towards this end a number of important conifer genes have been cloned. These findings raise the possibility of performing transgenic experiments with pine genes in 'model' plants, thus gaining some understanding of the function of a sequence before choosing to transfer it into pine tissue.

Transient expression of foreign genes in pine tissue has been achieved in a number of laboratories (Newton et al., 1992) and in some cases stable transformation has been achieved (Sederoff et al., 1986). The regeneration of a fertile transgenic plantlet, however, has proven more difficult but recent reports of success using gene gun technology (Newton et al., 1992) suggest that this goal is attainable, and ultimately should become routine.

The data presented here, together with that cited above, affirm the usefulness of molecular biological methods as tools to study the physiology of these formerly recalcitrant species. These data also indicate the potential for genetic modification of forest species to produce enhanced water deficit stress resistance.

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MOLECULAR AND CELLULAR EVENTS DURING ADVENTITIOUS ROOT INITIATION IN LOBLOLLY PINE CUTTINGS

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One of the principal obstacles to using rooted cuttings to implement clonal forestry with the southern pines is maturation. As the age of the donor plant increases, the rooting ability of its cuttings decreases. To better understand the causes of maturation, we are studying adventitious root formation in stem cuttings of loblolly pine. Our overall approach is to determine: (1) the molecular events that are necessary for root initiation and (2) how the root initiation pathway differs in cuttings from juvenile and mature plants. Endogenous or exogenous auxin is necessary, but not sufficient, to stimulate root meristem initiation. We have examined auxin transport and metabolism in hypocotyl cuttings which root at high frequencies and rapidly and in epicotyl cuttings which root at much lower frequencies and more slowly. Our data indicate that auxin transport and metabolism do not differ substantially between hypocotyl and epicotyl cuttings. Moreover, in hypocotyl cuttings, the presence of auxin is not limited to the cells which divide to form root meristems. Thus, the ability of a cutting to respond by forming roots is not just dependent on the availability of auxin, but by some other determinant of cellular competence.

One potential competence determinant is the ability of cells to respond to the presence of auxin by initiating gene expression. A class of auxin-responsive genes has been cloned from annual plants that are rapidly transcribed after auxin treatment. We have cloned genes from loblolly pine with sequence similarities to the auxin-induced genes from pea, soybean, mung bean and *Arabidopsis*. Preliminary experiments indicate at least one of the genes is induced by auxin in hypocotyl cuttings. Further studies will test the association between the expression of these genes and competence for root initiation.

Another critical step in the root initiation process is cell division. Studies from many organisms indicate that the cell cycle is under control of gene products such as cyclin-dependent kinases (cdks). We have cloned two cdks from loblolly pine which have sequence similarities with cdk1 and cdk2. Preliminary data indicate that one of these genes is expressed equally in hypocotyl and epicotyl cuttings. The other appears to be induced only in the hypocotyl cuttings after auxin treatment. Future experiments will test the localization of expression of the latter gene in root-forming cells and the expression over time in stem cuttings from mature donor plants. These experiments, plus those examining the role of auxin-binding proteins, should provide insight into the mechanisms of adventitious root initiation and its limitation by maturation.

REGULATION OF DEFENSE/REPAIR GENE EXPRESSION IN WOODY PLANTS IN RESPONSE TO WATER DEFICIT

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Abstract:--Four cDNA clones and two genomic clones of different members of a PI gene family have been isolated from *Atriplex canescens*. The sequence of the coding regions are very similar however promoter sequences and AU-rich sequences within the transcribed regions suggest different levels of regulation. Gene specific oligonucleotides reveal some of these differences and will facilitate gene regulation studies and, as part of a broader program, the assignment of physiological roles to individual family members. An Loblolly Pine mRNA of unusual structure encodes a protein similar to a number of chitinases. The 5'UTR of this mRNA accounts for almost half the molecule and possesses numerous inverted repeat and uORF, features found in many translationally regulated mRNAs. RT-PCR results which suggest additional transcripts possess some of these sequences. Cloning and sequencing of these gene fragments along with in vivo studies of the reported genes will illuminate the types of gene regulation which operate in pines.

Keywords: Gene regulation, Reverse Transcription-PCR, sequence specific oligonucleotides, upstream open reading frames, secondary structure.

INTRODUCTION

The study of plant gene expression in response to environmental stress has resulted in the elucidation of a number of biochemical defense and repair mechanisms (Skriver and Mundy 1990, Keen 1992). The application of that knowledge, through the isolation of genes, their modification and subsequent transfer into plant cells, has brought spectacular successes (Chrispeels and Sadava 1994). As recognition of the efficacy of biotechnologies has spread, the desire among researchers and corporate enterprises to avail themselves of new opportunities has broadened the field. An increasing numbers of researchers now conduct molecular experiments with plants and with their arrival, the less highly publicized technical problems of gene transfer and expression have begun to surface (Matzke and Matzke 1995). The recognition of the complexities of gene regulation has led researchers to seek more fundamental understandings of gene expression. In this paper we discuss the regulation of two gene families whose members display variable responses to environmental and hormonal cues. The sequence and structural features of the genes and

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mRNAs suggest tiers of regulation which would allow rapid modulation of protein synthesis in response to stress.

METHODS

Plant material and water deficit stress treatments. Saltbush (*Atiplex canescens* (Pursh Nutt.): Whole plants were desiccated after growth in germination medium (GM) for 4-6 weeks. Plants were left to desiccate on Whatman 3MM paper at 22^0C and 60% humidity under dim light following published protocols (Yamaguchi-Shinozaki and Shinozaki, 1993 a and b). For ABA treatment, plants were removed from GM and grown hydroponically in half strength either MS media or MS media supplemented with 10μM ABA for 14 hours.

Loblolly pine (*Pinus taeda* L.):Seedlings were full-sibling, resulting from the cross of S6PT2 and S6PT3 sources from east Texas. The seeds were sown in 5-liter cylindrical containers filled with a fritted clay medium adapted for pine seedlings (Meier et al. 1992). The seedlings were randomly separated into 7 groups, and water was then withheld from the seedling groups in staggered fashion. Seedlings were harvested pre-dawn on the same day at which point the water potentials were taken. (Chang et al 1995).

Molecular methods and cDNA library construction. For Atriplex a cDNA library was made from the leaves of seedlings with water potential - 0.94 MPa (Adair et al 1992). The pine library was made from the entire root tissue of an 8-month-old seedling with a water potential of -1.1 MPa (Cairney et al 1993). In both cases the vector was the λgt10-derived vector, Lambda-ZAP (Stratagene, La Jolla, CA).

Reverse Transcription-Polymerase Chain Reaction. One microgram of total RNA was used for Reverse transcription using MMLV Reverse Transcriptase (Promega, Madison, WI). One tenth of the reaction products were then used for PCR. Conditions were, 94^0C, 15 s(econds), 50-55^0C, 15s, 72^0C 30s, 35 cycles. Reaction products were transferred to membranes, probed with a labelled cDNA and the membrane scanned and signals quantitated.

DNA sequencing and homology comparison. DNA sequencing was performed by the dideoxy chain termination method (Sanger et al. 1977). Standard molecular methods were used in other cases (Sambrook et al. 1989). For comparing the putative proteins encoded by these pine cDNA clones with similar proteins from other organisms, sequences were aligned using the LaserGene program (DNASTAR, Madison, WI).

RESULTS AND DISCUSSION

Halophytes which survive long periods with little moisture, possess both physiological and biochemical adaptations which permit them to prosper under conditions where most plant would perish. A study of halophyte physiology may shed light on novel molecular mechanisms of stress tolerance or may reveal a source of new genes. In parallel projects we have chosen to study gene expression under water deficit in the woody desert shrub, Saltbush (*Atriplex canescens*) and the commercially important conifer, Loblolly Pine (*Pinus taeda* L.). In both cases cDNA libraries

were constructed from water stressed plants and differentially screened to isolate clones of genes whose steady-state mRNA levels fluctuate under water deficit. Details of clone isolation have been reported elsewhere (Adair et al 1992, Cairney et al 1993. Chang et al 1995).

An *Atriplex* clone, p23-3, isolated from a cDNA library by differential screening was shown to encode a protein with strong similarity to Proteinase Inhibitor-I (PI-I) from soybean and potato Figure 1). The clone proved distinctive in hybridizing to a broad mRNA band in Northern analysis. This signal appeared be divided between two principal bands, of about 0.5 and 0.7kb, suggesting closely related transcripts. By rescreening the library four additional cDNA clones which hybridized to p23-3 were isolated. DNA sequencing showed them to be 95% identical at the nucleotide level. They appeared to part of a multigene family. Most of these clones were around 0.4 kb however clone p8-3 was 0.65 kb and differed from the other clones, principally, in possessing a longer 3'UTR which contained a AU-rich region.

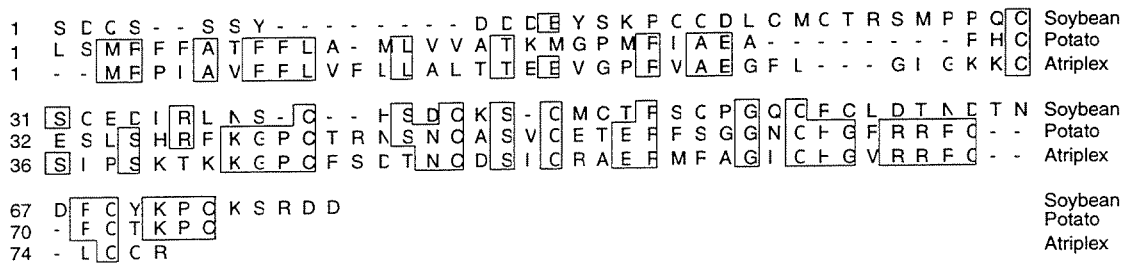

Figure 1. Comparison of amino acid sequences of proteinase inhibitor CII(Bowman Birk) of soybean, potato (p322, Steikema et al., 1988), and predicted polypeptide of Atriplex clone 23-3.

Since the translation product of these mRNA molecules was essentially the same, the presence of several transcript sizes within a given organ begs questions about their role. Several explanations are possible;

1. The PIs may differ in subtle but significant ways, fulfilling different roles in the cell, their cognate genes responding to different cues.
2. These transcripts may be produced in different cell types within an organ, each with a appropriate stability and translational efficiency.

The altered cytoplasmic conditions of water deficit may favor one transcript over another. A number of AU-rich sequences have been identified in the 3'UTRs of transcripts. These sequences have been assigned roles in mRNA instability (Green 1993), translational enhancement (Gallie 1993) regulated/cytoplasmic polyadenylation and deadenylation (Bachvarova 1992).

Attempts to purify the protein and determine its function are underway, this paper discusses advance made in the study of PI-I gene regulation.

Genomic Southern blots gave a complex pattern indicating many copies of the PI-I genes. To

shed light on the transcriptional regulation of these genes genomic clones were isolated. Two clones, pG12-95 and pG18-1 were sequenced. The coding regions and introns of both genomic clones were determined on the basis of nucleotide sequence comparison with the cDNA clones. Neither genomic clones matches a cDNA exactly; their putative exonic sequences show 95% identity to the cDNAs. Both pG12-95 and pG18-1 contain one intron of 931 and 422 bp in length, respectively. The first exon of both clones are identical in length, coding for 17 amino acids. Therefore, the introns of both genes vary at the 3' end in length.

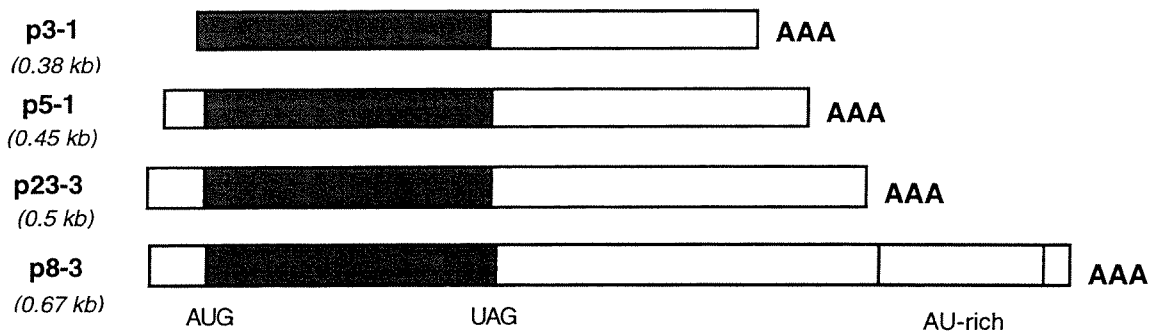

Figure 2. Schematic showing alignment and placement of open reading frames and additional sequence features of Atriplex PI-1-like cDNA clones (p3-1, p5-1, p23-3, p8-3).
Key: Gray area; open reading frame, Stippled area; AU-rich region, AAA: polyA tail.

Preliminary analysis of the promoters reveals a number of motifs which have been identified in other plants as transcription factor binding sites. These include ABA-Responsive Elements (ABREs).A number of unusual repeat structure are conspicuous, their function is being investigated. There is no AU-rich region in the second exon of pG12-95 however pG18-1, while differing in sequence from the other clones, contains a 3'AU-rich sequence identical to that of cDNA clone p8-3.

Multigene families contain many pseudogenes and sequence analysis alone cannot distinguish these from transcriptionally active form. Transgenic studies would indicate the inducibility of these genes however with large families of genes such experimentation is laborious and time consuming. We have sought a swift,sensitive and specific assay of gene expression through the use of specific oligonucleotides and Reverse-Transcription-PCR (RT-PCR). Oligonucleotides specific to each transcript and suitable for PCR were designed. Each should amplify a product of a characteristic size, facilitating identification. Preliminary assay using the cDNA and genomic clones showed that these primers displayed fidelity either with single templates or a mixture of templates.

RT-PCR experiments were carried out with RNA from Atriplex seedlings water stressed for varying periods of time. Each transcript showed some level of induction however the magnitude of the response and its' triggering point were greatly different. The same individual patterns were observed during ABA induction. These results suggest that our technique is sufficiently sensitive

to discriminate between individual members of a multigene family and at present these experiments are being repeated to establish levels of confidence with the assay. Such a technique will be of great use in determining which members are induced under different circumstances. Such assessment, in combination with other biochemical and physiological data, would allow workers to chose the most appropriate protein for their purposes from among a family of similar polypeptides. In gene regulation studies pseudogenes or genes whose level of induction is low, specific to a tissue or environmental signal, could be identified.

As part of a parallel project, examining gene expression in response to water deficit in Loblolly Pine, a cDNA library was constructed from the roots of five month old pine seedling which had been deprived of water for 11 days. The library was differentially screened using polyA+ RNA from control seedling (water potential, -0.4MPa) and stressed seedlings (-1.3MPa) and examples of inducible and repressible clones were selected (Cairney et al 1993). One clone, pLP6, is strongly expressed in the roots and stems of well-watered plants but mRNA levels decline rapidly as plants dehydrate. The same pattern is seen in needles although the absolute level of gene expression is much lower. A single transcript of 1.5kb is dectected, the same size as the cDNA suggesting that almost all the information in the mRNA is present in the cDNA clone.

The nulceotide sequence of pLP6 consists of 1488 nulceotides, concluding with a polyA tail. The longest open reading frame commencing with an ATG could encode a polypeptide of 216 amino acids, which has a predicted molecular weight of 24.2 kD and PI of 5.05. However this open reading frame does not start until nucleotide 721, almost halfway through the mRNA (Figure 3).

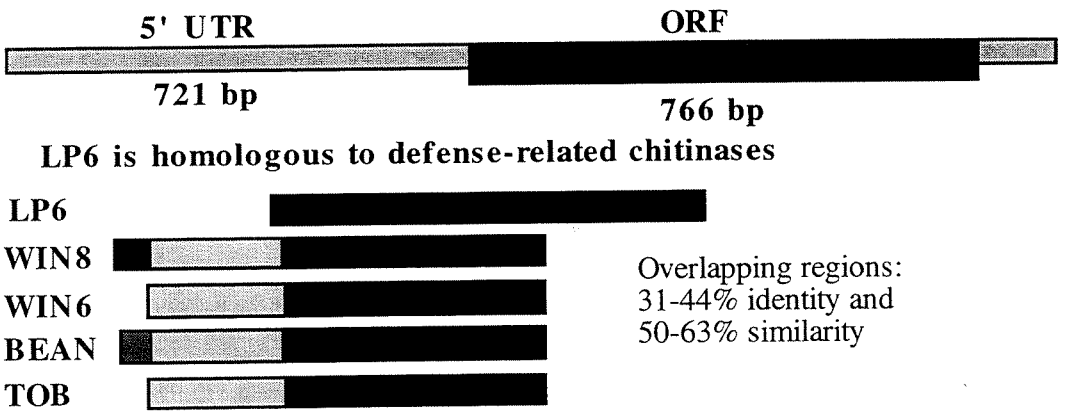

Figure 3. Schematic showing structure of pLP6 5'UTR and major ORF (above) and the alignment of the putative LP6 polypeptide and several Class I chitinases (below).

To demonstrate that pLP6 is not a "double clone" fortuitously hybridizing to an mRNA of similar size we repeated hybridization using a 500 bp 5' probe generated using a convenient EcoRI site and found a similar hybridization pattern . To eliminate any possibility of an abundant mRNA masking the expression of a rare transcript, we performed RT-PCR using primers near the 5' terminal of the cDNA and from within the open reading frame. A fragment of

predicted size was amplified and cloned. The sequence of approximately 150 nucleotides from either end of this molecule was determined, this is identical to the corresponding region of pLP6 (data not shown). These results confirm that pLP6 is derived from a single mRNA molecule.

The polypeptide encoded by pLP6 shows strong homology to a number of Class I Chitinases from bean, tobacco and poplar (Broglie et al 1986, Shinshi et al 1987, Parsons et al 1989) however the simililarity is only with the carboxy half of these proteins. The signal peptide, cysteine-rich chitin binding domain and Glycine/Proline rich "hinge" region, all located in the amino terminal portion of the Class I chitinases (Collinge et al 1993, Raikhel et a 1993) are absent from LP6. Neither the putative catalytic site nor the carboxy terminal sequence involved in translocation to the vacuole is present. In addition the pLP6 protein has a carboxy extension of 69 amino acids not present in any of the chitinases though no homolgies with any sequence in the gene bank could be detected. .

Since chitinases are wound-inducible we wounded 12 seedlings with pliers and chose seedlings from this group for assay 1h, 2h, and 6h after wounding. The water potentials of these plants were assayed and gene expression was compared to unwounded control plants harvested at the same time. Steady state RNA levels of pLP6 are greatly reduced 6h after wounding. Similar results have now been obtained for roots and needles (data not shown).

At present we have no information on the function of the putative LP6 protein. The physiological function of chitinases has long proved elusive however recently, with the elucidation of the biochemical nature of Nod factors (Dénarié and Cullimore 1993) and the demonstration that a protein capable of rescuing a developmentally blocked carrot embryo mutant was a chitinase (De Jong et al 1992), a role for chitinases in development is being suggested. It is possible that the LP6 protein fulfills some role in develpment and to investigate this possibility expression in early embryos is being investigated. The unusual structure of pLP6 may be explained if the protein is a 'proto-chitinase', such gene variants have been suggeted (Shinshi et al 1990).

The sequence of the 5'UTR of pLP6 revealed several inverted repeats which could form stem loop structures of moderate to high stabiliy (-11kcal/mol to -17kcal/mol). In addition eight upstream open reading frames (uORFs) were identified. Both these feature are found often in genes exhibiting post transcriptional regulation (Gallie 1993). The 600 nucleotide 5'UTR from Cauliflower Mosaic Virus posseses extensive secondary sturcture and several uORFs and recently a novel translation mechanism, a "ribosome shunt", was proposed to control expression of the downstream cistron (Fütterer et al 1993). At present we are cloning copies of the LP6 5'UTR into plant expression vectors to determine whether reporter gene expression can be influenced by this sequence or truncated and mutated variants.

CONCLUSIONS

Sequence anaysis and preliminary regualtory studies show stress genes are regulated at several levels. In vivo analysis and additional environmental and hormonal treatments will illuminate the relative contribution of each step and provide information for efficient biotechnological applications in forest species.

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ROOT AND SHOOT GROWTH OF SWEETGUM ROOTED CUTTINGS

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Abstract. Sweetgum (*Liquidambar styraciflua* L.) is one of the most widely adapted hardwood species in the southeastern U.S.. The productivity and value of sweetgum plantations may be increased by using genetically superior, rooted cuttings. Previously, methods have been developed to produce rooted cuttings of sweetgum and now the objectives are to fine tune the techniques and increase the size of the rooted cuttings for field planting. Five levels of nitrogen (0, 25, 50, 100, and 200 ppm N) with either ambient photoperiod or a 3-hour night interruption (with incandescent light) were imposed on four clones over five replications. The night interruption stimulated shoot growth, while nitrogen levels affected root and shoot growth differently. The same four clones were tested in five different potting media combinations (1:2:2 peat : perlite : vermiculite, 1:1 peat : perlite, 1:1 peat : sand, 1:1 peat : bark, 1:1 bark : sand by volume) over five replications. Even though there was poor survival in the media study (<50%), differences in root morphology existed. Some sprouts of two clones were chilled at 4°C for an additional month and then set and placed under intermittent mist. Root and shoot growth on these rooted cuttings were greater than those not exposed to additional chilling. Clonal differences existed in all the studies. Future studies can build on these results to produce high-quality sweetgum rooted cuttings for operational plantation establishment.

Keywords: *Liquidambar styraciflua*, stem cuttings, vegetative propagation, nitrogen, photoperiod, media, chilling.

INTRODUCTION

Sweetgum (*Liquidambar styraciflua* L.) is widely adapted to many different soils and sites in the southeastern United States. Approximately 2500 acres of sweetgum are planted annually in this region. It is an important raw material for forest products, such as pulp, veneer and lumber. Due to the increasing demand for hardwoods, it would be desirable to increase productivity using plantation forestry. One way of accomplishing this is by planting superior genotypes using vegetative propagation. Vegetative propagation in forestry is becoming a widely used method of reproduction. Large genetic gains can be made by establishing clonal plantations using rooted cuttings (Zobel and Talbert, 1984). Sweetgum is a species that roots well from cuttings, making it a good candidate for clonal forestry.

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Even though sweetgum cuttings root well, limitations currently exist to the production of sweetgum by rooted cuttings. To date, most rooted cuttings produced have not been as large as first year seedlings. Therefore, our first objective was to produce larger rooted cuttings.

It has been reported that dormant sweetgum shoots do not root well (Land and Cunningham, 1993). Previous research (Cunningham, 1989), showed cuttings collected in May and July rooted at higher frequencies than those collected in February and September. However, cuttings collected in July did not grow as large as May collected cuttings. It would be advantageous and more cost effective to obtain additional crops per year for production. We collected sprouts in both May and July to determine if larger cuttings can be produced by altering certain environmental conditions. Another objective was to amend or eliminate the time consuming and labor intensive procedures associated with propagation. We conducted a study to test the necessity of trimming versus not trimming the leaves. Another study involved a cold-storage treatment.

METHODS

Sweetgum sprouts were collected on May 17, and July 18, 1994 from the Union Camp Corporation cutting orchard in Bellville, Georgia. The cuttings were rooted and grown at the North Carolina State University Plant Science Research Greenhouses in Raleigh, North Carolina. Sprouts were prepared for setting by pinching back all the new growth, both terminal and lateral. Then, they were cut into two six-inch (15 cm) cuttings; apical = 1st 6" cutting, basal = 2nd 6" cutting. All leaves were trimmed back to approximately one-half the leaf surface area (except those cuttings used in the leaf surface area study). At least two leaves remained on each cutting. The bases of all prepared cuttings were treated with Hormodin 2 (0.3% IBA).

The cuttings were grown in Ray Leach™ tubes (Stuewe and Sons, Corvallis, Oregon) and set on benches in a greenhouse bay equipped with an intermittent mist system. The mist nozzles delivered 8 1/2 gallons (70 liters) of water per hour. A misting regime of 6 seconds every 20 minutes was used after an initial period of daily observations and adjustments. The cuttings remained in the mist house for 10 weeks. During the ninth and tenth weeks in the mist house, the misting frequency was gradually reduced before the cuttings were transferred to another greenhouse bay.

Four experiments were conducted in 1994. Four clones were used throughout the studies except for a cold-storage study in which only two clones were used. The first and largest of the four studies was the Nitrogen/Photoperiod Study. It was a split-plot design with 5 replications. There were 5 nitrogen levels (0, 25, 50, 100, and 200 ppm) and 2 light treatments (ambient photoperiod or a 3-hour night interruption with incandescent lights). These treatments were imposed on the cuttings the week following transfer to the non-mist greenhouse bay (11 weeks after the cuttings were stuck). The second study tested different rooting and growing media in a randomized complete block design with five replications. Five different media compositions (1:2:2 peat : perlite : vermiculite, 1:1 peat : perlite, 1:1 peat : sand, 1:1 peat : bark, 1:1 bark : sand by volume) were used to determine their effect on root (and shoot) growth. A third study

investigated the effects of different leaf surface areas using a split-plot design with five replications. Leaves on cuttings were either trimmed (leaves cut to approximately 1/2 leaf surface area) or left intact. The last study involved a cold-storage treatment of one month. Although there was no replication of chilling treatments, cold-stored cuttings were compared with cuttings treated in an otherwise identical manner (the 1 peat : 1 perlite composition of the media study). The sprouts for the cold-stored cuttings remained in a 4°C cooler for one additional month before preparing and setting.

All studies were terminated in December, 1994. Roots and new shoot growth were collected and their dry weights [dried at 70°C (150°F) for 48 hr.] obtained. Other variables measured included root collar diameters (mm), number of main roots counted, and shoot lengths (cm). Survival and rooting percentages were calculated for the treatments.

RESULTS AND DISCUSSION

The following results are preliminary and based on mean statistics. Analyses of variance are underway. Only the results of the May studies are reported in this paper as cuttings set in July rooted poorly. The rooting percentage for May, across all studies, was greater than 80%, while for July, the percentage was less than 40%.

Clonal differences were evident in all four studies. Clone 1 had the greatest root and shoot growth, as well as survival, except in the Nitrogen/Photoperiod study, where survival of this clone was the lowest. Clonal differences in rooting and short-term shoot growth may contribute to successful plantation establishment. These characteristics could be important for early assessment of potential clones for field planting.

In the Nitrogen/Photoperiod study, shoot growth increased as the level of nitrogen increased. Root growth, however, peaked at 25 ppm N. The optimal nitrogen level for a particular application will depend on the ideal root:shoot ratio. An intermediate range of 50 to 100 ppm N resulted in a fairly large and well-balanced rooted cutting. The night interruption promoted shoot growth, but root growth was unaffected by photoperiod. The larger shoots from the night interruption probably resulted from delayed budset.

Different media compositions resulted in differing root morphologies. Media did not, however, affect survival, which was generally poor for this study. This limits the conclusions that can be drawn from this study.

In the leaf surface area study, root growth was greater on cuttings with non-trimmed leaves, while shoot growth was greater on cuttings with trimmed leaves. Trimming the leaves did not increase rooting percentages. However, this study was too small to see the effects of these treatments as if they were to take place on an entire greenhouse bench. Overlapping leaves could restrict water from reaching the media and reduce air movement, which could potentially cause uneven moisture and fungal problems in a large-scale operation.

Cold-storing sprouts for one month resulted in an increase in both root and shoot growth. The control cuttings were those in the 1 peat : 1 perlite composition of the media study. It appears that a chilling treatment is beneficial, but further studies need to be done. The greater growth in chilled cuttings may be the result of the conditions in the greenhouse at the time of setting. Cold-storing sprouts prior to setting has potential, but needs further investigation.

Finally, across all studies, there were differences in performance between apical and basal cuttings. The basal cuttings had slightly greater root and shoot growth compared to the apical cuttings.

CONCLUSIONS

- Clonal differences exist in sweetgum cutting root and shoot growth characteristics.
- Higher N levels produce larger shoots. Lower N levels produce larger roots; a reasonable range is 50-100 ppm N.
- A 3-hour night interruption with incandescent lights promotes shoot growth.
- Leaf trimming does not affect rooting percentages, but needs to be investigated on a larger scale.
- Cold-storage of sprouts before setting cuttings appears to have potential, but needs further investigation.
- Basal cuttings produce larger rooted cuttings than apical cuttings.
- Cuttings collected in May perform better than those collected in July.

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ARRESTING PLANT MATURATION TO MAINTAIN HIGH PROPAGATION SUCCESS WITH AMERICAN SYCAMORE CUTTINGS

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Abstract.--Loss of rooting potential with maturation in sycamore limits clonal propagation of selected clones by conventional cuttings. By the time the clones can be identified in progeny tests, they have already lost much of their juvenility (and thus the rooting ability of their cuttings has declined). Data from four studies conducted during 1991-95 are presented to show how serial propagation can arrest the maturation process, thereby maintaining high propagation success until progeny tests are completed. Three-month survival and sprout growth in an on-going field trial of cuttings from different serial stages of propagation of the same clones are given.

Keywords: *Platanus occidentalis* L., vegetative propagation, serial propagation

INTRODUCTION

Clonal plantation forestry has a genetic advantage over seedling plantations by being able to utilize all of the superiority of a selected genotype, rather than just the additive component. Conventional unrooted hardwood cuttings, stecklings (rooted cuttings), or *in vitro*-derived propagules have been used, but conventional cuttings may be more cost competitive with seedling planting stock. The problem for forest geneticists is that rooting ability of vegetative propagules from many woody plant species declines with age of the seedling-derived mother plant (ortet) (Hackett 1988). By the time seedlings have been progeny tested and selected, they may have lost much of their potential to root. As a result, they cannot be clonally propagated with commercially acceptable efficiency.

Several methods have been successfully used with some species to arrest or reverse (rejuvenate) the maturation process in order to obtain large numbers of cuttings with high rooting potential. These include (a) severe pruning or hedging, (b) serial grafting of mature scions on juvenile rootstocks, (c) *in vitro* rejuvenation by serial subculturing of mature explants, (d) use of cuttings from adventitious origin, and (e) recovery of existing juvenile material and placement in stock block plantings with serial propagation (Hackett 1988).

American sycamore (*Platanus occidentalis* L.) will root easily from conventional cuttings taken from seedlings (Land 1983), but cuttings from mature woody tissue of this species are difficult to root (Hare and Land 1982). There is some evidence that severe hedging may partially rejuvenate cuttings from sprouts on mature grafts (Land *et al.* 1995). The objectives of the studies reported in the present paper were to determine if serial propagation [similar to method (e) above] can be used to (a) rejuvenate mature clones and (b) arrest

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the maturation of juvenile material, thereby maintaining high propagation success until progeny tests are completed.

METHODS

1991 Nursery Cutting Study

Cuttings were collected from one-year-old sprouts on eight-year-old hedged trees for a 1988 Nursery Cutting Study (Land *et al.* 1995), and the resulting stecklings were planted in a field test at Mississippi State University (MSU) on December 19, 1988. Some of the two-year-old stecklings were detopped (coppiced) to a 15-cm stump height on April 10, 1990.

Three rootstock types and six of the clones from the 1988 study were used as sources of 25-cm (10-inch) cuttings for the 1991 Nursery Cutting Study. These cuttings were planted in 2.8-liter pots in an irrigated nursery at MSU on March 20, 1991. The three rootstock types were (a) one-year-old sprouts on the three-year-old stecklings that had been coppiced in 1990; (b) limbs from the upper crowns of other three-year-old stecklings that had not been coppiced; and (c) three-year-old sprouts on the 11-year-old ortets that had originally provided the stecklings. The six clones came from six seedlings of Mississippi origin that had been obtained from the state nursery and planted for grafting rootstock at MSU. Since the same clones were used in all three rootstock types, the genetic makeup of the three types was the same.

The 25-cm cuttings were prepared by making the basal-end cut at 1.2 cm (one-half inch) below a bud node, were selected to have at least two nodes within the lower 20 cm of the cutting, and were chosen to have a basal diameter between 16 and 21 mm [based on results of Land *et al.* (1995)]. These cuttings were basally dipped for three seconds in 50% ETOH followed by a dip in 5% Captan powder, and they were planted with only the top 2.5 cm remaining above ground.

The experimental design in the nursery consisted of randomized complete blocks (RCB) with four replications. The three rootstock types served as treatments. A rootstock treatment plot within a replication contained 12 cuttings (two cuttings from each of the six clones). Measurements for number, height, and diameter of sprouts per cutting were taken monthly from May through July, again in September, and finally on November 23, 1991. Analyses of variance were conducted on a plot-mean basis to test for differences among rootstock types in percent sprouted cuttings (percent survival) and sprout height.

1993 Nursery Cutting Study

Twelve clones, representing two seedling ortets from each of six progeny families, were selected from a two-year-old (from seed) progeny test at MSU. Cuttings were taken from the lower limbs of the seedlings and cut into 25-cm lengths such that the first (lowest) bud node was within two cm above the basal cut. Basal diameter of cutting, height above ground of the limb/stem junction for the limb where the cutting was collected, length from basal end of cutting to second node up the cutting, and number of nodes per 25-cm cutting were measured at time of collection on March 1, 1993.

The cuttings were planted on March 1 in a MSU nursery containing six rows

spaced 90 cm (3 feet) apart, with rows raised to an average height of 30 cm above the furrows. Spacing between cuttings within rows was 30 cm, and overhead irrigation was provided daily. The nursery experimental design was a RCB with four replications and 12 cuttings (one per clone) in each replication. Replications ran across rows, so each replication consisted of two cuttings per row on each of the six rows. Measurements of number and length of sprouts per cutting were taken on May 1 and June 16, 1993, and sprout diameter was added to these measurements on March 10, 1994. The cutting measurements taken at time of collection were subdivided into classes and examined for effects on percent propagation success for the March 10 data.

1994 Nursery Cutting Study

Four origins of cuttings and 24 clones were used as sources of 25-cm cuttings, which were planted on March 10-14, 1994, in the same MSU nursery as the 1993 study. The four origins were: (a) one-year sprouts from the rootstocks in the 1993 study [12 clones]; (b) lower limbs (lower 1/3 of crown) of the same ortets that were used for the 1993 study, but ortets were now three years old [same 12 clones as for (a)]; (c) lower limbs (lower 1/3 of crown) of 12 new selections in the three-year-old seedling progeny test [12 different clones from (a) and (b)]; and (d) upper limbs (upper 1/3 of crown) of the 12 new selections [same 12 clones as for (c)]. Cuttings were prepared, as in the previous studies, to have the first node within two cm above the basal cut, and basal diameters between 11 and 19 mm were sought. Cuttings were measured for diameter, distance from root collar of ortet to base of cutting, and number of nodes per cutting.

Cuttings were planted on the day of collection to the same number of rows and at the same spacing as used for the 1993 study. A RCB design was used with five replications, four treatments (origins), and 12 cuttings per treatment plot (one cutting per clone). Number of sprouts per cutting, height of tallest sprout, and cumulative length of all sprouts per cutting were measured on June 22, 1994, and February 10-21, 1995. Diameter of the largest sprout was also measured on the February date. Regression analysis was used to evaluate the effect of distance of cutting origin from root collar on three-month propagation success. Analysis of variance on an origin plot-mean basis was used to test differences among the four origin treatments.

1995 Cutting Field Trial

Three of the 12 clones used in both the 1993 and 1994 studies, and four rootstock (RS) treatments, were used as sources of cuttings planted to a non-irrigated field site at MSU on March 7, 1995. One RS treatment (TRT#1) provided cuttings from one-year-old sprouts on the two-year-old rootstocks of the 1993 nursery. These rootstocks had originally been taken from two-year-old seedling ortets. The second RS treatment (TRT#2) utilized cuttings from one-year-old sprouts on the one-year-old rootstocks in the 1994 nursery study. These rootstocks had come from one-year sprouts on the one-year-old rootstocks (at that time) in the 1993 nursery. Thus, the cuttings represented material from the second cycle of serial propagation, but the original rootstocks still came from two-year-old ortets. The third RS treatment (TRT#3) was represented by cuttings from one-year-old sprouts on one-year-old rootstocks in the 1994 nursery. These rootstocks came from the same ortets as TRT#1 and TRT#2, but were collected at ortet age three. The final RS treatment (TRT#4) used the original ortet as the rootstock. Cuttings came from limbs in the live crown (upper half of tree) of

the 4-year-old trees. The same three clones were represented in all rootstock treatments, so the genetic makeup of the treatments was the same.

The field site was cultivated and subsoiled during the fall of 1994. The cuttings were collected, cut into 25-cm lengths with a bud node within two cm above the basal cut, measured for basal diameter, and planted on the same day. Spacing was 2.5 m (8 feet) between rows and 0.9 m (3 feet) within rows. The study has been cultivated monthly for weed control since planting, but no irrigation has been provided.

The experimental design consisted of a RCB with four replications, 12 clone-by-RS-treatment combinations, and three cuttings per combination plot within a replication. Cuttings were measured for number and length of sprouts on May 10 (two months) and May 31, 1995 (three months after planting). Analyses of variance to evaluate RS treatment differences were conducted on a plot-mean basis.

RESULTS AND DISCUSSION

The objective of the 1991 Nursery Cutting Study was to determine if serial propagation of cuttings from mature sycamore ortets would increase propagation success (*i.e.* "rejuvenate" mature material). Cuttings taken from one-year-old sprouts on the coppiced stecklings did sprout and survive significantly better than cuttings from either the upper limbs of non-coppiced stecklings or the three-year-old sprouts at the top of the 11-year-old ortets (Table 1). However, data from the original 1988 Cutting Study indicated that average survival for cuttings from one-year-old sprouts on the same six ortets at age eight was 32 percent at three months and 24 percent at nine months after being planted. The 29-percent survival obtained from the sprouts on the coppiced stecklings was essentially the same, indicating that the maturation process had only been arrested rather than reversed. Woody tissue from near the ground line (root collar) of the three-year-old steckling was still at the same level of maturation as the original cutting taken from the eight-year-old ortet, even though the top limbs on both the non-coppiced stecklings and the three-year-old sprouts of the ortets had continued to advance in maturation.

Subsequent objectives of the 1993-1995 studies have been centered on questions of what ages, what positions on the plant, and how many cycles of serial propagation are best for arresting maturation and maintaining rooting ability of selections in seedling progeny tests. Also important have been questions about when propagation success can be assessed after planting and about the height-growth pattern of sprouts.

Sprouted cuttings in June, three months after planting, provide a good assessment of success, since average results from the 1991, 1993, and 1994 studies all indicated only small declines in survival and no changes in rank from this time to the end of the growing season (Table 1). Ranks for sprout heights also did not change after the June measurement, and the average June height was approximately one fourth of the height at the end of the season. Land *et al.* (1995) have shown from monthly measurements in earlier tests that percentages of sprouted cuttings after two months (early May) are much higher than the percentages after three months, so conclusions should not be made before the first of June.

Table 1. Percent survival and length of tallest sprout at three months and one complete growing season after planting cuttings in four sycamore cutting studies at Mississippi State University.[a]

Study	Cutting Source	3-Mo. after Planted Survival (%)	3-Mo. after Planted Tallest Sprout (cm)	8-12 Mo. after Pltd Survival (%)	8-12 Mo. after Pltd Tallest Sprt (cm)
[1] 1991 Nursery	[a] 3-yr sprout on 11-yr-old ortet	6 a	5.8 B	4 b	49 A
	[b] upper limb on 3-yr-old steckling	15 a	6.2 B	10 b	55 A
	[c] 1-yr sprout on coppiced 3-yr steck.	29 a	9.5 A	29 a	64 A
[2] 1993 Nursery	[a] lower limb on 2-yr-old ortet	73	24.8	68	127
[3] 1994 Nursery	[a] 1-yr sprout on 1-yr rootstock from 2-yr-old ortet	87 A	55.7 a	87 A	165 a
	[b] lower limb on 3-yr-old ortet (same ortet as [a])	60 B	32.7 b	58 B	124 b
	[c] lower limb on 3-yr-old ortet (not same as [a])	68 a	41.0 A	67 a	147 A
	[d] upper limb on 3-yr-old ortet (same ortet as [c])	38 b	30.2 B	38 b	92 B
[4] 1995 Field	[a] TRT#1: 1-yr sprout on 2-yr rootstock#1 from 2-yr-old ortet	72 A	21.9 ab	--	--
	[b] TRT#2: 1-yr sprout on 1-yr rootstock#2 from 1-yr rootstk#1 from 2-yr-old ortet	89 A	23.5 a	--	--
	[c] TRT#3: 1-yr sprout on 1-yr rootstock#1 from 3-yr-old ortet	54 B	17.7 ab	--	--
	[d] TRT#4: upper limb on 4-yr-old ortet	14 C	16.0 b	--	--

[a] Means for the same trait (column) that are in the same study are not significantly different at the 0.05 probability level according to Duncan's Test if they are followed by the same letter.

Cuttings in the 1993 study from low limbs on two-year-old seedlings had higher propagation success than cuttings from low limbs on the same ortets at age three in the 1994 study ([2a] versus [3b] in Table 1). However, sprouts on the rooted cuttings of the 1993 study retained the higher rooting ability of the two-year-old ortets ([3a] versus [3b]). Furthermore, low limbs provided cuttings with

higher propagation success than upper limbs from the same trees ([3c] versus [3d]). These results probably arise from effects of maturation and distance of cutting origin from the root collar of the ortet, as discussed below.

There was a significant decline in propagation success of the limb cuttings in the 1994 study associated with the distance from the base of the cutting's position on the limb to the root collar of the ortet (Figure 1). Most cuttings

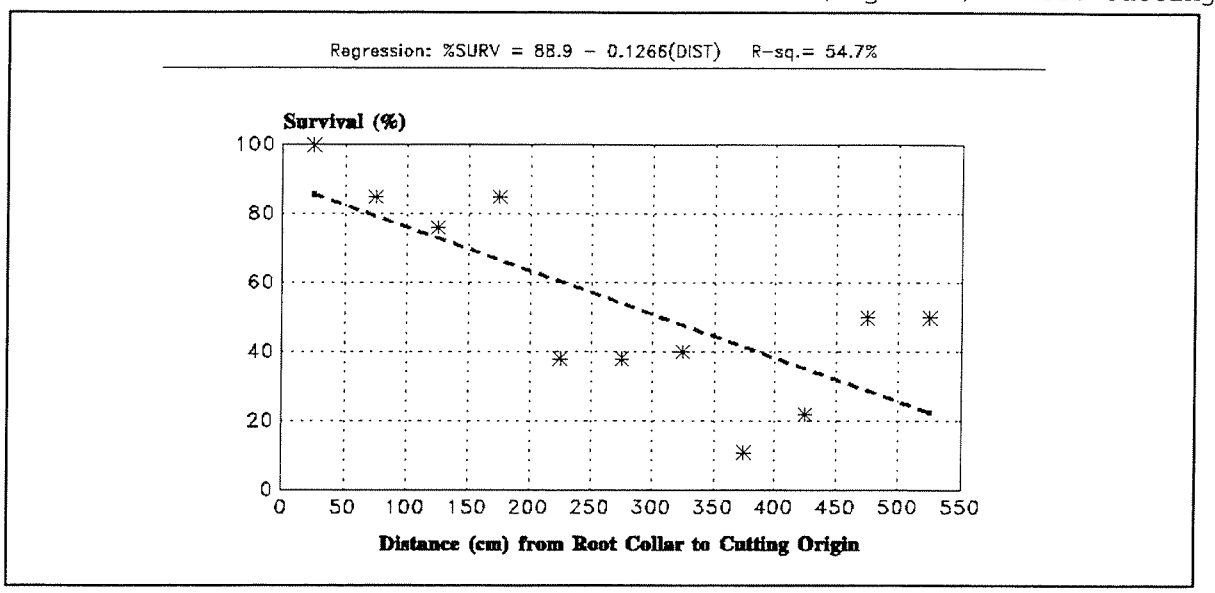

Figure 1. Three-month propagation success of cuttings in the 1994 Nursery Cutting Study from three-year-old trees as affected by distance of cutting origin from root collar.

from sprouts on the rootstocks in the 1993 study were close to the "root collar" and gave high survival with no effect of distance (Figure 2). The cuttings in the 1993 study also exhibited a decline in propagation success associated with increasing distance of source limb above root collar. Limbs originating within 40 cm of the root collar gave cuttings with 80-percent survival, while limbs from 41 to 80 cm above the root collar gave cuttings with only 55-percent success. Therefore, results for the 1994 study could be interpreted as follows. First, the better performance of treatment [3a] than [3b] in Table 1 for the same ortets arises because origins of [3a] were limbs closer to the root collar at age two than limbs of [3b] at age three. Lower limbs grew in diameter and length between ages two and three, and some died from shading. To get equivalent-sized cuttings from the two ages, collections had to be taken further from the root collar at age three, and survival declined. Serial propagation maintained the "closer-to-origin" effect of the collection at age two, thereby arresting the maturation effect associated with increased distance from root collar. Second, the better performance of [3c] than [3d] demonstrates the gradient in maturation within the plant. The lower limbs were more juvenile than the limbs from the upper crown, so that cuttings from the lower limbs were propagated with greater success.

The 1995 field trial was established to determine if cutting survival under non-irrigated field conditions would be consistent with nursery results, to examine how two cycles of serial propagation would affect cutting survival, and to evaluate performance of cuttings from the same clones when originally collected at ortet ages two, three, and four years. The three-month results in

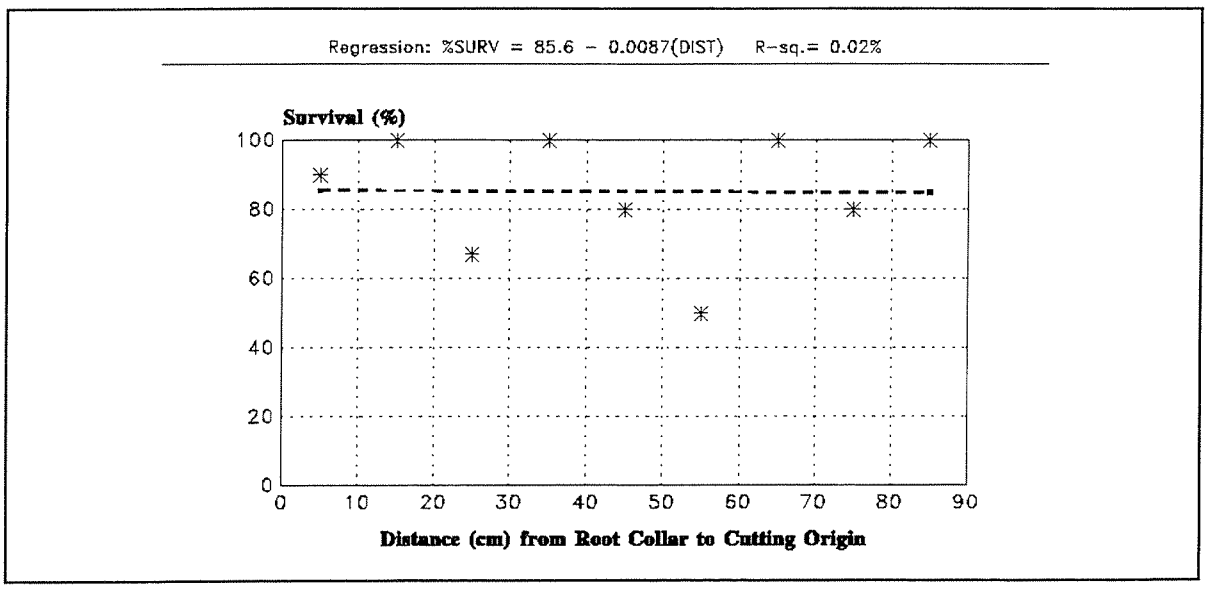

Figure 2. Three-month propagation success of cuttings from sprouts on one-year-old nursery rootstocks as affected by distance of cutting origin above the root collar.

Table 1 were reliable indicators of relative performance at the end of the growing season, as already noted for the first three studies, and survival results were consistent with nursery averages. Sprout heights were shorter than the nursery heights at three months, but this probably resulted from measurements being taken one to two weeks earlier than for the nursery studies. Cuttings from one-year-old sprouts on two-year-old rootstocks ([4a] in Table 1) did not differ significantly in propagation success from cuttings taken from one-year-old sprouts on the second serial cycle of rootstocks ([4b]), and survival was the same as obtained for one-year-old sprouts on the first serial cycle in the 1994 study ([3a]). Propagation success of cuttings from one-year-old sprouts on rootstocks taken from the three-year-old ortets [4c] was significantly poorer than for cuttings from rootstocks originating from the two-year-old ortets. This was clear evidence that serial propagation only arrested the maturation process, rather than reversing it. Thus, two serial cycles of propagation and up to two years of cutting harvest per rootstock cycle appear to maintain maturation at the stage of the original collection from the ortet. Cuttings from limbs of the four-year-old ortets [4d] had the poorest survival of all treatments. This probably resulted because lower limbs had died as crowns closed during the fourth year, so the only cutting sources were far from the root collar.

The overall effect of ortet maturation on propagation success of sycamore cuttings is summarized for all four studies in Figure 3. Sprouts maintained the maturity status of the limb source from which they were collected. Since most of the sprout-origin cuttings in the studies came from low limbs, sprouts and low limbs were combined in the figure. Also, propagation success was assumed to be 100 percent for a very young seedling (age zero). The figure illustrates (a) that rooting ability declined with increasing ortet age, (b) that the greatest decline occurred between ages two and four, when sources of cutting material (limbs) from near the root collar disappeared, and (c) that upper limbs were more mature than low limbs at the same ortet age, so that propagation success was less.

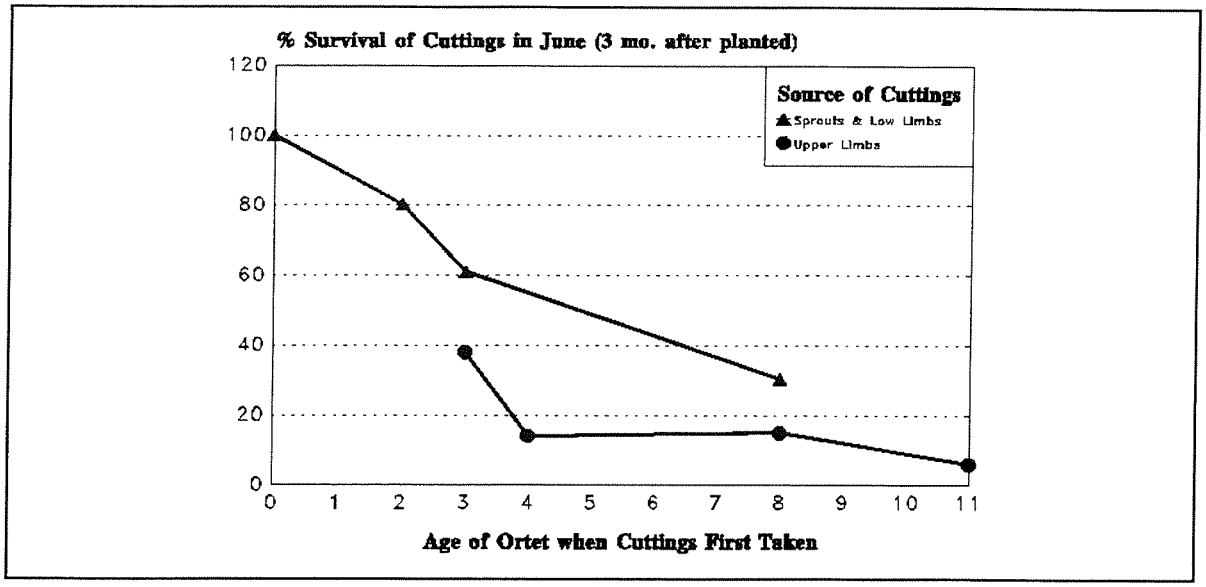

Figure 3. Effect of ortet age on propagation success of sycamore cuttings.

CONCLUSIONS

Serial propagation with cuttings arrests the maturation process of sycamore at the level of the original propagule taken from the ortet. The method does not rejuvenate the vegetative material. The maturation level varies within the ortet, being most juvenile for material (such as limbs) taken from near the root collar. As maturation increases, propagation success with conventional cuttings declines. Therefore, if it is undesirable to coppice selected individuals within progeny tests, serial propagation with cuttings from low limbs on young seedlings of no more than two years of age is required to maintain high rootability.

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GENERAL AND SPECIFIC COMBINING ABILITY FOR FUSIFORM RUST INFECTION IN SLASH PINE

T.D. Byram and W.J. Lowe[1]

Abstract.--General and specific combining ability for fusiform rust infection were calculated on a number of five and ten year old slash pine genetic tests. Average family heritability for rust infection at a single location was 0.54 at age five and 0.53 at age ten. General combining ability accounted for 81% of the genetic variation at both ages. Coefficients of genetic prediction between ages five and ten were 96% as large as age ten heritabilities indicating that selection at age five would be as efficient as delaying selection until age ten. Age five family heritabilities calculated for one set of parents classified as resistant at the USDA Forest Service Resistance Screening Center (RSC) averaged 0.33 across three tests with only 52% of the genetic variation attributable to general combining ability. This could be a sampling artifact caused by low infection levels as the susceptible checklots averaged only 31.7% infection. However, it is possible that screening at the RSC changed the population structure. If so, using variance components from control-pollinated genetic tests of unscreened parents to plan subsequent breeding strategies or to predict gains for parents screened at the RSC is inappropriate. Resolution of this question will require more field tests of parents classified as resistant to fusiform rust at the RSC.

Keywords: *Pinus elliottii* Engelm. var. *elliottii*, *Cronartium quercuum* (Berk.) miyabe ex Shirai f. sp. *fusiforme*, heritability, disease resistance

INTRODUCTION

Fusiform rust (*Cronartium quercuum* (Berk.) Miyabe ex Shirai f. sp. *fusiforme*) is the most devastating disease on slash pine (*Pinus elliottii* Engelm. var. *elliottii*) in the Western Gulf region of the United States. In Texas alone, 50% of young slash pine trees are infected (Lenhart et al. 1988) and it is likely that many of these individuals will eventually die (Nance et al. 1981) In fact, rust infection is so prevalent and mortality is such an important factor that the level of family infection at age five was determined to be the most reliable predictor of family volume per acre at age 15 (Lowe and van Buijtenen 1991). As a result, disease resistance has been identified as the most important trait for genetic improvement in the Western Gulf Forest Tree Improvement Program (WGFTIP) strategy for this species.

Approximately 1,000 first generation slash pine selections were initially identified by WGFTIP cooperators. Because slash pine is an exotic species in most of the Western Gulf region, the majority of these selections

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were made in plantations of unknown seed source. The selections were rust free, but no other attempt was made to ensure disease resistance. The initial breeding plan required the establishment of control-pollinated genetic tests in which evaluation would be based primarily on volume growth. A number of these tests were planted. However, as results from early open-pollinated genetic tests became available, the importance of improving disease resistance became obvious and a two-step testing strategy was developed. Parents would first be progeny tested at the USDA Forest Service Resistance Screening Center (Anderson and Powers 1985) and only successful candidates would be used in the subsequent long term breeding and testing program (Byram et al. 1991 p. 12). Control-pollinated field tests of progeny from parents classified resistant at the Resistance Screening Center (RSC) are currently being established. This has resulted in two populations of slash pine within the WGFTIP cooperative: 1) a population not screened at the RSC that received very little selection pressure for disease resistance and 2) a population which was screened at the RSC prior to the breeding and establishment of field trials.

Estimates of genetic parameters are important at all stages of a tree improvement program to aid in the development of breeding strategies and the prediction of genetic gains. Because variance components are population parameters, they can be safely applied only to the population from which they are derived. The objectives of this paper are to report heritabilities, the contribution of general combining ability to total genetic variation and the coefficients of genetic prediction between ages five and ten for fusiform rust infection levels in an unscreened population of slash pine. Data from one partial-diallel of parents classified resistant at the RSC are also reported for comparison.

MATERIALS AND METHODS

A summary of the data sets representing the first generation parents used in this study is given in Table 1.A. There were eleven genetic tests evaluated at age five, ten tests evaluated at age ten and nine tests for which comparisons were made between ages five and ten. These plantings represented 51 parents in six series with each series planted in one to three locations. The breeding scheme was a partial-diallel with each parent crossed with an average of 4.4 other parents (a range from a minimum of three crosses per parent to a maximum of ten). Field designs were completely randomized blocks with six to twelve blocks per location and six to ten trees from each cross planted in row plots in each block. Presence or absence of rust was evaluated at each measurement, and the percentage of trees infected in each plot was calculated for analysis. Trees killed by fusiform rust were scored as infected.

For a test to be included in this study, the average fusiform rust infection level of either the plantation or the two unimproved checklots had to be greater than 30%. Plantation averages exceeded 30% in all cases except for tests 387 and 424. In test 387, it was necessary to rely on the performance of the unimproved checklots as an indicator of exposure to fusiform rust at both measurement ages. The age five data for test 424 was deleted from the study because of insufficient rust infection. Analysis of variance was performed on untransformed percentages using GLM (SAS Inst. 1989); only tests with significant differences among crosses at the 0.10 level were analyzed further.

Percentage data were transformed using the inverse sine transformation (Steel and Torrie 1960 p. 158) before any additional analyses were conducted. General combining ability (GCA) and specific combining ability (SCA) were calculated on a single location basis using the computer program DIALL (Schaffer and Usanis 1969). Negative values and terms that were not significant at the 0.25 level on the basis of an F test were set to zero. DIALL was also used to calculate cross-products for the age-age comparisons.

Table 1. The number of parents, crosses and blocks in each field trial used in this study. Plantation rust infection levels are shown for ages five and ten.

A) Parents not screened at the RSC.

Test	Number of Parents	Number of Crosses	Number of Blocks	Average Rust Infection(%) Age 5	Average Rust Infection(%) Age 10
Series A					
351	11	20	10	43.1	44.0
352	11	20	10	31.1	38.0
387	11	23	12	26.7[1]	24.2[1]
Series B					
423	8	23	6	40.7	42.7
424	8	25	6	--[2]	38.0
Series C					
437	6	12	12	49.4	48.2
477	6	12	12	49.9	41.3
Series D					
438	8	19	12	75.3	74.2
439	8	19	12	51.4	55.4
464	8	19	12	62.9	69.2
Unrelated Tests					
259	11	29	12	45.5	--[3]
561	7	13	12	40.7	--[3]

B) Parents screened at the RSC.[3]

	Number of Parents	Number of Crosses	Number of Blocks	Average Age 5 Rust Infection(%) Test	Average Age 5 Rust Infection(%) Checklot
522	9	22	12	18.7	21.9
523	9	19	12	33.7	28.7
541	9	19	12	27.7	44.4

[1] The average infection of the susceptible checklots were 36.2% at age five and 32.1 % at age ten.
[2] Insufficient rust at age five (<30%).
[3] Age ten evaluations not yet available.

Negative cross-products were accepted. Family heritabilities were calculated according to van Buijtenen (1976) using the following formulae:

$$h^2_{fam} = \frac{\sigma^2_{GCA}}{\sigma^2_{GCA} + \sigma^2_{SCA} + \sigma^2_E / r}$$

where σ^2_{GCA} is the variance for general combining ability, σ^2_{SCA} is the variance for specific combining ability, σ^2_E is the error variance and r is the harmonic mean for the number of plots/family.

Coefficients of genetic predictions (CGP) were computed for infection levels between ages five and ten within individual locations (Baradat 1976). CGPs are generalized heritabilities indicating the fraction of the breeding value for one trait that can be manipulated by phenotypic selection on a second trait. The ratio of the CGP to the heritability can be used as an

indicator of early selection efficiency. CGPs are calculated as:

$$CGP = \frac{Cov(A_1, A_2)}{\sigma_{p1} * \sigma_{p2}}$$

where $Cov(A_1,A_2)$ is the additive covariance between traits A_1 and A_2, and σ_{p1} and σ_{p2} are the phenotypic standard deviations for each trait.

Three field tests of a group of parents classified as resistant at the RSC are included for comparison (Table 1.B). Overall rust infection levels were lower in this series. Test 522, with an average rust infection of 18.7% and a checklot infection rate of 21.9%, did not meet the criteria outlined above to ensure an adequate challenge by rust. It is reported here because of the limited amount of field data available for parents screened at the RSC; however, the results should be viewed with caution.

RESULTS AND DISCUSSION

Family heritabilities, their standard errors and the fraction of genetic variation attributed to GCA are summarized in Table 2. For the unscreened population, single location estimates of family heritability averaged 0.54 at age five with a range from 0.31 to 0.73. At age ten, family heritabilities averaged 0.53 with a range from 0.16 to 0.79. These values were extremely similar despite the fact that the age ten data included one test with insufficient rust infection to be included in the age five data (test 424) and lacked two other locations that have not yet reached age ten (tests 259 and 561). These figures compare favorably to the individual heritabilities calculated by Dieters as reported by Hodge et al. (1995). As one would expect, estimates of individual tree heritabilities were lower; however, estimates based on a large number of progeny tests did not vary greatly by test age. Evaluations at ages five, eight and eleven were 0.156, 0.148 and 0.146 respectively. Single location heritabilities have an upward bias because the genotype by environment interaction is ignored and the true heritabilities are expected to be lower.

Series D tests appeared to have substantially higher average rust infection levels (Table 1), lower heritability estimates and lower percentages of GCA than the tests in the other series (Table 2). This could be the result of environmental sampling. If the tests were planted in high rust infection years and locations, any inherit resistance may have been overwhelmed and genetic variation masked. However, it is likely that these parents are susceptible and have less GCA for disease resistance. Tests of open-pollinated seed from these parents at the RSC resulted in the elimination of five of the eight parents from any further use in the breeding population. Furthermore, test 437 from series C was planted adjacent to test 438 from series D in the same year and had a much lower average rust infection in the same environment (50% vs 75%). This was despite the fact that the checklot common to both tests had a similar infection level (60% vs. 66%).

A matter of practical importance for tree breeders is the fraction of the total genetic variation attributable to GCA. For the unscreened population, GCA was 81% of the total genetic variation at both ages (Table 2.A). Correlation coefficients among parental GCA estimates were compared across locations in all possible pairwise combinations (Table 3). These correlation coefficients were always large and positive. Relatively large family heritabilities, a high fraction of GCA to total genetic variation and moderate to high correlations between parental values all support the current breeding strategy. This strategy emphasizes the selection of parents with good general combining ability and assumes that there will be little important genotype by environment interaction.

Table 2. Family heritabilities, standard errors, GCA/GCA+SCA at ages five and ten for rust infection levels. Coefficients of genetic prediction between ages five and ten.

A) Parents not screened at the RSC.

	Age 5			Age 10			
Test	h^2	S.E.	GCA (GCA+SCA)	h^2	S.E	GCA (GCA+SCA)	CGP (Age 5/10)
Series A							
351	0.63	0.30	1.00	0.64	0.31	1.00	0.63
352	0.57	0.29	1.00	0.54	0.26	1.00	0.48
387	0.31	0.17	1.00	0.16	0.24	1.00	0.30
Series B							
423	0.53	0.30	1.00	0.58	0.32	0.86	0.56
424[1]	----	----	----	0.44	0.26	0.70	----
Series C							
437	0.73	0.44	0.84	0.79	0.46	0.91	0.76
477	0.53	0.38	1.00	0.73	0.44	1.00	0.49
Series D							
438	0.41	0.27	0.47	0.37	0.26	0.41	0.38
439	0.41	0.27	0.46	0.48	0.29	0.54	0.44
464	0.46	0.29	0.52	0.58	0.32	0.65	0.52
Unrelated Tests[2]							
259	0.67	0.30	0.74	----	----	----	----
561	0.64	0.38	0.86	----	----	----	----
Ave:	0.54		0.81	0.53		0.81	0.51

B) Parents screened at the RSC.[2]

522	0.47	0.27	0.80[3]
523	0.53	0.31	0.76
541	0.00	0.16	0.00[4]
Ave:	0.33		0.52

[1] Test 424 was not used at age five because the average infection levels for the plantation and susceptible checklots were only 23.2% and 15.3%, respectively.
[2] Tests 259, 561, 522, 523 and 541 have not yet reached age ten.
[3] Insufficient rust infection to ensure that the entries were adequately challenged (test average = 18.7%, susceptible checklots = 21.9%).
[4] General combining ability was not significant at the 0.25 level although families were significantly different at the 0.10 level on GLM.

Coefficients of genetic prediction (CGP) between ages five and ten were very similar in magnitude to the age ten heritabilities (Table 2.A). The nine data sets for which comparisons could be made had an average CGP of 0.51 with a range from 0.30 to 0.76. Test 387 had a larger CGP between ages five and ten than the heritability at age 10. This may be the result of the low precision of the age ten heritability estimate which had a standard error larger than the estimate. Selection on age five data would be expected to be more productive than direct selection at age ten in this test. The average CGP was 96% as large as the age ten heritability. Therefore, selection at age five can be expected to be as efficient as direct selection at age ten when infection levels are above 30% and statistically significant.

Table 3. Pearson correlation coefficients between parental GCA estimates for all possible pair-wise comparisons of tests.

Tests	Correlation Coefficients	Probability>|R|
Age 5		
351-352	0.79	0.004
351-387	0.57	0.069
352-387	0.61	0.047
437-477	0.83	0.039
438-439	0.76	0.029
438-464	0.96	0.001
439-464	0.85	0.008
Age 10		
351-352	0.72	0.012
351-387	0.66	0.026
352-387	0.56	0.076
423-424	0.85	0.007
437-477	0.93	0.007
438-439	0.85	0.007
438-464	0.96	0.001
439-464	0.87	0.004

The five-year-old results from a series of nine parents classified as resistant at the RSC are reported in Table 2.B. One location had less rust infection than desirable for the evaluation of resistance. In test 522, the average family infection levels ranged from 7 to 32% with a plantation mean of 18.7%. In this series of tests, heritability averaged 0.33 with a range from 0.0 to 0.53. Test 541 had no significant GCA effects. A number of explanations are possible for the apparent differences between the genetic parameters for the screened and unscreened populations. This data represents only one set of parents and may be biased due to sampling. Rust infection levels are low and may be inadequate to allow reliable expression of genetic effects. Another possibility is that screening at the RSC altered the population structure; therefore variance components will be different in this new population. Investigation of these hypothesis will require additional field data from genetic tests of parents screened at the RSC.

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

Steve McKeand and Floyd Bridgwater[1]

Abstract. In the North Carolina State University - Industry Cooperative Tree Improvement Program with loblolly pine (*Pinus taeda* L.), data from 21 test series (two 6-tree diallels planted in 4 test locations per series) at age 6 years from throughout the Southeast were analyzed for height, straightness, and fusiform rust incidence. Individual-tree and family-mean heritabilities, genetic correlations, and genetic gains were estimated for all three traits. Individual-tree heritabilities for height were $h^2_{NS}=0.22$ and $h^2_{BS}=0.30$. Half-sib family mean heritabilities (narrow-sense) were 0.72, 0.78, and 0.73 for height, rust infection, and straightness score, respectively. On average, genetic correlations were essentially zero among the traits, but ranged between -1 and 1 for different diallels.

Gains from family selection were compared for height, straightness, and fusiform rust incidence. For selection intensities of $i=1.00$, percent gain estimates were 2.5, 4.4, 7.7, respectively. The economic impact from gains of this magnitude are discussed for each trait.

Keywords: Breeding, genetic correlations, heritability, *Pinus taeda* L., selection

Cooperators in the NCSU Tree Improvement Program have been actively breeding and testing over 3000 plantation selections and over 700 second generation selections since the early 1980's. The breeding is complete and by 1996 all progeny tests will be planted. Selections were bred in 6-parent disconnected half-diallels and the resulting progeny were planted in balanced test series (4 tests per series) each comprised of progeny from two or on occasion three diallels. Each full-sib family is planted in a 6-tree plot and is replicated 6 times in each of the 4 tests (see Talbert et al. 1981 for details). As of March 1995, measurement and analyses have been completed in 21 test series at age 6 years, with 44 separate diallels represented (Table 1). As the analyses of the diallel tests have proceeded and we gained some experience with the selection procedure some interesting and useful trends and relationships have developed. Highlights of analyses and interpretations are summarized.

Relationships Between Genetic Parameters and Test Characteristics

Several informative relationships among progeny test characteristics and estimates of genetic parameters have been identified. The estimates of individual-tree heritabilities (both narrow-sense and broad-sense) appear to be in line with other estimates for height (Cornelius 1994). The average narrow-sense heritability (individual basis) was .22, while the comparable broad-sense heritability average was .30. The average narrow-sense family mean heritability estimates for height, fusiform rust infection, and straightness score were 0.72, 0.73 and 0.78 respectively (Table 1).

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Table 1. Average height, % fusiform rust infection, and % survival and heritability estimates for different traits in the 8 Test Areas within the Cooperative.

Test Area[1]	# Test Series	# Diallels	Averages			Individual Tree Heritabilities for Height		Family Mean Heritabilities		
			Height	Rust	Survival	h^2_{NS}	h^2_{BS}	Ht.	Rust	Strt
1	3	6	17.9′	1.7%	95%	.31	.38	.85	.	.84
2	0	0								
3	4	9	20.8	12.7	82	.08	.12	.46	.72	.58
4	4	8	19.6	31.9	88	.22	.34	.72	.81	.89
5	6	13	21.3	23.3	93	.31	.39	.85	.87	.76
6	2	4	17.4	3.0	87	.12	.25	.57	.	.49
7	2	4	17.5	44.2	81	.18	.24	.84	.85	.76
8	0	0								
Total	21	44	19.7	19.8	89	.22	.30	.72	.73	.78

[1] Test Areas are: 1 = VA, 2 = Coastal NC, 3 = Coastal SC, 4 = Coastal GA & FL, 5 = Lower Gulf, 6 = Upper Gulf, 7 = Piedmont GA & SC, 8 = Piedmont NC.

The estimates for narrow sense heritability (individual basis) ranged between 0 and 0.93. Reasons for this large range of estimates include: 1) Genetic sampling, there were real differences in the genetic variance included in the 44 samples of 6 parents comprising the diallel mating groups. 2) The variation in heritability estimates were at least partially explained by variation in test precision (as measured by the coefficient of variation (CV) based on rep x family effects) and by average test survival. If test precision was relatively low (CV > 8%) then the likelihood of having a high heritability was relatively low (Figure 1). Likewise if survival was below 85%, there were very few estimates of heritability greater than 0.20 (Figure 2).

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

There was no relationship between average height and any estimate of heritability (Figure 3). This is an important "non-relationship". From this, we conclude that we are just as likely to see strong genetic effects on low site index sites as on high site index sites. The average height for a test series varied by almost 25% (from 15.6 feet to 19.2 feet) at age 6

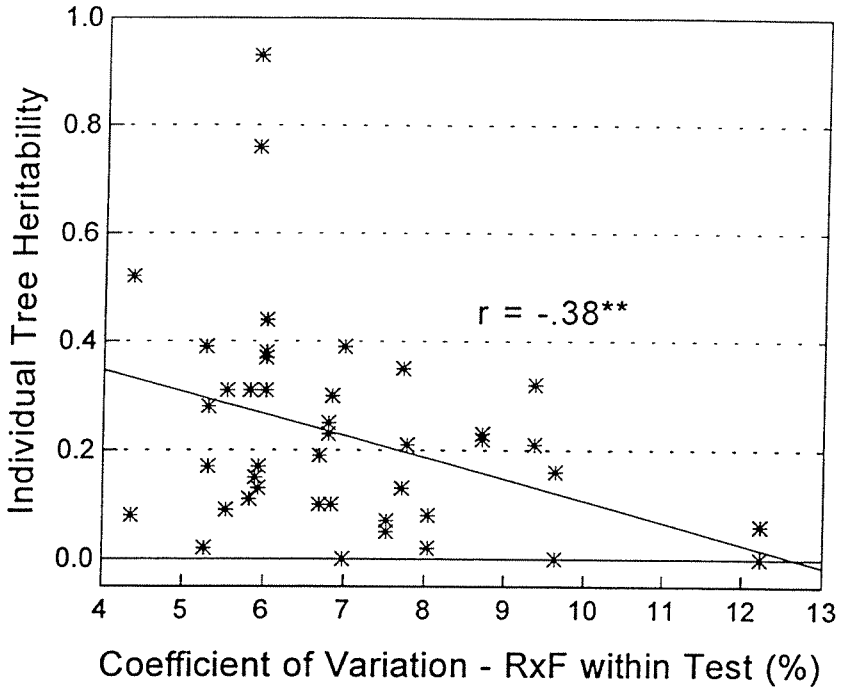

Figure 1. Relationship between estimates of individual-tree narrow-sense heritabilities for height at age 6 years and estimates of pooled coefficients of variation (rep x family mean basis) for height.

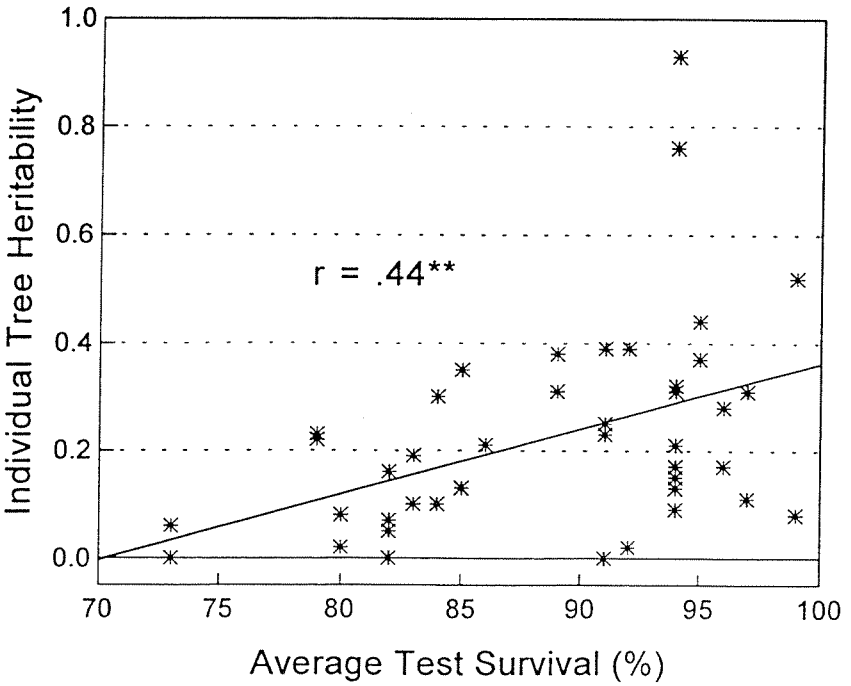

Figure 2. Relationship between estimates of individual-tree narrow-sense heritabilities for height and average test survival at 6 years.

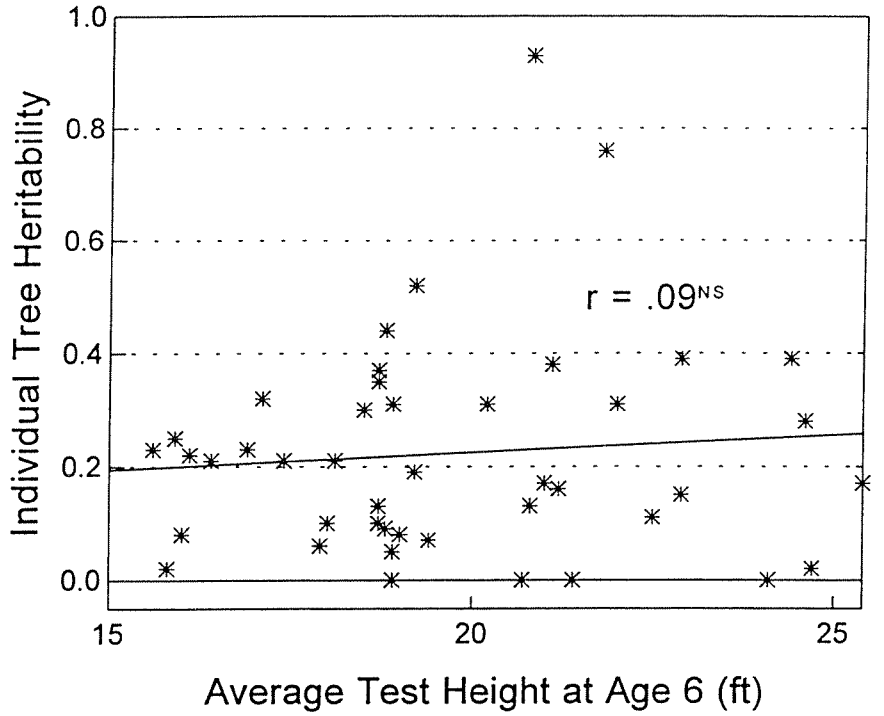

Figure 3. Relationship between estimates of individual-tree narrow-sense heritabilities for height and average test height at age 6 years.

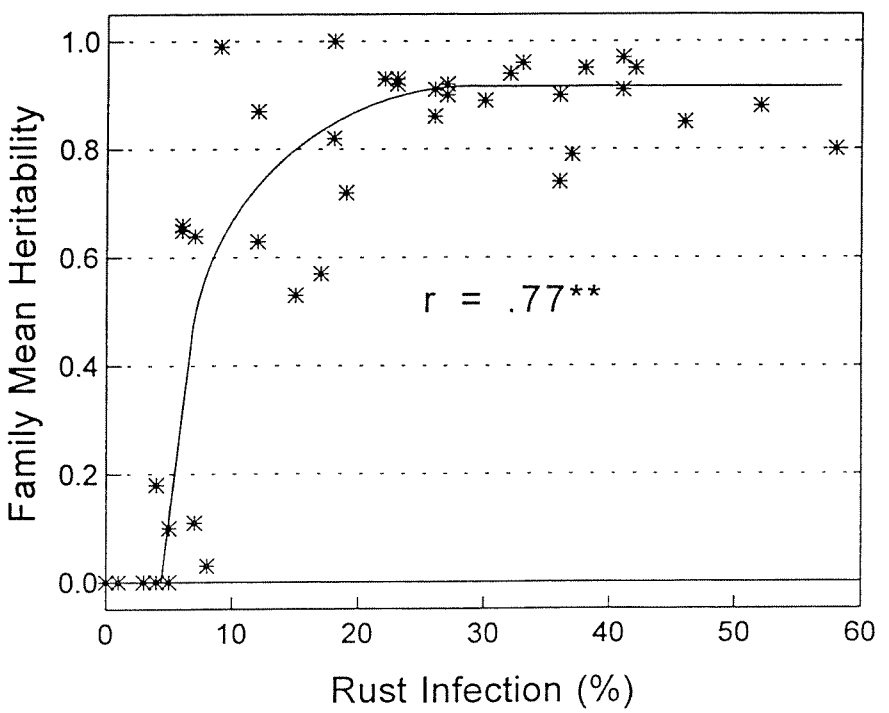

Figure 4. Relationship between estimates of family-mean narrow-sense heritabilities for percent fusiform rust infection and average rust infection level at the test at 6 years.

years. When establishing progeny tests, the use of agricultural fields was encouraged, not because of the potential for fast growth, but because in general the site uniformity on agricultural fields is much better than what is routinely encountered on cut over sites.

The most useful relationship between heritability and site factors was for fusiform rust infection (Figure 4). When rust levels were very low (< 5%) there was very little chance of detecting significant family differences (h^2 based on family means usually near 0). With rust levels of 5% - 20%, the estimates of h^2_F were much more variable than at levels of rust above 20%. The consistency of heritability estimates when rust was above 20% is cause for confidence in these estimates. For routine analyses of diallel tests and OP tests in the Cooperative, rust performance is estimated only when average fusiform rust infection equals or exceeds 20%.

Genetic Correlations Among Traits

In a multi-trait selection index, the relationships or associations among the traits included in the index have a major impact on the gains for any individual trait of interest. For example if there is a strong, unfavorable relationship between height and straightness, selecting for height would degrade stem quality. Across the different diallels, the genetic correlations varied widely:

Genetic Correlation for:	Low	High	Average
Height - Rust Infection	-0.93	0.96	-0.20
Height - Straightness	-1.08	0.98	0.05
Rust - Straightness	-0.93	0.93	0.08

When there is a favorable correlation between height and rust infection or between height and straightness (i.e., negative correlation is favorable since high values for rust and straightness score are bad), selecting trees has been relatively easy. When there is a strong unfavorable genetic correlation, it has been difficult to find tall, straight, rust-free trees. Fortunately the average genetic correlations were either slightly favorable (i.e., r = -0.20 for height and rust infection) or were essentially zero for height and straightness. Again, with the small genetic samples included in each diallel, the correlation can vary widely, but the expectation for the average of all correlations is about zero.

Genotype by Environment Interactions

In past studies, we have seen very little evidence for important genotype by environment interaction (GxE) at the half-sib family level. For example, in the Good General Combiner trial, half-sib families were remarkably stable across a wide range of sites that encompass large differences in site productivity (Li and McKeand 1989, McKeand et al. 1990). In the diallel tests, we have the opportunity to assess GxE for both half-sib and full-sib families. Full-sib families might be expected to display a higher degree of interaction with the environment than half-sib families since there is less genetic variance within each family (i.e.,

a lower level of buffering to environmental variations) and more of the genetic variance exists among families. Additionally, since GxE at the full-sib family level is caused by both additive and non-additive effects.

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

For half-sib families: $K = \frac{1}{4}\sigma^2_{AxE} / \frac{1}{4}\sigma^2_{A}$

For full-sib families: $K = (\frac{1}{2}\sigma^2_{AxE} + \frac{1}{4}\sigma^2_{NAxE}) / (\frac{1}{2}\sigma^2_{A} + \frac{1}{4}\sigma^2_{NA})$

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

The average K-statistic for half-sibs was 0.31 meaning that the GxE variance was only about one-third the genetic variance. As we have found in other trials, the family by environment or specifically the additive genetic by environment variance is of little practical concern. On the other hand, the GxE for full-sibs is higher (K = 0.54), apparently this is due to the contribution of non-additive effects to the GxE. Never the less, the magnitude of GxE variance for full-sibs is still only about half the magnitude of the genetic variance, and it appears to be of small practical importance. More analyses will be conducted to determine if any significant rank changes occur for full-sib families.

Gain Estimates

Genetic gains from family selection for height, % fusiform rust, and straightness score were estimated using the average family mean heritabilities (h^2_F) and average phenotypic standard deviations of family means (σ_F) for each trait:

$$\text{Gain} = i\, h^2_F\, \sigma_F$$

For a selection intensity of i = 1.0, the expected means of progeny from selected parents (e.g. deployment of the best open-pollinated families from a seed orchard of these clones) would be:

Trait	Current Family Mean	Mean After Selection
Height	19.73′	20.17′
% Rust	34.8%	24.6%
Straightness[1]	51.0%	57.5%

[1] Straightness is percentage of trees above average for straightness.

Larger gains are possible with higher selection intensities, but these gains illustrate the relative gains possible in the three traits used in selection. These gains do not reflect the gain over

unselected populations or over the previous generation. When more tests are available from more Test Areas, and family means can be compared to the same check seedlots, we will estimate gains over unselected populations and the previous generation.

In summary, the analyses of 44 diallels in 21 test series and the initial third-cycle selection work has led to several conclusions:

1. Planting tests on uniform sites is essential if reasonable levels of heritability and genetic gains are to be achieved.

2. Test survival below 85% usually results in low heritability estimates.

3. Genetic differences in height are not related to site index, but genetic differences in rust resistance can only be detected if rust infection exceeds 20%.

4. Genetic correlations among traits vary because of the sampling effect with small diallels, however, on average the correlations are near zero, which indicates that the traits are inherited independently.

5. Genotype by environment interaction effects are relatively minor.

As more diallel data become available, these estimates of variance components and gains will be updated on a regular basis. Ultimately an excellent data base for each of the Test Areas in the Cooperative will be available for us to fine-tune testing and selection procedures.

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HERITABILITY ESTIMATES FOR LOBLOLLY PINE WOOD SPECIFIC GRAVITY BASED ON CONTROL-POLLINATED GENETIC TESTS

W. J. Lowe and T. D. Byram[1]

Abstract. Specific gravity is an important wood quality trait that affects both the quality and quantity of pulp and solid wood properties. To determine the heritability and age-age relationships for specific gravity, increment cores were collected at DBH (1.4 m) from four control-pollinated loblolly pine genetic tests that were at least 20 years old. These tests contained 93 families representing 23 different parents. Unextracted specific gravity was determined on core segments that included the first five years from the pith, rings six to 20 years and the total core. The estimates of general combining ability were significant for all age segments in each test; however, none of the estimates for specific combining ability were significant. Family heritability estimates for the first five years from the pith averaged 0.58 and ranged from 0.38 to 0.75 among the four genetic tests. For the six to 20-year core segments, family heritability estimates averaged 0.73 and ranged from 0.62 to 0.79. The coefficient of genetic prediction between the two age segments averaged 0.50. Selection at age five for specific gravity would be approximately 68 percent as efficient as direct selection to increase age six to 20-year specific gravity.

Keywords: *Pinus taeda* L., density, coefficient of genetic prediction.

INTRODUCTION

Specific gravity is an important wood quality trait that affects the forest products industry. Increasing specific gravity can impact both the quantity and/or quality of pulp and solid wood products. Both selection (van Buijtenen 1962, McKinley et al. 1982, Talbert et al. 1982, Williams and Megraw 1994) and silvicultural treatments (Megraw 1985, Zobel and van Buijtenen 1989) can influence wood specific gravity.

Wood specific gravity for loblolly pine (*Pinus taeda* L.) has been reported to be under moderate to high genetic control (review Zobel and van Buijtenen, 1989, pp. 259-261). However, most of these studies were based on open-pollinated genetic tests of relatively young ages (ten years or less). Talbert et al. (1982) reported ten-year results from a control-pollinated loblolly pine genetic test. They concluded that juvenile wood specific gravity was under strong additive genetic control ($h^2 = 0.84$) and that a genotype by environment interaction was of little importance. The lack of any meaningful genotype by environment interaction for loblolly pine wood specific gravity was confirmed by Byram and Lowe (1988) and Jett et al. (1991).

Wood specific gravity has not been commonly used as a selection criteria in developing breeding populations for loblolly pine in the southeastern United States. The lack of juvenile-mature relationships and different economic importance for various products have hindered the incorporation of

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wood specific gravity into selection criteria. Recently, increased consideration is being given to the development of breeding populations that include specific gravity as a selection criteria. Reliable juvenile mature relationships are needed to incorporate wood specific gravity into a breeding strategy. Williams and Megraw (1994) reported moderate to high positive age-age relationships for loblolly pine using several techniques.

The objectives of this study were to determine the heritability pattern and age-age relationship of wood specific gravity for the first 20 years in control-pollinated genetic tests of loblolly pine and evaluate the efficiency of early selection.

MATERIAL AND METHODS

Samples were collected from four control-pollinated loblolly pine genetic tests located in Ashley County, Arkansas. The genetic tests ranged from 25 to 33 years in age. All of the tests were established using a randomized complete-block design with families planted in block plots. The number of replications varied from three to six and the number of seedlings planted for each control-pollinated family per replication ranged from 9 to 64 among the tests. All of the tests had been thinned at least once.

Unextracted wood specific gravity was determined on 11 mm diameter bark to bark increment cores that were collected at DBH (1.4 m). Knots, resin pockets or other visible abnormalities were avoided. The cores were divided into two age segments: 1) the first five years from the pith, and 2) 6 to 20 years. The maximum moisture content procedure as described by Smith (1954) was used to determine specific gravity for each age class. Specific gravity was also determined for the total core. Approximately 20 cores were collected from each control-pollinated family in each test. A total of 93 families representing 23 different parents were sampled. The number of crosses ranged from three to twelve per parent utilizing a partial-diallel mating scheme in each genetic test.

Variance components, including general combining ability (GCA) and specific combining ability (SCA) were estimated using DIALL (Schaffer and Usanis 1969). Heritability and the coefficient of genetic prediction were determined on a family basis. Family heritability was calculated as (van Buijtenen 1976):

$$h^2_{fam} = \frac{\sigma^2_{GCA}}{\sigma^2_{GCA} + \sigma^2_{SCA} + \sigma^2_{\epsilon}/r}$$

Where σ^2_{GCA} is the general combining ability variance, σ^2_{SCA} is the specific combining ability variance, σ^2_{ϵ} is the error variance, and r is the harmonic mean of the number of replications. Nonsignificant ($p \leq .25$) and negative estimates were set to zero.

Baradat (1976) defined the coefficient of genetic prediction (CGP) between two traits as follows:

$$CGP = \frac{Cov(xy)}{P(x)\,P(y)}$$

Where Cov(xy) is the additive genetic covariance between x and y; P(x) and P(y) are the phenotypic standard deviation of x and y, respectively.

Pearson correlation coefficients were calculated among GCA estimates for

parents that occurred in more than one test (SAS Institute 1989).

RESULTS AND DISCUSSION

The DIALL analysis for each test indicated significant effects for GCA ($p \geq .05$) for all of the age classes but no significant SCA for any of the classes (Table 1). The lack of significance of SCA for the first five year core segments supported the conclusion of Talbert et al. (1982) that non-additive variation was unimportant in the inheritance of juvenile wood specific gravity for loblolly pine. The same pattern of genetic variation (no significant SCA) was evident in this study for both the whole core (20 years) and older age segment (6-20 years). This study supports the conclusion that wood specific gravity is mainly influenced by additive genetic effects.

Table 1. Variance components[1] for wood specific gravity by genetic test and age class.

Variance Component[2]	Test Number 102	103	123	258
A. First Five-Years.				
Replications	0.008	0.071	0.004	0.023
GCA	0.235	0.038	0.100	0.103
SCA	0.005	0.000	0.000	0.000
Error	0.222	0.360	0.398	0.250
h^2_{fam} (S. E.)	0.75(.34)	0.38(.27)	0.50(.32)	0.67(.36)
B. Age 6 to 20 Years.				
Replications	0.126	0.006	0.018	0.035
GCA	0.156	0.126	0.212	0.120
SCA	0.008	0.000	0.008	0.000
Error	0.135	0.198	0.478	0.179
h^2_{fam} (S. E.)	0.75(.34)	0.79(.40)	0.62(.37)	0.77(.40)
C. Total Core.				
Replications	0.047	0.047	0.002	0.016
GCA	0.151	0.086	0.149	0.086
SCA	0.000	0.000	0.003	0.000
Error	0.108	0.212	0.287	0.162
h^2_{fam} (S. E.)	0.81(.36)	0.71(.37)	0.66(.38)	0.73(.37)

[1]All variance components were multiplied by 1000.

[2]All estimates of GCA were significant at the 5 percent level of confidence. None of the SCA estimates were significant at the 25 percent level of confidence.

Family heritability estimates for the first five year core segments averaged 0.58 and ranged from 0.38 to 0.75 among the four tests (Table 1). These estimates were smaller than the estimate of 0.84 reported by Talbert et al (1982) for juvenile wood. Because unextracted specific gravity was determined on older trees in this study, differential resin deposits may have contributed to reducing the heritability estimates. Heritability estimates ranging from 0.55 to 0.76 for extracted specific gravity at age three were reported by Williams and Megraw (1994). Again, their estimates tend to be slightly larger than those obtained in this study; however, their estimates were based on extracted samples.

Average heritability estimates for the 6 to 20 year segment and the total core increased to 0.73 (range 0.62 to 0.79) and 0.73 (range 0.66 to 0.81) among the four tests, respectively. These heritability estimates are biased upwards because they are based on single genetic tests, and the genotype by environment interaction cannot be determined. This bias should not be a major factor because genotype by environment interactions are reported to be negligible for wood specific gravity in loblolly pine (Byram and Lowe 1988, Jett et al. 1991, and Williams and Megraw 1994). These heritabilities indicate that loblolly pine wood specific gravity is under strong genetic control and can easily be manipulated in a breeding program.

The CGP values between the first five year and the 6 to 20 year core segments ranged from 0.44 to 0.52 among the four tests and average 0.50 (Table 2). These estimates indicated that approximately 68 percent of the gain made by direct selection for wood specific gravity on the 6 to 20 year core segment could be made by selecting for specific gravity in the five year core. The effect of a common environment could have biased these estimates. Average values for the CGP's increased to 0.60 and 0.70 when the specific gravity for the first five year core segment and the 6 to 20 year core segment were compared to the total core specific gravity. Repeated measurements on a sample collected from a single tree contain biases because of a common environment and an autocorrelation effect. The GCP values for both age segments with the total core specific gravity are inflated because of these biases.

Table 2. Coefficients of genetic prediction (CGP) for wood specific gravity among age classes by genetic test.

	Test Number			
Traits	102	103	123	258
First 5 Years and 6 to 20 Years	0.51	0.51	0.52	0.44
First 5 Years and Total Core	0.69	0.51	0.57	0.61
6 to 20 Years and Total Core	0.72	0.74	0.64	0.69

Although represented by different crosses, seven parents were in common between tests 102 and 103. Correlation coefficients among specific gravity GCA estimates for parents in both tests were significant for all age classes (Table 3). This is important because the GCA estimates for wood specific gravity for the common parents were based on a different set of crosses in each genetic test. Furthermore, These correlations are free from both the biases of autocorrelation and a common environment because the cores were collected from different genetic tests.

Table 3. Correlation coefficients among wood specific gravity GCA estimates for seven common parents among age classes in genetic tests 102 and 103.

	Test 102		
Test 103	First Five Years	Years 6 to 20	Total Core
First five years	0.82*	0.93**	0.96**
Years 6 to 20	0.76*	0.94**	0.94**
Total Core	0.82*	0.93**	0.96**

*Significant at the five percent level of confidence.
**Significant at the one percent level of confidence.

The large positive correlations among parents across genetic tests indicate that the bias due to a common environment should not be a major factor inflating the CGP values. Both the CGP values and correlations indicate that selection based on juvenile wood specific gravity would be effective in changing mature wood specific gravity in loblolly pine. The results of this study support the conclusions of Williams and Megraw (1994) describing the efficiency of early selection for wood specific gravity. The large positive correlations among GCA estimates for parents in different genetic tests support the reported lack of any meaningful genotype by environment interaction (Byram and Lowe 1988, Jett et al. 1991).

SUMMARY

Four control-pollinated loblolly pine genetic tests greater than 20 years in age were sampled to determine the inheritance pattern of wood specific gravity and explore age-age correlations. Eleven mm diameter increment cores were collected bark to bark at DBH (1.4 m) on the sample trees. Unextracted wood specific gravity was determined on the first five rings from the pith, rings 6 to 20, and the total core. The results of the study were as follows:

1. Loblolly pine wood specific gravity was mainly controlled by additive genetic effects. Average family heritability across the four genetic tests was 0.58 for the first five year core segments and 0.73 for both age segments 6 to 20 and the total core specific gravity.

2. Specific combining ability was not important in the inheritance of wood specific gravity for any age class.

3. Early selection would be an efficient procedure to improve mature wood specific gravity in loblolly pine. According to this study, selection on juvenile specific gravity (five years from the pith) would be 68 percent as efficient as direct selection in improving specific gravity at age 6 to 20 years.

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AGROBACTERIUM TUMEFACIENS-MEDIATED TRANSFORMATION OF POPULUS DELTOIDES LEAF SECTIONS

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Abstract. Several factors, including Agrobacterium tumefaciens (At) exposure times and concentrations, were varied in efforts to increase transformation efficiency. Leaf sections of Populus deltoides clone C175, collected from shoot cultures, were inoculated with At strain LBA4404 carrying binary vector pBI121. Included in the vector were the selectable marker gene (NPTII) for kanamycin (K) resistance and the reporter gene (uidA) for beta-glucuronidase production. Transformants were identified by selection on medium containing 50 mgK/L and confirmed by histochemical staining for uidA expression. Exposure to At for 120 min proved more effective than shorter times, and elevated concentrations gave more transformants than lower ones. Long exposure times and high concentrations, however, tended to reduce shoot formation. Selection of putative transformants with 50 mgK/L proved workable, but this level clearly inhibited regeneration. The selection process was therefore modified to include culture on nonselective medium for 14 days before transfer to selective medium. This gave higher transformation frequencies than otherwise obtained, apparently a result of transformed calli enlarging and organizing sufficiently to develop on selective medium.

Keywords: Cottonwood, Poplar, Organogenesis, uidA Gene, Gene Transfer.

INTRODUCTION

Populus species and hybrids are among the fastest growing and most commercially important forest trees in the world. Eastern cottonwood (P. deltoides) (Pd) is especially noted for rapid growth and desirable pulping and papermaking properties. Significant genetic improvement has been obtained via classical selection and breeding, and the species is regenerated and planted vegetatively. More rapid and specific improvement, however, may be obtained by insertion of genes for traits not available in the species.

Genetic transformation has become almost routine with a variety of dicotyledonous plants, including a number of Populus species and hybrids. Indeed, the genus has proven to be a model for insertion of genes having commercial value (Chandler 1995). Much of this research, however, was performed with taxa other than Pd or its hybrids (e.g. Fillatti et al. 1987). Transformation has also been accomplished with hybrids between Pd and other species; e.g, P. trichocarpa (Parsons et al. 1986, De Block 1990, Wang et al. 1994) and P. nigra (Charest et al. 1992, Devantier et al. 1993). Against this background, we sought to devise a transformation protocol for Pd, with the intent of extending it from clones noted for ease of manipulation in culture to elite clones of commercial value.

In our earlier work (Stephens and Dinus 1994), a gene for enhanced auxin synthesis (Klee et al. 1987) was inserted into a model Pd clone (C175). Transformation frequencies, however, were low, and transgenic plants were not recovered. Accordingly, research was continued with a benign marker gene, the

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uidA reporter gene, to improve protocol efficiency. The present report describes results from three experiments in which several factors hypothesized as important to transformation efficiency were evaluated. Included were: preincubation treatments (Fillatti et al. 1987, Tsai et al. 1994, Confalonieri et al. 1994), At exposure time, and At concentration (Confalonieri et al. 1994). Effectiveness of two antibiotics for clearing cultures of residual At contamination and of culture on nonselective medium for a short time before transfer to selective medium were also tested. Adjustment of these several factors resulted in increased transformation frequencies and recovery of transgenic calli, primordia, and shoots.

MATERIALS AND METHODS

Plant Materials. Three experiments were conducted to evaluate factors influencing genetic transformation and regeneration of transgenic plants from eastern cottonwood clone C175 (Dr. S.G. Ernst, University of Nebraska-Lincoln). This model clone, easily manipulated in culture, was maintained in continuous shoot cultures to supply leaf explants over the long term.

Transformation and Regeneration. A leaf section system developed by Uddin et al. (1990), and modified for transformation by Shorter (1991) was used for transformation and regeneration. Procedures used in research reported here have been described by Dinus (1992) and Dinus and Stephens (1994). Five leaf sections were used per petri plate or replication; numbers of plates varied among experiments and treatments as indicated below.

Transformation was done with At Strain LBA4404 containing the binary vector pBI121. Included in the vector were the selectable marker gene (NPTII) for kanamycin (K) resistance coupled to the NOS promoter and the uidA reporter gene linked to CaMV35S (Clonetech Laboratories Inc.). At was cultured in YM broth (Lin 1994) at 30°C for three days, sedimented by centrifugation at 2500 rpm for 5 min, resuspended in standard leaf section medium, and diluted to the concentrations used for transformation.

Antibiotics. Selection of putative transformants was done on medium containing 50 mgK/L. Earlier research with C175 (Shorter 1991) showed that K levels as low as 30 mg/L halt development of nontransformed C175 leaf sections. In the present research, 50 mg/L was used as a safety margin and as recommended by Clonetech Laboratories Inc. Lethal dose assays (Shorter 1991) showed that carbenicillin (CA) concentrations as high as 500 mg/L did not harm C175 leaf sections, and were reasonably effective at clearing cultures of residual At. This concentration was used in the present research until questions arose about efficacy of CA. In response to such questions, cefotaxime (CE) was also evaluated in a lethal dose assay (Dinus et al. 1995). At growth was stopped by 250 mg/L or more, without apparent detriment to C175 leaf sections. Utility in C175 transformation trials, however, was not evaluated until experiments reported herein. All antibiotics were obtained from Sigma Chemical Co., St. Louis, MO. Other changes made to the aforementioned protocols are described below in the context of individual experiments.

Identification and Confirmation of Transformation. Callus, primordia, and/or shoots surviving on selective media were counted as putative transformants. Assays for expression of the uidA gene were performed as per the histochemical methods of Jefferson et al. (1987). Small portions of putatively transformed

calli or leaves were used for assay. Nearly all putative transformants were free of At contamination. The few suspected of being contaminated were rinsed with 70 percent ethanol three times and sterile distilled water twice before assay. When color developed within a few minutes or between plant cells, candidates were not counted as transformed. In sum, plant materials counted as confirmed transformants included only those showing complete expression at the end of the lengthy culture periods noted above. Those showing transient or chimeric expression, or responses due to At contamination, were excluded.

Trial 1. The first experiment evaluated efficacy of the At exposure time (5 min) and concentration (10^9 cfu/mL) used in our earlier research (Shorter 1991). Protocols described above were followed with one exception. Several authors working with *Populus* hybrids and species have incubated explants for 24-48 hr before exposure to At in order to foster explant growth (Fillatti et al. 1987) or to cull unhealthy explants (Tsai et al. 1994). Accordingly, half of all leaf sections used in this trial, regardless of subtreatment, were incubated on standard leaf section medium for 24 hr in darkness prior to At exposure.

Subtreatments are described below. A control (-At-K) was included to verify that leaf sections developed normally (N = 30). To quantify effects of At and the transformation process on regeneration, leaf sections were exposed to At but not K (+At-K) (N = 110). This treatment also was intended to produce putatively transformed shoots for later selection on shoot growth medium containing K. Results from this latter aspect will be reported elsewhere. A +At+K subtreatment was used to assay yields of transformants resulting from selection immediately after exposure to At (N = 110).

Percentages of leaf sections forming callus, primordia, and harvestable shoots were recorded weekly for the first 63 days of culture and at roughly 3 week intervals through 277 days. Putative transformants were assayed for uidA expression at the end of the trial.

Trial 2. The second experiment tested effects of longer At exposure time (30 min) and a lower concentration (10^8 cfu/mL). As a secondary objective, utility of CE for clearing cultures of residual At contamination was compared to that of CA.

Protocols described above were used with one change; half of the leaf sections, regardless of subtreatment, were cultured on medium supplemented with 500 mgCA/L and half on medium containing 250 mgCE/L. As in the foregoing trial, leaf sections were divided among three subtreatments: -At-K control (N = 30); +At-K (N = 150); and +At+K (N = 150).

Percentages of leaf sections forming callus, primordia, and shoots were recorded weekly for the first several weeks to establish that development was proceeding normally. Observations continued at roughly 6-week intervals thereafter. The trial was terminated after 213 days of culture, when putative transformants from the +At+K subtreatments were assayed for uidA expression.

Trial 3. The third experiment compared effects of varying At exposure times (30 versus 120 min) and concentrations (10^7 versus 10^{10} cfu/mL). Subtreatments used in the aforementioned trials were included, and contrasted

with another that provided for culture on nonselective medium for 14 days before transfer to selective medium (+At+KP). The intent was to allow time for development to start, thereby permitting transformed cells to accumulate and differentiate to an extent sufficient to resist the debilitating effects of K and of the dying cells surrounding them.

Protocols were the same as those used earlier, except for changes described immediately above. Leaf sections were divided among 13 treatment combinations as follows: -At-K (N = 15); +At-K, 4 combinations of exposure times and concentrations (N = 15 per combination); +At+K, 4 combinations (N = 30 per combination); and +At+KP, 4 combinations (N = 30 per combination).

Percentages of leaf sections forming callus, primordia, and harvestable shoots were recorded weekly for the first few weeks, and at roughly four week intervals through the 91st day of culture. The experiment was then terminated, and putative transformants were collected from +At+K and +At+KP treatments for uidA assay and regeneration.

RESULTS AND DISCUSSION

Trial 1. Results from the first trial showed that incubation on standard leaf section medium for 24 hr before exposure to At did not provoke differential responses. Accordingly, results were averaged over all explants given each subtreatment.

In retrospect, this finding is not surprising. Though such practices are used with some frequency (Tsai et al. 1994, Confalonieri et al. 1994), few data are available to substantiate efficacy of this extra step in transformation protocols. Also, effectiveness of such treatments would seem dependent upon their being sufficiently long to ensure that development not only starts but also gets well underway. The time course of development for control leaf sections (Figure 1) shows that callus formation began in the first few days of culture. Primordia, first visible manifestation of organized meristematic centers, however, appeared 14 to 21 days later. Thus, incubation to ensure that development is not hindered by exposure to At and/or selective medium probably should span the first 14 to 21 days of culture. Such treatment also seems best applied after At exposure but before transfer to selective medium. This would permit transformed cells to multiply before dying cells surrounding them interfere with development. This approach was tested in Trial 3; the outcome is described below.

Development on +At-K leaf sections was delayed relative to those given the control treatment (Figure 1, Table 1). Percentages of explants forming callus and primordia eventually reached control levels, but shoot formation and elongation were delayed and reduced relative to controls. Thus, exposure to At and other aspects of the transformation protocol appear disruptive to regeneration.

Development on explants given the +At+K treatment was slow; callus was not evident until the 56th day of culture. Primordia and shoot formation were inhibited (Table 1) even though fair numbers of calli survived and continued to grow. Midway through the trial, a number of leaf sections showing promise were removed from this medium, cultured on nonselective medium to foster

Figure 1: Percentage of Explants from Trial 1 Having Callus (—●—), Primordia (—■—), and Shoots (—▲—) Through Day 63; A (-At-K) and B (+At-K)

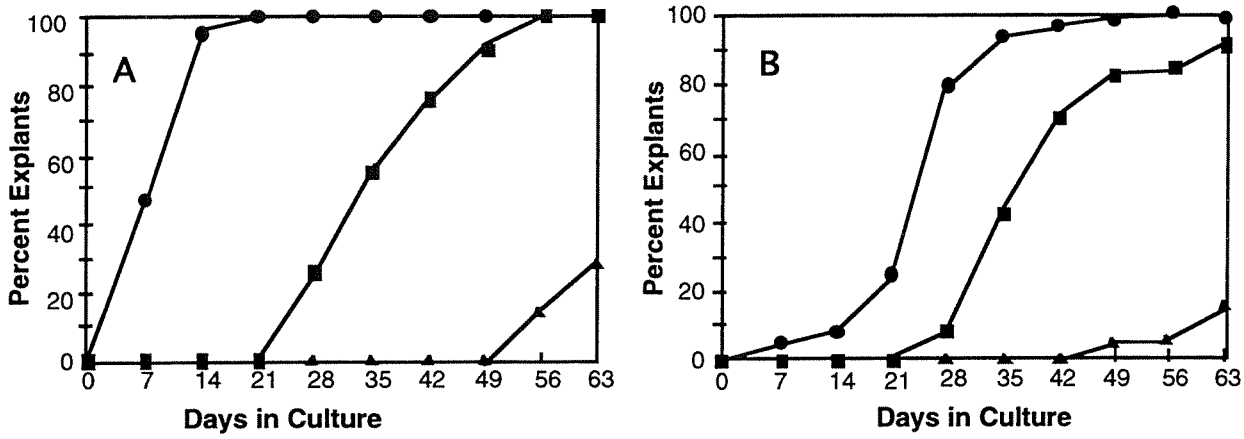

Table 1: Development on C175 leaf sections 277 days after exposure to At (10^9 cfu/mL) for 5 min and cultured on medium supplemented with 500 mgCA/L.

Treatment	Percent of Leaf Sections Forming		
	Callus	Primordia	Shoots
-At-K	100	100	94
+At-K	100	97	9
+At+K	38	0	0

development, and then returned to selective medium. They may not have survived if left on selective medium. As a result, percent of +At+K explants forming callus (Table 1), and therefore percent putative transformants (Table 2), is inflated by roughly 9 percentage points.

As noted above, all +At+K calli that survived through end of the trial were regarded as putative transformants (Tables 1 and 2). Only 5 percent showed expression of the uidA gene, and only 1 percent were rated as confirmed transformants. Thus, this short AT exposure time did not produce high frequencies of lasting transformation despite the relatively high At concentration, a finding in line with other recent investigations in which, depending on species and explant, exposure times ranged from 20 (Confalonieri et al. 1994) to 240 min (Wang et al. 1994).

Trial 2. Findings from the second trial, an effort to increase transformation rates via a longer At exposure time, mirrored those of the first trial. Frequencies of control explants forming callus, primordia, and shoots reached 100 percent quickly, regardless of CA and CE treatment.

Performance of +At-K leaf sections was similar to that of controls (Table 3), except that shoot formation was somewhat lower. Though small, this differential response confirms that At exposure and/or the transformation process reduces regeneration potential. Differences between responses to CA and CE, however, were nominal.

Table 2: Putative and confirmed transformation frequencies as affected by At exposure time (ET) and concentration (Conc).

At ET and Conc. (min. and cfu/ml)	Transformation, Percent of Available Leaf Sections Putative	Confirmed
Trial 1: 5, 10^9	38	1
Trial 2: 30, 10^8, CA	13	1
CE	19	1
Trial 3: 91 Days On Selective Medium		
30, 10^7	10	0
10^{10}	37	0
120, 10^7	36	10
10^{10}	27	10
14 Days on Nonselective + 77 Days On Selective Medium		
30, 10^7	27	10
10^{10}	33	10
120, 10^7	43	3
10^{10}	47	13

Explants given the +At+K treatment also formed callus and primordia with some frequency (Table 3), higher than in the first experiment. Shoot formation was also higher. No differences were apparent between responses to CA and CE.

Frequency of putative transformants averaged 16 percent; CA and CE produced similar outcomes (Table 2). All transformants, putative and confirmed, were calli; none of the primordia or shoots surviving on selective medium were transgenic. Only 3 percent of surviving calli showed uidA expression, and only an average of 1 percent were counted as confirmed transformants. These few transformants were distributed equally between CA and CE treatments. Thus, increasing At exposure from 5 to 30 min, and slightly reducing concentration to a level considered desirable by other workers (e.g., Confalonieri et al. 1994) did not raise transformation frequencies above levels noted earlier.

Regardless of subtreatment, differences between CA and CE treatments were minor, thereby confirming that CE does not interfere with regeneration from C175 leaf sections (Table 3). Indeed, when all subtreatments are considered, CE may have had a slight advantage in that numbers of putative transformants were slightly higher than for CA, an outcome possibly associated with lesser interference by residual At.

Residual At contamination was not as severe a problem in this experiment as in our earlier research. Midway through the present experiment, percentages of contaminated explants ranged from 43 to 60 percent, with +At+K explants most affected. Contamination levels were similar on CA and CE medium. With time, however, the margin between antibiotics widened,

Table 3: Development of C175 leaf sections 213 days after exposure to At (10^8 cfu/mL) for 30 min and cultured on media supplemented with 500 mgCA/L or 250 mgCE/L.

Treatment	Percent of Leaf Sections Forming		
	Callus	Primordia	Shoots
Carbenicillin			
-At-K	100	100	100
+At-K	97	94	82
+At+K	13	3	3
Cefotaxime			
-At-K	100	100	100
+At-K	100	99	100
+At+K	19	7	1

particularly in +At+K subtreatments. Within this subtreatment, 57 percent of explants cultured on CA experienced At contamination at one time or another as opposed to only 39 percent of those given CE. Taken together, such findings confirm those from dose/response assays (Dinus et al. 1995), and demonstrate the utility of using CE in the future.

Trial 3. Treatments tested in the third trial yielded definite increases in transformation frequencies (Tables 2 and 4). The trial was terminated after 91 days. Development generally was better than in Trials 1 and 2, and results from them showed little advantage to longer culture periods.

Table 4: Development of C175 leaf sections as affected by varying At exposure times and concentrations after 91 days of culture.

Treatment	Percent of Explants Forming		
	Callus	Primordia	Shoots
At-K: Control	100	100	40
+AT-K: 30 min, 10^7	100	100	47
10^{10}	100	100	47
120 min, 10^7	100	100	67
10^{10}	100	100	20
+AT+K: 30 min, 10^7	10	3	0
10^{10}	37	0	0
120 min, 10^7	36	3	3
10^{10}	27	10	3
+AT+KP: 30 min, 10^7	27	10	0
10^{10}	33	10	0
120 min, 10^7	43	17	0
10^{10}	47	10	0

Control explants formed callus and primordia at frequencies similar to those noted above (Tables 1, 3, and 4). Shoot production was reduced relative

to earlier experiments, but explants were healthy and yields were expected to increase beyond those noted at 91 days.

Leaf sections given the +At-K treatment performed similarly to controls (Table 4). Though somewhat inconsistent across treatments, shoot production tended to decline with increasing exposure time and concentration. Concentration appeared to have the greater effect. The fact that shoot production was not greater highlights the need for At exposure times and concentrations that maximize transformation without reducing regeneration.

Explants given the +At+K treatment formed callus, primordia, and shoots at similar or slightly higher frequencies than in earlier trials (Tables 1, 3, and 4). The overall increase in survival and development suggests that greater At exposure times and concentrations produce higher frequencies of transformation, even though they reduce regeneration potential to some extent.

Culture for 14 days on nonselective medium before transfer to selective medium increased numbers of +At+KP explants forming callus and primordia. Although shoot formation did not increase relative to +At+K subtreatments in this and other experiments, overall development was enhanced, and the improvement appears associated primarily with longer exposure time.

Similar trends were apparent for percentages of putative and confirmed transformants (Table 2), and yields from +At+KP subtreatments were greater than those from +At+K subtreatments in this and the other two experiments. In addition, frequencies varied directly with exposure time and concentration, with longer exposure time having the more pronounced effect. Concerns that +At+KP putative transformants would be largely transient and/or chimeric were not realized. When averaged over all subtreatments, the difference between confirmed and putative transformants was only slightly greater for the +At+KP subtreatments.

Collectively, findings from the third trial indicate that longer exposure times (120 min) and modest concentrations (perhaps, 10^8) offer much promise for raising transformation efficiency without reducing regeneration potential. That the +At+KP treatment tended to foster development and produced higher frequencies of both putative and confirmed transformants further indicates importance of allowing development to start and proceed for some time before challenging putative transformants with selective medium. This procedure apparently allows transformed calli to form and accumulate meristematic centers sufficiently organized to survive and develop.

Clearly, selection with K is far from ideal, and research on transformation would benefit from availability of a more benign marker gene. Increasing public concern about placing genetically altered trees resistant to antibiotics in the environment emphasizes need for such markers. Until such markers are developed, efficient regeneration of transgenic Pd plants will require some further adjustment of K concentrations in selective media. More importantly, treatments similar to the +At+KP tack used here also seem a workable means to circumvent the barrier posed by selection with K. While this approach resulted in slightly more escapes and partial or transient transformants, overall yields of confirmed transformants were greater and obtained in far less time than with other treatments. Trials to reevaluate

the procedure and to test other time periods on nonselective medium are underway.

Transgenic calli from Trial 3 have been transferred to media designed to force development of shoots. Also, leaves from cultures containing primordia and/or shoots have been harvested for multiplication/regeneration on standard leaf section medium. Shoots have been rooted, and are being multiplied via the leaf section protocol. These materials will be used to again verify transformation via histochemical and polymerase chain reaction assays for uidA gene expression and presence, respectively, and to check for any abnormalities in morphology and growth.

Results are also being used to effect transformation with and to study expression of a gene for enhanced auxin synthesis (Klee et al. 1987) in Pd clone C175, and to extend transformation to elite clones of commercial value.

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EFFECTS OF ACETOSYRINGONE, PH AND CONCENTRATION OF *AGROBACTERIUM TUMEFACIENS* ON PUTATIVE TRANSIENT GUS GENE EXPRESSION IN *POPULUS*

Xin Y. Li and Feng H. Huang[1]

Abstract. -- Efficiency of transient GUS gene transfer mediated by *Agrobacterium tumefaciens* has been studied on *Populus* hybrid NC-5331 leaf explants. Included in this study were three factors which may affect the gene transfer efficiency: concentrations of acetosyringone (0 to 100 µM), dilution of the bacterium (25 and 50 times) and pH (5.5 to 6.4). All three factors were very important to achieve high efficiency gene transformation in the poplar. Interactions among the factors obviously existed. However, several tendencies were evident: 1) exogenous acetosyringone did not always enhance the gene transformation frequency, which was dependent on its concentration and the other factors; 2) acetosyringone preferred higher pH for higher transformation efficiency; 3) the most beneficial range of acetosyringone was between 25 and 75 µM, depending on the other factors; 4) dilution of the bacteria (overnight culture) 50 times, in most cases, resulted in higher transfer rate than the 25 times under the same conditions.

Key words: acetosyringone, *Agrobacterium tumefaciens*, gene transformation efficiency, GUS, pH, *Populus*.

INTRODUCTION

The soil bacterium *Agrobacterium tumefaciens* has the ability to transfer, insert and express a particular segment of DNA in the cell genome on all tested dicotyledonous and some monocotyledonous plants due to a tumor-inducing Ti plasmid. The segment of the Ti plasmid DNA is called transferred or T-DNA. The transfer of T-DNA is dependent upon the *vir* or virulence region of Ti plasmid as well as genes on the bacteria chromosomes (Hille *et al.*, 1984; Douglas *et al.*, 1985). The induction of transcription of the *vir* region is mediated by signal molecules such as acetosyringone (AS, 3',5'-dimethoxy 4'-hydroxyacetophenone), a phenolic compound (Bolton *et al.*, 1986; Ashby *et al.*, 1988). However, an inhibiting effect by AS on the growth of certain strains of *A. tumefaciens* has been noticed. Further studies indicated that inhibition by AS was accompanied by the accumulation of avirulent mutants (Fortin *et al.*, 1992).

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Researchers have reported on gene transformation in several poplar (*Populus sp.*) species (Parsons *et al.*, 1986; Fillatti *et al.*, 1987; De Block, 1990; Wilde *et al.*, 1992). The highest frequency of *Agrobacterium*-mediated gene transfer approximately 40% has been achieved on a poplar clone (De Block, 1990). In this experiment, three factors, AS, pH and the bacteria concentration were investigated in an attempt to achieve higher gene transfer efficiency in a hybrid poplar.

MATERIALS AND METHODS

Bacteria strain and its preparation

The *A. tumefaciens* strain ASE-9749 with binary vector plasmid (pMON9749, obtained from Monsanto Co., St. Louis, MO) was used in this study. This plasmid carries an intact *vir* region and four chimeric genes, including three antibiotic (kanamycin, spectinomycin, and chloramphenicol) resistant genes and a β-glucuronidase (GUS) gene. The strain, stored at -20 °C, was streaked on solidified LB medium supplemented with 50 mg/l kanamycin, 75 mg/l spectinomycin and 25 mg/l chloramphenicol for 2 days at 28 °C, and then grown overnight in LB liquid medium on "Roto-torque" at 100 rpm. The bacteria concentration was determined at OD_{600}. Before inoculation, the liquid culture was diluted 25 or 50 times with LB liquid medium supplemented with 0 to 100 µM AS. The pH of LB liquid culture media was adjusted to 5.5, 5.6, 5.8, 6.1 or not adjusted (pH 6.4) in different experiments. Then the bacteria solutions were incubated for 3 hr at 28 °C before mixed with leaf segments.

Plant materials and putative gene transformation

The *in vitro* grown hybrid poplar line NC5331 (*Populus nigra* L. var. *betulifolia* Torr X *P. trichocarpa* Torr and Gray) was used as plant material. Leaf segments were mixed with bacteria liquid culture for 1 to 2 min and then placed on a co-culture solid medium (MS supplemented with 0.2 mg/l kinetin, 0.5 mg/l 2,4-D, 20 g/l sucrose and 6 g/l agar; pH was 5.5, 5.6, 5.8 or 6.1 depending on experiments and treatments). After 2 days of co-cultivation at 25°C in darkness, the leaf segments were transferred to the selection medium (co-culture medium supplemented with 60 mg/l kanamycin, 100 mg/l cefotaxime and 200 mg/l carbenicillin; pH 5.8). Petri dishes were para-film sealed and incubated at 25°C in darkness.

The histochemical procedure of GUS activity was performed according to Jefferson *et al.* (1987). The calli or survived leaf segments were assayed with X-gluc (5-bromo-4-chloro-3-indolyl-β-glucuronide) in 6 weeks. The small pieces of calli about 50 mg or whole leaf segments were mixed with 100 µl X-gluc at 37°C for overnight. The blue color indicated the putative GUS gene expression. The number of transformed explants was determined based upon at least one visible blue spot on each leaf segment or callus under a dissecting microscope. The percentage of transformed from total explants was calculated as the putative gene transformation efficiency.

Experimental Design

Three experiments were conducted in this study. In the first experiment, the influences of AS concentrations and dilution times of the bacteria solution were investigated. The pH of the

bacteria solution and the co-culture medium were 6.4 and 5.8, respectively (Table 1). In the second experiment, only the dilution at 50 times was used. The effects of AS concentrations and pH of the bacteria solution on putative gene transfer efficiency were determined. In the last experiment, possible interactions among AS concentrations, bacteria concentration and the pH of bacteria solution and co-culture medium were investigated. The pH of the bacteria solution and co-culture medium were adjusted to the same level in each treatment (Table 1).

In each treatment, three petri dishes were used; each contained 15 to 21 leaf segments (Table 1). The pH of the bacteria solution containing AS and that of the co-culture media were adjusted as designed. After the solution was cultured overnight, its OD_{600} value was about 0.45. It was diluted 25 or 50 times dependent on the experiments. The AS was filter-sterilized to the bacteria solution.

RESULTS

Experiment 1

Addition of AS promoted the putative GUS gene transformation with bacteria solution diluted 50 times but inhibited transformation with the bacteria dilution of 25 times (Table 1). However, the putative gene transformation efficiency gradually decreased with the increasing of AS concentration regardless of the bacteria concentration. Without the supplement with AS, the dilution of 25 times achieved 22.2% gene transfer frequency while the dilution of 50 times did not achieve transformation (Table 1). The enhanced putative gene transformation frequency with the assistance of AS at the dilution of 50 times was achieved without reducing pH of the bacteria solution or co-culture media.

Experiment 2

Only the bacteria dilution of 50 times was used in this study, and the pH of that solution was adjusted while the pH of co-culture medium was maintained at 5.8. Under these conditions, the impact of pH was clear in that the addition of AS only slightly increased the putative transformation efficiency when the pH of bacteria solution was adjusted to 5.6 while it significantly improved the putative transformation at pH 6.4 (Table 1). Higher AS concentration did not enhance or decreased the putative transformation efficiency at pH 5.6 or 6.4, respectively. Addition of AS combined with higher pH could promote putative *Agrobacterium*-mediated gene transfer compared with no supplement with AS. Also this experiment confirmed that putative gene transfer efficiency could be increased with the assistance of AS when the bacteria solution was diluted 50 times and its pH was at 6.4.

Experiment 3

Surprisingly, the highest putative transformation frequency of 82% was achieved at pH 5.8 with the bacteria dilution of 50 times and without AS addition (Table 1). Regardless of the bacteria concentration, higher concentration of AS slightly increased the putative gene transformation efficiency at pH 5.5. At pH 5.8, the different bacteria dilutions produced completely different outcomes in that the 50 times dilution resulted in significantly higher transfer

rate at AS 0 and 25 μM than the solution diluted 25 times. At pH 6.1, addition of AS decreased putative transformation rate under the dilution of 25 times compared with no AS treatment, but putative transformation increased with increasing the AS concentration. The addition of AS at 25 to 75 μM significantly enhanced transfer efficiency with the bacteria dilution of 50 times at pH 6.1 compared with no addition of AS. This confirmed the results in experiment 1 that addition of AS could increase the putative transformation efficiency at the dilution of 50 times at higher pH. The low transformation efficiency achieved at pH 5.5 was similar to the results at pH 5.6 in the experiment 2.

DISCUSSION

AS is an inducer of the virulence region, which can mediate the T-DNA transfer. In nature, wounded plant cells contain defined signal molecules, such as AS (Stachel *et al.*, 1986). Thus, addition of AS should enhance gene transfer efficiency. This has been demonstrated in some species (Sheikholeslam and Weeks, 1987; Owens and Smigocki, 1988; Godwin *et al.*, 1991). Our results showed that AS, in most cases, did enhanced the putative gene transformation efficiency but the extent of influence was dependent upon its concentration, the pH and the bacteria concentration. An AS level up to 200 μM is not considered to be significantly toxic to *Agrobacterium* cells (Stachel *et al.*, 1985). However, in our investigation, more than 75 μM AS rarely promoted the gene transfer. Similar results have been reported on carrot (Guivarc'h *et al.*, 1993). It was not surprising that the addition of AS did not always raise the transformation frequency because wounding of tobacco cells is known to induce more than a 10-fold increase of AS in cell exudate (Stachel *et al.*, 1985). We do not know exactly how high the concentration of AS was that explants were exposed to. The non-effect with AS addition has also been demonstrated on other species (Godwin *et al.*, 1991). In addition, decreased transformation efficiency by AS may be associated with the accumulation of avirulent mutants as indicated by Fortin *et al.* (1992).

The AS-mediated *vir* gene induction increases with the decreasing of pH from 6.2 to 5.1 (Stachel *et al.*, 1986). The optimal induction of *vir* gene is attained when pH is lower (Stachel *et al.*, 1986) than those commonly used in plant tissue culture medium (pH 5.8 to 6.0). However, Godwin *et al.* (1991) indicated that AS-assisted gene transfer frequency was higher at pH 5.5 to 5.8 than at pH 5.2. They suggested that actual pH around explants was depressed by leakage of cell contents into the medium, and hence the optimal pH was reached on the less acidic media. In our work, supplement with AS at lower pH only slightly increased the gene transfer efficiency. Higher pH from 5.8 to 6.4 was preferred by AS to promote the gene transformation. The gene transfer with AS at pH higher than 6.1 was rarely reported. Based on our study including three experiments, AS could enhance the gene transfer at higher pH from 5.8 to 6.1 or even 6.4. This may be due to the interaction between plant and bacteria which optimized the microenvironment. Without addition of AS, pH was also important to gain a higher gene transfer frequency. Over 20% putative transformation efficiency was achieved only at pH higher than 5.8 depending on the concentration of bacteria solution.

The impact of the bacteria concentration on gene transfer has rarely been reported. Based on the experiments 1 and 3, the bacteria concentration was critical to attain a higher efficiency of putative GUS gene transformation. This may be attributed to the negative effects of higher

bacteria concentration on the growth of explants and the pH change induced by the growth of bacteria.

Overall, from the three experiments, several common tendencies were evident. First, exogenous AS did not always enhance the gene transfer, e.g. with the dilution of 25 times in experiment 1, with the dilution of 25 times at pH 6.1 in the experiment 3, and with the dilution of 50 times at pH 5.6 in experiment 3. The bacteria concentration and pH both were important to affect the *Agrobacterium*-mediated transformation by addition of AS. Second, AS, in most cases, preferred medium to high pH (5.8 to 6.4) to raise the putative gene transfer efficiency. With reducing pH even only in the bacterium solution, the gene transfer efficiency decreased under the same conditions, e.g. in the experiment 2 and experiment 3. Third, the bacteria solution diluted 50 times usually produced higher putative gene transfer frequency than that diluted 25 times under the same conditions, especially with the addition of AS. Fourth, the most beneficial range of AS was between 25 and 75 μM, but was dependent on the other factors, especially the bacteria concentrations.

Southern blotting will be tested in the future to provide further evidence that these putative GUS gene expressions are true. The putatively transformed calli had potential to be regenerated. The stable gene transformation efficiency will be determined.

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Table 1. Effects of acetosyringone, pH and concentration of *A. tumefaciens* on the putative GUS gene transformation efficiency in hybrid poplar NC-5331.

AS (μM)	pH of BS[1]	pH of CM[2]	*A. tumefaciens* dilution times	Transformed[3] explants/total	Transformation efficiency %
Experiment 1					
0	6.4	5.8	25	10/45	22.2
25	6.4	5.8	25	2/45	4.4
50	6.4	5.8	25	2/45	4.4
75	6.4	5.8	25	0/45	0
100	6.4	5.8	25	0/45	0
0	6.4	5.8	50	0/45	0
25	6.4	5.8	50	23/45	51.1
50	6.4	5.8	50	13/45	28.9
75	6.4	5.8	50	6/45	13.3
100	6.4	5.8	50	2/45	4.4
Experiment 2					
0	5.6	5.8	50	3/45	6.7
30	5.6	5.8	50	5/45	11.1
60	5.6	5.8	50	5/45	11.1
0	6.4	5.8	50	1/45	2.2
30	6.4	5.8	50	35/45	77.8
60	6.4	5.8	50	15/45	33.3
Experiment 3					
0	6.1	6.1	25	12/52	23.1
25	6.1	6.1	25	2/57	3.5
50	6.1	6.1	25	7/52	13.5
75	6.1	6.1	25	8/50	16.0
0	6.1	6.1	50	0/52	0
25	6.1	6.1	50	32/49	65.3
50	6.1	6.1	50	23/54	42.6
75	6.1	6.1	50	31/55	56.4
0	5.8	5.8	25	0/58	0
25	5.8	5.8	25	3/54	5.6
50	5.8	5.8	25	4/63	6.3
75	5.8	5.8	25	0/57	0
0	5.8	5.8	50	41/50	82.0
25	5.8	5.8	50	19/47	40.4
50	5.8	5.8	50	3/57	5.3
75	5.8	5.8	50	3/47	6.4
0	5.5	5.5	25	0/59	0
25	5.5	5.5	25	1/54	1.9
50	5.5	5.5	25	4/52	7.7
75	5.5	5.5	25	4/45	8.9
0	5.5	5.5	50	0/50	0
25	5.5	5.5	50	2/52	3.8
50	5.5	5.5	50	1/54	1.9
75	5.5	5.5	50	3/45	6.7

[1] represents bacteria solution.
[2] represents co-culture medium.
[3] at least one blue spot showed in each survived leaf segment or callus.

GENETICALLY ENGINEERING PLANTS WITH A GENE FOR MERCURIC ION REDUCTION AND RESISTANCE

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Abstract. We are engineering a bacterial gene encoding mercuric reductase for the production of plants with the ability to electrochemically reduce toxic, ionic mercury. Bacterial mercury resistance operons have been described in detail (Summers, 1986). One gene contained in the polycistron is *merA*, which codes for mercuric ion reductase. This enzyme catalyzes the reduction of toxic Hg^{++} to far less toxic Hg^{o}. The coding sequence of the bacterial gene is very GC-rich, possibly preventing observable expression in transgenic plants. We designed a *merA* sequence that contained codons more typical of highly expressed plant genes. Overlap Extension-PCR (OE-PCR) was used to modify the codons in a 9% block of coding sequence and to alter the non-coding regulatory sequences. *Agrobacterium*-mediated transformation was used to produce *Arabidopsis* having the modified gene, *merA9*. These plants are capable of germinating and growing to seed set on medium containing up to 100 μM mercuric chloride, whereas control seeds fail to grow on 25 μM Hg^{++}. Full length mRNA was detected in mercury resistant plants using northern blot hybridization. Mercury-resistant transgenic *Arabidopsis* seedlings were observed to evolve Hg^{o} vapor from buffered $HgCl_2$ at up to three times the rate of control seedlings. We are continuing to investigate the effect of further sequence modification for the optimization of *merA* gene expression in transgenic plants. Ultimately, we intend to develop transgenic tree species transformed with modified *merA* constructs. We have developed reliable and efficient protocols for tissue culture propagation, plant regeneration and gene transfer for yellow-poplar (*Liriodendron tulipifera*). Our progress towards the production of *merA* transgenic yellow-poplar is discussed.

Keywords: phytoremediation, genetic engineering, mercury resistance, *Arabidopsis thaliana*, *Liriodendron tulipifera*.

INTRODUCTION

The use of plants to stabilize, reduce or detoxify aquatic and terrestrial pollution is known as phytoremediation. Many terrestrial environments are naturally high in phytotoxic metallic compounds (Alloway, 1990) and when combined with sites contaminated with heavy metals from man-made sources this creates pollution hazards that are very difficult and expensive to remediate (Nriagu and Pacyna, 1988). Some plant species have developed the ability to thrive on many of these sites by hyperaccumulating the metals and sequestering them away from sensitive physiological processes (Baker, 1989). These plant taxa have been suggested as possible phytoremediation "crops" (Baker et al., 1994), though their implementation in the near term is hampered by inadequate understanding of the physiological, biochemical and genetic complexity of hyperaccumulation. Molecular genetic techniques have been used to characterize and manipulate plant genes involved in heavy metal tolerance or uptake and should aid in their eventual application

(Zhou and Goldsbrough, 1994). Biotechnology may also allow the utilization of novel, foreign genes from organisms having metabolic pathways for the processing of toxic heavy metals (Stomp et al., 1993). Our laboratory has been involved in this latter approach for the development of potentially phytoremediative species using a bacterial gene for ionic mercury detoxification.

Bacterial mercury resistance operons have been described in detail and one gene of this operon ,*merA* , codes for mercuric ion reductase (Summers and Sugarman, 1974; Summers, 1986). This enzyme catalyzes the reduction of toxic, ionic mercury to volatile, elemental mercury having far lower toxicity. Early attempts to confer Hg^{++} resistance to plants using the wildtype *merA* gene were unsuccessful (Thompson, 1990). Petunia plants were transformed with plant expression vectors containing the *merA* gene. These plants remained mercury sensitive and no full length gene product could be detected. Analysis of the *merA* DNA sequence revealed a high GC (up to 75%) nucleotide bias (Barrineau et al., 1984), in contrast to the ~50% GC content of common, highly expressed plant genes (Murray et al., 1989). We hypothesized that the highly GC-skewed codon usage was extremely unfavorable for plant gene expression machinery, and therefore sequence modification would confer *merA* gene activity and ionic mercury resistance in plants. This strategy has been successful for improving the expression other foreign genes in transgenic plants (Fischoff et al., 1987). We have designed an altered DNA coding sequence for the *merA* gene that retains the coded amino acid sequence of the enzyme, but optimizes the codon usage, lowers the GC-bias to 45-55%, and alters the noncoding regulatory regions of the gene for efficient expression in plants. A directed mutagenesis strategy is being used to develop stepwise versions of modified *merA* gene constructs for transformation and analysis in plants species.

Ultimately, we intend to develop transgenic tree species transformed with modified *merA* constructs. Trees possess many desirable characteristics for a putative phytoremediative species. Their long life, deeply mining root system, and large reservoir of non-living woody tissues seem optimal for the removal, storage and remediation of heavy metal contaminants from the soil. We have developed reliable and efficient protocols for tissue culture propagation, plant regeneration and gene transfer for yellow-poplar (Merkle and Sommer, 1986; Wilde et al., 1992). Our progress towards further *merA* modification and transgenic tree development will be reported. Additionally, the theoretical phytoremediative benefits and potential advantages of *merA*-expressing tree species will be discussed as part of our long term goals for this project.

MATERIALS AND METHODS

Overlap extension (OE)-PCR for modification of *merA* .

The OE-PCR procedure is essentially as described by Ho et al. (1989). Terminal primers were designed to alter the sequences up- and downstream of the coding sequences to optimize for gene regulation in plants. The internal primers were designed to change the nucleotide coding sequence of the wildtype *merA* gene to codons more common to plants genes. Two halves of the gene were amplified separately using internal/terminal sense/antisense primer pairs. The two products were then gel purified, annealed and primed by the two external oligonucleotides, 5'S and 3'N, to complete each successive, modified *merA* fragment. The fragments were cleaved at designed restriction ends, *Bam HI* and *Pst I*, and ligated into the same sites of the cloning vector pBluescriptSKII (Strategene, La Jolla, CA). To screen against PCR-error mutants, the pBS-*merA* molecules were transformed into a Hg^{++}-supersensitive, *merA*-deficient *E.coli* strain. Only ligation products complementing *merA*- and conferring resistance to 200 μM Hg^{++} on replica plates were selected.

Agrobacterium-mediated transformation of modified *merApe.*

The *BamHI/PstI* fragment of *merA9* was subcloned into the compatible site in the binary plant expression vector, pVSTI (Malik and Wahab, 1993), and transformed into the

Agrobacterium strain LBA4404. This strain was then used to inoculate *A.thaliana* root explants and regenerate selected, genetically transformed plants as described in Marton and Browse (1991).

Determination of Hg^o evolution by *merA9-A.thaliana*.

Transformed *merA-Arabidopsis* lines and control lines lacking the *merA* gene were sterilized and germinated on GB5 medium containing no growth regulators or selective reagents (e.g. Hg^{++} or kanamycin). Twelve to 15 day old seedlings were weighed and placed in the side-arm reaction tube connected to the Jerome 431 mercury vapor analyzer. This apparatus accurately measures and displays evolved, elemental mercury drawn from either standard curve or experimental samples. The plants were assayed in 2 ml of 25 μM $HgCl_2$, 50 mM phosphate, pH 6.8, with Hg^o evolution sampled each minute over a 10 minute period.

Hg^{++} resistance seed germination assays.

merA9-A. thaliana and control seeds were surface sterilized and transferred to modified GB5 medium plates containing 0, 25, 50, 75, or 100 μM $HgCl_2$; wrapped with Parafilm and incubated at 24°C with 16 hr day cycle. Mercury resistance was defined as full expansion of true leaves and elongation of true roots by greater than half of the seeds sown upon medium containing Hg^{++}.

Detection of *merA* mRNA in transgenic *A.thaliana* using northern blot analysis.

Total RNA was isolated from stem and leaf tissues of 15-20 day old seedlings using a phenol/SDS/LiCl extraction protocol (Ausubel et al., 1987). The samples were run on an agarose-formaldehyde gel and blotted to Nitran filters. The filters were washed at moderate stringency in 1X RNA Hybridization Mix with 40% deionized formamide at 48°C (Hightower and Meagher, 1985) using the *merA9* 1.7 kb insert as radioactively labeled probe. To quantify the sample loadings and the *merA* hybridized band, the blots were stripped and re-hybridized with a probe for 18s rRNA. The sample loading quantification and subsequent normalized adjustment factors for the positive *merA* hybridization bands were determined using PhosphoImager analysis (Molecular Dynamics, Inc.).

Transformation and regeneration of *merA*-yellow-poplar.

Embryogenic cultures of yellow-poplar (*Liriodendron tulipifera*) were established and transformed as described in Merkle and Sommer (1986) and Wilde et al.(1992). To summarize, immature seed explants were cultured on growth medium containing plant growth regulators with subculturing at three week intervals. After 1-2 months proembryogenic masses (PEMs) developed from zygotic embryos. PEMs will develop into somatic embryos when size fractionated (38-140 μm) and transferred to hormone-free medium. For transformation, the fractionated PEMs were collected on filter paper, transferred onto growth medium and bombarded with DNA-adsorbed microprojectiles for each of the *merA* constructs using the Biolistic transformation system as directed by the manufacturer (Du Pont, Wilmington, DE). After two days bombarded cultures were transferred to growth medium containing 100 mg/l kanamycin to select for cells transformed with the selective marker gene also contained on the plant expression vector.

RESULTS AND DISCUSSION

merA Gene Modification

Overlap-Extension PCR (OE-PCR) was used to generate modified nucleotide sequences of the *merA* gene. In the initial *merA* modification, *merApe0*, only the flanking regions have been altered to include 5' and 3' untranslated plant and *E.coli* regulatory sequences. Each construct ID is shortened, for example *merApe0* to *merA0,* when transformed into plants. *MerApe9* has had its

coding sequence changed to more typical plant codon usage within a **9%** block of the reading frame. The *merApe0* through *merApe38* modifications have been completed. Six more OE-PCR rounds are required to complete *merApe47* to *merApe100*. Note that all these sequences encode a normal MerA protein, since only conserved changes are made to more typical *E.coli* and plant codons. *E.coli* transformants for each *merA* modification prepared to date were analyzed for their ability to reduce ionic mercury to volatile, elemental mercury. Each stepwise alteration of the *merA* sequence allowed increased Hg^o evolution by the transformed cells (data not shown).

Table 1. Comparative *merA9* gene transcription and whole plant expression. Shown are relative rates of *merA9* mRNA abundance, ability to evolve elemental Hg, and resistance to ionic Hg-containing growth medium by *merA9*-transformed *A.thaliana* lines and control lines. The three *Arabidopsis* control lines: RLD, ACT7/GUS, and 35S/GUS; do not contain any form of *merA* construct. All others are independently transformed lines or sublines for the *merA9* gene.

Plant Line	Relative *merA* mRNA[1]	Relative Hg^o Evolution[2]	Hg^{++} Resistance[3]
1A	0.86	2.87	+++
1B	1.00	3.07	++++
1C	0.78	2.75	+++
5B	0.49	2.17	++++
2A	0.51	1.70	++++
4D	0.32	1.33	+
7A	0	0.93	--
RLD	0	1.00	--
ACT7/GUS	0	1.21	--
35S/GUS	0	1.06	--

[1] Relative *merA* mRNA values are based upon quantified northern blots of total RNA using *merA9* probe. *MerA* mRNA values were normalized by secondary labeling with a probe for 18s rRNA to account for uneven loading of the samples on the agarose gel.

[2] Relative Hg^o evolution rates by whole plants of total Hg^o for a 10 minute sampling period to determine ng Hg^o evolved/mg plant/min. Values are normalized against the wildtype strain, RLD, as equal to 1.

[3] Hg^{++}resistance as determined by plant growth assays. Growth was defined as germination and production of true roots and true, expanded leaves. Symbols represent >50% germination and continued growth and development at up to the following [Hg^{++}]: -- = 0 μM, + = 25 μM, ++ = 50 μM, +++ = 75 μM, ++++ = 100 μM Hg^{++}.

Development and Characterization of *merA-Arabidopsis* plants

In an indirect assay for MerA enzymatic activity in transgenic *merA9-A.thaliana*, 12-15 day-old seedlings were examined for their ability to evolve Hg^o from a buffered 25 μM Hg^{++} solution. The assays were performed with 10-30 mg of plant material (6-10 seedlings), for ten minutes with mercury vapor analyzer sampling each minute. The *merA9* lines evolved Hg^o at

approximately three times the rate of the control (*merA-*) lines (see Table 1). These data demonstrate the substantial gain of function conferred by the *merA9* gene in many of the transformed lines. Several sublines derived from Kan^R/Hg^R selected transformants display little or no evidence of *MerA* enzyme activity, probably due to Mendelian segregation of the gene from these sublines.

merA9-A.thaliana and control seeds were placed on Hg^{++}-containing medium and assayed for resistance. The controls failed to germinate on $[Hg^{++}] > 50$ µM, though seeds occasionally germinated and grew briefly at lower levels. The *merA9* seeds germinate and grow vigorously to flowering at up to 100 µM. Of special note is the slightly less vigorous growth by the *merA* plants on mercury-free (0 µM) medium. We suspect that this may be due to low-level affinity of the MerA enzyme for other divalent metallic cations, and may be disruptive of normal metabolic processes involving ions such as $Zn2^+$, Cu^{2+} or Mg^{2+}.

Northern blot analysis of *merA9-A.thaliana* lines was performed for detection and quantitative comparison of *merA9* mRNA. Total RNA extractions of T3 generation *merA9* plants were run on agarose-formaldehyde gels, transferred to filters and hybridized with radioactively labeled *merA9* insert. We obtained *merA9* -specific bands for most of the transgenic lines, with only nonspecific background staining visible in a few of the *merA9* sublines and the three control lines. Rehybridization with a probe for the 18S rRNA band served to quantify the loading of the samples for relative *merA9* mRNA abundance determination. As shown in Table 1, *merA* mRNA values are closely related to both level of mercury resistance and relative mercury evolution among the transgenic and control plants.

This research demonstrates that sequence modification of a region of the *merA* gene was sufficient to confer ionic mercury resistance to the plant species *Arabidopsis thaliana*. We intend to continue the analysis of each of the currently modified *merA* constructs in plant systems, especially in light of the increased Hg^{++}-reducing ability observed in *E.coli* with each successive version of the gene. We will further characterize the genetic makeup of the *merA9* plant lines by genomic Southern blot analysis to assist in our understanding of the variation among lines and the segregation patterns between sib sublines. We are also preparing a series of metabolic uptake analyses to elucidate the effect that the gene may have upon normal metallic ion metabolism, especially in the absence of Hg^{++}. We speculate the poorer growth of the *merA* lines may be due to effects upon the normal metabolism of other divalent metal cations, such as Mg^{++}, Ca^{++}, and Zn^{++}.

We are most interested in the development of *merA-Liriodendron*. We feel that phytoremediation may be most effectively performed in such a system due to specific characteristics of trees. Trees are deeply soil mining plants and would be more capable of penetrating a greater underground volume than most hyperaccumulator species. The majority of aerial mass in a tree is non-living tissues - perhaps ideal for accumulation and sequestration of hazardous substances. In addition to the long life span of trees, their prolonged juvenile period allows for 10 or more years before the issue of outcrossing by a genetically engineered species is a problem. We are confident that we will be capable of obtaining transgenic *Liriodendron* for the *merA* constructs using protocols developed in our lab for genetic transformation and regeneration of yellow-poplar. Our preliminary success with *Arabidopsis* has encouraged us to continue to develop *merA-Liriodendron* and to determine their effectiveness for the reduction of toxic, ionic mercury ion in laboratory studies. We feel that this research has great potential for the development of an effective pilot phytoremediative system.

As we continue to characterize our *merA* plant systems, issues relevant to the use of these organisms in pollution control and abatement projects become important for consideration. There remains concern about the introduction of transgenic plants into the environment, though much of this fear will probably diminish as their safety is demonstrated in field trials and the value added characteristics of genetically engineered products are realized. Additionally, genetic tools are becoming increasingly available to prevent the unwanted escape of transgenic germplasm into wild populations (Goldberg et al., 1993). We can apply the genetic control of pollen and seed sterility with our *merA* plants, as well as further ensure no escape occurs by harvesting of these trees prior to sexual maturity. Concerns may also be raised about the deliberate evolution of gaseous mercury from polluted sites into the atmosphere. Precautions should certainly be taken to avoid releasing excessive levels of elemental mercury near population centers, but the substantially greater hazard is to ignore the high concentrations of highly toxic, ionic mercury at polluted sites. Furthermore, mercury-contaminated areas are continually volatilizing elemental mercury due to soil chemical reactivity and biological processes by microbial and plant populations adapted to such sites (Barkay et al., 1992), and thus even an aggressive phytoremediation program on selected areas would not significantly effect the current levels of atmospheric mercury. Therefore, we propose that the use of *merA* trees could be used to accelerate this process on our most urgent and sensitive sites and alleviate the hazards to local habitats and adjacent water supplies.

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TOPWORKING YOUNG SCIONS INTO REPRODUCTIVELY-MATURE LOBLOLLY PINE

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Abstract.--Scions from young trees ranging in age from 1-5 years were grafted into the upper and lower crowns of reproductively-mature loblolly pine seed orchard trees. The scions were randomly collected from a 12-clone, first-generation mix of families used as a check lot in Weyerhaeuser Company's progeny tests. Scions for tree ages of 2-5 years were collected from four different progeny test sites. The 1-year-old scions were collected from nursery-bed seedlings. Ten scions of each age class were grafted into ramets of four second-generation clones in Weyerhaeuser Company's seed orchard at Lyons, GA. On each ramet, five grafts were in the lower crown and five were in the upper crown. A total of 200 grafts were completed in February 1994.

In March 1995, scions were measured for shoot growth, number of branches, number of female strobili, and number of pollen clusters. Survival of grafts in the upper crown was 97%. Female strobili were produced on scions from all age classes and ranged from 21% of age one grafts to 80% of the age four surviving grafts. A total of 247 female strobili were produced on 53 grafts in the upper crown. No female strobili were produced in the lower-crown grafts.

Pollen did not occur on any of the 1-year-old grafted scions. In the lower crown, scion ages from 2-5 years produced pollen clusters on 33 of the 75 surviving grafts. The percent of grafts with pollen ranged from 15% on age 2 scions to 75% on age 5 scions. Pollen was less frequent in the upper crown but did occur on age 3-5 grafts.

Topworking allows tree breeders to produce female strobili one year after selection on scions collected from trees that are 1-5 years old. Pollen can be produced in one year on topworked scions in lower crown from scions that are collected from trees 2-5 years old. The topworking procedure can be used to greatly reduce the generation interval in loblolly pine and to accelerate the breeding cycle for genetic improvement.

Keywords: *Pinus taeda*, female strobili, pollen, tree breeding, seed orchard, grafting

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INTRODUCTION

The breeding cycle in forest trees is the time required from the selection of a genotype in one generation until the selection of a new genotype from the succeeding generation. This breeding cycle depends on the generation interval of the species (time required to produce adequate seed for testing and selection) and the age that genetic selections can be made from the next generation. Obviously, tree breeders want to reduce the time required for the cycle. However, because forest trees typically have a long, non-flowering juvenile stage of development, the breeding cycle can be delayed for as long as 10 to15 years.

Topworking, or the grafting of young scions into mature trees, has been used in an attempt to reduce the generation interval in conifer species. Robinson and Wareing (1969) grafted scions from both juvenile seedlings and nine-year-old trees of European larch (Larix decidua) and Japanese larch (Larix leptolepis) on to aged and flowering shoots of older trees. There was very little cone initiation in juvenile scions of either species but some of the nine-year-old material did produce both female and male strobili.

Barnes and Bingham (1963) also attempted to induce flowering of seedling scions by grafting into the crowns of older trees. The scions were five years old when grafted into 28-year-old mature trees, but none of the scions produced strobili. Other methods tested also proved unsuccessful in inducing or stimulating early strobilus production in western white pine.

Greenwood and Gladstone (1978) demonstrated that both male and female strobili could be produced on scions from one-year-old loblolly pine (Pinus taeda L.) seedlings topworked throughout the crowns of large seed orchard trees. Two to three years after topwork grafting, 50 percent of the surviving grafts produced male strobili. Female strobili occurred on about 20 percent of the scions. Topworking has not been used for tree breeding because an accelerated breeding schedule of 5 years was developed by Greenwood (1993) using a greenhouse environment to stimulate early female and pollen production for breeding purposes.

Burris and Williams (1991) further reduced Greenwood's five-year breeding schedule to four years by applying flower stimulation treatments in the same year that scions were grafted to greenhouse rootstocks. Female strobili were produced one and two years after grafting but pollen was not available until 26 months after grafting. The time required for pollen production was reduced to 13 months by Bramlett et al. (1995) using a surrogate pollen induction (SPI) method. In SPI, scions from selected individual trees in five-year-old progeny tests were topworked into the lower crown of ramets in a second-generation seed orchard. The seed orchard trees had been established for 8 years and were producing abundant pollen in the lower crown. Topworked scions produced pollen strobili on 57% of the surviving grafts 13 months after grafting, and produced enough pollen for breeding four of the five selected individuals.

Based on the success of SPI, a similar method was tested to produce female strobili for breeding purposes. We observed limited female strobili production on scions grafted into the upper

crowns of receptor clones in 1993[2]. The objective of the 1994 study was to evaluate the strobilus production on young scions topworked into the upper and lower crowns of reproductively-mature seed orchard trees.

MATERIALS AND METHODS

Genetic Material

Scions were collected from trees that ranged in age from one to five years. Scions from each age class were part of a 12-clone, first-generation mix of trees used as a check lot in Weyerhaeuser Company's progeny tests. This mix had an equal number of seedlings from each of 12 clones, thus, each age class represented identical genetic material. Obviously, individual trees selected for scion collection could not be identified as an individual family, but the composite sample represented a minimum of 15 trees from the same genetic sources. Scions from tree age classes two-five were collected from progeny test sites. Scions from age class one were collected from seedlings growing in a nursery bed.

Four clones were selected in Weyerhaeuser Company's second-generation loblolly pine seed orchard at Lyons, GA. Receptor clones were 10 years from seed orchard establishment at the time of topworking in February 1994. The seed orchard is intensively managed with annual fertilization, mowing, herbicide application, and pest management. The receptor clones were known to be good female and pollen strobili producers.

Grafting Method

Scions were grafted in February 1994. The scion was prepared by removing all needles and making axial cuts starting just below the terminal bud on opposite sides. A wedge was created exposing cambium layers on both sides of the scion. A total of 10 rootstock branches were chosen, 5 in the upper crown and 5 in the lower crown, for each receptor tree. An axial cut was made just below the terminal bud of each rootstock branch reaching into the pith area. This cut continued downward for another three to four inches and the prepared scion was inserted into the slit. After matching at least one side of the cambium layers of the scions to the cambium layers of the exposed rootstock slit, the scion was secured in place with a rubber budding strip wrapped in an overlapping spiral pattern. Hot wax (175-200°F) was applied to the completed graft for protection from desiccation. Two to four weeks after the graft emerged through the wax, the rootstock branch was cut back and the rubber budding strip removed.

Experimental Design

A split-plot experimental design was used with receptor clones considered blocks and scion age the treatment variable. A whole plot was an individual ramet of the receptor clone with crown location as the split plots and individual branches as observations within the subplots. The receptor clone was considered a random variable and the treatment (age class) was a fixed variable.

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[2]Data on file, U.S. Forest Service, Macon, GA.

Survival of scions in both the upper and lower crown locations was recorded 13 months after grafting. Shoot elongation and the number of branches that developed in 1994 were recorded in December of 1994. The number of new shoots in spring of 1995 was recorded in March 1995.

The number of female strobili was recorded on each surviving scion in March 1995. Pollen strobili were recorded as clusters on individual shoots and ranged from a single strobilus to 25 strobili per individual shoot. The observation date for male and female strobili was about two weeks after maximum receptivity and pollen release.

The data was analysed using the SAS (SAS Institute, Cary, NC) procedure for mixed models. Contrasts were computed for all possible comparisons (10) of the five scion age classes for both the upper and lower crown levels. For each response variable tested, mean separation was computed at the 5% level of probability. This value gives a rigorous t-test for significant differences between individual means as the comparisonwise error rate (CER) is equal to the experiment error rate (EER) divided by the number of comparisons. Thus, for means to be significantly different CER = EER/C or .05/20 = 0.0025. All contrast tests were completed with CER = 0.0025.

RESULTS AND DISCUSSION

Survival and Shoot Growth

The survival of topworked scions was excellent for all age classes. Ninety-seven percent of the grafts in the upper crown survived one year after grafting, and 91% of the grafts on the lower crown survived (Table 1).

Table 1. Survival and growth of young scions topworked into the upper and lower crowns of reproductively-mature loblolly pine seed orchard trees.

RESPONSE VARIABLE	CROWN LEVEL	SCION AGE				
		1 YR	2 YR	3 YR	4 YR	5 YR
		(%)				
Survival	Lower	80a	95a	95a	100a	85a
	Upper	95a	100a	95a	100a	95a
		(in)				
Shoot lengh (December 1994)	Lower	6.0a	7.4a	6.4a	6.7a	5.4a
	Upper	15.8a	18.0a	18.3a	17.8a	14.6a

The amount of shoot growth on grafts in the upper crown was phenomenal during the 1994 growing season with an average length of 16.9 inches. Some grafts, in the most favorable locations in the upper crown, grew more than 30 inches (76cm) in length during the summer of 1994.

Position in the tree crown had a very pronounced effect on the growth of the grafted scion. Grafts placed directly on large primary branches in the upper crown produced the greatest shoot growth. In general, large branches originating from the buds at the base of the annual shoot, were the most vigorous branches in the tree crown and produced the greatest shoot growth.

Shoot growth in the lower crown was significantly less than in the upper crown with an average of 6.4 in. per branch. These lower crown grafts were on secondary and tertiary branches and associated with much less shoot growth, in general, in the lower crown.

The grafted scions exhibited juvenile growth characteristics even though they were in the upper crown of second-generation seed orchard trees. Although we did not quantify these juvenile characteristics, we observed that the needles were shorter, smaller in diameter, and frequently showed some winter chlorosis that would be characteristic of seedlings in comparison to mature foliage. Grafted scions from young trees also appeared to have more branches per shoot length than adjacent branches from the interstock. Thus, in appearance, the young scions looked like a seedling growing in the top of a large tree crown.

Number of Branches

As the amount of flower production may be a function of the number of shoots, we counted the number of shoots per branch in December of 1994 and again in March of 1995 (Table 2). The 1994 branches contained multiple buds, so the number of 1995 branches increased substantially from the 1994 count.

Table 2. Number of branches on young scions topworked into the upper and lower crowns of reproductively-mature loblolly pine seed orchard trees.

RESPONSE VARIABLE	CROWN LEVEL	SCION AGE				
		1 YR	2 YR	3 YR	4 YR	5 YR
		(no)				
Number of branches (December 1994)	Lower	1.9a	3.6a	2.2a	2.2a	2.3a
	Upper	8.2a	7.9a	5.9a	5.6a	4.7a
Number of branches (March 1995)	Lower	5.8a	6.3a	4.5a	4.6a	2.6a
	Upper	31.6a	30.8a	17.8ab	15.4b	12.8b

There was no statistical difference between crown levels or among scion ages for the number of branches in the upper or lower crown in December 1994. In 1995, the mean of 4.8 branches per shoot in the lower crown was significantly different from the mean of 21.7 shoots per branch in upper crown level. And, in the upper crown, the younger age scions also had more

branches per shoot than the older age scions. Scion age one grafts averaged 31.6 shoots compared to scion-age five grafts with 12.8 shoots per branch.

Strobili Production

In the upper crown, scions from all age classes produced some female strobili one year after grafting. None of the 91 surviving grafts in the lower crown produced female strobili on any scions regardless of the age of the collected scion. Scion age influenced both the frequency and number of female strobili produced. Of 20 attempted grafts with age one scion material, 17 grafts survived and 5 of those produced a total of 11 female strobili (Table 3). In contrast, all 20 attempted grafts from age 4 scion material survived and 16 grafts (80%) had female strobili for a total of 90 strobili.

One-year-old scions in either the upper or lower crown did not produce pollen on any grafts. However, scions collected from 2-year-old trees produced pollen strobili on 3 of the 19 surviving grafts (15%) in the lower crown. Ages 3-5 scions produced pollen in both the upper and lower crown. For age 5 scions, 75% of the surviving grafts produced pollen strobili in the lower crown and 52% produced pollen in the upper crown (Table 3).

Table 3. Percentage of live grafts with strobili and number of female and male strobili on young scions topworked into reproductively mature loblolly pine seed orchard trees.

RESPONSE VARIABLE	CROWN LEVEL	SCION AGE 1 YR	2 YR	3 YR	4 YR	5 YR
		(%)				
Live grafts w/ female strobili	Lower	0a	0a	0a	0a	0a
	Upper	21b	70a	59a	80a	52ab
Live grafts w/ male strobili	Lower	0c	15bc	26b	55a	75a
	Upper	0b	0b	31ab	25ab	52a
		(no)				
Number female strobili (total no.)	Lower	0a	0a	0a	0a	0a
	Upper	11a	62ab	53ab	90b	31ab
Pollen clusters (total no.)	Lower	0a	6a	11a	20a	19a
	Upper	0a	0a	17a	6a	20a

The mechanism of strobili induction on young topworked scions into reproductively-mature trees is not clearly understood. It appears that the location of the grafted scion in the tree crown is the major factor. Furthermore, it appears that the flower-promoting stimulus, perhaps GA, is produced locally in the reproductively-mature branches and is translocated to the newly-grafted material. Bramlett et al. (1995) found that branch girdles had no effect on the survival or

the number of pollen clusters produced per graft on branches that were already heavy pollen producers. This indicated a localized stimulation factor for pollen induction or perhaps transportation in the xylem, but not in the phloem, as the phloem would be blocked by the branch girdle.

Nineteen-ninety-five was considered a good flowering year and this obviously favored abundant female and male strobili production on the topworked scions. It is certainly plausible that a reduced effect would be observed in poorer flowering years and perhaps a greater response in bumper years. Our interstocks were not screened as high flower producers. Perhaps interstocks exist that are even more conducive to the promotion of male and female strobili. Further research is needed to evaluate many interstocks with the objective of identifying one or more specific interstocks that are especially effective in the promotion of strobili on topworked grafts.

We offer no rational explanation for the apparent, but not statistically significant, reduction in female strobili produced on age five scions compared to age four scions. This appears to be an artifact of the data and is possibly related to the random selection of the scions from the family mix. Or, by chance, the scions could have been grafted to less vigorous branches in the 5-year-old treatment. A larger study is needed with known genetic identification over a range of age classes to separate the components of variance attributed to scion age vs. scion genotype.

CONCLUSIONS

Topworking young selections into reproductivley-mature tree crowns greatly reduces the generation interval in loblolly pine. We think that the amount of strobili production observed on topworked scions was conservative because not all scions in the upper crown were grafted on the most vigorous branches, nor did we totally release the grafted scion by taking out all competing branches.

The scions that grew and flowered best were grafted on vigorous primary branches or on secondary branches with the tip of the primary branch removed. In this study only light pruning around the graft was done when the grafts were released by cutting away the tip of the branch distal to the graft location. Based on our observations of the 1994 topworked scions, we recommend that grafts for tree breeding be made, whenever possible, on the major branches in the crown. Typically, these branches are from the basal whorl of branches for a given year of stem growth. If grafts cannot be completed on the primary branch because of a large difference between scion and branch diameter, graft onto a secondary branch and prune out the primary branch tip. When the grafts are released, also cut away other branches that may compete with the grafted scion. The objective is to give the graft maximum opportunity to develop into a major branch within the tree crown.

The fact that pollen strobili were produced in the upper crown grafts suggests a somewhat different strategy than our original approach. We grafted scions in the upper crown for female strobili and scions in the lower crown for pollen production. The problem of using the lower crown is that even though these grafts consistently produced pollen, shoot development was rather restricted. For example, we observed an average shoot growth of 6.4 inches in the lower crown grafts compared to 16.9 inches in the upper crown. This greater growth is also associated with

more branches and thus more flowering points the next year. Our recommendation is to graft in the mid crown on vigorous primary branches for pollen production. We suggest that 5-10 grafts be made for each cross where the selection will be used as a female parent, and about 5 grafts in the mid crown if the selection will be used as a pollen parent.

The utility for breeding very young genetic selections that have been topworked into reproductively-mature seed orchard trees remains speculative until validated with actual results. Previous to this study, female strobili had not been produced one year after grafting on scions collected from one-year-old seedlings. In this study, female strobili were produced on one-year old seedlings but pollen was not produced until scions were from two-year old seedlings. It would appear feasible to select as early as age two and complete the breeding one year later. This would then shorten the generation interval to a minimum of three years and the breeding cycle to five years for loblolly pine. This procedure assumes that early genetic evaluation procedures are valid for two-year-old progeny. Otherwise, the minimum breeding cycle using topworking is three years plus the age of selection from progeny tests.

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ANALYSIS OF GROWTH, FORM AND BRANCHING TRAITS IN AN F2 POPULATION OF THE *Pinus elliottii* x *Pinus caribaea* INTERSPECIFIC HYBRID USING RAPD MARKERS

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Abstract.--The developmental biology of the haploid conifer megagametophyte has been exploited using dominant RAPD markers to construct a reference genetic map for an individual *Pinus elliottii x Pinus caribaea* F1 hybrid tree forming part of a three generation pedigree. This map incorporates 186 markers across 17 linkage groups. At 1595cM, the map is estimated to cover 80% of the genome at an average marker density of 8.6cM. Using this reference genetic map with phase known, genotype information obtained using dominant RAPD markers in the F2 population was successfully used to determine full genotype classification. This genotype information was used to map QTL for five growth and form traits and seven branching traits in six year old trees. A total of 57 putative QTL were identified for all 12 traits examined. Considerable overlap existed between QTL identified for under and overbark diameter and bark thickness, and between QTL for branch angle, regularity of branch spacing, ramicorn number, and occurrence of double leaders, suggesting common genetic control of physiologically related traits. Detailed analysis of QTL for bark thickness indicated cryptic genetic variation not evident from the phenotype of either parent, as well as additive, dominant, overdominant and underdominant modes of gene action. Loci whose effects are stable across environments and specific to particular environments were also indicated. An approach was developed to accumulate confidence in QTL results obtained from small populations: potentially a common limitation to QTL mapping in forest trees. The implications of these findings for both conventional tree breeding and marker assisted breeding are discussed.

Keywords: Genetic mapping, QTL mapping, RAPD, genotyping, F2 intercross, interspecific hybrid, tree breeding, *Pinus elliottii* x *Pinus caribaea*.

INTRODUCTION

The F1 hybrid between *Pinus elliottii* and *Pinus caribaea* is the most economically important forest plantation species in sub-tropical Queensland, Australia. The performance of this hybrid is at least equal, and often superior to the pure species of both its parents in all commercially important traits. Breeding and improvement programs over the past thirty years have achieved significant gains in yield and stem quality of both the parental species, and identified parents with specific hybridizing ability (Nikles and Newton 1991). Gains achieved through breeding have been matched by the development of an operational vegetative propagation system, and practices to maintain juvenility. These combined developments are poised to be capitalized on by a shift from family to clonal forestry (Haines and Walker 1993a).

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In the context of this advanced breeding and propagation program, future gains will be facilitated by a precise understanding of the specific effects and interactions of alleles from each parental species at quantitative trait loci (QTL) influencing commercially important traits. Such knowledge may potentially influence the efficiency and rate of improvement in each parental species, selection of parents from each species for hybridization, within family selection of F1 or F2 hybrid individuals for clonal propagation, and the capacity for prospective breeding of superior hybrids from advanced hybrid individuals.

In an appropriate pedigree, genetic mapping provides a systematic framework for the investigation and quantitation of allelic effects and interactions. In an F2 self or intercross population, the effect of substituting both one and two alleles at a given locus may be followed with co-dominant molecular markers, and hence both additive gene effects and dominance may be quantified. For this reason, Paterson et al. (1991b) refer to an F2 self, segregating in the classical 1:2:1 Mendelian ratio for the three possible genotypes, as the 'ideal' population for studying gene dosage effects (or gene action) in diploids. This contrasts with testcross or half-sib populations which only permit characterization of the effect of a single gene substitution at any given locus (Paterson et al. 1991b)

Although the 'ideal' QTL mapping population for studying gene dosage effects and intra-allelic interactions is rare in most commercial tree breeding programs, such a population was available within the Queensland Forest Service tree breeding program. This population thus provided a unique opportunity for QTL investigation in forest trees. The size of this F2 population was restricted to just 54 individuals, far below the few hundred to few thousand estimated to be required to detect QTL effects of 1% to 5% of the phenotypic variance (Weller 1992). Yet this unique population represented a valuable model to gain a preliminary indication of the architecture of the most significant loci influencing a range of quantitative traits in forest trees, the mode of action underlying these loci, an appreciation of the value of an F2 population for QTL mapping and guidance for the experimental design of future studies. Finally, it provided the opportunity to develop an approach to extend the utility of dominant RAPD markers to provide co-dominant genotype information (Dale and Teasdale 1995).

MATERIALS AND METHODS

Experimental Pedigree

A unique three generation pedigree comprising selfed F2 progeny of an F1 individual from an interspecies cross, was identified within the Queensland Forest Service hybrid breeding and evaluation program. *Pinus elliottii* E1-023, planted around 1934, was used as the maternal parent, and *P. caribaea* CH6-029, planted around the early 1950s, was used as the pollen or paternal parent. Both parents were selected for intercrossing on the basis of their superior growth and form characteristics relative to other individuals within each species. The F1 hybrid family produced from this cross was planted at Beerwah in 1962. A ramet of one of the plus trees selected from this F1 family, EH4, was selfed to provide F2 progeny, planted at Beerwah (24) and Tuan (32) in May/June of 1987. Of the seedlings planted, 23 remain surviving at Beerwah, and all 32 have survived at Tuan.

Site Description

Temperature and rainfall patterns were similar between sites. Soil type at Beerwah is a deep Red earth, rated as plantability category 'A'. Soil type at Tuan is a Lateritic podzolic, rated as plantability category 'B', inferior to the soil type at Beerwah. Prior to establishment of the experiment, the Tuan site was an improved pasture. Beerwah is a second rotation site, having been under *P. elliottii* and *Pinus taeda* since 1932.

Assessment of Traits

All F2 trees were measured in June 1993 at six years of age for the following quantitative and quasi-quantitative traits: i) over and underbark diameter at breast height, ii) height, iii) average bark thickness, iv) stem straightness, v) number of ramicorns, vi) number of double leaders, vii) average branch angle and viii) regularity of whorl spacing (co-efficient of variation for whorl spacing expressed as a percentage). Branching traits were measured on all branches between breast height (1.3m) and 3/4 of total tree height.

Genetic Mapping of the F1 Hybrid, EH4

A total of 520 RAPD primers (Operon™ kits A to Z, 20 primers per kit) were screened using the F1 hybrid, EH4, and its two parents, E1-023 and CH6-029. RAPD reactions were performed, and marker segregation data collected, on a set of 92 megagametophytes, commencing with the primers generating the highest number and quality of putative polymorphic markers. Linkage analyses for the construction of a genetic map from marker segregation data were performed using Macintosh MapMaker V1.0 (Proctor et al. 1990).

Genotyping of the F2 Population

The dense genetic map constructed for EH4 was used as a reference to select markers for the purpose of determining their pattern of segregation in the F2 progeny of this individual. Where possible, pairs of markers closely linked in repulsion were selected with a spacing of around 20cM between marker pairs. Alternatively, alternating maternal and paternal markers were selected at around 10cM intervals along each linkage block. When feasible, markers were selected to minimize the number of primers and hence RAPD reactions required.

Inference of Fully Classified Genotypes

F2 genotype data was arranged in a spreadsheet with individuals in columns and markers in rows. Markers were arranged in the same order as they occurred in each linkage group. Three contiguous columns were generated for each individual. Maternal genotypes were aligned in the first, and paternal in the second. By reference to a diagram of each linkage group, genotype was inferred for unknown regions of each linkage group within each individual, by assuming a chiasma to occur midway between known parental genotypes of opposite phase. The fully classified inferred genotype of each linkage block within each individual was entered in the third column. This portion of the spreadsheet was later extracted to compile a database suitable for QTL analysis with MapMaker

Proportion of Parental Genome Inherited

Based on the inferred fully classified genotype data, HyperGene™ software (Young and Tanksley 1989, 1991) was used to calculate the proportion of parental genome comprising each F2 individual, the overall average of each parental genome inherited in the F2 population, and the proportion of each F2 individual heterozygous and homozygous for the alternative parental alleles.

Quantitative Trait Analysis

Co-segregation analysis of genotype data with phenotype data to identify and characterize QTL for each trait assessed, was carried out by interval mapping (Lander and Botstein 1989). This procedure was performed using MapMaker/QTL V1.1 (Lincoln et al. 1992). A LOD threshold of 2.6, corresponding to a nominal significance level of about $P = 0.001$ per test or $P = 0.05$ for the entire genome, was used to declare the presence of significant QTL.

RESULTS

Site Effects on Trait Characteristics

Table 1 summarizes for both the Beerwah and Tuan sub-populations, the trait means and co-efficient of variation for all traits examined.

Table 1. Mean, co-efficient of variation and site effects for growth, form and branching traits in selfed F2 progeny of the *P. elliottii x P. caribaea* hybrid.

Site	Tuan		Beerwah		Difference Between Sites
	Mean	Co-efficient of Variation	Mean	Co-efficient of Variation	
Average Bark Thickness (mm)	18.3	17.4	19.0	15.8	NS
Over Bark Diameter (cm)	14.2	12.9	16.4	10.9	*SIG
Under Bark Diameter (cm)	10.6	10.8	12.6	11.8	*SIG
Height (m)	8.2	9.9	10.6	9.7	*SIG
Straightness	2.0	50.0	3.1	27.5	*SIG
Ramicorns per Tree	0.4	186.2	0.3	207.3	NS
Double Leaders per Tree	0.1	387.1	0.0	-	NS
Branch Angle (^{o} from vertical)	69.0	10.2	71.9	11.7	NS
Regularity of Whorl Spacing	0.6	30.9	0.5	42.8	NS

Genetic Mapping of the F1 Hybrid, EH4

Linkage analysis of 232 Mendelian markers produced a haploid linkage map for the F1 hybrid of *P. elliottii x P. caribaea* incorporating 186 markers across 17 linkage groups varying in size from 7 to 175cM, and comprising a total haploid map length of 1595cM. This map is estimated to cover 80% of the genome at an average density of 8.6cM.

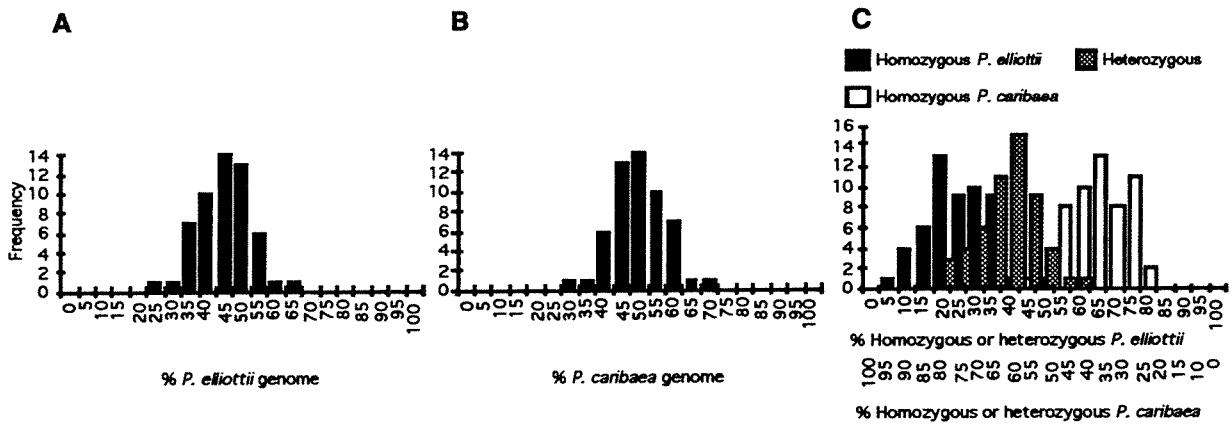
Genotyping of the F2 population and Inference of Fully Classified Genotypes

A total of 139 markers from the megagametophyte derived genetic map of EH4 were used to genotype its F2 progeny at an average density of 11.5cM (Dale et al. 1995). Linkage analysis using fully classified genotype data for the F2 generation revealed marker order throughout the genome to generally remain identical to that of the megagametophyte derived reference map for EH4, except for closely spaced markers. Overall map size differed by 8% between the haploid megagametophyte and diploid F2 populations.

Proportion of each Parental Genome Inherited in the F2 Population

Figure 1, parts a and b, illustrate the frequency distribution for the total proportion of *P. elliottii* and *P. caribaea* genome respectively, inherited in their F2 progeny. On average, the F2 population is comprised of 48.3% *P. elliottii* genome and 51.7% *P. caribaea* genome. This does not differ significantly (P = 0.05) from the expected 1:1 ratio. The minimum proportion of each parental genome inherited by any individual in the F2 was 29.2% and 34.5% for *P. elliottii* and *P. caribaea* respectively. The maximum proportion inherited was 65.5% and 70.7% respectively. In addition, the F2 population conformed to the expected 1:2:1 proportion of alleles heterozygous and homozygous for each parental genotype (Figure 1, part c).

Figure 1. Frequency distributions for: A) the percentage of *P. elliottii* and B) the percentage of *P. caribaea* inherited in their F2 intercross progeny, and C) the percentage of genome heterozygous or homozygous for each parental type.



Identification of Putatively Significant QTL

Simple, unverified results of single locus interval mapping using MapMaker/QTL software detected putative QTL exceeding the minimum LOD threshold of 2.6 for each of the five growth and form traits and four branching characteristics examined. Genomic regions influencing these traits are illustrated in Figure 2a for growth and form traits and in Figure 2b for branching traits. Figure 3 displays the QTL likelihood plots for bark thickness at Tuan, Beerwah and the two sites combined, for one linkage group carrying a region putatively influencing this trait.

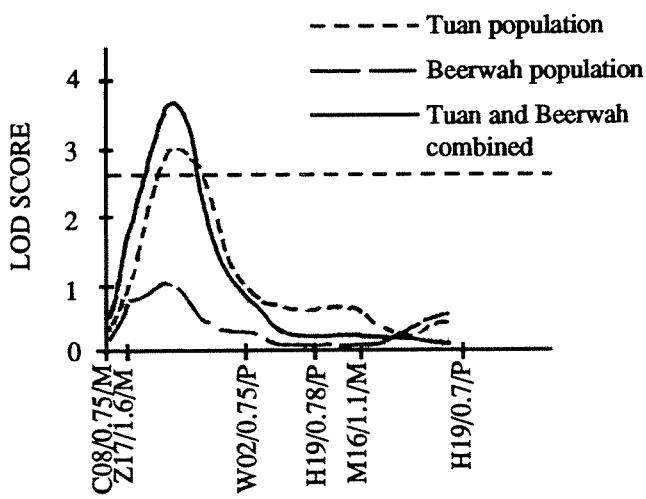
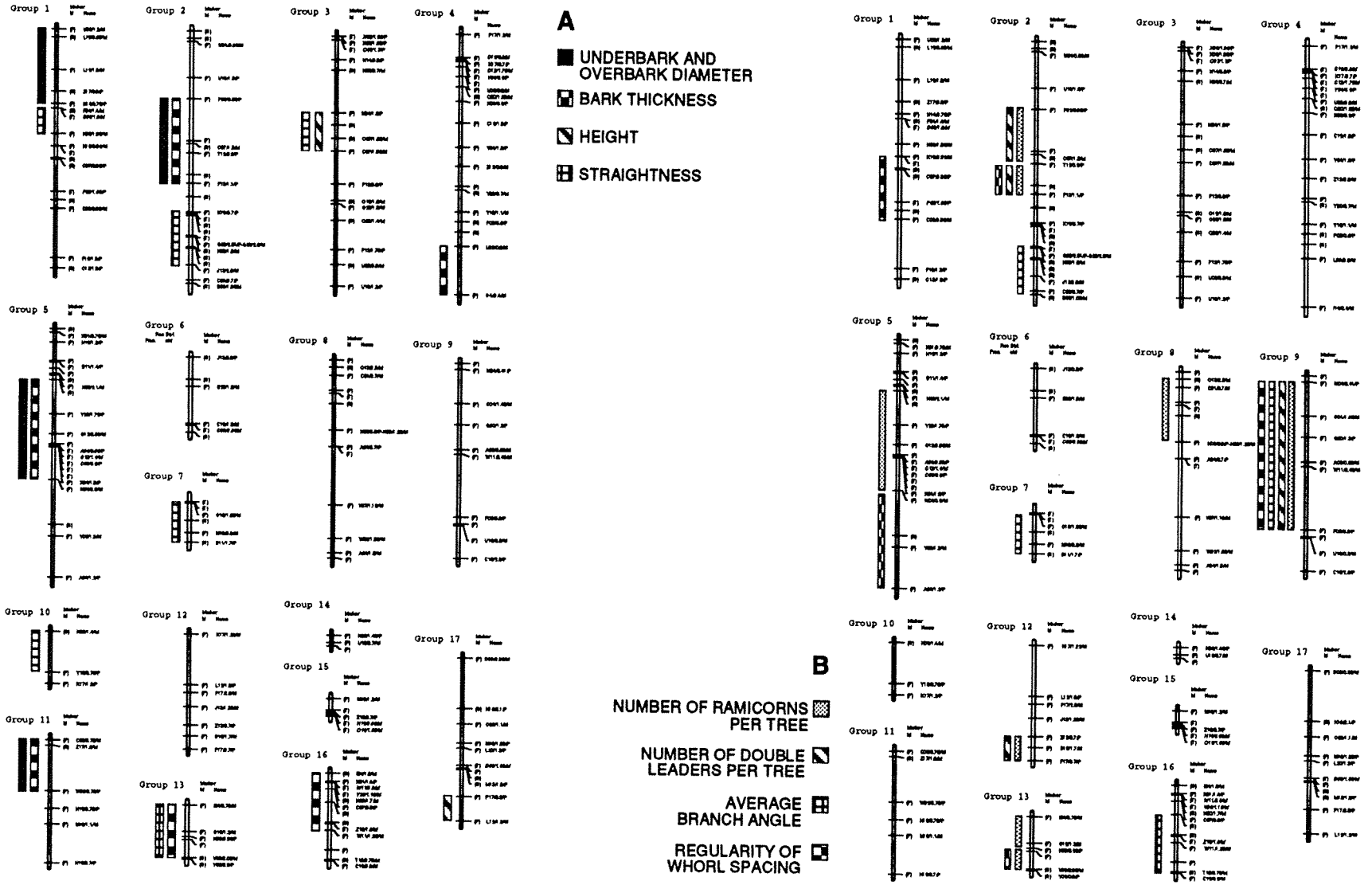


Figure 3. QTL liklihood plots indicating the LOD scores for bark thickness along linkage group 11 of the *P. elliottii x P. caribaea* interspecific hybrid individual, EH4. Likelihood plots are shown for the Tuan and Beerwah sub-populations of the F2 progeny to EH4, and for the the entire F2 family combined across both sites. The horizontal line at LOD 2.6 indicates the threshold for declaration of a QTL.

Using the notation 'X(Y)' to denote numbers of significant QTL (X) and numbers of genomic regions (Y) showing a consistent rise in the LOD score plot for each data set, the following numbers of putative QTL were detected for each trait: average bark thickness, 3(4); diameter at breast height over bark, 2(2); diameter at breast height under bark, 0(4); height, 0(2); straightness, 3(3); number of ramicorns, 6(2); number of double leaders, 4(0); average branch angle, 1(3); and whorl spacing regularity, 3(2).

Figure 2. Putative genomic regions influencing: A) growth and form traits, and B) branching traits in the *P. elliottii x P. caribaea* interspecific hybrid.



Similarity of QTL Between Physiologically Related Traits

Considerable overlap in the set of putative QTL influencing physiologically related traits is evident from Figure 2, parts a and b. Bark thickness, over bark diameter and under bark diameter, all share three putative QTL. Similarly, ramicorns and double leaders share four QTL. These two traits also share a putative QTL with branch angle and whorl spacing regularity.

Detailed Analysis of QTL Effects

Cryptic genetic variation not predicted by the phenotype of either parent was evident among branching QTL. The most significant QTL explaining number of ramicorns (LOD =12.2), double leaders (LOD = 78.1), branch angle (LOD = 2.0), and whorl spacing regularity (LOD= 3.6), all co-incided in the same genomic region, and all displayed an underdominant mode of gene action.

Analysis of the Tuan and Beerwah sub-populations for bark thickness indicated four putative QTL expressed in both environments. For each of these QTL, the LOD score for the Tuan and Beerwah sub-populations roughly summed to that for the combined data set. In contrast, the remaining three QTL identified for bark thickness were expressed in only one environment, and their LOD scores for the combined data set were less than those for either Tuan and/or Beerwah independently.

Three of the putative QTL for bark thickness displayed an additive mode of gene action, this trait being increased by *P. caribaea* alleles for one locus, and *P. elliottii* alleles for the other two. A dominant mode of action was displayed by two other QTL, bark thickness being increased by *P. caribaea* alleles at one locus, and *P. elliottii* alleles at the other. The remaining two QTL both displayed an underdominant mode of gene action, with the heterozygous genotype having thinner bark than the homozygote for either parent.

DISCUSSION

Inference of Fully Classified Genotypes from Dominant RAPD Data

With the availability of a megagametophyte derived reference map, this study has demonstrated that the limitations imposed by the dominant nature of RAPDs may be overcome to permit this technically simple and relatively cost effective marker system to be efficiently applied to the genotyping and analysis of diploid F2 populations. In terms of map resolution, dominant RAPD markers of alternating phase will provide genotype information equivalent to a co-dominant marker map of 62.5% the marker density. The average spacing of 11.5cM between RAPD markers in the present map thus provides a level of genotype precision slightly greater than an equivalent co-dominant marker map of 18.4cM average marker density. This precision is in the order suggested by Lander and Botstein (1989) for detection of QTL, and is unlikely to limit the precision of QTL location given the small population size (Darvasi et al. 1993).

Proportions of each Parental Genome Inherited in the F2 Population

Although both average genome and genotype composition in the selfed progeny of EH4 corresponded to the expected 1:1 and 1:2:1 Mendelian ratios, considerable variation was found in the proportion of parental genome inherited by specific individuals. The minimum composition of *P. elliottii* genome in any F2 individual was just 29.2%. Similarly, the minimum composition of *P. caribaea* genome for any individual was 34.5%. Both these extreme individuals thus have genetic compositions not greatly removed from that expected for an average backcross individual.

The results for both average genome composition and genome composition of extreme individuals correspond very closely with similar studies in tomato (Paterson et al. 1991b). As suggested by Paterson and co-workers, the broad variation found for proportion of each parental genome may provide an opportunity for marker assisted selection (MAS) in F2 and backcross hybrid families, both inbred and outbred. The range of parental genome combinations possible in F2 and backcross populations may potentially produce a proportion of individuals which transgress the performance of F1 hybrids. F2 and backcross hybrids are not presently favoured for operational plantation establishment due to the greater variability they exhibit relative to F1 hybrids (Garth Nikles, pers. comm.). However, the capacity to genotypically characterize F2, backcross and later generation hybrids, and to select genetically superior individuals, may change this perspective. Through clonal propagation of superior genotypes or transgressive segregants (De Vicente and Tanksley 1993), both the additive and nonadditive components of genetic variation inherent in such individuals would be captured. This strategy would also serve to speed the operational deployment of superior germplasm. As genetic markers become a more integral part of forest tree breeding programs, consideration should thus be given to breeding populations showing wide variability, in contrast to the more traditional goal of breeding for uniformity.

Identification of Valid QTL in Small Populations

Numerous putative QTL may be identified by 'black box' application of MapMaker/QTL software, but validation of these and rejection of spurious QTL is necessary to achieve a realistic picture of QTL architecture. This is particularly so for small population sizes (Knapp et al. 1992), and is likely to remain a persistent problem in the application of QTL analysis to forest trees where existing pedigrees, often limited in number and designed for conventional tree breeding purposes, must necessarily be employed. Given the small population size involved in this study, only QTL of large effect will be statistically significant, even though real QTL below the statistically significant threshold may be segregating. Conversely, small population size may result in detection of spurious QTL and biased estimates of QTL effects (Knapp et al. 1992).

Using the results for bark thickness to address this problem, it is evident that there is a consistent trend in the LOD curve between independent populations for some putative QTL. The putative QTL for bark thickness on linkage group 11 provides a suitable example (Figure 3). This putative QTL has a LOD of 1.04 in the Beerwah sub-population and 2.98 in the Tuan sub-population. Although the Beerwah sub-population is not significant, the probability that this same peak occurs in both independent data sets by random chance is $10^{1.04} \times 10^{2.98} = 10^{4.02}$, equivalent to a LOD score of 4.02, a statistically significant value given the appropriate LOD threshold for declaration of a QTL in the present species is 2.6.

Extending this proposition to other QTL for bark thickness, it becomes possible to construct a model for the architecture of this trait composed of environmentally stable, statistically significant and non-significant QTL, plus statistically significant, environment specific QTL. The former are characterized by supporting trends in the data from independent sub-populations, while the latter are specific results from a particular sub-population, unsupported by any other data. This model is illustrated in Figure 4.

In employing the model for bark thickness QTL presented in Figure 4, marker assisted breeding might potentially pursue the alternative strategies of selection for broadly adapted individuals, or selection of individuals adapted to specific sites (Burdon 1977). In the former case, one might select individuals carrying the appropriate alleles for QTL 3, 4, 5 and 6. In the latter, one might select for individuals carrying the same alleles as previously, but in addition for the Tuan site, the appropriate allele of QTL 1.

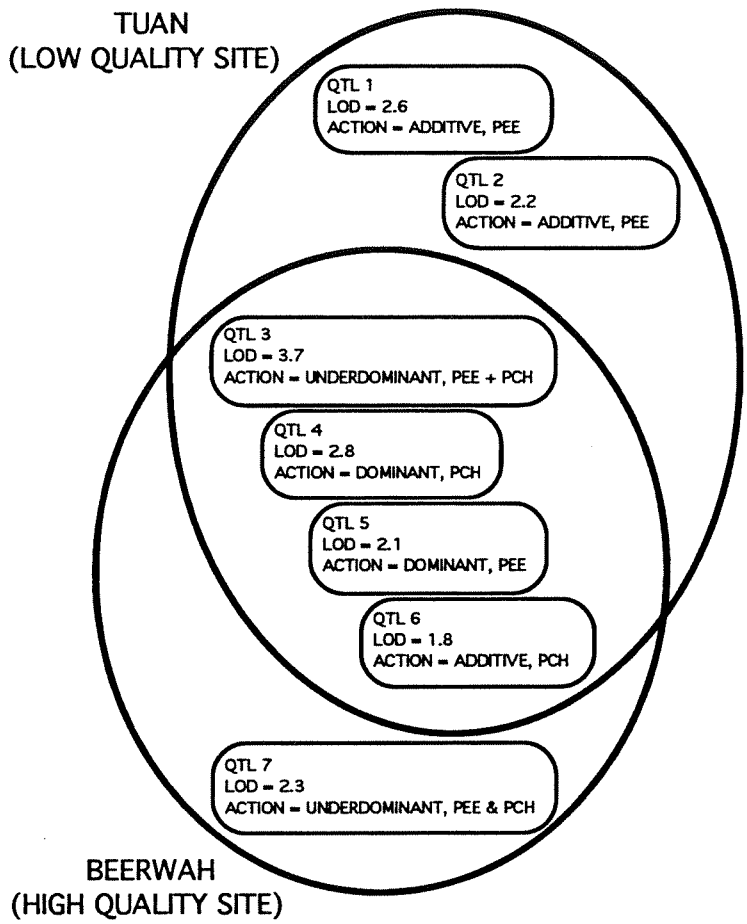


Figure 4. Model for the architecture of bark thickness QTL. This model comprises environmentally stable loci expressed at both Tuan and Beerwah, and evironment specific loci for both sites. For environment stable QTL, LOD score values below 2.6 are considered strongly indicative of putative QTL, since a consistent peak occurs in the likelihood plot for both independent sub-populations (a low probability event by chance alone). In addition, for environment stable QTL, the direction of allelic substitution effect and mode of gene action remain the same for both sub-populations and the combined population. For environment specific QTL, LOD score values over 2.6 are considered significant, but LOD score values below 2.6 provide weak, unsupported evidence for the presence of a QTL.

Similarity of QTL Among Physiologically Related Traits

Commonality among QTL controlling physiologically related traits may also add support to their validity. Bark and wood cells are each laid down in the stem of a tree by bidirectional differentiation of the cambium. An overlap of three QTL between bark thickness, overbark and underbark diameter (Figure 2a) is, therefore, not unexpected from a physiological perspective. These QTL comprise three of the four putatively identified above as environmentally stable QTL for bark thickness on the basis of consistency between sub-populations.

Similarly, QTL for branching characteristics display considerable commonality (Figure 2b). Measurements on numbers of ramicorns and double leaders, average branch angle and regularity of whorl spacing were all physically independent. Yet there exists commonality of loci between ramicorns and double leaders (4) ramicorns, double leaders and whorl spacing regularity (2) and ramicorns, double leaders, whorl spacing regularity and branch angle (1). Of these four branching traits, ramicorns and double leaders each violate the assumptions of normality in their trait distributions, and equal variance between alternate genotype classes (Weller 1992). This has led to inflated LOD scores of 12 and 78 respectively. In isolation, little confidence could be given to the QTL results for ramicorns and double leaders. However, commonality of QTL between related traits adds substantial weight to the validity of these results.

The results for growth and branching QTL appear to indicate strong genetic relationships between physiologically related traits. This alone could have valuable application in selection of

trees for conventional breeding programs. For example, ramicorns are rare on trees at Tuan, but common at Beerwah, while double leaders are common at Tuan and absent from Beerwah. However, these two traits share four putative QTL, each of which displays a consistent mode and direction of gene effect between sub-populations. Hence, it is possible that ramicorns and double leaders may be different manifestations of a similar genotype under different environmental influences. This may also explain low heritability estimates in the order of 0.04 to 0.08 reported for double leaders or 'forking' in *Pinus* species (Cotterill and Dean 1990). A practical application of this result in tree breeding may be to consider ramicorns and double leaders as a single trait, and give equal weight to selection against these. Further, as branch angle and whorl spacing regularity appear genetically related, these traits could also be factored into a selection index. Trees with irregularly spaced whorls and steeply angled branches, may give rise to progeny with an unacceptably frequent occurrence of ramicorns or double leaders under altered environmental conditions.

Mode of QTL Effects

For the range of the growth, form and branching traits examined, observed QTL effects included full dominance, additivity, overdominance and underdominance, with both parental alleles and their heterozygous combination variously increasing the value of the traits. This complex mode of cryptic genetic effects, where phenotype of the parental species is not a clear indicator of their progeny's performance, has been reported for a range of traits in other species (De Vicente and Tanksley 1993; Paterson et al. 1991a). Nor is the variety in mode of gene effects unusual. In summarizing the results of six studies, Beckmann (1991), found reports of QTL effects ranging from additivity, through dominance to overdominance.

Interestingly, each of the four QTL for bark thickness identified in Figure 4 as environmentally stable, display a consistent mode of gene action in each sub-population and the combined data set. This consistency is observed when each QTL is analyzed individually, and as a multilocus model. In contrast, the effects of bark thickness QTL identified as environment specific display varying modes of gene action between sub-populations. This result may provide an additional indicator to the validity of both occurrence and mode of effect for putative environmentally stable QTL.

The occurrence of cryptic variation in genotype effects appears to be inversely related to heritability (Paterson et al. 1991a). For such traits, the capacity of MAS to guide selection of parents and control allelic combinations created in their progeny, should prove more efficient in breeding than conventional phenotype based selection (Lande and Thompson 1990), particularly in view of the long generation intervals typical for most forest trees.

CONCLUSIONS

1. Dominant RAPD markers can be used to efficiently provide co-dominant genotype information in F2 populations of gymnosperms.

2. Molecular marker characterization of the broad distribution of parental genome composition in F2, backcross and other advanced generation populations holds significant potential for identification and clonal propagation of superior genotypes, and for introgression of desirable traits from related species.

3. Restricted population sizes characteristic of mature tree breeding families may not necessarily be a limitation to discovery of environmentally stable QTL, and initial application of MAS to forest trees.

4. Similarity of QTL among physiologically related traits may provide guidance for conventional phenotypic selection, particularly for traits strongly influenced by environment.

5. Guided breeding using molecular markers has the capacity to identify and exploit valuable alleles with effects not predicted by their parental phenotype, potentially increasing the efficiency of breeding and rate of genetic gain, particularly for traits of low heritability.

ACKNOWLEDGEMENTS

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CLONAL VARIATION IN FOUR-YEAR-OLD LOBLOLLY PINE IN COASTAL NORTH CAROLINA

Lewis John Frampton, Jr.[1] and Dudley A. Huber[2]

Abstract. -- Four clonal field trials of loblolly pine rooted cuttings were established in coastal North Carolina during 1990 and 1991. From measurements of these trials, age four variance components as well as full-sib family and clonal best linear unbiased predictions (BLUPs) for height, diameter at breast height (DBH), wood specific gravity and branching characteristics were calculated via restricted maximum likelihood (REML) techniques.

Age four full-sib family mean heritability estimates for height, DBH, wood specific gravity and branching traits were: 0.33, 0.53, 0.59 and 0.73-0.76, respectively. Clonal mean heritabilities for the same traits were considerably higher: 0.52,0.67, 0.74 and 0.80-0.90, respectively. Genetic gains (BLUPs) for the best full-sib family for age four height, wood specific gravity and number of branches/height were 5.2, 5.2 and 8.5%, respectively, over the mean of the second generation full-sib families being tested. Similar genetic gains for the best 5% of the clones were 7.9, 8.9 and 13.0%, respectively.

Although based on young trees, these data corroborate expectations that, when operationally feasible, clonal production of loblolly pine will offer substantial benefits over full-sib family production.

Keywords: *Pinus taeda*, clonal forestry, vegetative propagation, rooted cuttings, genetic variation, heritability, genetic gain, BLUPs

INTRODUCTION

Worldwide recognition of the benefits of clonal forestry has given rise to operationally successful clonal forestry programs in some species (Ahuja and Libby 1993). Although no operational clonal forestry programs currently exist for loblolly pine, industrial, governmental and academic research programs are underway to develop vegetative propagation technology for loblolly pine toward this end. The two most promising clonal propagation systems are 1) the use of rooted cutting technology for clonal testing and operational production coupled with a micropropagation system for maintenance of juvenile stock material and 2) somatic embryogenesis technology for clonal testing and operational production with cyrostorage techniques to maintain juvenility stock material (Handley et al. 1995).

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Although some general knowledge about the field growth and development of loblolly pine vegetative propagules relative to seedlings exists (Frampton 1987, Frampton and Foster 1993), little information is available concerning the field performance of loblolly pine clones. In 1989, the within-clone specific gravity variation for a single tissue-cultured loblolly pine clone was described (Frampton and Jett 1989). Based on wood samples from three sites in coastal Georgia and Florida, at age five years, the within-site variation in wood specific gravity for a single clone was 29% that of the open-pollinated family from which it was derived. In 1993, five-year-old data was reported for five clonal field trials of loblolly pine rooted cuttings in coastal Alabama, Georgia and Florida (McRae et al. 1993). Height, DBH, stem volume and the presence or absence of fusiform rust galls were measured. Strong clonal effects were present for all traits measured and some G X E interaction was detected for the growth traits. Select clones performed substantially better than commercial checklots for both growth and rust resistance at three sites.

In 1990, Weyerhaeuser's Loblolly Pine Rooted Cuttings Project developed a scoping initiative to assess the field performance of clones of loblolly pine rooted cuttings. From 1990 to 1992, a series of eight clonal rooted cutting field trials were established. Trials were established in each of the three Southern Timberlands regions existing at the time: four in North Carolina, two in Mississippi/Alabama and two in Arkansas/Oklahoma. This report describes some of the early findings from the four trials established in coastal North Carolina.

MATERIALS AND METHODS

Propagation

During 1987 and 1988, a loblolly pine hedge orchard was established at the Weyerhaeuser seedling nursery near Magnolia, AR. The seedlings planted in 1988 were from 20 second-generation full-sib crosses and 1 unimproved checklot. The crosses included in the hedge orchard were those demonstrating the fastest field growth based on data available at the time and also having sufficient available seed. The hedge orchard seedlings were regularly sheared and subjected to an intensive management regime involving irrigation, fertilization and pest control to produce cuttings for a research propagation program.

Cuttings collected from these hedges were rooted in a propagation greenhouse at Weyerhaeuser's Southern Forestry Research Center in Hot Springs, AR. Cuttings for the 1990 and 1991 plantings were collected and set in the winters of 1989 and 1990, respectively. The greenhouse was equipped with a gantry irrigation and injection system, a fog system, supplemental lighting, a wet-wall and fan cooling system and a forced air bottom-heat system. Cuttings were rooted in a peat:perlite medium in 10 cm^3 Ray Leach Supercells. Upon rooting, cuttings were hardened-off in an outdoor holding area prior to planting. Containerized seedlings were also cultured in the greenhouse for these field trials.

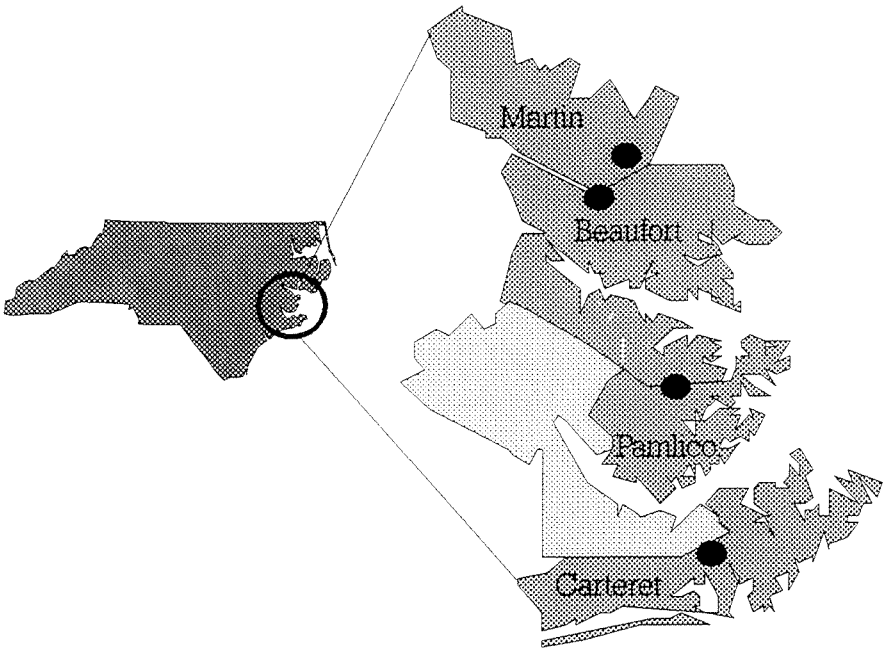


Figure 1. *Approximate locations of four loblolly pine clonal field trials in coastal North Carolina.*

Field

Two trials were established in May of 1990 in Pamlico and Martin Counties and two trials were established in April of 1991 in Beaufort and Carteret Counties (Figure 1). These sites represented organic-based, mineral-based and transition coastal plain soils. These cut-over sites were operationally site-prepared, bedded and received phosphorus fertilization prior to planting. After establishment, herbicides were applied either aerially or via back-pack sprayers during the first and/or second growing season(s) to reduce weed competition.

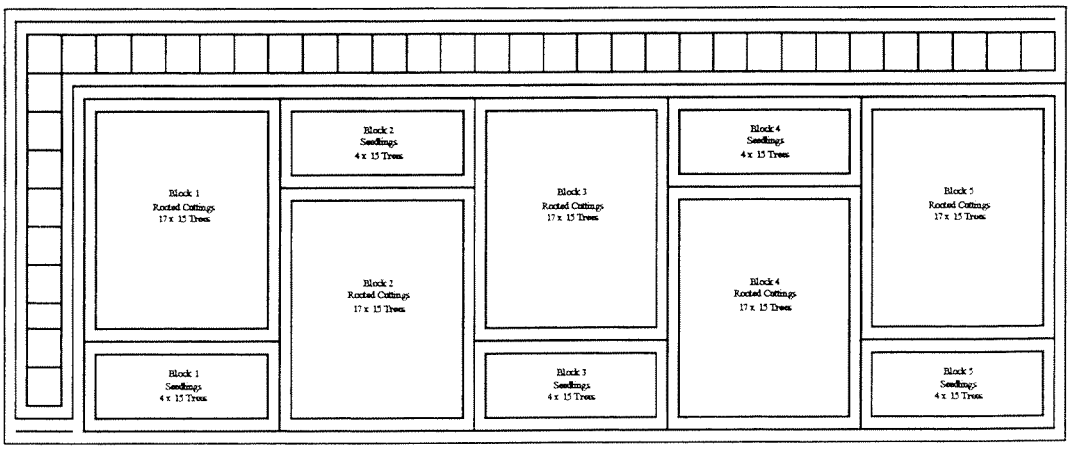


Figure 2. *Generalized map of four loblolly pine clonal field trials in coastal North Carolina.*

Table 1 . *Design of 4 loblolly pine clonal field trials located in coastal North Carolina.*

Location	Pamlico and Beaufort Counties, NC	Martin and Carteret Counties, NC	Combined
Establishment Date	May 1990	April 1991	
# Full-Sib Families	16 in Blocks 1-5 29 in Block 6	16 in Blocks 1-5 28 in Block 6	18 Total 14 in Common
# Clones/Family	9-11 in Blocks 1-5 13 in Block 6	15 in Blocks 1-5 27 in Block 6	
# Clones/Block	1 in Blocks 1-5 9 in Block 6	1 in Blocks 1-5 9 in Block 6	347 Total 63 in Common
# Total Trees	1061	1452	2513

Studies established within the same year were identical except for the randomization pattern (Table 1, Figure 2). Studies planted across years contained some common genetic material. All studies involved a randomized complete block design. Blocks 1-5 included a single tree plot of each clone from each full-sib family represented in the study. Block 6 consisted of nine-tree square plots of a sub-sample of the clones in blocks 1-5. A split-plot of seedlings was established in each of blocks 1-5 and seedlings were also included in the nine-tree square plots of block 6. The seedlings were established to compare propagule types but, due to lack of seed, were not of the same families as the rooted cuttings and were not included in any of the analyses in this report.

Total tree height was measured on the trees prior to planting (age "0") and after each of the first four growing seasons in the field. After the fourth growing season, diameter at breast height (DBH), wood specific gravity of a bark-to-bark increment core collected between 30-60 cm above ground line, the number of branches/height, the number of whorls/height and a branch angle scale (0=0°, 1=15°, 2=30°, 3=45°, 4=60°, 5=75° and 6=90° from horizontal) were assessed.

Data Analysis

For each trait analyzed, full-sib family and clonal best unbiased predictors (BLUPs) as well as variance components were estimated via restricted maximum likelihood (REML) techniques using the following model (and ignoring the plot layout of block 6):

$$y_{ijklmn} = \mu + l_i + b_j(l)_i + g_k + g_l + s_{kl} + c_m(s_{kl}) + lg_{ik} + lg_{il} + ls_{ikl} + l_i\, c_m(s_{kl}) + e_{ijklmn}$$

where,

y_{ijklmn} = n^{th} observation of the mth clone of the kl^{th} family in the jth block of the ith location,

μ = overall mean,

l_i = random variable location ~ $NIID(0,\sigma^2_{location})$,

$b_j(l)_i$ = random variable block within location ~ $NIID(0,\sigma^2_{block(location)})$,

g_k and g_l = random variables female and male general combining ability (GCA),

respectively ~ $NIID(0,\sigma^2_{gca})$,

s_{kl} = random variable specific combining ability (SCA) ~ $NIID(0,\sigma^2_{sca})$,

$c_m(s_{kl})$ = random variable clone within full-sib family ~ $NIID(0,\sigma^2_{clone(family)})$,

lg_{ik} and lg_{il} = random variables location interaction with female and male GCA, respectively ~ $NIID(0,\sigma^2_{location*gca})$,

ls_{ikl} = random variable location interaction with SCA ~ $NIID(0,\sigma^2_{location*sca})$,

$l_i\, c_m(s_{kl})$ = random variable location interaction with clone within full-sib family ~ $NIID(0,\sigma^2_{clone(family)})$,

e_{ijklmn} = random error ~ $NIID(0,\sigma^2_{error})$.

Full-sib family and clonal mean heritability estimates were calculated as follows:

$$h^2_{FS\ Family\ Mean} = (2\sigma^2_{GCA}+\sigma^2_{SCA})/(\sigma^2_{location}/l+\sigma^2_{block(location)}/lb+2\sigma^2_{GCA}+\sigma^2_{SCA}+\sigma^2_{clone(family)}/c +2\sigma^2_{location*GCA}/l+\sigma^2_{location*SCA}/l+\sigma^2_{location*clone(family)}/lc +\sigma^2_{error}/lbcn)$$

$$h^2_{Clonal\ Mean} = (2\sigma^2_{GCA}+\sigma^2_{SCA}+\sigma^2_{clone(family)})/(\sigma^2_{location}/l+\sigma^2_{block(location)}/lb+2\sigma^2_{GCA}+\sigma^2_{SCA}+ \sigma^2_{clone(family)}+2\sigma^2_{location*GCA}/l+\sigma^2_{location*SCA}/l+\sigma^2_{location*clone(family)}/l+\sigma^2_{error}/lbn).$$

where,

l = number of locations (4)
b = number of blocks/location (6)
c = number of clones/family/block/location (harmonic mean = 8.66)
n = number of ramets/clone/family/block/location (harmonic mean = 2.15).

Among full-sib family and clonal genetic correlations among traits were estimated by calculating correlations among the respective BLUPs. Genetic gain estimates for the best 5 and 1% of the clones was calculated for height, wood specific gravity and number of branches/ht at age four by averaging the best 17 and three clones (out of 347), respectively, for each trait. The full-sib family and clonal BLUPs for these three traits were also linearly combined into a selection index using arbitrary weights as follows:

$$\text{Index Value}_i = 4\ HT_i + 2\ SG_i - BRHT_i$$

where,

Index Value = index value for the ith full-sib family or clone
HT = standardized age four height BLUP for the ith full-sib family or clone
SG = standardized age four wood specific gravity BLUP for the ith full-sib family or clone
BRHT = standardized age four branches/ht BLUP for the ith full-sib family or clone.

RESULTS AND DISCUSSION

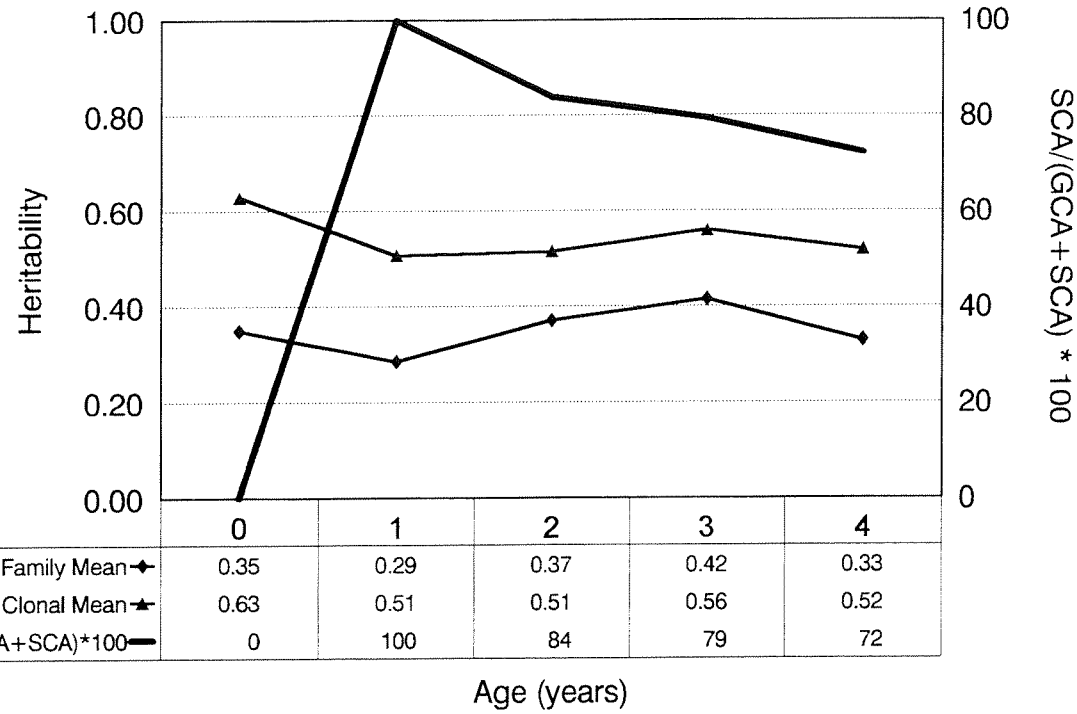
Genetic Variation and Heritabilities

Due to the lack of a mating design among the parents of the full-sib crosses in this study, the power in partitioning the genetic variation between GCA and SCA was low. Further, since these estimates were based entirely on measurements of rooted cuttings, if the variation among full-sib families or clones was increased as a result of the rooted cutting process, these propagation or C-effects may have biased some variance component and heritability estimates in the following discussion.

The allocation of genetic variance between general and specific combining ability for height dramatically changed from the greenhouse to age four in the field (Table 2, Figure 3). All the genetic variation detected for greenhouse rooted cutting height was GCA. After one growing season in the field, this allocation was reversed so that all detectable genetic variation was SCA. From years 1 through 4 , the SCA proportion of the genetic variation for height steadily decreased. After four growing seasons, DBH, branch angle and the number of whorls/height displayed a moderate to high proportion of SCA relative to the total genetic variation (Figure 4). No SCA variance was detected after four growing

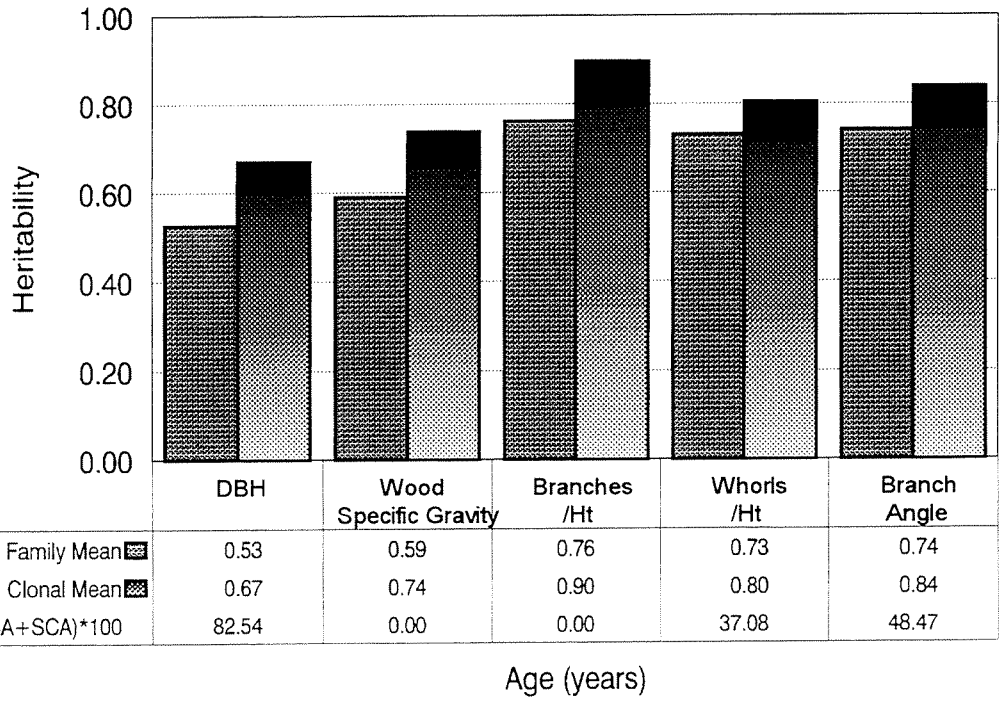
Table 2. *Estimated generalized least squares mean, and restricted maximum likelihood variance components for 10 traits. Data are from four loblolly pine clonal field trials in coastal North Carolina.*

			Estimated Variance Component								
Trait	Age (Years)	Mean	Location	Block(Location)	GCA	SCA	Clone(Family)	Location*GCA	Location*SCA	Location*Clone	Error
Height (m)	0	0.174	0.000990	0.000034	0.000087	0.000000	0.000370	0.000053	0.000000	0.000124	0.000910
Height (m)	1	0.480	0.009550	0.000421	0.000000	0.001158	0.002160	0.000303	0.000000	0.001376	0.016810
Height (m)	2	1.511	0.039980	0.000387	0.000978	0.005049	0.007540	0.000932	0.000000	0.004660	0.098798
Height (m)	3	2.710	0.052650	0.008597	0.002046	0.007870	0.014820	0.001439	0.000000	0.011007	0.209718
Height (m)	4	4.058	0.126740	0.013012	0.004364	0.011326	0.032520	0.000604	0.000000	0.026619	0.355218
DBH (cm)	4	5.996	0.248740	0.077539	0.016554	0.079806	0.160640	0.018706	0.000000	0.074711	2.110472
Specific Gravity	4	0.381	0.000500	0.000005	0.000105	0.000000	0.000170	0.000001	0.000000	0.000007	0.000370
# Branches/Whorl	4	8.592	0.148500	0.076771	0.082053	0.000000	0.321820	0.000000	0.009005	0.027827	2.051350
# Whorls/Height (m)	4	2.660	0.010440	0.002915	0.005998	0.003535	0.019340	0.000276	0.000000	0.010561	0.152330
Branch Angle (0-6 scale)	4	1.411	0.028980	0.006109	0.018405	0.017314	0.055710	0.002317	0.010662	0.000000	0.504060



	0	1	2	3	4
Full-Sib Family Mean	0.35	0.29	0.37	0.42	0.33
Clonal Mean	0.63	0.51	0.51	0.56	0.52
SCA/(GCA+SCA)*100	0	100	84	79	72

Figure 3. *SCA variance proportion, full-sib family and clonal mean heritability estimates for height in four loblolly pine clonal field trials in coastal North Carolina.*



	DBH	Wood Specific Gravity	Branches /Ht	Whorls /Ht	Branch Angle
Full-Sib Family Mean	0.53	0.59	0.76	0.73	0.74
Clonal Mean	0.67	0.74	0.90	0.80	0.84
SCA/(GCA+SCA)*100	82.54	0.00	0.00	37.08	48.47

Figure 4. *SCA variance proportion, full-sib family and clonal mean heritability estimates for several traits at age four in four loblolly pine clonal field trials in coastal North Carolina.*

seasons for wood specific gravity and number of branches/height.

The full-sib family and clonal mean heritabilities for height decreased from 0.35 and 0.63, respectively, coming out of the greenhouse to 0.29 and 0.51, respectively, after the first growing season in the field (Figure 3). Both types of heritabilities increased after the second and third growing seasons in the field but then dropped after the fourth growing season. Four-year-old DBH and wood specific gravity displayed moderately high full-sib family mean heritabilities (0.53 and 0.59, respectively) and higher clonal mean heritabilities (0.67 and 0.74, respectively). Full-sib family mean heritabilities for four-year-old branching characteristics were high (0.73-0.76) with clonal mean heritabilities higher yet (0.80-0.90).

Unlike many heritability estimates, those reported here were estimated across four locations and therefore, not biased by genotype x environmental interactions. It should also be noted that the full-sib family and clonal mean heritability estimates reported apply to coastal North Carolina sites and further, such heritabilities vary as a function of the testing design (i.e., number of sites, block/sites, etc.).

The growth (height and DBH) heritability estimates were moderate while wood specific gravity was slightly higher and the branching characteristics showed the highest degree of genetic control. Observations in a clonal demonstration area adjacent to the Pamlico County trial corroborate these quantitative results. While some uniformity for growth is evident within clonal blocks in this demonstration area, the transition between clonal blocks is easily distinguishable by obvious changes in branching habit.

Genetic Correlations

The height age-age genetic correlations were mostly high for both full-sib families and clones (Table 3). The height age-age correlation between consecutive years increased over time and was very high between ages three and four (0.96 and 0.94, respectively). This suggests that both family and clonal height rankings were stabilizing. As expected, full-sib family and clonal genetic correlations between DBH and height were also high (0.76 and 0.80, respectively, at age four). A negative genetic correlation between height and wood specific gravity was detected for both full-sib families and clones (-0.39 and -0.20, respectively, at age four) suggesting that faster growing trees produce less dense wood. On the other hand, a beneficial correlation was detected between wood specific gravity and number of branches/height (-0.31 and -0.16, respectively, for full-sib families and clones).

Genetic Gains

The lack of a mating design among the parents of the full-sib crosses did not adversely effect full-sib family and clonal BLUP precision since the parental GCA estimates were recombined with the cross SCA for these estimates. Propagation or C-effects may have influenced these gain estimates. However, if these effects exist and if they

Table 3. *Full-sib family (above diagonal, n=18) and clonal (below diagonal, n=347) genetic correlations among 10 traits in loblolly pine. Data are from four loblolly pine clonal field trials in coastal North Carolina. Probabilities below correlations assume BLUPs are known without error.*

Trait		Height	Height	Height	Height	Height	DBH	Specific Gravity	Branches/ Height	Whorls/ Height	Branch Angle
	Age	0	1	2	3	4	4	4	4	4	4
Height	0		**0.63** 0.0050	**0.58** 0.0117	**0.66** 0.0028	**0.63** 0.0051	**0.68** 0.0017	**-0.54** 0.0218	**0.48** 0.0418	**0.13** NS	**0.018** NS
Height	1	**0.60** 0.0001		**0.96** 0.0001	**0.93** 0.0001	**0.86** 0.0001	**0.77** 0.0002	**-0.19** NS	**0.20** NS	**0.047** NS	**0.14** NS
Height	2	**0.47** 0.0001	**0.86** 0.0001		**0.95** 0.0001	**0.86** 0.0001	**0.72** 0.0007	**-0.65** NS	**0.15** NS	**0.028** NS	**0.17** NS
Height	3	**0.47** 0.0001	**0.79** 0.0001	**0.92** 0.0001		**0.96** 0.0001	**0.75** 0.0003	**-0.28** NS	**0.15** NS	**-0.036** NS	**0.27** NS
Height	4	**0.42** 0.0001	**0.71** 0.0001	**0.84** 0.0001	**0.94** 0.0001		**0.76** 0.0002	**-0.39** 0.1066	**0.027** NS	**-0.22** NS	**0.29** NS
DBH	4	**0.46** 0.0001	**0.67** 0.0001	**0.75** 0.0001	**0.79** 0.0001	**0.80** 0.0001		**-0.39** 0.1115	**0.23** NS	**-0.0098** NS	**-0.18** NS
Specific Gravity	4	**-0.23** 0.0001	**-0.049** NS	**-0.0089** NS	**-0.15** 0.0035	**-0.20** 0.0002	**-0.24** 0.0001		**-0.31** NS	**-0.088** NS	**-0.18** NS
Branches/Height	4	**0.13** 0.0140	**0.022** NS	**-0.024** NS	**-0.019** NS	**-0.14** 0.0111	**0.033** NS	**-0.16** 0.0032		**0.86** 0.0001	**-0.11** NS
Whorls/Height	4	**0.0022** NS	**-0.065** NS	**-0.12** 0.0217	**-0.17** 0.0014	**-0.31** 0.0001	**-0.14** 0.0074	**-0.049** NS	**0.79** 0.0001		**-0.062** NS
Branch Angle	4	**0.045** NS	**0.074** NS	**0.036** NS	**0.091** 0.0894	**0.063** NS	**-0.20** 0.0001	**-0.16** 0.0028	**-0.068** NS	**-0.025** NS	

Table 4. *Genetic gain (best linear unbiased predictions) based on selection for three traits independently and in a selection index. Gains are for four-year-old loblolly pine in coastal North Carolina. Highlighted boxes include gains for trait under selection.*

	Height (m)		Wood Specific Gravity		Branches/Height (#/m)		Index Value	
	Gain	% Gain	Gain	% Gain	Gain	% Gain	Gain	% Gain
Selection for Height Mean = 4.06 m								
Best Full-Sib Family	0.21	5.2	-0.025	-6.6	0.44	-5.1	0.34	--
Best 5% of Clones	0.32	7.9	-0.002	-0.5	-0.24	2.8	1.53	--
Best 1 % of Clones	0.41	10.1	-0.030	-7.9	0.18	-2.1	1.39	--
Best Clone	0.44	10.8	-0.032	-8.4	0.12	-1.4	1.56	--
Selection for Wood Specific Gravity Mean = 0.381								
Best Full-Sib Family	0.07	1.7	0.020	5.2	-0.12	1.4	0.46	--
Best 5% of Clones	-0.02	-0.5	0.034	8.9	-0.13	1.5	0.11	--
Best 1 % of Clones	0.05	1.2	0.042	11.0	-0.11	1.3	0.40	--
Best Clone	0.08	2.0	0.044	11.5	0.32	-3.7	0.07	--
Selection for Number of Branches/Height Mean = 8.59 branches/m								
Best Full-Sib Family	0.07	1.7	0.010	2.6	-0.73	8.5	1.02	--
Best 5% of Clones	0.13	3.2	0.001	0.3	-1.12	13.0	1.67	--
Best 1 % of Clones	0.23	5.7	0.016	4.2	-1.54	17.9	2.50	--
Best Clone	0.06	1.5	0.010	2.6	-2.03	23.6	2.29	--
Selection for Index Value Mean = 0.00								
Best Full-Sib Family	0.07	1.7	0.010	2.6	-0.73	8.5	1.02	--
Best 5% of Clones	0.24	5.9	0.008	2.1	-0.91	10.6	1.92	--
Best 1 % of Clones	0.32	7.9	0.022	5.7	-1.20	14.0	2.54	--
Best Clone	0.35	8.6	0.021	5.5	-1.31	15.3	2.75	--

are transmitted through the commercial clonal production process in a manner similar to the clonal production for these field trials, then these predicted gains properly reflect achievable results. The genetic gains in the following discussion are expressed as a percent of the mean of the second generation families which were tested.

The predicted genetic gains (BLUPs) for age four height and wood specific gravity were both 5.2% when selecting the best full-sib family (Table 4). When selecting the best 5% of the clones, the gains for these traits were 7.9 and 8.9%, respectively. Despite different heritabilities, these predicted gains for height and wood specific gravity were fairly similar due to a greater degree of variation present among the full-sib families and clones for height than for wood specific gravity. Predicted genetic gains for the number of branches/height were 8.5% and 13.0% when selecting the best full-sib family and best 5% of the clones, respectively. When selecting for individual traits, a genetic loss was generally incurred in other trait(s) due to the negative correlations among some traits. Using the selection index, the best 5% of the clones produced considerably greater height gain than did the best full-sib family while maintaining a similar genetic gain in wood specific gravity and reducing the number of branches/height (Table 4).

The best 5% of the clones represented 17 clones. No relatedness restrictions were placed on these selections. Clones from nine full-sib families were selected among the best 5% of the clones for height growth. Clones from six full-sib families were selected for wood specific gravity and number of branches/height. Using the index, eight full-sib families were represented in the best 17 clones.

These results demonstrate that clonal selection can yield considerably greater gains than full-sib family selection. Further, this increased genetic gain may be achieved at selection intensities that allow for more genetic diversity than would be achieved via full-sib family deployment. When multiple traits are combined in a selection index, "correlation breaker" clones or a group of complimentary clones can be selected to achieve gains not available through full-sib family selection. Additional advantages of cloning including better matching genotypes to sites and improving raw material uniformity were not addressed in this paper but will certainly further enhance the benefits derived from future loblolly pine clonal forestry programs.

CONCLUSIONS

Much effort is currently underway to develop commercially feasible methods to clonally propagate loblolly pine. While based on juvenile assessments, the results of this study suggest that the genetic gains resulting from practicing clonal forestry with loblolly pine will more than compensate for theses efforts.

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ROLE OF MAJOR GENES FOR RESISTANCE IN THE LOBLOLLY PINE-FUSIFORM RUST FOREST PATHOSYSTEM

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Abstract. The genetic basis of disease resistance in forest trees is poorly understood, but for long-lived trees to survive the resistance must be durable. Forest tree disease resistance traditionally has been viewed as polygenic (controlled by many genes each with small effect), and most tree breeding programs have used quantitative approaches to define disease resistance. In contrast to this, examples of host-pathogen specificity in some forest pathosystems suggest that major resistance genes play a role, but convincing evidence for Mendelian inheritance of major gene resistance in forest trees is rare. In studying the loblolly pine-fusiform rust (*Pinus taeda-Cronartium quercuum* f.sp. *fusiforme* [*Cqf*]) pathosystem, we recently identified and mapped with RAPD markers a major gene for fusiform rust disease resistance in a pedigree of loblolly pine family 10-5 across three generations. While inheritance of this resistance gene appears to be simple, disease expression is a complex trait which is environmentally modulated. A new approach of complex trait dissection with molecular markers has allowed us to quantify an environmental component of disease phenotype which confounded previous analyses. Existing phenotypic data suggest that other major genes for fusiform rust resistance are present among various loblolly pine families. We hypothesize that fusiform rust disease resistance in loblolly pine is controlled by major resistance genes, that resistance gene and corresponding virulence gene frequencies are low and that resistance genes have an associated resistance cost. We propose to use molecular (RAPD) markers and a complex-trait approach to determine the extent and role of major resistance genes in the endemic loblolly pine-fusiform rust forest pathosystem by addressing each of these hypotheses. Demonstration of a role for major genes in forest tree disease resistance will advance basic understanding of forest pathosystems for both the pathology and the ecology communities. Fusiform rust disease is the most economically damaging forest tree disease in the southern US, and this research will directly impact and probably redefine tree breeding efforts against this disease.

MICROPROPAGATION OF *PAULOWNIA ELONGATA*

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Abstract. Stem apex explants including two or three nodes were taken from year-old *Paulownia elongata*[1] stock plants and placed onto culture media that differed in auxin and cytokinin concentrations. Basal culture medium was Murashige and Skoog (1962 Physiol Plant 15:473-497), the auxin was naphthaleneacetic acid (NAA), and the cytokinin was benzylaminopurine (BAP). The NAA:BAP concentration (mg/l) combinations tested were: 0:1.0, 0.2:2.0, 0.2:4.0, 1.0:5.0. Four weeks after culture initiation the 0 NAA:1.0 BAP treatment was clearly inferior to the others as judged by the number of shoots produced per explant. After subculture and an additional four weeks of growth the number of shoots suitable for rooting (*i.e.* > 0.5 cm) was greatest on 0.2 NAA:4.0 BAP and differed significantly among all treatments ($p < 0.01$). Shoot proliferation varied greatly among genotypes within treatment. The bases of shoots ranging from 0.5 to 1.5 cm were dipped into rooting powder consisting of 0.2% NAA. Rooting took place in the tissue culture growth room in plastic boxes containing a peat-based, soilless rooting medium. Rooting frequency of over 95% was obtained within two weeks regardless of the tissue culture medium upon which the shoots were produced. Transfer of rooted shoots to the greenhouse resulted in a plantlet mortality rate of <1%. An additional two subcultures using nodal segments from a subset of genotypes on 0.2 NAA:2.0 BAP or 0.2 NAA:4.0 BAP showed that it is possible to routinely produce >100 rootable shoots per explant after three 4-week periods in culture. However, the number of shoots produced per nodal segment decreases with each subculture.

Treatment NAA:BAP (mg/l)	Shoots/Explant Week 4	Shoots/Explant Week 8	# Shoots to Root	% Root
0 : 1.0	2.9 b	6.6 d	578	96
0.2 : 2.0	3.7 a	17.2 b	1152	100
0.2 : 4.0	4.2 a	21.3 a	2002	98
1.0 : 5.0	3.9 a	9.9 c	1053	98

Values within a column followed by the same letter are not different at the 0.05 level according to Duncan's Multiple Range Test.

Keywords: Princess tree, tissue culture, vegetative propagation, rooting

[1] Plant material used is designated *Paulownia elongata carolinia* (patent pending) by Carolina Pacific International Inc. who provided the explants to conduct this research.

GENETIC MAPPING OF QUANTITATIVE TRAIT LOCI FOR WOOD BASIC DENSITY IN *Pinus radiata* USING RAPD MARKERS

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Abstract: Quantitative trait mapping in *Pinus radiata* was carried out in a controlled-cross half-sib family forming part of an operational tree-breeding program. This family structure required the identification of RAPD markers fitting the pseudo-testcross configuration between the common parent and other contributing parents. The half-sib family structure was successfully used to identify three loci influencing a high heritability trait: wood basic density. These three loci were found to be clustered within a single linkage group, but separated by at least 48cM. All three loci for wood density showed a consistent direction of allelic substitution effect in the background of the half-sib family studied. Determination of the phenotypic variance explained by each locus was not satisfactorily resolved: most probably a consequence of restricted population size. Indications for full-sib family specific effects within the half-sib family, and for genotype x environment interaction at specific loci eroded the resolving power of half-sib family QTL analyses, and the usefulness of this family structure for marker based predictions on the performance of individuals with particular genotype configurations. These results indicate that average loci effects determined through half-sib families of moderate size may be useful for marker assisted breeding. However, potentially the most valuable application of genetic markers in forestry will involve selection of superior genotypes as embryos, young seedlings or juvenile trees for vegetative multiplication and clonal deployment. This application will require QTL analyses to be based on large full-sib families within each site of interest. RAPD marker technology should enable marker/QTL associations to be established in the most important families of interest to an operational plantation program, overcoming the problem of linkage equilibrium.

Keywords: Genetic mapping, QTL mapping, RAPD, half-sib, wood basic density, tree breeding, *Pinus radiata*.

MORPHOLOGICALLY DIFFERENTIAL CELL CULTURES FROM SPECIFIC ORGANS OF EMBRYONIC ALEPPO AND LONGLEAF PINES

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Abstract. Radicle tips, radicles, hypocotyls, hupocotyl apices and cotyledons were separately cut from mature embryos of aleppo and longleaf pines. Tissue cultures were grown from each cut fragment on a modified MS medium supplemented with 2,4-D (2 mg/l) and BAP (1 mg/l), and compared to those grown from the collective chopped tissues of the entire embryo. Tissues were incubated in the dark at 22C, and subcultured to freshly prepared medium of the same composition at biweekly intervals.

Radicle tips, radicles, and hypocotyl apices produced unique and dissimilar cell cultures; those from radicle tips were white to pale grey, and with limited viability. Those from radicles were white and friable through prolonged subculture. Cultures from hypocotyl apices commonly showed cell clusters of three different phenotypes, including pale yellow, white, and pale grey. The white and grey clusters assumed a nodular appearance with time and progressive growth in a prominent mucilagenous liquid matrix which provided a viscid appearance to the smooth, rounded cultures. The pale yellow clusters and cultures developed exclusively from them were similar to those grown from the mid- to low hypocotyl or cotyledons, and were irregular in shape, yellow and friable. The morphological integrity of each of these cultures remained stable through 5 months of subculture, though cultures from cotyledon bases and hypocotyl apices continually generated variable amounts of the smooth, viscid culture types distributed throughout the otherwise pale yellow, friable matrix, presumably from residual apical fragments within the cultures. Relative growth, physiology and whole-plant regenerative potential of the different cell types are now being studied.

Keywords: Pinus palustris Mill., Pinus halepensis Mill., callus, tissue culture, conifer.

High-frequency induction of adventitious shoot formation from hypocotyl segments of *Liquidambar styraciflua* L. by thidiazuron

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Abstract. The effects of thidiazuron on adventitious shoot formation from hypocotyl segments of *Liquidambar styraciflua* were tested either alone or in combination with 2,4-dichlorophenoxyacetic acid. The combination of 0.01 mg/L 2,4-D with TDZ stimulated bud production up to 1 mg/L TDZ and gave the most buds at 1 mg/L of TDZ with 0.01 mg/L of 2,4-D. Lower concentrations of TDZ stimulated shoot production, generating the most shoots at 0.1 mg/L of TDZ with 0.01 mg/L of 2,4-D. The hinderance of TDZ on shoot elongation was overcome by transfer of shoot cultures to a shoot proliferating medium lacking TDZ or containing NAA and BA in addition to TDZ. Transferred bud and shoot clumps were subjected to two different culture systems, solid culture and liquid culture. The performance of shoot proliferation in liquid culture was significantly improved compared to that in solid culture. In addition to in vitro rooting, we attempted to establish ex vitro rooting to save labor and time. On the basis of our results, ex vitro rooting is believed to be a reliable system for rooting and acclimatization of adventitious sweetgum shoots.

Key words: Adventitious shoot, ex vitro rooting, *Liquidambar styraciflua*, thidiazuron.

LACCASE AS A TARGET FOR DECREASING LIGNIN CONTENT

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Abstract. Studies show that laccases (p-diphenol:O_2 oxidoreductase, EC 1.10.3.2) are specifically expressed in lignifying xylem from a variety of vascular plants where they may play a role in the deposition of lignin. Laccases are members of a highly conserved class of metalloenzymes, the "blue" copper oxidases, which includes ascorbate oxidase and ceruloplasmin. Plant laccases can be fractionated into two isoform classes on the basis of their isoelectric point. These different pI forms may be involved in discrete functions in the cell. cDNAs encoding laccase have been isolated from libraries prepared from 1) suspension-cultured cells of *Acer psuedoplatanus* (sycamore maple) and 2) cambial/lignifying zone tissue of *Liriodendron tulipifera* (yellow-poplar). The *A. psuedoplatanus* full-length cDNA clone encodes an acidic form of laccase and represents an highly abundant message in the suspension-cultured system. The *L. tulipifera* cDNA clone encodes a basic form of the enzyme and its message level is comparatively low. Although the cloned genes show more homology to one another than with any other gene sequence in the gene database, their sequences are only 45% identical (65% similar) at the amino acid level and they do not cross-hybridize at low stringency. As would be expected, the amino acid ligands for binding of the catalytic copper atoms are completely conserved between these two genes and most other blue copper oxidases. To test the hypothesis that laccase is involved in the deposition of lignin, we prepared an antisense construct of the yellow-poplar gene which contained the 5'-untranslated region and 12% of the amino-terminal coding sequence. The antisense laccase sequence was fused to the nominally constitutive CaMV35S viral promoter. The construct was introduced into embryogenic cultures of yellow-poplar by microprojectile bombardment, and over 60 kanamycin-resistant cell lines were recovered. These cell lines are currently being analyzed for antisense gene expression. This research was supported by Georgia Pulp and Paper Consortium grant PP96-FS3.

Keywords: *Acer psuedoplatanus*, *Liriodendron tulipifera,* genetic engineering, laccase, lignin, pulp bleaching.

RECOVERY OF YELLOW-POPLAR (*Liriodendron tulipifera*) EMBRYOGENIC MATERIAL FOLLOWING CRYOPRESERVATION

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Abstract. Recovery of yellow-poplar proembryogenic masses (PEMS) after freezing in liquid nitrogen was tested. In preliminary experiments, six treatments were used to determine the optimum combination of pretreatments and cryoprotectants for recovery of PEMs after cryopreservation. Pretreatments consisted of transferring sample material to normal induction medium supplemented with 0.4 M sucrose or a control that was transferred to normal induction medium (0.12 M sucrose) for 24 hours. For cryoprotection, samples were immersed in induction medium with either 0%, 5%, or 10% DMSO before freezing. Samples were slowly frozen at a rate of -1°C min^{-1} to -70°C using Nalgene™1°C Freezing Containers and then plunged directly into liquid nitrogen (-196°C) where they remained for over 80 days. They were thawed in a 40°C water bath for 20 minutes and washed with fresh induction medium before re-suspension in normal culture conditions. Recovery and growth was measured every three weeks by packed cell volume.

Keywords: *Liriodendron tulipifera*, cryopreservation, tissue culture, somatic embryogenesis, long term storage.

A PRELIMINARY SURVEY OF MITOCHONDRIAL VARIATION IN LOBLOLLY, LONGLEAF, SLASH, AND SHORTLEAF PINES

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Abstract. We have studied mitochondrial DNA restriction fragment length polymorphism in a total of 62 individuals from nine populations of loblolly, five populations of longleaf, four populations of slash, and two populations of shortleaf pines. Mitochondrial variants were visualized by Southern hybridizations of *Eco*RI restriction fragments from total cellular pine DNA with a [^{32}P]-labelled *coxII* probe from white spruce. We found a total of three mitochondrial variants in our preliminary survey. All 18 loblolly and all 14 slash pines sampled shared the same variant. Two of the shortleaf and 25 of the longleaf individuals also shared this common variant. The remaining two shortleaf pines shared a second mitochondrial variant, while the third variant occurred in only a single longleaf pine. Although the sample sizes were small, it is noteworthy that we observed mitochondrial variation only among populations. We found no variation within populations, even though, *e.g.*, we sampled 18 individuals from one of the longleaf populations. These data are consistent with the results of theoretical and other empirical studies of maternally inherited genetic markers, *i.e.* such markers tend to have high population subdivision. Increased population sample sizes, as well as more intensive sampling of southern pine mitochondrial genomes with additional endonuclease/probe combinations, may reveal mitochondrial markers that would be useful for fingerprinting southern pine populations. If so, these markers would provide valuable information for germplasm improvement and conservation programs.

Keywords: *Pinus echinata* Mill., *Pinus elliottii* Engelm., *Pinus palustris* Mill., *Pinus taeda* L., maternal inheritance, population subdivision.

TRENDS IN VARIANCES AND HERITABILITIES WITH STAND DEVELOPMENT OF TROPICAL PINES

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Abstract: Phenotypic and additive variances as well as narrow-sense heritability for cumulative height were determined from assessments of tropical pine (*Pinus caribaea* Morelet, P. *chiapensis* (Mart.) Andresen and P. *tecunumanii* (Schw.) Eguiluz et Perry) trials established in South America and South Africa by the CAMCORE Cooperative. Stem height was analyzed from data collected on open-pollinated families to determine additive genetic and phenotypic variances. Variances based on arithmetic as well as log-transformed values are presented. Log-transformed phenotypic and genetic variances decreased over time reflecting the onset of intergenotypic competition and compensatory growth. Arithmetically derived variances increased over time as the trial became older but the rate of increase for phenotypic variance was greater than for additive variance. Individual narrow-sense heritability, changed over time but without showing any definite trend by species. It appeared that variance trends reflected the varying ontogenetic changes during the development of the stand which could be indicated by size rather than by age. Breeding strategies in tropical pines have to consider the increasing intensity of environmental effects as the stand matures that cause a decline of genetic variances in a higher rate than the phenotypic variances. Furthermore, it is hypothesized that a phenotypic trait in a given ontogenetic stage of the individual is, presumably, under temporal control of a set of genes that changes as the temporal environmental conditions change.

USDA FOREST SERVICE, FOREST HEALTH RESISTANCE SCREENING CENTER DISPLAY

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Abstract. The Resistance Screening Center (RSC) is operated by the Forest Health Unit of the USDA Forest Service, Southern Region, State and Private Forestry. The Center is located at the Bent Creek Experimental Forest near Asheville, NC. The Center evaluates pine seedlings for resistance to fusiform rust (caused by *Cronartium fusiforme*) and pitch canker (caused by *Fusarium annosum*) as a service to tree improvement specialists, seed orchard managers, scientists, government agencies, research institutions, universities, and private industry. Testing enables clients to obtain information on the relative resistance of their materials in much less time than is possible in field progeny tests. The RSC provides information on resistance in 8 to 11 months, depending on species and disease, whereas field progeny tests require 4 or 5 years. By using information from these tests, trees producing rust-resistant progeny are identified.

Keywords: Resistance Screening Center, resistance, fusiform rust, pitch canker

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