A Strategy for the Third Breeding Cycle of Loblolly Pine in the Southeastern U.S.

By S. E. **McKeand**[1]) and F. E. **Bridgwater**[2])

(Received 9th June 1998)

Abstract

A strategy for the North Carolina State University - Industry Cooperative Tree Improvement Program's third-cycle breeding for loblolly pine (*Pinus taeda* L.) was developed to provide genetic gain in the short-term as well as to maintain genetic diversity so that long-term genetic gains will also be possible. Our strategy will be to manage a hierarchy of three populations, each at a different level of intensity. The mainline population will consist of about 160 selections that are available to each cooperator in a given geographic region (i.e. recruitment population). These populations will be managed as subdivided breeding populations (40 sublines of 4 trees each) primarily to provide for long-term genetic gain and diversity The most intensively selected and managed hierarchy will be the elite populations. A highly selected group of trees (approximately 40 selections) will be managed to provide short-term genetic gain for each member's program. A third hierarchy will be the genetic diversity archives managed to preserve and breed genotypes with extreme breeding values for individual traits (not necessarily for all traits combined) as an insurance population for environmental or *selection* criteria changes in future generations.

The improved efficiency of this breeding strategy along with the reduction in population sizes compared to the current program, will result in a substantial reduction in effort by individual cooperators, The increase in selection intensity from reducing the population sizes and the increased rate of breeding made possible by mating fewer trees will substantially increase gains in subsequent generations. While the most intensive effort will be devoted to those populations providing immediate genetic and financial gain, the long-term well-being of the genetic resource will be maintained by judicious management of all three hierarchies.

Key ***words:*** Elite breeding populations, genetic gain, mating designs, ***Pinus taeda*** L., testing designs.

FDC: 165.3; 165.41; 165.6; ***232.13;*** 174.7 ***Pinus taeda;*** **(756).**

Introduction

As the North Carolina State University - Industry Cooperative Tree Improvement Program progressed toward the third cycle of breeding, an efficient, cost-effective breeding strategy was developed to ensure both short- and long-term benefits for

---

[1]) Geneticist and Professor, Cooperative Tree Improvement Program, Box 8002, Department of Forestry, North Carolina State University, Raleigh, NC 27695-8002, USA.

[2]) Research Geneticist and Adjunct Professor, USDA Forest Service, Southern Forest Experiment Station, Texas A&M University, College Station, TX 77843-2585, USA.

Cooperative members. A primary criterion for the breeding strategy was that it be flexible for current and future breeders. Decisions made in the third-cycle program will influence all future loblolly pine breeding within the Cooperative's working area, and options available to future breeders should not be constrained by the methods employed in the next cycle. In addition, the strategy had to be flexible in accommodating the diverse objectives of the membership. While members share in supporting the mission and objectives of the Cooperative, there is a diversity in product goals, aggressiveness of programs, and investment level that must be accommodated. Additionally, the plan had to provide enough flexibility to permit the incorporation of new information.

In this paper we present a summary of the options considered for the breeding strategy, a brief background of the current breeding strategy, details of the strategy for the third breeding cycle, research and development needs for implementing the strategy, and some options for future cycles.

Overview of Breeding Strategies

The different types of breeding strategies used in forest and crop improvement will be briefly described, and the main advantages and disadvantages of each strategy will be emphasized. Detailed reviews of breeding strategies can be found in BRIDGWATER and FRANKLIN (1985), LI and WYCKOFF (1994), NAMKOONG et al. (1988), NIKLES (1992), SHELBOURNE (1986), WHITE (1992), and WHITE et al. (1993).

There are numerous systems of breeding currently in use in tree and crop improvement programs. Recurrent selection for general combining ability (GCA) is the most commonly used system for forest trees, while selection for both GCA and SCA (specific combining ability) is more common in outcrossed agronomic species. The type of production system (i.e., open-pollinated seed orchards, full-sib families, F_1 hybrids, clones, etc.) will drive the type of breeding system employed, but the breeding program is considered separately from the production side of tree improvement. The breeding program feeds material into the operational program, and all gain from one generation to the next is derived from the breeding program. The production program is considered separate and distinct from the breeding population.

Breeding for GCA

The simplest recurrent selection schemes rely upon open-pollinations (OP) or pollen mix (PMX) breeding among selected trees. This has been proposed for *Pinus radiata* to reduce costs in the large mainline breeding program in Australia (COTTERILL et al., 1988). In some hardwood species where control-pollinations are prohibitively expensive, only partial pedigrees are maintained through OP matings (MCKEAND and BEINEKE, 1980; PURNELL and KELLISON, 1983). The best individuals from the best OP or PMX families are selected and allowed to pollinate with other selects to establish the next cycle. The primary advantages of this system are low cost and acceptable gain in the absence of high levels of non-additive genetic variance.

Most breeding programs have employed control-pollinated mating designs to maintain full-pedigrees (BRIDGWATER and FRANKLIN, 1985). There are three primary reasons for this. First, the relative proportions of additive and non-additive genetic variances were largely matters for speculation when tree breeding programs began, and genetic tests of controlled matings were required to determine this ratio. Second, breeders anticipated from studies of self-pollinations of forest trees and evidence from other crops that inbreeding depression would present problems. By keeping complete pedigrees, co-ancestry would be known and could be managed. Finally, any hope of using non-additive variance through sexual propagation required that male and female parents be known if good specific crosses were to be repeated.

The same principles are involved in selection and breeding programs for full- and partial-pedigree designs. The best individuals from the best families are selected and intermated in some control-pollinated design. The advantage of using CP matings in the breeding strategy include:

- gain is higher than in OP designs since selection on both female and male parent can be practiced, and
- deleterious effects from inbreeding can be managed more readily.

The objective of recurrent selection for GCA is to increase the frequency of favorable alleles from one generation to the next, thus accumulating genetic gain from one generation to the next. Individuals are selected because they presumably have a higher frequency of favorable alleles than non-selects. The higher the degree of additive genetic variance (or narrow-sense heritability), the greater the likelihood that selected trees do indeed have more favorable alleles than non-selects.

Partial-pedigree (OP or PMX) breeding schemes were not evaluated in great detail. Despite cost savings and potential for genetic gain (e.g. COTTERILL et al., 1988), loss of full-pedigree information in the breeding population would be permanent, or at best be very expensive to reconstruct using biochemical markers. Since the long-term effects of inbreeding are not well known for loblolly pine, it is essential that full pedigrees be maintained if inbreeding is to be managed. The use of partial pedigrees was considered to be unacceptable given the criteria for evaluating our strategies.

Use of a full-pedigree breeding system does not necessarily mean that an expensive control-pollinated (CP) mating design (e.g. large diallels) must be used. Mating designs which complement each other could be used cost-effectively to meet different objectives (e.g. OP or PMX families for GCA estimates and a limited number of CP families to generate full-pedigree selections for the next generation of breeding).

Breeding for SCA and GCA

In recurrent selection and breeding programs to advance GCA, there is no attempt to change or advance SCA from one generation to the next. However, SCA can be readily utilized in the production population (i.e., specific crosses which exhibit good SCA and GCA can be deployed operationally or specific clones deployed), even though there is no attempt to increase the likelihood of getting good specific combinations in the next generation. If SCA is large, then breeding schemes to advance both SCA and GCA may be warranted. Methods for exploiting non-additive genetic variance in the long-term have not been widely used in tree breeding but have been extensively used for various agronomic crops. These methods advance individual lines or populations for subsequent inter-crossing in order to realize SCA effects.

Heterotic F_1 crosses among inbred lines offer a method for' using specific combining ability. This method typically employs several cycles of inbreeding with selection to create homozygous lines, which are then crossed to permit selection among the hybrids. This method has not been used for intraspecific improvement of forest trees for several reasons including, the long generation times, the necessity of inbreeding for several generations before outcrossing (ORR-EWING, 1976), and the difficulty of producing inbred lines due to inbreeding depression in traits affecting fecundity (FRANKLIN, 1969).

The main drawback to breeding systems which advance SCA is that they are invariably more expensive than systems that advance only GCA. If SCA effects are large, the added expense may be justified. It is difficult to evaluate the relative importance of SCA and GCA for different traits for loblolly pine, because of the extreme variability in the ratios of SCA/GCA (McKeand et al., 1986; McKeand and Bridgwater, 1995; Li et al., 1996). Another complicating factor, discussed by Byram and Lowe (1986), is the declining ratio of SCA to GCA for height with age. If early estimates (<10 years) are not reliable, then the value of SCA is in question. Recent analyses of a rotation-age heritability study indicate that the ratio of additive to non-additive variance changes dramatically with age (Balocchi et al., 1993). At young ages (<10 years) the non-additive variance as high as 4.4 times the additive genetic variance but was only $^{1}/_{4}$ the level at age 25 years. The Cooperative is currently accumulating a large data base that will allow for estimation of GCA and SCA at different ages and in different geographic regions in the mainline breeding populations (Li et al., 1996). We must understand the effects that stand development, geographic location, genetic sampling, and varying test management procedures have on estimates of genetic variances over time. Most installations of the study have only recently been established (oldest is 1984), so results will not be available for several years to answer the question of age effects on SCA/GCA estimates.

Managenent of Inbreeding

The effects of inbreeding on loblolly pine are not thoroughly known. In general, inbreeding in outcrossed species results in reduced growth, survival, and fecundity, and this is the case for loblolly pine (see Sniezko and Zobel, 1988 and Williams and Savolainen, 1996 for a general review). Preliminary analyses of six-year data from the Cooperative's Inbreeding Study indicate that for every 0.1 increase in the inbreeding coefficient (F value) there is approximately a 5% reduction in height growth (McKeand and Jett, 1995).

The deleterious effects of inbreeding can be avoided in commercial plantations in two ways. First, coancestry can be managed in the breeding population and in the production population if pedigrees are known. Ultimately, all closed populations must become completely inbred, but the rate at which that occurs is under the breeder's control if pedigrees are known. Secondly, multiple breeding sublines can be incorporated as part of the breeding strategy (Burdon et al., 1977). This method requires that all matings occur within sublines and that trees for reforestation be derived from crosses among individuals from different sublines. Thus, all individuals in plantations will be derived from crosses among unrelated individuals. There has been considerable interest in sublining and it has been incorporated into several tree breeding programs (Li and Wyckoff, 1994; Lowe and van Buijtenen, 1986; Matheson, 1983; McKeand and Beineke, 1980; Purnell and Kellison, 1983; van Buijtenen and Lowe, 1979; White et al., 1993).

Most tree breeders are concerned primarily with the negative consequences of inbreeding on the vigor of individuals in production populations. However, there are also many benefits expected from inbreeding (Lindgren, 1976). Inbreeding schemes have been used very effectively in agronomic crops (e.g. Hallauer and Miranda, 1988). Maize breeders have not only used selfing to produce inbred lines for selecting for hybrid vigor (non-additive genetic variation) but also for increasing additive genetic variance in their populations. Baker and Curnow (1969) emphasize that replicate populations (sublines) will diverge because of genetic drift, and this divergence can be an important source of genetic variation. As stated by Falconer and MAckay (1996) when considering only additive genetic variance, "... the total genetic variance in the whole population is the sum of the within-line and between-line components, and is equal to (1+F) times the original genetic variance. Thus, when inbreeding is complete, the genetic variance in the population as a whole is doubled, and all of it appears as the between-line component." This simplified example is what happens to additive genetic variance in the absence of selection or any other forces that would change gene frequencies. The opportunity to increase genetic gain by simply having more genetic variance to exploit, especially among lines, is an incentive to have numerous small sublines.. There is no inherent reason (other than the loss in fecundity) why tree breeders should not consider the benefits of directed inbreeding in the management of breeding programs.

Hierarchy of Breeding Populations

Most tree improvement programs have utilized one large breeding population for a given breeding region. In recent years there has been interest in establishing another hierarchy in the population structure (Kannenberg, 1984, Greenwood et al., 1986; Cotterill et al., 1988; Mahalovich, 1990). In its simplest form, a subset of the large breeding population is selected and a new "elite" breeding population is established. The benefits of an elite population breeding program in consort with a larger mainline program include:

1. More intensive selection and hence more gain can be realized for specific traits.
2. Depending on product goals, different traits can be emphasized in a selection index for each elite population.
3. Multiple elite populations can be established within a breeding region depending on product goals and environmental variation.
4. The elite population size will be smaller so breeding can progress more rapidly than in the larger mainline program.

The use of elite breeding populations for the Cooperative's third cycle was highly desirable since this strategy permitted a significant increase in selection intensity by utilizing only the top clones in a recruitment population to produce propagules for plantation establishment rather than all clones in a region. The average current recruitment population within any region is about 600 genotypes (Anonymous, 1982). If an elite population of 40 individuals were used, the selection intensity for 40 out of 600 is 1.937. The selection intensity benefits derived from reducing the population size from 600± will occur only once, since populations will not likely be reduced in size in future generations. Another major advantage of breeding elite populations is rapid generation turnover. Breeding only 40 entries is expected to require substantially less time than the larger number of entries in the mainline, thus gain per year will be higher (Mahalovich, 1990) and figure *1*.

Population Sizes

The size of the mainline breeding population is the driving force behind many of the breeding strategy decisions. The appropriate breeding population size depends upon the trade-offs between short-term and long-term gain. Short-term gain is maximized by intensive selection and, hence, small population size. Long-term gains are maximized by milder selection and larger population sizes.

Population size basically affects the risk of losing favorable alleles through genetic drift, and this is a more serious problem for those alleles that are at a low frequency. The loss of alleles affects the progress possible in breeding programs. The problem of choosing population size basically "reduces to one of

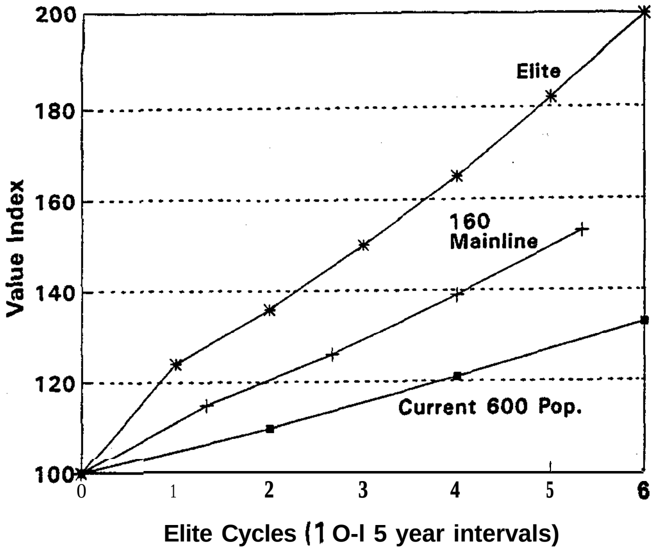

Figure I. - Expected gain over time for different breeding populations. Gain per generation is assumed to be 10% for each population, but the generation turnover is faster in the elite population compared to the mainline populations of 160 for the third-cycle program and the current population of about 600 trees per recruitment population.

deciding on the minimum effective population size that will avoid the major effects of drift, that is, that will retain most of the genetic potential in the population" (RAWLINGS, 1970).

For alleles that are not under selection (but may be valuable if selection goals change in future generations), the population size necessary to maintain these alleles with a high probability is higher than for those alleles under selection (KANG, 1979). Again, the appropriate population size depends upon the gene frequency and the desired probability of not losing the allele.

WHITE (1992) summarized many of the critical issues that must be considered when deciding on population sizes for breeding. "Theoretical studies show that 20 to 50 members may be large enough to sustain substantial progress from selection for several generations of breeding. Larger breeding populations are desired if: 1) *There* is relatedness among selected members such that the effective population size is much less that the census number, 2) Many traits are being bred for in the program, 3) The plans call for using intensive efforts to achieve large gains from selection in the first few generations of breeding, and 4) The breeding population is serving the dual functions of gene conservation and providing flexibility to changing conditions (e.g., to retain neutral rare alleles in the population)."

The optimal number of families to initiate a breeding program is a complex function of costs, expected gains, heritability, and desired effective number of families after selection. Under a range of assumptions where the desired effective number of families is 100 and heritabilities < 0.25, starting with about 300 parents (150 unrelated full-sibs) seems reasonable (LINDGREN et al., 1997b). Of course, if costs are allowed to increase or the cost of additional entries is small, then the optimal number of initial families would be much larger.

Fortunately, the Cooperative managed relatively large populations in the first two breeding cycles. NAMKOONG et al. (1988) also emphasized that one of the benefits of maintaining a relatively large population size in the early generations of breeding was to move low frequency alleles into the intermediate frequencies where future selection will be more effective.

Background of the NCSU Cooperative

Concept of Breeding Regions with Loblolly Pine

While the concept of designating breeding regions appears straightforward, in practice, assigning loblolly pine selections to specific breeding regions in the Cooperative is not practical. In a shared breeding program with a species that is adapted to and will be planted over a range of environments, a breeding region can be defined as a geographic area with a breeding population of trees where environmental factors and product goals are similar enough to warrant using the same selection criteria and breeding goals. In loblolly pine, geographic variation for most traits is large and typically clinal in nature from north to south and from the interior Piedmont to the Coastal Plain (KRAUS et al., 1984; SCHMIDTLING, 1994). Clinal variation implies continuous variation in both environmental and genetic effects. The problem with assigning trees to "distinct" breeding regions is that no distinct boundaries exist for any breeding population; the variation is continuous. Two cooperators whose land bases are 150 km apart north to south may have 75% of their genotypes in common. The other 25% for each cooperator would come from further north or south. In essence there is a continuum of "breeding regions" among cooperators from Virginia to Louisiana and from the' Coastal Plains to the interior Piedmont and Highlands (*Figure 2*).

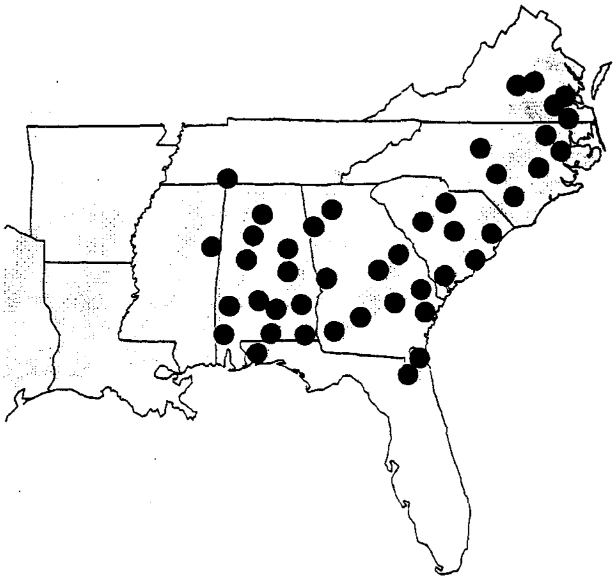

Figure 2. - Location of breeding programs within the Cooperative. There are currently 22 members of the Cooperative, but 44 separate breeding programs exist since many members operate in more than one geographic region. The natural range of loblolly pine is shaded.

Another factor concerning "breeding regions" is that trees from the local provenance may not be the best for that region. Results from the Southwide Pine Seed Source Study (WELLS, 1983) and other trials (e.g. MCKEAND et al., 1989) indicate that sources such as Atlanta Coastal NC and SC and Livingston Parish, LA perform very well compared to local material over a broad range of sites. Unfortunately, there are currently inadequate data to "fine tune" decisions concerning optimum seed source allocation. Data from the Cooperative's Plantation Selection Seed Source Study (Anonymous, 1987) will be available in 10 to 15 years to guide such decisions. Breeding populations may be restructured at that time.

Status of the Breeding Populations in the NCSU Tree Improvement Program

The Cooperative's 2^{nd}-cycle breeding populations incorporated a total of 720 second-generation parents and approximately

3300 phenotypic selections from unimproved plantations (Again, for any one Cooperative member- in a given region, approximately 600 of **these** parent trees are available.). The plantation selection effort was initiated in 1975 to augment, the relatively small and genetically less diverse second-generation population. Second-generation parents had unacceptably high co-ancestry due to the tester mating design used in first-generation breeding. The two sources of selections will be merged in the third cycle of breeding. See WEIR and ZOBEL (1975) and Anonymous (1982) for details.

The six-parent, disconnected half-diallel mating design being used in the second-cycle program was adopted in the late 1970's (**TALBERT,** 1979). Trees **from** two (rarely three) diallels were planted together in a test series comprised of four test plantations (two locations in each of two years). Each test consists of six blocks with six-tree plots of each cross in each block. A total of 144 seedlings per full-sib family was planted in each test series (**TALBERT** et al., 1981). Check **seedlots (a** local seed orchard mix and three unimproved seed collections from local and adjacent geographic areas) were included in each test series as standards by which diallels in different. test series can be compared (Anonymous, 1984). Field test establishment was completed in 1996.

Height, diameter, straightness, and fusiform rust, infection is measured for all trees in each test series at age six years (**MCKEAND,** 1988). A selection index which uses relative genetic and economic weights to assign values to each tree is used to select those individuals which maximize harvest value (**BRIDGWATER** and **SQUILLACE,** 1986; **LI** et al. 1996). Wood samples from select trees are taken to determine specific gravity values to combine with the other traits to determine which trees will be included in seed orchards and used for future breeding programs (**JETT** and TALBERT, 1982).

The first of the third-generation selections were made in 1991. Selection will continue on the schedule in figure 3. The majority of third-generation selections (approx. 70%) will be made after 1998, and all selection should be completed by 2003. Third-cycle breeding can commence around 1999 when about 50% of new selections will be available and pollen mixes are composited.

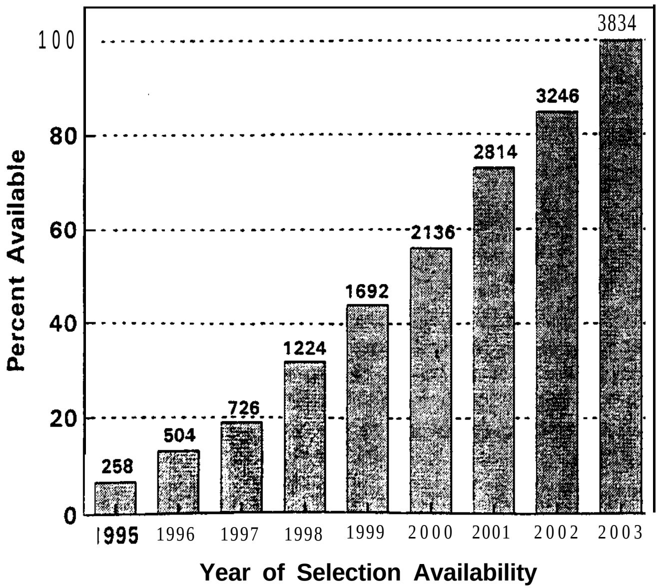

Figure 3. - Availability of third-cycle selections each year from current diallel tests. The actual number of parents from which selections can be made with B-year data is on each bar.

Third-Cycle Strategy

The total. breeding program is composed of local programs for each member in a region. There are 44 such local breeding programs, the geographic centers of these are illustrated in *figure* 2. Adjacent local breeding programs share material and responsibilities to a high extent (cf. the breeding region section) so that efforts are not. duplicated. We have chosen to describe the breeding program mainly from the local perspective.

Mainline Population

Given the long-term nature of the Cooperative's breeding program and uncertainties about future selection pressures (**KANG** and NIENSTAEDT, **1987),** a third-cycle breeding population with an initial population size of 160 unrelated selections available for each Cooperative member in a region (i.e. **a** recruitment population) is reasonable. While this population size is much smaller than the **600±** selections currently available, the reduction to 160 selections is warranted because:

- Mainline breeding populations will be composed of local selections only. For future generations, selections from adjoining or even distant geographic regions may be combined in breeding populations.
- The **600±** selections will have been tested, so that estimates of breeding values for each parent will be available. Had this cycle started with untested selections, 160 trees with unknown breeding values would be a bigger risk and probably unacceptable.

Since breeding responsibilities and financial burdens are shared in the Cooperative, each member will be responsible for breeding, testing, and selecting about one-fourth to one-sixth of the 160 clones available to a cooperator's program.

Both parental and progeny selections will be used in the third-cycle breeding program. The best 160 parents based on breeding value estimates from the diallel tests and low **coancestry** will be utilized. Until conclusive evidence is available **from** the Plantation Selection Seed Source Study and other seed source studies, recruitment populations will be composed of trees from local geographic origin.

The number of **sublines** and the number of entries per **subline** is primarily dictated by the size of the breeding population and the number of unrelated clones needed to establish orchards. With 160 selections, and **subline** sizes of 4 clones, 40 **sublines** would be available for establishment of orchards or other types of production populations. There are several potential benefits from making the **sublines** as small as possible:

- Inbreeding within a line will increase more rapidly, thus increasing the total genetic variance from which to make gain (**FALCONER** and MACKAY, 1996).
- The 40 **sublines** allow for exploitation of the increased genetic variance (1+F) among lines, since selections **from only** the best lines would be used for orchard establishment each generation.
- Alleles can be **fixed** faster in small populations (**KANG,** 1991).
- If selection criteria change in future generations, small **sublines** will be more responsive to selection (**NAMKOONG** et al., 1988).
- Breeding and testing each **subline** will be more rapid with small sublines. Each can be managed as a discreet unit.
- Small **sublines** can be combined into larger **sublines** in the future. If alternative population management strategies are deemed appropriate for future breeding cycles, the populations can be readily restructured. This is perhaps the greatest advantage of opting for very small **sublines** sizes. Choosing very small **subline** size initially may seem radical, but it is a very conservative alternative. If large **sublines** were formed

initially, it would be practically impossible to reduce them in size after only a few generations of breeding. Beginning with very small sublines allows the greatest flexibility in the long term.

- A minimum population size or status number (e.g. LINDGREN et al., 1997a) of size 20 can be maintained even after many generations.

Some of the potential disadvantages of maintaining small sublines include:

- Inbreeding may be too rapid so that alleles (both favorable and unfavorable) are fixed too rapidly and selection is not effective.
- Inbreeding will reduce fecundity sufficiently so that seed-production forbreeding and operational needs is very difficult and more expensive.
- Recombination is restricted to within lines in the breeding population.
- Sublines will be eliminated due to genetic sampling, drift, and/or selection.

Several of the key points above can be illustrated by computer modeling of different subline sizes. The alternatives we examined subdivided a population of 160 into 40 sublines of 4 each and 10 sublines of 16 each. We generated a population of 160 using the method of BRIDGWATER et al. (1993). Each individual in the population had 64 loci, each with alleles at equal frequencies. The model for gene action was completely additive. Assignment to the sublines was at random. Once sublines were formed, matings were made in a partial diallel mating scheme within sublines. Six crosses were made in each 4-tree subline in the 40-subline strategy Matings were done two different ways in the 16-tree sublines. First, the 16 parents in each subline were assigned to four 4-parent diallels (i.e. 4x4) and the 6 crosses were made among the parents. For comparison, all 120 crosses were also made among the 16 parents within each subline. One hundred progeny were generated per cross and the best phenotype was selected from the best *crosses; only* one individual per cross was selected. The best crosses were determined based on the average of their breeding values. Sufficient numbers of individuals were selected in each cycle to regenerate the subline size. Coancestry was restricted only in that self-fertilized individuals were not selected. We ran the simulation for 20 generations with 30 replicate runs per generation. Each simulation was run 10 times with different random assignments to form sublines.

Population additive variance for the larger 10-subline strategy mated in 4-parent diallels was essentially constant through generation 3, then declined to generation 20 (*Figure 4a*). The population additive variance for the 40-subline strategy increased from generations 1 through 6, then declined at a much lower rate than for the 10-subline strategy. After 20 generations, the 10-subline strategy had declined to about one-sixth of the initial level while the 40-subline strategy was still marginally higher than the initial variance. Making more crosses in the 16-parent sublines resulted in a faster rate of decline in the population additive variance than mating the 16 parents in 4-parent diallels (4x4) because the selection intensity was higher when more crosses were made. The difference in population additive variance between the 10- and the 40-subline strategies occurred largely because of the changes in the additive variance among sublines (Figure *4b*). The 10-subline strategy increased through generation 8, then declined to generation 20. The among-subline additive variance for the 40-subline strategy increased through generation 14 then plateaued at a value more than 6 times greater than the 10-subline strategy. When parents in the 10-subline strategy were mated in 4-parent diallels, the between, subline additive variance increased through generation 5 then declined to generation 16 where it plateaued until generation 20 at the same level as the

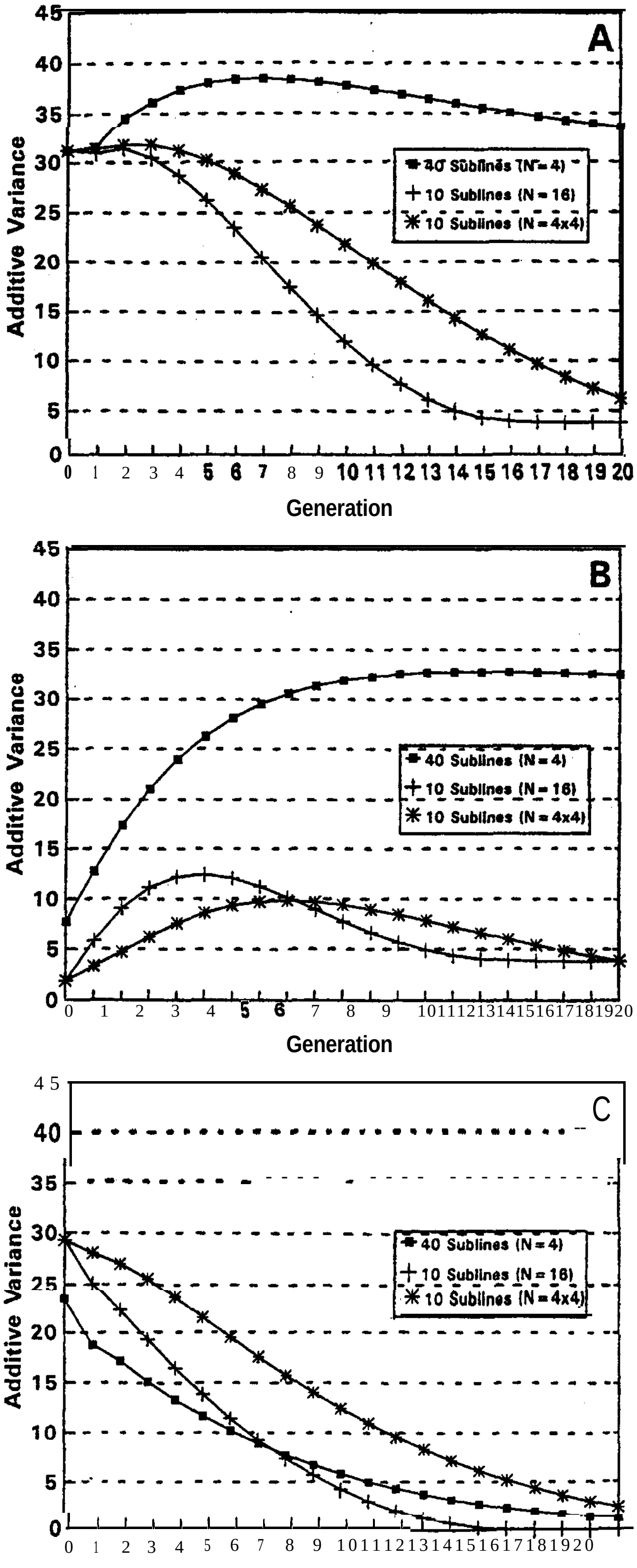

Figure 4. – Additive genetic variance in a population of 160 trees over 20 generations for three different subline strategies (40 sublines of 4 trees each – all possible matings, 10 sublines of 16 trees each – all possible matings, and 10 sublines of 16 trees each – mated as 4 diallels of 4 trees) as estimated by computer modeling (BRIDGWATER et al., 1993). The total genetic variance (A) is the sum of among subline variance (B) and within subline variance (C).

10-subline strategy which mated parents in 4-parent diallels. The within-subline variance was always greater for the 10-subline strategy with 4-parent diallels than either of the other alternatives (*Figure 4c*) The 10-subline strategy with 16-parent diallels was initially greater than the 40-subline strategy, but after converging at generation 8 was greater for the 40-subline strategy.

Inbreeding increased faster with smaller sublines as expected (*Figure 5a*). The 40-subline strategy reached F = 0.5, equivalent to one generation of self-fertilization, at generation 7, two generations sooner than the 10-subline strategy with 16-parent diallels. The 4-parent diallel mating scheme within the 16-parent sublines had a much lower rate of increase in mean inbreeding than either other strategy The average inbreeding was lower for the Pparent diallel mating scheme than for the 16-parent mating scheme in the 10-subline strategy because selection was not as intensive as when all possible matings were made among all 16 parents.

The status number declined rapidly for both the 40- and the 10-subline strategies through generation 7, then began to plateau (*Figure 5b*). The status number is the size of a non-inbred population of unrelated individuals following random mating that would give rise to a population of progenies with the same average inbreeding coefficient as the population under consideration (LINDGREN et al., 1997a). As expected, each population ultimately reached a status number equal to half the number of initial sublines.

Mean breeding values increased more rapidly for the lo-subline strategy than the 40-subline strategy and were about 40% greater for the 10-subline strategy after 20 generations (*Figure 6a*). The difference in gain is primarily due to the greater selection intensity in the 16-parent sublines. When more crosses were made in the 16-parent sublines the increased selection intensity resulted in greater genetic gains which plateaued at generation 16.

With the subline strategy all mating and selection is within sublines in the breeding population (BURDON et al., 1977). Thus, the results of our simulation are those we expected from small population theory (FALCONER and MACKAY, 1996). Of immediate concern to breeders is the fact that larger subline

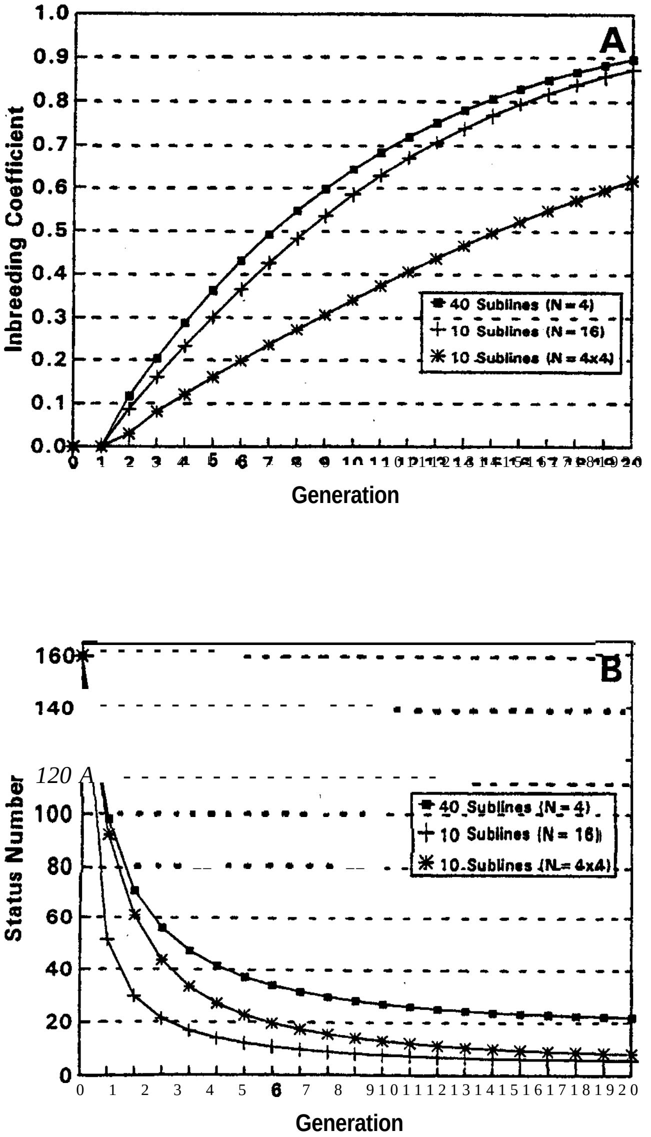

Figure 5. - Average inbreeding coefficient within subline over 20 generations (*Figure A*) and status number (*Figure B*) for three different subline strategies (40 sublines of 4 trees each - all possible matings, 10 sublines of 16 trees each - all possible matings, and 10 sublines of 16 trees each - mated as 4 diallels of 4 trees) as estimated by computer modeling (BRIDGWATER et al., 1993).

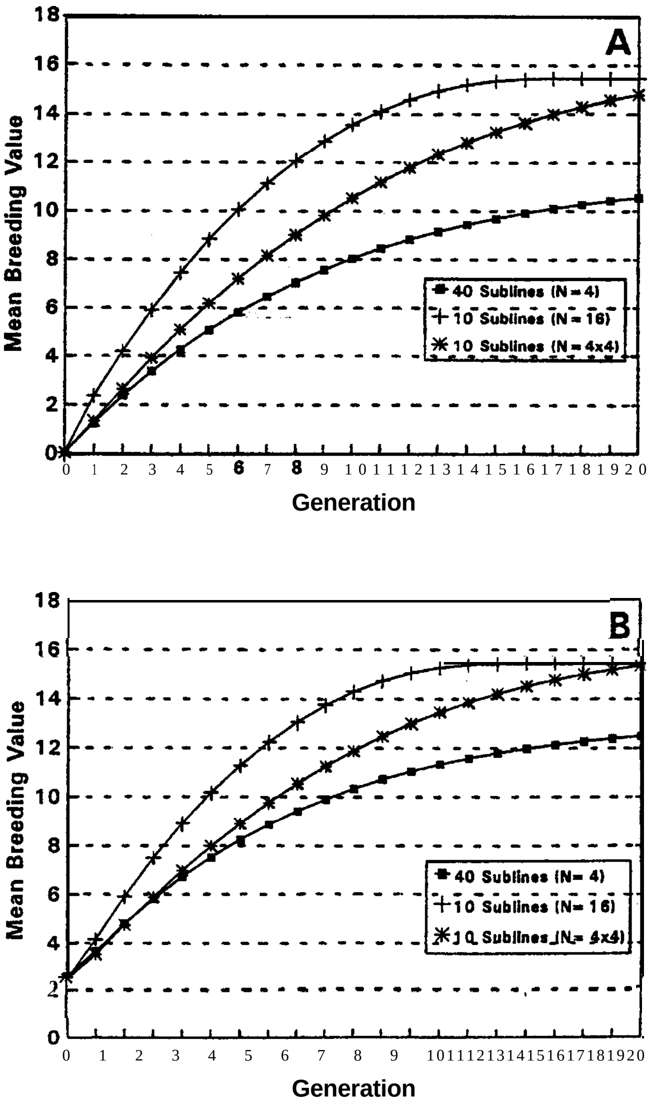

Figure 6. - Mean breeding values over 20 generations for three different subline strategies (40 sublines of 4 trees each - all possible matings, 10 sublines of 16 trees each - all possible matings, and 10 sublines of 16 trees each - mated as 4 diallels of 4 trees) as estimated by computer modeling (BRIDGWATER et al., 1993). The average for the entire breeding population is depicted in figure *A*. The average for the top 10 parents is in figure *B*.

sizes distribute more of the total variance within sublines (Figure *4c*). Thus, selection intensity is greater within sublines resulting in greater increases in breeding values (*Figure 6a*) and less rapid increases in coancestry in the short term (Figures *5a* and *5b*). Conversely, smaller subline sizes distribute more of the variance among sublines 4b) resulting in smaller increases in mean breeding value and more rapid increases in coancestry within sublines. The advantages of smaller subline sizes are long-term. Smaller subline sizes maintain the total population variance longer than larger subline sizes, which is desirable from a conservation viewpoint. Smaller subline sizes defer some of the potential genetic gains from selection within sublines. Making more crosses within sublines for any choice of subline size will increase the selection intensity, the expected genetic gain and the rate of inbreeding (Figures *6a* and *5a*).

There are three factors that deserve further discussion from a practical tree breeding viewpoint. The *first* is the difference in rate of increase in coancestry between the two strategies. We emphasize that the more rapid increases in coancestry within sublines with the smaller subline sizes was not reflected in the genotypic means since we have adopted an additive genetic model. However, this is not important in terms of genetic gains that may be expected from plantations established from utilization populations such as seed orchards since utilization populations will be constructed by selecting unrelated individuals from different sublines. Inbreeding depression will be a factor in tree improvement because increasing coancestry will result in inbreeding depression in vigor and fecundity of parents used to produce propagules for plantation establishment, and reduced seed yields implies greater cost. The second practical point is that the differences in mean breeding values for the populations may be ameliorated when selections are made to construct an elite breeding population or a utilization population. For example, if a minimum of 10 unrelated parents are required to establish a seed orchard, one individual must be chosen from each of the 10 sublines with the larger subline strategy. However, with the smaller subline strategy, among-subline selection is also possible. Thus, the difference in average breeding value for the best 10 parents from different strategies is not as great as the difference in mean breeding value for the breeding populations (*Figure 6b*). *In* our simulations, the difference was reduced by about one-half after 20 generations. Finally, increasing the number of crosses made and thus the entire effort in the breeding and testing programs increased the rate of gain. Making five times more crosses (1200 vs. 240) in the 16-trees subline strategy produced average breeding values at generation 14 equivalent to those at generation 20 for the l&parent sublines mated in B-parent diallels.

Establishment of Sublines

A sublining system is not only effective for managing inbreeding and genetic variance, but it is also very effective for managing a series of small, homogeneous populations of trees ordered along a continuous environmental gradient. There are no discrete "breeding regions" in the Cooperative, but a continuous series of populations (*Figure 2*). Since environmental factors which affect genotypic performance are continuous, different selection criteria can be utilized in a continuous fashion in managing the sublines. For example, the importance of fusiform rust resistance gradually changes both N-S and E-W. The economic weight for rust resistance can be changed in a selection index for sublines occurring along rust gradients. Sublining also simplifies breeding, since each subline will be managed by a single Cooperative member. The logistical problems of exchanging selections, pollen, seeds, and seedlings for breeding, tasting, and selection will be eliminated.

The first four parents selected with "high" breeding values will form a subline. Subsequent sublines will be constituted as suitable selections are made. The third-cycle breeding program will commence about 1999 when about 40% to 50% of the third-cycle selections are available (*Figure 3*).

The mating design for the sublining system will be a complementary design that combines pollen-mix mating to estimate GCA's and control-pollinated matings for within-family selection. VAN BUIJTENEN and BURDON (1990) and BURDON and VAN BUIJTENEN (1990) emphasize the value of pollen mix designs combined with control-cross designs. A pollen mix of 30± average selections that differ for different regions in the Cooperative will be used for GCA estimation of parents. Control-pollinated families will be used only to provide phenotypes for within-family selection.- Sii crosses among 4 parents in each sublime will be made. Seedlings from the control-pollinations will be planted in non-replicated lull-sib family blocks, and the best phenotypes will be selected. The crosses from which selections will be made will be determined by the results of the pollen mix tests. Crosses with the highest expected mid-parent value and highest population merit (LINDGREN and MULLIN, 1997) will be identified, and the best individual phenotype(s) within those crosses will be selected.

As each new parent is selected for inclusion in the breeding population, it will be established in a breeding orchard and "top grafted" into the crowns of mature trees and intensively managed for strobilus production. With stimulation, female strobili can be produced very early on loblolly pine (BRAMLETT, 1997; GREENWOOD, 1981). Enough females should be available so that breeding with the stored pollen mix can begin 2 to 3 years after grafting. All pollen-mix seeds should be available 6 years following grafting.

Information from numerous field trials will be used to determine the optimum age to select among full-sib families based on pollen mix tests. Selection of individuals within full-sibs is expected to be most efficient at a later age than selection based on PMX tests (BALOCCHI et al., 1994). Therefore, these crosses must be made as soon as both pollen and females are available from the selected parents. Pollen production from young grafts is usually not as precocious as female production, so full-sib crosses will usually be made after the PMX crosses.

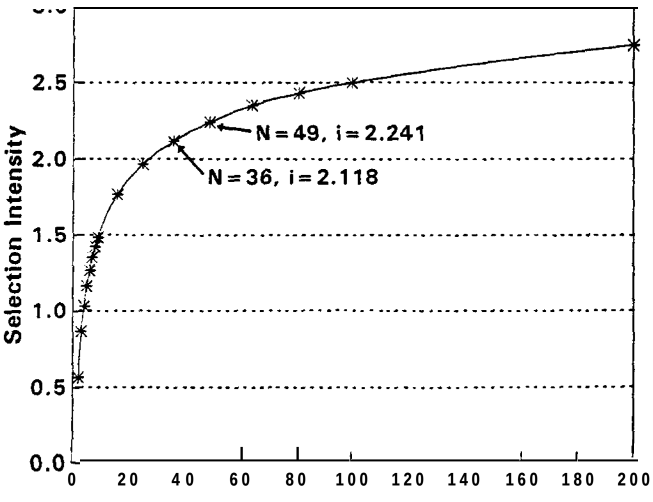

Figure 7. - Selection intensity for selecting one tree in a **block plot of various sizes.**

Table 1. — Idealized time line for breeding activities in the mainline population for a local program in the third cycle[1]).

Year	Activity
0	Select and graft 160 selections into breeding orchard.
5	Pollen-mix (PMX) cross the 160 genotypes.
6	Plant PMX tests to estimate breeding values of the 160 parents.
8	Structure the 160 genotypes in 40 sublines and complete the 6 crosses among the 4 parents in each subline.
10	Complete planting all the full-sib families in blocks.
16	Complete selection of superior individuals from full-sib families that have highest breeding values and population merit based on polycross tests.
16	Start next cycle.

---

[1]) For simplicity and illustration, we have assumed that all 160 parents will be available at one time. Third-cycle selections will actually be available over a 9 year period (*Figure 3*).

As soon as seeds are available, a block plot of at least 36 individuals will be installed for each cross. The selection intensity of selecting one tree out of 36 trees appears to be about optimal (Figure 7).

Below and in table 1 is a summary of activities for the mainline breeding program. For simplicity, it is assumed that all 160 selections will be available at the same time. From figure 3 it is obvious that this will not be the case. Selections will be available over a nine year period and will be assigned to different sublines in a chronological order.

a. Polycross all 160 selections. This can be done 2± years after selection since females are typically produced first and the pollen mix will be available. Plant in field tests at year 6.

b. Assign the 160 clones to 40 sublines of 4 parents each in a chronological order. Assign 4 trees to each subline based on: 1) relatedness – every effort will be made to minimize relatedness among sublines by putting related trees, especially siblings, in the same subline; 2) the physical location of the parent trees (i.e. make sure only one member is responsible for a subline); and 3) flowering (i.e. first trees that flower are assigned to a subline).

- Make the 6 crosses among the 4 parents in each subline.
- Finish planting the full-sib crosses in at least 36-tree block plots about year 10. Larger plots will increase the selection differential, but the expected gains with the addition of more individuals increase at a slower rate as numbers increase. Make final selections about year 16.

With the time difference within a generation of when selections will be available *(Figure 3)*, our strategy is similar to the concept of the "rolling front" breeding strategy of BORRALHO and DUTKOWSKI (1996) and appears to have many of the advantages. In the rolling front strategy, rather than wait for all the selections within a generation to be available, the best trees are mated together each year regardless of the generation from which they come. The 4-tree sublines will likely be bred at different rates, and at any one time, sublines will vary as to the number of generations of breeding. Overall, breeding should not be delayed for the entire mainline population due to a few Slow flowering clones.

Elite Populations

A significant benefit of the reduced size and effort in the mainline program is that more emphasis can be devoted to the elite populations where short-term gain and financial benefit will be substantial. The additional gain to be obtained from breeding only the most highly selected trees for specific geographic regions (e.g. COTTERILL et al., 1988; WILLIAMS and HAMRICK, 1996) was the main attraction to including elite populations into our hierarchy of breeding populations. There is a substantial increase in selection intensity from utilizing only the best parents in a recruitment population rather than all 160 trees.

Numerous options are available for management of elite populations. Rather than developing one elite population breeding strategy for all cooperators, elite populations will be customized for each cooperator or subsets of cooperators. Individual members or groups of members will decide if and how their elite populations will be managed. Populations can be structured using local or non-local genotypes to utilize only general combining ability effects or both general and specific combining ability effects. Since one of the main benefits of elite populations is that breeding cycles can be completed more rapidly than in the mainline, any strategy adopted must have fast generation cycling as a feature.

One option we are evaluating is the use of inbreeding to rapidly increase homozygosity and the frequency of favorable alleles as well as to increase additive genetic variance among sublines. Another advantage of very rapid inbreeding (e.g. selfing) is that the among-family selection intensity for selfed families is greater than for bi-parental crosses. With selfing, the best parent based on GCA performance is mated with itself and can be selected without also selecting another parent with a poorer genotype.

While selling theoretically offers the most rapid gain, there are several problems to be addressed. Getting an adequate number of selfed seeds (200±) will be difficult or impossible for many clones. Based on past selfing trials, selfing will only be feasible for about 50% of the clones. Thus, if an elite population of 40 parents is utilized, selfed seeds from about 20 parents may be obtained at a reasonable cost. Selfing may also increase homozygosity so rapidly that selection may not have a chance to operate. Slower inbreeding such as full-sib mating or half-sib mating could be more effective in the long run.

For the second and future cycles in the elite population, there are numerous options available for advancing to the next generation. Selfing can continue and S_2 offspring from the selected S_1's can be produced. Progeny from outcrossed trees could be selfed to create new S_1's. For milder levels of inbreeding that might allow selection to operate more effectively, crosses between full-sibs or crosses among half-sibs can be made. There are also numerous options for crosses among all parents within the elite population (e.g. among S_1's or among any selections within or among sublines would be possible).

Additionally, there is no reason to consider the elite populations as "closed". Breeding values are expected to overlap in the mainline and elite populations for at least a few cycles (i.e. heritabilities are relatively low and selection is far from perfect). Each cycle, the best genotypes from the mainline population in the same geographic area or from other elite and mainline populations can be infused into the elite population if the breeding values meet selection criteria. Infusion of new genotypes will also enhance the genetic variation in the elite populations.

To increase gain in the short run, as many crosses as possible should be made among the 40 parents in the elite population. While a 40-tree diallel is not realistic (780 crosses and 40 selfs), if 8-tree diallels are used, then 140 crosses plus 40 selfs would generate a large population from which to make substantial gain. If future breeders desire to maintain a sublining system within the elite population, related trees from within each diallel can be maintained within lines. The full-sib and' half-sib crosses could be one way of maintaining the genetic integrity of a subline. In fact, the full-sib crosses described above can be considered as 2-tree sublines from within the diallels. The selfs are nothing more than one-tree sublines if S_2's are made.

Elite populations are expected to cycle faster than the mainline since there are $1/4$ the number of trees as in the mainline, and more effort till be devoted to the elite population. With fewer trees to breed in the elite populations, pollen mix crosses and the full-sib matings should be completed at least 3 years faster than in the mainline population (Figure 1).

Genetic Diversity Archives

Recognition of the importance of conserving forest genetic resources is increasing (NRC, 1991; NAMKOONG, 1997). We have made no attempt to elaborate on the economic, biological, or ethical needs for gene conservation, but the extremely diverse and rich gene pool for loblolly pine must be preserved and managed to ensure that a healthy genetic resource will exist in the long term. The fully-pedigreed populations that the Cooperative has developed and will continue to develop are an invaluable resource for breeders and forest scientists today and for the future. For long-term preservation of genetic diversity in loblolly pine populations, an additional management strategy (apart from the mainline breeding program) will be the judicious use of Genetic Diversity Archives to preserve all existing selections being bred in diallels as well as many future selections. First, archives or clone banks will be used to preserve the gene pool that the Cooperative amassed in the 1950's through the 1980's. Almost 4000 trees have been selected from natural stands and unimproved plantations throughout the Cooperative's working area (Maryland to Mississippi). The vast majority of selections from this enormous genetic sample still exists in seed orchards or clone banks. A concerted effort has been made to preserve these selections in the genetic diversity archives. If environmental or selection criteria change dramatically in the future, these archives will be valuable if mainline breeding populations do not contain the genes that breeders desire. In future breeding cycles, rather than lowering the selection intensity in the mainline population to ensure the maintenance of rare alleles or rare allelic combinations, up to 150 "unique" genotypes (in addition to the 160 in the mainline) will be selected from each recruitment population for inclusion in the archives. These trees will have unique and extreme values for different traits (e.g. very large volume but relatively poor straightness or very high wood specific gravity and relatively poor rust resistance). If future breeders find that collection of open-pollinated seeds or additional crossing among clones in the archives is warranted, all trees will be available for this effort.

Genetic resources are also available from other loblolly pine breeding programs in the South and from around the world. The Western Gulf Forest Tree Improvement Program and the U.S.D.A. Forest Service both have advanced-generation breeding programs that are based on different gene pools. Where loblolly pine has been used as an exotic (e.g. Argentina, Brazil, China, France, South Africa, Zimbatwe), there are also breeding programs and potential genetic resources. Natural populations of loblolly pine in the South will continue to exist for selection in the unlikely event that desirable alleles are not available in breeding or archived populations.

The Cooperative has taken a very conservative approach to managing and preserving the genetic resources of our loblolly pine populations. NAMKOONG (1984) argues that hierarchical populations are not necessary for managing genetic diversity. The multiple population breeding strategy in itself is a gene conservation strategy. "As a part of breeding population management, gene conservation implies much more than preserving one generation's allelic array, In this context, gene conservation is identical to long-term breeding or populational gene management and implies wide population sampling as a start for designed multiplicity, and continued development of population diversity as environmental and economic demands change." (NAMKOONG, 1984). We have elected to add the genetic diversity archives as an additional low-cost insurance of diversity.

Workloads

A critical criterion for evaluation of breeding strategies is the workload required to complete a cycle of improvement. In *table* 2, the breeding and testing efforts for the Cooperative's 2nd-cycle program (the current 6-parent-diallels) are compared with the 3rd-cycle program (both the mainline and elite strategies). For a typical Cooperative member, there are currently 100 selections bred in 6-parent-diallels resulting in 250 crosses planted in 30 ha to 35 hectares of actual test trees in the 2nd-cycle. For the 3rd-cycle program, there will be a significant overall reduction in effort. Depending on how many cooperators share in the breeding and testing efforts in a given area, each organization would be responsible for breeding and testing between 16 and 40 parents for the mainline program and between 4 and 12 parents for a single elite population. The number of crosses, including polycrosses, will be 58 to 154 per cooperator, or about 1/5 to 3/5 the number of crosses in the 2nd-cycle program. The testing program will consist of 4 ha to 8 hectares of tests per cooperator, or about 1/8 to 1/4 the size in the and-cycle.

Every effort to keep the logistics as simple as possible in the field have been made. While the management and coordination of the different populations (especially elite populations with potentially different breeding strategies) will be complex, the actual breeding, testing, and selection activities by cooperators will be much simpler than the diallel system currently in use.

Additional Issues

A final issue concerns the compatibility of the third-cycle breeding plan with new technologies in forestry and genetics. If, for example, vegetative propagation of mature loblolly pine becomes feasible, breeders may want to reconsider breeding for specific combining ability as well as for general combining

Table 2. — Comparison of breeding and testing efforts for the NCSU-ICTIP for the Second- and Third-Cycle Programs.

Criterion for Comparison	Second-Cycle	Third-Cycle	
		Mainline	Elite[1]
Population size for a local Cooperative member			
Recruitment	**600±**	160	40
Parents to breed	**100±**	16-40	**4 -** 12
Breeding			
#Crosses / member	250	**PMX:** 16-40	
		CP: 24 - 60	**Selfs:4** - 12
			CP: 14-42
Testing[2]			
#tests / member	32	**PMX: 1 - 2**	
		Block 24 - 60	Blocks: 18 - 54
#hectares of tests / member (assume 3m x 3m spacing)	32	**PMX: 2 - 4**	
		Blocks: 1 - 2	Blocks: 0.6 - 1.7

---

[1]) Assume an elite population will have 5 diallels of 8 trees each.

[2]) Assume for PMX tests, there are two series (early and late selections) each with 80 families. Each of 4 tests has 30 seedlings per family. Assume block plots have 36 trees total per cross.

ability in the mainline program. The *small* **sublines** lend themselves very well to a paired **sublining** strategy or a reciprocal recurrent selection strategy to advance SCA and GCA (**McKeand** et al., 1986). Another option available to any cooperator is to advance SCA and GCA in elite populations.

Vegetative propagation could also change and enhance the testing and selection program. If individuals within families can be clonally replicated, the within family heritability would be much higher, and gain from within family selection would be increased. Additionally, to exploit SCA and GCA more fully in production populations, additional crossing among parents and planting their full-sib progenies in replicated field trials may be desirable.

New developments in molecular genetics can also be incorporated into the Cooperative's breeding strategy. If molecular marker aided selection (MAS) for quantitative traits becomes feasible (e.g. Williams and Neale, 1992; **O'Malley** and **McKeand, 1994**), the line breeding and particularly the inbreeding in the sublines will be a major benefit. Marker aided selection depends upon the maintenance of linkage disequilibria between the marker loci and the quantitative trait loci (**Lande** and **Thompson,** 1990). Line breeding as opposed to random mating among individuals is very effective at maintaining linkage disequilibria for multiple generations, and enhancing the efficiency of MAS (**O'Malley** and **McKeand, 1994**).

For other biotechnologies such as recombinant DNA to be applied to forest tree populations, breeders must have a better understanding of the genetic control of specific traits. The elite populations lend themselves to very intensive genetic experiments where breeders can intensively study a few, highly selected individuals to conduct the necessary physiology and biochemistry experiments.

Acknowledgments

The authors were members of a task force whose other members were **Ralph** C. **Bower,** MacMillan Bloedel, Inc.; **John** A **Hendrickson, Kimberly-Clark** Corporation (formerly Scott Paper Company); Barbara G. **McCutchan,** Westvaco Corporation; C. **William Mueller,** Tenneco Packaging Inc.; **Robert** C. **Purnell,** International Paper Company; Robert J. Weir, **NCSU-ICTIP;** and **Marvin** H. Zoerb, Jr., Union Camp Corporation. We gratefully acknowledge their contribution as well as others who gave generously of their time and experience to help develop this breeding strategy.

Literature

Anonymous: 26th Annual Report. N.C. State University-Industry Cooperative Tree Improvement Program. 72p. (1982). -Anonymous: 28th Annual Report. N.C. State University-Industry Cooperative Tree Improvement Program. 68 p. (1984). — Anonymous: Strategic plan for loblolly pine. N. C. State. University-Industry Cooperative Tree Improvement Program. 55 p. (1987). -Baker, L. H. and **Curnow,** R. N.: Choice of population size and use of variation between replicate populations in plant breeding programs. Crop Sci. **9:555–560** (1969). — Balocchi, C. E., Bridgwater, F. E. and Bryant, R.: Selection efficiency in a nonselected population of loblolly pine. For. Sci. **40:452–473** (1994). — Balocchi, C. E., Bridgwater, F. E., Zobel, B. J. and **Jahromi,** S.: Age trends in genetic parameters for tree height in a nonselected population of loblolly pine. For. Sci. **39:231–251** (1993). — **Borralho,** N. M. G. and **Dutkowski,** G. W.: A 'rolling-front' strategy for breeding trees. P. 317-322. *In:* Dieters, M. J., **Matheson,** A. C., **Nikles,** D. G., **Harwood,** C. E. and **Walker,** S. M. (*eds.*). Tree Improvement for Sustainable Tropical Forestry. **Proc.** QFRI-IUFRO Conf. Caloundra, Queensland, Australia. 27 Oct. to 1 Nov., 1996. (1996). — **Bramlett,** D. L.: Genetic gain from mass controlled pollination and topworking. J. For.

95(3):15–19 (1997). — BRIDGWATER, F. E. and FRANKLIN, E. C.: Forest tree breeding: strategies, achievements and constraints. p. 36-48. *In:* Attributes of trees as-crop plants. CANNELL, M. G. R. and J. E. JACKSON feds.), Titus Wilson & Son, Ltd., Cumbia. Great Britain. 592 D. (1985). — BRIDGWATER, F. E. and SQUILLACE, A. E.: Selection indexes for forest trees. p. 17-20. *In:* Southern Coop. Ser. Bull. No. 309. Advanced Generation Breeding of Forest Trees. Proc. of a Workshop on Advanced Generation Breeding. Current Status and Research Needs, June 6 to 7, 1984. Baton Rouge (1986). — BRIDGWATER, F. E., WOODBRIDGE, W. C. and MAHALOVICH, M. F.: Computer modeling of a sublining breeding system. p. 327-333. *In:* Proc. 22nd South. Forest Tree Improv. Conf., Atlanta, GA. (1993). — BURDON, R., SHELBOURNE, C. J. A. and WILCOX, M. D.: Advanced selection strategies. p. 1133-1147. *In:* Proc. of Third World Consultation On Forest Tree Breeding, IUFRO, Canberra, Aust. Vol. 2. (1977). — BURDON, R. D. and VAN BUIJTENEN, J. P.: Expected efficiencies of mating designs for reselection of parents. Can. J. For. Res. 20:1664–1671 (1990). — BYRAM, T. D. and LOWE, W. J.: General and specific combining ability estimates for growth in loblolly pine. p. 352–360. *In:* Proc. IUFRO Conf. Joint Meeting of Working Parties on Breeding Theory, Proaeny Testing, Seed Orchards. Williamsburg, VA *(1986).* — COTTERILL, P., DEAN, C., CAMERON, J. and BRINDBERGS, M.: Nucleus breeding: a new strategy for rapid improvement under clonal forestry. *In:* Proc. IUFRO Meeting on Breeding Tropical Trees. Pattaya, Thailand (1988). — FALCONER, D. S. and MACKAY, T. F. C.: Introduction to quantitative genetics. Longman, Essex, England. 464 p. (1996). — FRANKLIN, E. C.: Inbreeding-as a means of genetic improvement of loblolly pine. p. 107-115. *In:* Proc. 10th South. Forest Tree Improv. Conf.. Houston, TX. (1969). — GREENWOOD, M. S.: Reproductive development in loblolly pine. II. The effect of age, gibberellin plus water stress and out-of-phase dormancy on long shoot growth behavior. Amer. J. Bot. 68:1184–1190 (1981). — GREENWOOD, M. S., LAMBETH, C. C. and HUNT, J. L.: Accelerated breeding and potential impact upon breeding programs. p. 39-42. *In:* Southern Coop. Ser. Bull. No. 309. Advanced Generation Breeding of Forest Trees Current Status and Research Needs, June 6 to 7, 1984. Baton Rouge, LA (1986). — HALLAUER, A. R. and MIRANDA, Fo., J. B.: Quantitative genetics in maize breeding. 2nd Ed. Iowa Univ. Press, Ames. 468 p. (19881. — JETT, J. B. and TALBERT, J. T.: The place of wood specific gravity in the development of advanced-generation seed orchards and breeding programs. South. J. Appl. For. 6:177–180 (1982). — KANG, H.: Long-term tree breeding. p. 66–72. *In:* Proc. 15th South. For. Tree Impr. Conf., June 19 to 21, 1979. Miss. St. Univ. (1979). — KANG, H.: Recurrent selection, mating design, and effective population size. p. 129-143. *In:* Proc. 21st South. For. Tree Impr. Conf., June 17 to 20, 1991. Knoxville, TN (19911. — KANG, H. and NIENSTAEDT, H.: Managing long-term breeding stock. Silvae Genetica 36:30–39 (1987). — KANNENBERG, L. W.: Utilization of genetic diversity in crop breeding. p. 93-109. *In:* Plant Genetic Resources. C. W. YEATMAN, D. KAFTON and G. WILKES (eds.). Westview Press, Boulder (1984). — KRAUS, J. F., WELLS!, 0. 0. and SLUDER, E. R.: Review of provenance variation in loblolly pine (*Pinus taeda* L.) in the southern United States. p. 281-317. *In:* Provenance and genetic improvement ***strategies*** in tropical forest trees. R. D. BARNES and G. L. GIBSON (eds.). Commonw. For. Inst., Oxford, England and For. Res. Cent., Hirare, Zimbabwe (1984). — LANDE, R. and THOMPSON, R.: Efficiency of marker-assisted selection in the improvement of quantitative traits. Genetics 124:743–756 (1990). — LI, B., MCKEAND, S. E. and WEIR, R. J.: Genetic parameter estimates and selection efficiency for the loblolly pine breeding the south-eastern U.S. P. 164-168. *In:* DIETERS, M. J., MATHESON, A. C., NIKLES, D. G., HARWOOD, C. E. and WALKER, S. M. (*eds.*). Tree Impmvement for Sustainable Tropical Forestry. Proc. QFRI-IUFRO Conf. Caloundra, Queensland, Australia. 27 Oct. to 1 Nov., 1996 (1996). — LI, B. and WYCKOFF, G. W.: Breeding strategies for ***Larix decidua, L. leptolepis*** and their hybrids in the United States. For. Gen. 1:65–72 (1994). — LINDGREN. D.: Inbreeding and coancestry. P. 49-72. *In:* Proc. IUFRO Joint Meeting of Working Parties on Population and Ecological Genetics, Breeding Theory, Biochemical Genetics and Progeny Testing. Bordeaux, France (1976). — LINDGREN, D., GEA, L. D. and JEFFERSON, P. A.: Status number for measuring genetic diversity For. Gen. 4:69–76 (1997a). — LINDGREN, D. and MULLIN, T. J.: Balancing gain and relatedness in selection. Silv. Gen. 46:124–129 (1997). — LINDGREN, D., WEI, R.-P. and LEE, S. J.: How to calculate optimum family number when starting a breeding program. For. Sci. 43:206–212 (1997b). — LOWE, W. J. and VAN BUIJTENEN, J. P.: The development of a sublining system in an operational tree improvement program. p. 352-360. *In:* Proc. IUFRO Conf. Joint Mtg. of Working Parties on Breeding Theory, Progeny and Seed Orchards. Williamsburg, VA (1986). — MAHALOVICH, M. F.: Modeling positive assortative mating and elite populations in recurrent selection programs for general combining ability. PhD Thesis, North Carolina State Univ., Raleigh. 129 p. (1990). — MATHESON, A. C.: Strategy and tactics. p. 4.1-4.13. *In:* Radiata Pine Breeding Manual. A. C. MATHESON and A. G. BROWN (eds.). CSIRO, Canberra, Aust. (1983). — MCKEAND, S. E.: Optimum age for family selection for growth in genetic tests of loblolly pine. For. Sci. 34:400–411 (1988). — MCKEAND, S. E. and BEINEKE, W. F.: Sublining for half-sib breeding populations of forest trees. Silvae Genetica 29:14–17 (1980). — MCKEAND, S. E. and BRIDGWATER, F. E.: Variance component and genetic gain estimates from 6-year-old diallel tests of loblolly pine. P. 197–203. *In:* Proc. 23rd South. For. Tree Impr. *Conf.*. Asheville, NC (1995). — MCKEAND, S. E., FOSTER, G. S. and BRIDGWATER, F. E.: Breeding systems for pedigree-controlled production populations of loblolly pine. p. 53-61. *In:* Southern Coop. Ser. Bull. No. 309. Advanced Generation Breeding of Forest Trees Current Status and Research Needs, June 6 to 7, 1984. Baton Rouge, LA (1986). — MCKEAND, S. E. and JETT. J. B.: Effect of inbreeding on superior loblolly pine. Abstract in CTIA/WFGA Conference. Evolution and Tree Breeding. Victoria, BC. Aug. 28 to Sept. 1. 1995. (1995). — MCKEAND, S. E., WEIR, R. J. and HATCHER, A. V.: Performance of diverse provenances of loblolly pine throughout the southeastern United States. South. J. Appl. For. 13:46–51 (19891. — NAMKOONG, G.: Strategies for gene conservation in forest tree breeding. p. 79-89. *In:* Plant Genetic Resources -A Conservation Imperative. C. W. YEATMAN, D. KAFTON, and G. WILKES (eds.). AAAS Selected Symposium 87. Westview Press, Inc., Boulder, CO. 164 p. (1984). — NAMKOONG, G.: A gene conservation plan for loblolly pine. Can. J. For. Res. 27:433–437 (1997). — NAMKOONG, G., KANG, H. and BROUARD, J. S.: Tree breeding: principles and strategies. Springer-Verlag, NY. 180 p. (1988). — NIKLES, D. G.: Hybrids of forest trees: the bases of hybrid superiority and a discussion of breeding methods. P. 333-347. *In:* Proc. IUFRO Conf. S2.02–08, Breeding Tropical Trees. Solving tropical forest resource, concerns through tree improvement, gene conservation and domestication of new species. Oct. 8 to 18, 1992. Cartagena and Cali, Colombia (1992). — NRC: Managing global genetic resources - forest trees. Committee on Managing Global Genetic Resources: Agricultural Imperatives, Subcommittee on Managing Plant Genetic Resources, Forest Genetic Resources Work Group: Board on Agriculture, National Research Council. National Academy Press, Washington, DC. 228 p. (1991). — O'MALLEY, D. M. and MCKEAND, S. E.: Marker assisted selection for breeding value in forest trees. Forest Genetics 1:231–242 (1994). — ORR-EWING, A. L.: Inbreeding Douglas-fir to the S_3 generation. Silvae Genetica 25:179–183 (19761. — PURNELL, R. C. and KELLISON, R. C.: A tree improvement program for southern hardwoods. p. 90-98. *In:* Proc. 17th South. Forest Tree Improv. Conf.. Athens, GA (1983). — RAWLINGS, J. O.: Resent status of research on long and short-term recurrent selection in finite populations - choice of population size. p. 1-15. *In:* Proc. Second Mtg. of the Working Group on Quantitative Genetics. Section 22 IUFRO. Aug. 18 to 19, 1969. Raleigh, NC (1970). — SCHMIDTLING, R. C.: Use of provenance tests to predict response to climatic change: loblolly pine and Norway spruce. Tree Physiology 14:805–817 (1994). — SHELBOURNE, C. J. A: New approaches-in breeding radiata pine. P. 269-284. *In:* Proc. IUFRO Conf. Joint Meeting of Working Parties on Breeding Theory, Proaeny Testing, Seed Orchards. Williamsburg, VA (1986). — SNIEZKO, R. A. and ZOBEL, B. J.: Seedling height and diameter variation of various degrees of inbred and outcross progenies of loblolly pine. Silvae Genetica 37:50–60 (1988). — TALBERT, J. T.: An advanced-generation breeding plan for the N.C. State University-Industry Pine Tree Imomvement cooperative. Silvae Genetica 28:72–75 (1979). — TALBERT, J. T., BRIDGWATER, F. E. and LAMBETH, C. C.: Genetic testing manual. N.C. State University-Industry Cooperative Tree Improvement Program. 37 p. (1981). — VAN BUIJTENEN, J. P. and BURDON, R. D.: Expected efficiencies of mating designs for advanced-generation selection. Can. J. For. Res. 20:1648–1664 (1990). — VAN BUIJTENEN, J. P. and LOWE, W. J.: The use of breeding groups in advanced generation breeding. p. 59-65. *In:* Proc. 15th South. Forest Tree Improv. Conf.. Starkville, MS (1979). — WEIR, R. J. and ZOBEL, B. J.: Managing genetic resources for the future, a plan for the N. C. State-Industry Cooperative Tree Impmvement Program. p. 73-82. *In:* Proc. 13th South. For. Tree Improve. Conf.. Raleigh, NC (1975). -WELLS, 0.0.: Southwide pine seed source study - loblolly pine at 25 years. South. J. Appl. For. 7:63–71 (19831. — WHITE, T.: Advanced-generation breeding populations: size and structure. P. 208-222. *In:* Proc. IUFRO Conf. S2.02–08, Breeding Tropical Trees. Solving tropical forest resource concerns through tree improvement, gene conservation and domestication of new species. Oct. 8 to 18, 1992. Cartagena and Cali, Colombia (19921. -WHITE, T. L., HODGE, G. R. and POWELL, G. L.: An advanced-generation tree improvement plan for slash pine in the southeastern United States. Silvae Genetica 42:359–371 (1993). — WILLIAMS, C. G. and HAMRICK, J. L.: Elite populations for conifer breeding and gene conservation. Can. J. For. Res. 26:453–461 (1996). — WILLIAMS, C. G. and NEALE, D. B.: Conifer wood quality and marker-aided selection: a case study. Can. J. For. Res. 22:1009–1017 (1992). — WILLIAMS. C. G. and SAVOLAINEN, 0.: Inbreeding depression in conifers: Implications for breeding strategy. For. Sci. 42(1): 102-117 (1996).