indication that breeding programs should concentrate on individual and family sampling within well-adapted provenances for optimum genetic gain on desirable traits.

Acknowledgements

This work was performed at the Institute of Forest Genetics, Placerville, California. The authors are grateful to P. Hodgskiss for technical assistance and to F. Bergmann, A. Kremer, F. T. Ledig and E. Teissier du Cros for helpful comments on the manuscript. This study was made possible by a grant from the French Ministry of Foreign Affairs (Bourse Lavoisier).

References

Bergmann, F. and Kownatzki, D.: The genetic variation pattern of silver fir (*Abies alba*) in Europe monitored from enzyme gene loci. Proc. IUFRO-Tannensymposium, Zvolen, 3 to 5 September 1987, 21—26 (1987). — Brown, A. H. D.: Enzyme polymorphism in plant populations. Theor. Popul. Biol. **15**, 1—42 (1979). — Cavalli-Sforza, L. L. and Edwards, W. F.: Phylogenetic analysis: models and estimation procedures. Evolution **21**, 550—570 (1967). — Conkle, M. T., Hodgskiss, P. D., Nunnaly, L. B. and Hunter, S. C.: Starch gel electrophoresis of conifer seeds: a laboratory manual. U.S.D.A. Gen. Tech. Rep. PSW-64 (1982). — De Lumley, H.: Cadre chronologique absolu, paléomagnétisme, chronologie paléontologique et botanique, esquisse paléoclimatique, séquences culturelles. In: "La préhistoire française", Vol. 1. De Lumley (edit.). Editions du CNRS, Paris, 5—23 (1976). — Diebel, K. E. and Feret, P. P.: Isozyme variation within the Fraser fir (*Abies fraseri* (Pursh) Poir.) populations on Mont Rogers, Virginia: lack of microgeographic differentiation. Silvae Genet. **40**(2), 79—85 (1991). — Fady, B., Arbez, M. and Ferrandès, P.: Variability of juvenile Greek firs (*Abies cephalonica* Loud.) and stability of characteristics with age. Silvae Genet. **40**(3/4), 91—100 (1991). — Fady, B., Arbez, M. and Marpeau, A:. Geographic variability of terpene composition in *Abies cephalonica* and *Abies* species around the Aegean: hypotheses for their possible phylogeny from the Miocene. Trees **6**, 162—171 (1992). — Fady, B. and Conkle, M. T.: Segregation and linkage of allozymes in seed tissues of the hybrid Greek fir *Abies borisii regis* Mattfeld. Silvae Genet. **41** (4—5), 273—278 (1992). — Hamrick, J. L.: Isozymes and the analysis of genetic structure in plant populations. In: "Isozymes in plant biology". D. E. Soltis and P. S. Soltis (edits.). Dioscorides press, Portland, Oregon. 87—105 (1989). — Hemrick, J. L. and Godt, M. J.: Allozyme diversity in plant species. In: "Plant population genetics, breeding and genetic resources". A. H. D. Brown et al. (edits.). Sinauer Associates, Sunderland, Mass.. 43—63 (1989). — Jacobs, B. F. and Werth, C. R.: Genetic relationships in *Abies* (fir) of eastern United Staates: an electrophoretic study. Can. J. Bot. **62**, 609—616 (1984). — Koedam, A.: Composition of the volatile leaf oil from Greek Fir (*Abies cephalonica* Loud.). Fitoterapia **52**, 25—30 (1981). — Kormutak, A., Bencat, F., Rudin, D. and Sezyedyazdani, R.: Isoenzyme variation in the four slovakian populations of *Abies alba* Mill. Biologia (Bratislava) **37**(5), 433—440 (1982). — Ledig, F. T.: Heterozygosity, heterosis and fitness in outbreeding plants. In: "Conservation biology: the science of scarcity and diversity". M. E. Soulé (edit.). Sinauer Associates, Sunderland, Mass., 77—104 (1986) — Liu, T. S.: A monograph of the genus *Abies*. Dept. of Forestry, College of Agric., National Taiwan Univ., Taipei, Taiwan, 608 p. (1971). — Mitsopoulos, D. J. and Panetsos, C. P.: Origin of variation in Fir forests of Greece. Silvae Genet. **36**(1), 1—15 (1987). — Müller-Starck, G.: Genetic differences between "tolerant" and "sensitive" beeches (*Fagus sylvatica* L.) in an environmentally stressed adult forest stand. Silvae Genet. **34**(6), 241—247 (1985). — Neale, D. B. and Adams, W. T.: Inheritance of isozyme variants in seed tissues of balsam fir (*Abies balsamea*). Can. J. Bot. **59**, 1285—1291 (1981). — Neale, D. B. and Adams, W. T.: Allozyme and mating-system variation in balsam fir (*Abies balsamea*) across a continuous elevational transect. Can. J. Bot. **63**, 2448—2453 (1985). — Nei, M.: Molecular population genetics and evolution. North-Holland Publishing, Amsterdam (1975). — Nei, M.: Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics **89**, 583—590 (1978). — Panetsos, K. P.: Monograph of *Abies cephalonica* Loud. Ann. For. Zagreb, **7**(1), 1—22 (1975). — Papapetrou-Zamani, A. and Psarianos, P.: Views on the paleogeographic evolution of Aegaeis. Ann. Géol. Pays Hell. **29**, 187—194 (1977). — Philippson, A.: La techtonique de l'Egeide. Ann. Géogr. **7**, 112—141 (1898). — Plessas, M. E. and Strauss, S. H.: Allozyme differentiation among populations, stands and cohorts in Monterey pine. Can. J. For. Res. **16**, 1155—1164 (1986). — Pons, A.: Les changements de la végétation de la région méditerranéenne durant le Pliocène et le Quaternaire en relation avec l'histoire du climat et de l'action de l'homme. Webbia **38**, 427—439 (1984). — Scaltsoyannes, A., Panetsos, K. P. and Zaragotas, D.: Genetic variation of Greek fir as determined by isozyme analysis and its relation to other Mediterranean firs. Proceedings of the third E. E. C. meeting "daptation, breeding and silviculture of Mediterranean Firs", Avignon (France), 11 à 15 juin 1990. EEC Publisher (EUR 13491), Luxembourg, 99—116 (1991). — Schroeder, S.: Outcrossing rates and seed characteristics in damaged natural populations of *Abies alba* Mill. Silvae Genet. **38** (5—6), 185—189 (1989). — Shee, K. L.: Segregation of allozyme loci in megagametophytes of Engelmann spruce and subalpine fir. Genome **30**, 103—107 (1988). — Shea, K. L.: Genetic variation between and within populations of Engelmann spruce and subalpine fir. Genome **33**, 1—8 (1990). — Swofford, D. L. and Selander, R. B.: BIOSYS-1: a FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. J. Heredity **72**, 281—283 (1981). — Wright, S.: The interpretation of population structure by F-statistics with special regard to systems of mating. Evolution (Lawrence, Kans.) **9**, 395—420 (1965).

An Advanced-Generation Tree Improvement Plan for Slash Pine in the Southeastern United States

By T. L. White, G. R. Hodge and G. L. Powell

Dept. of Forestry, University of Florida,
Gainesville, FL, 32611, USA

(Received 18th August 1993)

Abstract

The Cooperative Forest Genetics Research Program has adopted an advanced-generation breeding strategy designed to maximize genetic gain in the short term and provide for continued improvement in the long term, while maintaining a broad genetic base for gene conservation. The breeding population consists of 933 members with an effective population size of 625. The population is divided into 4 strata based on genetic quality, with the top stratum functioning as an elite population. Clones in higher strata of the breeding population will receive more emphasis for both breeding and progeny testing, while those in the bottom stratum will serve to maintain a broad genetic base and infuse potentially valuable genes into upper stratum. Superimposed on the quality stratification is a division into 2 superlines, with each superline composed of 12 breeding groups (for a total of 24). These sub-divisions of the population will allow long-term breeding, while

indefinitely maintaining the ability to create production populations of outcrossed progeny through directed controlled pollination or wind-pollinated seed orchards. A complementary mating design will be used, with polymix progeny tests to assess breeding values, and unreplicated full-sib plots used for within-family selection. The plan calls for a reduction in per cooperating member workload from 60 replicated tests on 98 ha for the first generation, to 2.5 tests on 9 ha for the advanced generation.

Key words: Breeding strategy, *Pinus elliottii*, advanced generation, progny testing, effective population size, breeding population, production population.

Introduction

The Cooperative Forest Genetics Research Program (CFGRP) is composed of 13 private and public organizations engaged in genetic improvement of slash pine (*Pinus elliottii* ENGELM. var *elliottii*). Mass selection from natural stands began in the mid-1950s and approximately 2500 selections were grafted into clonal seed orchards (GODDARD, 1980; WHITE et al., 1986). Wind-pollinated seed orchard seed from most selections were established in progeny tests during the 1960s. Between 1965 and 1985, some 2700 different full-sib crosses between first-generation parents were made, and full-sib progeny tests were established for the purpose of selecting the best individuals within the best families. This completed the first-generation of breeding and testing.

The CFGRP is beginning the second-generation of slash pine improvement, and this paper documents the system of breeding, testing and deployment strategies designed to meet 3 objectives: 1) maximize short-term genetic gain (in both breeding and production populations) in a cost-effective and logistically-feasible manner, 2) provide for near-maximum long-term genetic gain, and 3) maintain a broad genetic base to provide for gene conservation and flexibility to unforeseen changes in environment, technologies and product goals.

Developing advanced-generation breeding strategies is not an exact science; thus, any strategy is dynamic and will evolve and change over time. We agree with SHELBOURNE et al. (1986) that, "... a breeding strategy is a battle plan to assure near optimal genetic gains in the short- and long-term in the face of many uncertainties. ... because intuition and subjective judgement play no small part in it (i. e., strategy development), it is rightly viewed as an art". Therefore, it is important for tree improvement programs to document strategies at key stages to set forth the constraints, premises and logic upon which the strategies are based.

For our second-generation slash pine tree improvement program, this paper describes 1) CFGRP biological and organizational premises, 2) the logic of several key features of our tree improvement program, 3) the structure and formation of the breeding population, 4) the management (mating and testing) of the breeding population, and 5) the workload involved in implementing the program. We focus on the second-generation spanning 1987 through 2008, because we believe it is undesirable to make firm plans any farther into the future (KANG, 1979b).

Biological, Genetic and Organizational Premises

End-Product and Forest Management Objectives

Slash pine in the southeastern United States is currently used mainly for pulp and paper products and this will likely continue for the foreseeable future (The South's Fourth Forest, 1988). Plantations are planted at densities of approximately 1800 trees per hectare and managed for a 20- to 25-year rotation. Precommercial and/or commercial thinnings are rare, and the mean annual increment ranges from 5 m^3/ha-yr to 15 m^3/ha-yr. The slash pine plantation estate operated by CFGRP members is approximately 4 million hectares located in north Florida and coastal plain portions of Georgia, Alabama and to a lesser extent Mississippi. Currently, CFGRP seed orchards produce enough improved seed to reforest approximately 150,000 hectares annually (WHITE, 1992b; POWELL and WHITE, in press).

Genetic Premises

The 2 most important target traits for the CFGRP have been and will likely continue to be volume growth and resistance to fusiform rust, *Cronartium fusiforme* BERK. MIYABE ex. SHIRAI f. sp. *fusiforme*. Fusiform rust is the most important disease in the southeastern U. S. causing an estimated $ 35 million dollars of damage annually (ANDERSON et al., 1986). Minor traits include wood specific gravity, straightness, branch quality and resistance to the pitch canker fungus (*Fusarium monoliforme* var. *subglutinans*). Individual tree heritabilities for volume growth are estimated to be 0.08 at age 5 and 0.16 at ages 10 and 15 (HODGE and WHITE, 1992a). Heritability for rust resistance is somewhat higher than for volume growth. On sites with overall rust infection between 35% to 85%, heritabilities are approximately 0.30 (SOHN and GODDARD, 1979). Selection of uninfected individuals on high hazard sites has yielded a realized heritability estimate of 0.35 (HODGE et al., 1990). The 2 traits, growth and rust resistance, are essentially uncorrelated genetically (WHITE and HODGE, 1988). For both individual and parental selection, optimal selection ages in genetic tests are between 8 and 12 years for volume growth (WHITE and HODGE, 1992). It has proven useful to assess rust earlier, at 5 years of age, because: 1) by age 5, on the majority of slash pine sites there is enough rust infecton that heritabilities are high, 2) by age 5, infection of rust-susceptible and intermediate families are near maximum (GRIGGS and SCHMIDT, 1977), and 3) measurement of infection is particularly easy at this age.

Current information indicates that specific combining ability for growth declines with age (BYRAM and LOWE, 1986), and so the strategy focuses on recurrent selection for general combining ability. Presently, there are no early selection techniques for volume growth, and the greenhouse screening for rust resistance (ANDERSON et al., 1983) is not precise enough to obviate the need for field testing (DE SOUZA et al., 1992). Thus, genetic testing will rely on field tests measured at 5 and 8 years of age (WHITE and HODGE, 1992).

Very little genotype x environment (g x e) interaction exists within the slash pine range for rust resistance (SCHMIDT and GODDARD, 1971; GODDARD and SCHMIDT, 1979). For volume growth, a moderate amount of g x e exists with Type B genetic correlations between test locations (BURDON, 1977) from 0.60 to 0.70 (HODGE and WHITE, 1992a). Further, the entire slash pine estate has a very homogeneous climate (PRITCHETT and COMERFORD, 1983; LOHREY and KOSSUTH, 1990). For these reasons, the entire plantation estate of 4 million hectares will be considered a single zone both for breeding and deployment.

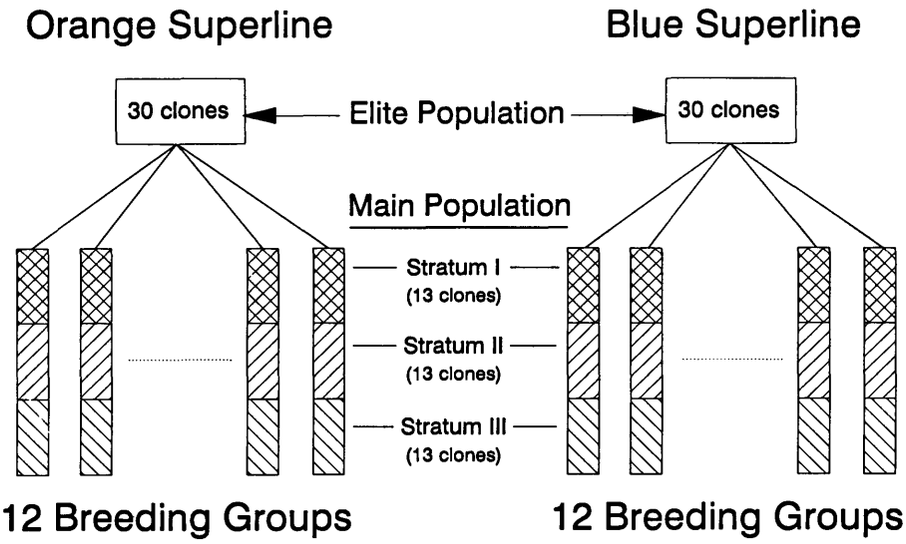

Figure 1. — Schematic diagram of the breeding population for CFGRP advanced-generation slash pine. The census number of N = 933 selections is divided in half into 2 superlines, orange and blue, each one consisting of an elite population of 30 selections and a main population of 12 breeding groups. There are 39 selections per breeding group stratified based on genetic quality into 3 strata (I, II and III with selections of the highest quality in stratum I).

Biological Characteristics

Slash pine grafts easily and flowering occurs on a sporadic basis 4 to 6 years after grafting; grafted seed orchards reach 50% of full production at about age 10 and nearly full production by 15 (POWELL and WHITE, in press). All propagules for operational plantations are currently seedlings; however, propagation by rooted cuttings of young material is feasible and may well be operational for some organizations within the next decade. Controlled pollination is relatively easy to effect, with one isolation bag resulting in an average of 50 to 100 sound seed.

As with most conifers (BRIDGWATER and FRANKLIN, 1985; GRIFFIN, 1989), slash pine suffers inbreeding depression (GANSEL, 1971; LAYTON and GODDARD, 1983; WHITE et al., 1986). Thus, inbreeding will be avoided in seed orchard seed destined for operational plantations.

Organizational Decisions

The following decisions were made jointly by members of the CFGRP: 1) all breeding and testing efforts will be conducted cooperatively with free exchange of genetic material among members, 2) production populations for producing propagules for operational plantations will be developed by each member individually, 3) most members plan to rely on wind-pollinated seed orchards as the primary type of production population for the foreseeable future and the strategy must attempt to maximize gain for this option, 4) some members may use controlled crosses or vegetative propagation as the primary form of producing propagules for operational deployment and the strategy must also provide for near optimum gain for this option, 5) the CFGRP is a primary custodian of the slash pine gene pool and thus will take a prominent role in gene conservation, 6) complete bi-parental pedigrees will be maintained for all selections in the breeding population (thus eliminating the sole use of open-pollinated and polymix mating designs), and 7) strategies can call for quite complex mating and testing designs as long as the workload is reasonable, logistics are feasible and the program can be completed in a timely fashion.

Overview of the CFGRP Slash Pine Breeding Strategy

The CFGRP slash pine breeding strategy entails recurrent selection for general combining ability (GCA) (SHELBOURNE, 1969; NAMKOONG et al., 1989, p. 44) because evidence suggests only small amounts of specific combining ability (SCA) and also because CFGRP members currently plan to use wind-pollinated clonal seed orchards as the primary type of production population. This section highlights the essential aspects of the strategy, while the underlying rationale, specific details of each phase and citations to the literature are presented in subsequent sections.

A major component of the strategy is the 3 types of structure used to sub-divide the breeding population (*Figure 1*). First, the 933 selections in the breding population are sub-divided horizontally into 24 breeding groups of 39 selections per group. Selections within a breeding group are unrelated to selections in any other breeding group in order to allow complete avoidance of inbreeding in seed from wind-pollinated clonal seed orchards established with 1 clone from each group (*Figure* 2). Wind-pollinated seed orchards are the main type of production populations for most CFGRP members. Second, 2 superlines (labeled orange and blue) exist; each superline consists of a main population containing 1/2 of the breeding population (12 breeding groups) and an elite population of the top 30 selections from the 12 breeding groups forming that superline. More emphasis (in terms of breeding and testing) will be placed on the members of the 2 elite populations, and CFGRP members using control-pollinated families for deployment to operational plantations will be able to avoid inbreeding by intermating among elite members of the 2 different superlines. Finally, in the main population each breeding group is stratified vertically into 3 strata (of 13 selections per stratum in each breeding group) based on predicted genetic worth (*Figure 1*). Stratum I of each breeding group contains the top 13 selections (out of 39), stratum II the middle third and stratum III the bottom third. The strategy calls for placing increasing emphasis on selections in higher strata (more full-sib crosses and better GCA testing in *Figure 2*).

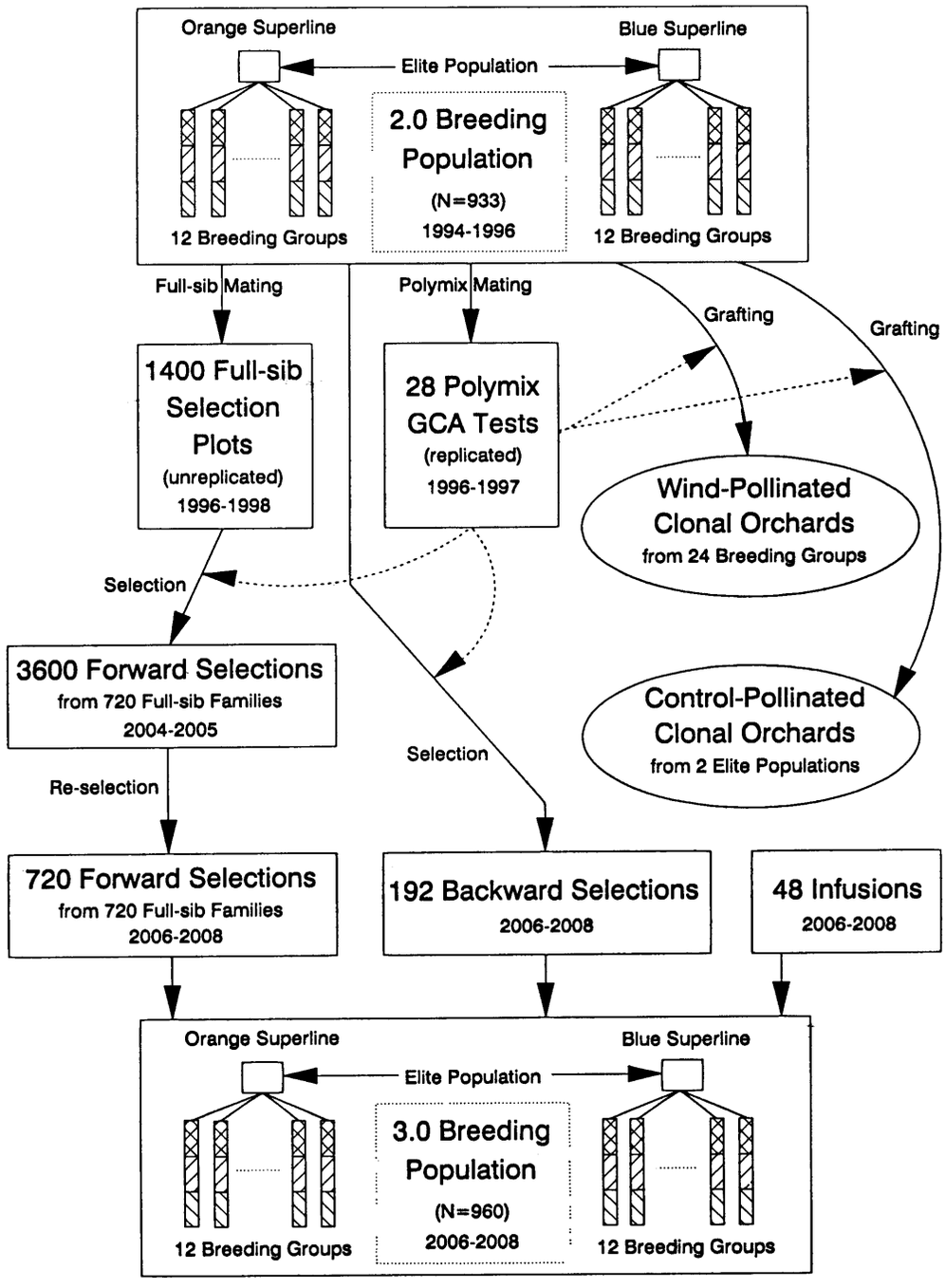

Figure 2. — Schematic diagram of CFGRP second-generation slash pine breeding strategy, 1994 to 2008. The complementary mating designs call for breeding of the selections from 1994 to 1996 followed by establishment of both polymix progeny tests (to estimate GCA) and full-sib selection plots (for making within-family, forward selections) in 1996 to 1998. Selection of the third-generation breeding population is scheduled for 2004 to 2008, and breeding will then commence directly in the full-sib selection plots in 2006.

The breeding strategy is based on complementary mating designs in which randomized, replicated progeny tests (formed with a single pollen mix) are used to estimate GCA values of selections in the breeding population, and unreplicated full-sib selection plots (formed by controlled-crosses among selections in the second-generation breeding population) are used to make forward selections for the third-generation breeding population (*Figure 2*). Only 360 of the 933 selections in the second-generation breeding population will be tested in the polymix GCA tests: those that are in the upper strata and/or are not well-tested for GCA in first-generation progeny tests. Data from these second-generation polymix tests will be combined with data from first-generation open-pollinated and control-pollinated progeny tests and used to develop unified rankings for all second-generation selections using best linear prediction. These rankings will be used both to select clones to include in production populations (wind-pollinated and control-pollinated seed orchards in *Figure 2*) and to select the third-generation breeding population (forward and backward selections in *Figure 2*).

Most full-sib mating will consist of crosses among selections within the same breeding group in order to retain the unrelatedness among breeding groups. The exception will be crosses among members of the elite population which will be restricted to crosses within the same superline. Each full-sib family will be planted in an unreplicated plot containing 50 to 100 seedlings (full-sib selection plots in *Figure 2*). Forward selections will be made from the best family plots (based on the GCA tests) to form the largest portion of the third-generation breeding population. Each breeding group will be reconstituted with 40 selections for a census number of N = 960 in the third generation.

CFGRP members may establish production populations at any stage during the breeding cycle. Most members plan to use clonal grafted seed orchards relying on wind-pollination to form the seed deployed to operational plantations (*Figure 2*). Having 24 breeding groups ensures that these orchards can always be established (even in subsequent generations) with 24 unrelated clones. CFGRP members planning to deploy control-pollinated seed to operational plantations (e. g., the hedged artificially-pollinated orchards of BUTCHER, 1988, or the meadow orchards of CARSON et al., 1990) may select and intermate top clones from the two elite populations with complete avoidance of inbreeding.

Key Features of the Program

More Emphasis on Superior Material

In advanced-generation breeding programs each selection in the breeding population has a predicted genetic worth based on its own performance and that of its relatives and ancestors in previous genetic tests. To make maximum short-term genetic gains (especially in terms of material deployed to operational plantations), it is important to concentrate progressively more effort on material of increasingly larger predicted genetic worth (LINDGREN, 1986; LINDGREN and MATHESON, 1986). This general concept permeates several aspects of our program: 1) when forming the breeding population, progressively more relatives of a given selection were allowed for increasingly better selections, 2) the breeding population is structured into main and elite fractions with only the top 60 selections (out of 933) included in the elite population (*Figure 1*), and 3) the main population is further stratified into three strata based on predicted genetic worth (*Figure 1*).

Stratification into an elite and main population was originally used in sheep breeding (JAMES, 1977, 1978; HOPKINS, 1978) and more recently introduced into tree breeding (COTTERILL, 1989; COTTERILL et al., 1989). The purpose is to focus efforts and energies of the program (in terms of breeding and testing) on the very best selections in the program, i. e., those included in the elite population (see review by WHITE, 1992). The terms elite and nucleus are used interchangeably in the literature to refer to the selections included in the top fraction (MAHALOVICH and BRIDGWATER, 1989). In our strategy, the elite population is managed much more intensively than the main population (details later). Also in future generations, the very best selections from the main population will be moved up and used in the elite population. This serves to broaden the base of the elite population in future generations and increase long-term gain. Even though it also increases long term gain to move some selections down from the elite to the main each generation (COTTERILL, 1989; COTTERILL et al., 1989), this will not be possible in our scheme without causing some relatedness among breeding groups of the main population.

Perhaps new to forest tree breeding is further stratification of the main population into 3 strata for the purpose of placing progressively less emphasis (in terms of breeding and testing) on progressively lower strata (*Figure 1*). This is a logical extension of the sub-division of the breeding population into elite and main populations and also follows the concept of placing more emphasis on better material as embodied by LINDGREN (1986). In our strategy the 312 selections in stratum I (13 selections x 24 breeding groups, *Figure 1*) are those of highest predicted genetic worth. These clones are more likely to be included in production populations in the near future and to contribute selections to the next generation's elite population; thus, we have chosen to breed them and test them more intensively compared to selections in strata II and III. Less emphasis is placed on selections in stratum II, and there is only minimal effort directed at the bottom stratum retained mainly for gene conservation and for the future infusion of potentially valuable alleles into higher quality material. In a situation with an infinite supply of capital, it would be possible to test and breed all selections very intensively. Our decision to place progressively more emphasis on selections of higher predicted genetic worth is an economic and logistical decision to focus more effort on selections of higher near-term economic value.

Use of Random and Mixed Model Analytical Techniques

Often genetic effects are treated as random variables for estimation of variance components and heritabilities, and then as fixed effects for estimating family means (WHITE and HODGE, 1989, chapter 2). Consistent treatment of genetic effects as random variables leads to three analytical methods (selection index, best linear prediction, BLP, and best linear unbiased prediction, BLUP) that have several important manifestations in our slash pine program. First, selection index methods were used 1) for developing testing designs (WHITE and HODGE, 1991, 1992), 2) for determining optimal selection ages (WHITE and HODGE, 1992) and 3) for maximizing gain from selection for both breeding and production populations (FALCONER, 1981; BULMER, 1985; COTTERILL, 1986; COTTERILL and JACKSON, 1989; COTTERILL and DEAN, 1990). Second, BLP methods facilitated prediction of precise parental breeding values for first-generation selections in messy progeny tests, e.g., for selections with offspring in different numbers of crosses or test locations or with offspring in tests of different ages quality, soil types, etc. (HENDERSON, 1963, 1984; WHITE and HODGE 1988, 1989). Third, these techniques lead naturally to use of overlapping generations as used in animal breeding (VAN VLECK et al., 1987) and advocated for tree improvement (LINDGREN, 1986), because selections from all generations can be ranked on a comparable basis. Thus, in making selections for our breeding population we were indifferent to whether a selection was first generation (i.e., backward) or second generation (i.e., forward); both types were selected to maximize gain subject to constraints on relatedness. Finally, the precision of the predicted genetic worth was estimated for each selection, and in our case some selections in the breeding population (e.g., well-tested backward selections) require little or no further testing, thus reducing the workload.

Intentional Inbreeding Among Breeding Population Members

In the breeding population, forced inbreeding combined with selection can be used to purge deleterious recessive alleles from the population thereby increasing genetic gain (STRICKBERGER, 1968; LINDGREN and GREGORIUS, 1976; HALLAUER and MIRANDA, 1981; VAN VLECK et al., 1987; NAMKOONG et al., 1989). While forced inbreeding is a very promising technique theoretically, empirical evidence is scant as to the extra benefits (above recurrent selection for GCA) and potentially negative side effects (e.g., reduced fecundity) (DICKERSON and LINDHE, 1977; HALLAUER and MIRANDA, 1981); therefore, inbreeding is incorporated into our strategy, but is not a main facet of it.

Choice of superior progenitors as the focus of inbreeding is important to maximize gain (NAMKOONG et al., 1989), so this slash pine strategy calls for directed matings among related selections mainly for members in the top $\frac{1}{3}$ of the breeding population. Top members in the breeding population (i.e., in the top 3% to 5%) will be selfed (LINDGREN, 1986), but selfing may lead to a too rapid increase towards homozygosity resulting in less gain (HALLAUER and MIRANDA, 1981). So, other types of related matings (i.e., among full-sibs, among half-sibs and backcrosses) will also be used, on a limited basis, for superior selections mainly in the top third of the breeding population.

Structured Breeding Population and Complementary Mating Designs

As described above, the breeding population is divided into the elite and main populations and the main population is further stratified into 3 strata based on genetic quality to facilitate placing more emphasis on selections of higher genetic quality. Superimposed on this vertical stratification is the horizontal breeding group structure (VAN BUIJTENEN, 1976; BURDON et al., 1977; VAN BUIJTENEN and LOWE, 1979; MCKEAND and BIENEKE, 1980; BURDON and NAMKOONG, 1983; WHITE, 1992a) designed to manage inbreeding in the production population (*Figure 1*).

With such a highly structured breeding population, use of complementary mating designs, CMD (VAN BUIJTENEN, 1976; VAN BUIJTENEN and LOWE, 1979; LOWE and VAN BUIJTENEN, 1986) was adopted to: 1) provide flexibility for different mating and testing designs for different strata of the breeding population, 2) facilitate the use of forced inbreeding, 3) provide a cost-effective mating and testing approach (cf "Generation Interval and Workload"). Polymix (PM) progeny tests are not always optimal (BURDON and VAN BUIJTENEN, 1990), but are generally quite efficient for ranking selections (BURDON and SHELBOURNE, 1971; LINDGREN, 1977; VAN BUIJTENEN and NAMKOONG, 1983; VAN BUIJTENEN and BRIDGWATER, 1986; BURDON and VAN BUIJTENEN, 1990; BRIDGWATER, 1992). Thus, the CFGRP will use replicated PM progeny tests for this purpose. The complementary full-sib matings done for purposes of forward selection will not be planted in replicated trails (thus requiring fewer seed per cross and allowing more flexibility with recalcitrant crosses); these crosses will include both outcrosses and related crosses of various degrees of inbreeding.

Replication of the full-sib selection plots for statistical purposes is not needed in this complementary scheme because, in the presumed absence of specific combining ability, the genetic worth of the full-sib families can be predicted as the mid-parent of the GCAs from the replicated polymix tests. Once good full-sib families are identified in this manner, full-sib selection plots allow within-family selection of the best phenotype(s) within a family.

Structure and Formation of the Breeding Population

Structure and Size Considerations

After reviewing theoretical and practical implications regarding breeding population size and structure (WHITE, 1992a), we adopted a breeding group structure for the breeding population. By constraining all relatedness to occur within breeding groups (synonymously called sublines), inbreeding can be completely avoided in seed destined for operational deployment by choosing members from different (and hence unrelated) breeding groups to form seed orchards (VAN BUIJTENEN, 1967; BURDON et al., 1977; VAN BUIJTENEN and LOWE, 1979; MCKEAND and BIENEKE, 1980; BURDON and NAMKOONG, 1983). All breeding groups are approximately equivalent in terms of average breeding values for all traits and are bred for the same objectives using the same selection criteria.

For CFGRP cooperators planning on using wind-pollinated clonal seed orchards as the primary type of production population, a minimum of 9 breeding groups is required to ensure at least 30 m separation among relatives (i.e., ramets of a clone or relatives from the same breeding group) at orchard spacings of 10 m by 10 m in systematic designs (LOWE and VAN BUIJTENEN, 1986; HODGE and WHITE, 1992b). However, more breeding groups are desirable to allow selection among groups (BAKER and CURNOW, 1969); in particular, orchard roguing involving removal of 50% to 75% of the clones seems beneficial in a wide range of circumstances (COTTERILL and JACKSON, 1989). In other applied breeding programs based on wind-pollinated seed orchards, the breeding population is commonly divided into 15 to 30 breeding groups (VAN BUIJTENEN and LOWE, 1979; KANG, 1979b; MCKEAND and BIENEKE, 1980; PURNELL and KELLISON, 1983). The slash pine breeding population is structured into 24 breeding groups, because in addition to the theoretical considerations, use of 24 breeding groups is convenient logistically: most CFGRP cooperators will physically manage (for breeding purposes) 2 breeding groups.

Theoretical studies indicate that each breeding group should have an initial effective inbreeding population size of N_e = 10 to 40 to support a breeding and selection program with appreciable gain for several generations (KANG, 1979a; BAKER and CURNOW, 1969; NICHOLAS, 1980; MAHALOVICH and BRIDGWATER, 1989; review by WHITE, 1992a). If there is considerable relatedness among selections, then the census number, N, (i.e., total number of selections per breeding group) would need to be larger, say double at N=20 to 80. When selecting the slash pine breeding population, we targeted the middle of these ranges (N_e = 20 to 25 and N = 40).

With 24 breeding groups and say 40 selections per breeding group, the target size of the slash pine breeding population would be 960 selections. This is larger than many applied tree improvement programs that have census numbers of 200 to 400 (reviewed by WHITE, 1992a). A breeding population of this size and structure should meet all 3 objectives identified in the Introduction: providing for near maximum short-term gains without sacrificing potential long-term gains, and also providing considerable flexibility and gene conservation.

Formation of the Breeding Population

The process of selecting candidates for inclusion in the breeding population is fully documented in HODGE et al.

(1989). Briefly, material potentially available for inclusion in the advanced-generation population consisted of: 1) 2373 original first-generation selections, called backward selections, 2) forward selections of the best individuals from 2700 different full-sib families generated from crosses among first-generation selections, and 3) a number of promising (but generally untested) clones which could be included as infusions into the population.

Candidates in the first 2 categories were ranked in a unified ranking on the basis of their predicted genetic worth using best linear prediction (BLP) and selection index theory. Based on progeny test data, breeding values for 15-year volume (VOL) and rust resistance (R50) of 2373 original first-generation parents were predicted using BLP (WHITE and HODGE, 1988). Breeding values for forward selections were estimated as the mean of the parental breeding values (from the previous step) plus an incremental increase in breeding value to account for gain from within-family selection (BV_w). BV_w for each of the 2700 full-sib families was calculated assuming selection of the tallest rust-free individual in a single progeny test with 30 trees per full-sib family. Predicted breeding values of VOL and R50 for forward selections calculated in this manner are directly comparable to those predicted for first-generation selections.

Next, using a growth and yield computer program that quantifies the effect of rust infection on yield (NANCE et al., 1983), economic weights for VOL and rust breeding value (R50) were estimated (HODGE et al., 1989). Since the economic weight estimates were rather imprecise, and the importance of the 2 traits may differ among cooperative members (and over time), 3 indices were developed that had different relative weight on growth and rust: I_B, I_G and I_R where I_B is aggregate genotypic value (also called genetic worth) when rust and growth were given their estimated economic weights, I_G placed primary emphasis on growth, and I_R placed primary emphasis on rust resistance. All 5073 first- and second-generation candidates for the breeding population (2373 original first-generation selections and a forward selection from each of 2700 full-sib crosses) were ranked according to these three indices. Generally, if a candidate was outstanding for any of the 3 indices, it was included in the breeding population. This is similar to the multiple index selection strategy outlined by NAMKOONG (1976), and results in development of breeds for growth, rust and both traits *within* each breeding group (CARSON et al., 1990).

During the selection process an attempt was made to balance the objectives of maximizing genetic gain and minimizing relatedness in order to maintain a broad genetic base. Thus, the maximum number of relatives allowed was 7, and this number decreased with decreasing predicted genetic value (in keeping with the principle of placing more emphasis on clones with higher breeding values). For example, a parent (say parent A) with very high aggregate genotypic values (I_B, I_G, I_R) was allowed to contribute genes to the selected population by inclusion of the parent itself (the original selection A) and inclusion of 1 or 2 forward selections from each of 2 to 4 full-sib crosses of A with other parents (up to a total of 7 relatives with a maximum of 2 selections from the same full-sib family). In contrast, an above average, but not outstanding parent (say B), might contribute genes from one forward selection from each of 2 full-sib crosses of B with other parents, or a selection from one full-sib cross and the original selection (i.e., 2 relatives). Finally, an average parent (C), usually contributed genes only from one forward selection from a full-sib cross of C with a better parent.

A large number of new selections called "infusions" were also included in the breeding population, primarily in order to broaden the genetic base (HODGE et al., 1989) (*Table 1*). The infusions originated from a variety of sources, and the majority (76%) of the infusions were included in the population because they likely posses favorable alleles for rust resistance. Most of these were "rust-free" selections, trees which had no rust infection in stands with over 90% of the trees infected. Realized gains tests have demonstrated that this approach has yielded significant genetic gains in resistance (HODGE et al., 1990). In addition, the rust-free selections were artificially screened for rust resistance (ANDERSON et al., 1983), and only the top third of the rust-free selections were included as infusions. The remainder of the infusions are selections which are superior for minor traits (high gum production or pitch canker resistance), or were selections from other slash pine improvement programs (e.g., United States Forest Service and Zimbabwe).

Composition of the Breeding Population

The advanced-generation breeding population for slash pine currently has a census number of 933 members (*Table 1*). Note that 395 members of the population are original first-generation selections (i.e., backward selections) whose predicted aggregate breeding values (I_B, I_G or I_R) were high enough to warrant inclusion (395 out of 2373 represents the top 17% of the first-generation selections). Similarly, 318 members of the population are forward selections from the better of the 2700 full-sib families. A total of 220 infusions complete the breeding population (*Table 1*). Overall, 850 unrelated individuals contributed genes to the breeding population, and the entire population has an inbreeding-effective population size of $N_e = 625$; thus, the population has a broad genetic base as intended.

There is substantial genetic improvement in this population compared to the first-generation selected population (*Table 1*). After first-generation mass selection, there was a 10% predicted gain in volume per tree at 15 years in row-plot tests (corresponding to 7.0% in volume per ha at age 20) and no gain in rust resistance over unimproved checks (HODGE et al., 1989). Average predicted breeding values for the second-generation selected population indicate a 20% gain (compared to unimproved material) in volume per tree (17% in volume per ha), and a substantial increase in rust resistance, with only 35% rust infection expected when unimproved material would incur 50% infection (*Table 1*).

The 24 different breeding groups contain approximately 39 clones each. All related selections were always assigned to the same breeding group, and infusions were distributed across groups. The 24 groups have similar average breeding values for rust resistance and volume growth. With a census number of 39, the typical breeding group has an inbreeding-effective population size of $N_e = 26$, with a range from 18 to 37.

One to 5 clones per breeding group were designated for inclusion in 1 of the 2 elite populations for a total of 60 clones (*Figure 1*). The 24 breeding groups form the main population and are divided into 2 groups of 12, designated the orange and blue superlines, with each superline supporting (for the purpose of enrichment in future generations) an elite populations of 30 clones. Average breeding value varies markedly among strata of the breed-

Table 1. — Census size (N), effective population size (N_e), ratio of N_e/N and average genetic gain in volume (VOL) and rust resistance (R50) for the advanced generation breeding population of slash pine sub-divided in two different ways: by type of selection, and by quality segment or stratum. Strata I, II and III are the top, middle and bottom third of the breeding population based on predicted genetic worth.

Group	N	N_e	N_e/N	VOL[a]	R50[b]
Backward	395	395	1.00	19.4	37.9
Forward	318	187	0.59	21.2	28.7
Infusions	220	210	0.95	7.3	33.9
Elite[c]	60	47	0.78	34.6	20.6
Stratum I	311	174	0.56	27.9	30.7
Stratum II	312	266	0.85	17.2	35.6
Stratum III	310	288	0.93	6.3	35.2
Total	933	625[d]	0.67	17.0	33.8

[a]) Percent gain above unimproved material at 20 years on a per unit area basis taking only growth improvement into account. Percent gain calculated following WHITE et al. (1988) and HODGE et al. (1989) as:

% gain = ((volume breeding value +4.21)/3.83 — 1) x 0.75

where the individual tree volume breeding values are calculated as in WHITE and HODGE (1988).

[b]) Expected rust infection on progeny of selected clones on a site where unimproved material incurs 50% infection.

[c]) Clones included in the elite population are also included in the stratum I population.

[d]) N_e = 625 is calculated as the sum of the N_e of the 24 breeding groups. This assumes that selections in different breeding groups will not be intermated. If the 933 selections are assumed to be one large intermating population, N_e = 600.

ing popultion, as does the relative contribution to the effective population size (*Table 1*). In particular, note that the strata with higher mean breeding values have smaller N_e/N ratios as a result of allowing more relatedness for selections with higher breeding values. Thus, the broad genetic base and gene conservation functions of the breeding population are being served more by the lower strata so that short-term genetic gain can be realized from the strata of higher genetic quality.

Management of the Breeding Population

Clone Bank Establishment

Selection of the advanced-generation breeding population was completed in 1990 and 4 to 12 ramets of each selection were grafted into clone banks (HODGE et al., 1989, 1990). Each of the 13 CFGRP members will manage 1 or 2 breeding groups for breeding and testing purposes, and all selections from those groups were grafted into a clone bank near breeding facilities. In addition, for insurance purposes, forward selections were also grafted into another member's clone bank. Backward selections are already present in first-generation seed orchards. A typical member's clone bank contains 100 clones (80 from 2 breeding groups plus approximately 20 of other members' forward selections).

In future generations, the unreplicated full-sib plots being used for within-family selection (*Figure 2*) will also be used as breeding facilities once the selections have been made. This obviates the need to use clone banks in future generations, thus saving the time (5 to 8 years) and associated cost. This was not possible in the current generation because selections were scattered many kilometers apart. By planting all full-sib selection plots near breeding facilities, each member will be able to begin breeding shortly after selection at age 8 to 10 yrs.

Polymix Testing

In order to reduce costs and streamline logistics, only 360 of the 933 selections will be tested in polymix tests. Whether or not a particular selection will be progeny tested depends on 1) its superiority based on its predicted breeding value, and 2) the precision of the breeding value prediction, i.e. how well tested it was in the first generation. For higher ranking clones there is some probability that in the near future the parent or its offspring could be included in production populations, thus it is important to have very precise breeding value predictions on these clones. Both backward selections tested in only a few

locations and forward selections (which have never been progeny tested, thus only parental information is available) have less precise breeding value predictions than predictions for well-tested backward selections. In other words, there is more error associated with predictions based on less data, and there is higher probability that the true breeding value is significantly higher or lower than the prediced breeding value.

The 360 clones in polymix progeny tests are all 60 elite clones and 300 clones from the main population: 1) 110 stratum I forward selections (these are all of the forward selections from full-sib families from the top third of the breeding population), 2) 100 stratum I backward selections (first-generation selections ranked in the top third) that were in 5 or fewer tests in the first-generation, 3) 50 stratum II backward selections (middle third) that were in 2 or fewer tests and 4) 40 stratum II forward selections with the average number of tests for their parents less than or equal to 2. Clones ranked in the middle third are only progeny tested if they were very poorly tested in the first generation because only then is there a significant chance that the true breeding value is high enough to warrant inclusion in a third-generation production population. Finally, stratum III clones (the bottom third of each breeding group), which are primarily infusions, will not be polymix tested in this generation unless an early selection test becomes operational. This is another example of placing more emphasis on higher ranking material: all elite clones will be tested, 210 stratum I clones will be tested, 90 stratum II clones will be tested and no stratum III clones will be tested.

The pollen mix will be composed of pollen from 30 parents with breeding values near the average of the second-generation breeding population. Pollen was collected in 1992 and breeding for the polymix tests will be conducted in 1994, 1995 and 1996. Clone banks will be 6 to 8 years old during this period and should provide sufficient levels of both pollen and female flowers for breeding purposes (Powell and White, in press). However, when expedient, CFGRP members will also breed backward selections present in first-generation seed orchards. In any case, breeding for the polymix tests will not extend past 1995. At this point, clones that have not yet flowered will not be included in these tests.

The polymix tests will be planted over a 2 year period (1996 and 1997, *Figure 2*). At each test location, the test design will be randomized complete block design with four blocks and 5 trees per family in non-contiguous plots (White and Hodge, 1992). Polymix families will be divided into sets of about 30 to minimize replication size, and sets will be nested within blocks. Families from the same breeding group will always be in the same set because those are the comparisons of most importance (since all breeding of the main population is conducted within breeding groups).

The 60 elite clones will be divided into two sets of 30 polymix families (30 from the orange line and 30 from the blue line), and all 60 families will be tested at each of 14 locations. For slash pine volume growth (White and Hodge, 1992), it is necessary to replicate progeny tests over 12 locations in order to achieve 97.5% of the efficiency of testing over 15 locations (assuming the RCB design, 4 replications, 5 trees/family/rep.). Efficiency refers to precision of breeding value predictions, and hence, accuracy of parental or family selection. To achieve 95% efficiency, 6 locations are needed (White and Hodge, 1992). Planting 14 locations allows some operational falldown to the 12 tests necessary for 97.5% efficiency, and also means that each member of the CFGRP will plant one polymix test of the elite population on their timberlands (2 large cooperative members will plant 2 tests). In addition, the tests will be allocated to sites of different quality or site index which may facilitate "regionalization" (Johnson and Burdon, 1990), in this case a sub-division of the production population in order to take advantage of predictable genotype x environment interaction in slash pine associated with site quality (Allard and Bradshaw, 1964; Hodge and White, 1992). The 300 clones in the main population will be polymix tested at each of 7 locations. This allows some operational falldown to 6 locations needed to ensure 95% testing efficiency (White and Hodge, 1992).

Full-Sib Selection Plots: Mating and Field Design

There will be 2 types of crosses for the full-sib selection plots: 1) among members of the elite population (these crosses are within superlines but across breeding groups), and 2) among members of the main population (these crosses are within breeding groups). Breeding will be conducted in both clone banks and first-generation seed orchards (for backward selections) from 1994 through 1996 (*Figure 2*). Among elite selections, 150 crosses will be made: all 60 clones will be selfed and each clone will be crossed with 3 others. Among the main population, 52 crosses per breeding group will be made, for a total of approximately 1250 crosses. The crossing pattern will be kept flexible by identifying possible crosses within different categories. The number of crosses per category will be designated, but the specific crosses will be made according to convenience. In this way we attempt to avoid problems with clones recalcitrant to flower, or specific crosses which are not compatible, and also simplify logistics by making crosses for which pollen and/or female strobili are available.

Noting that in each breeding group there are 13 clones in each stratum (top, middle and bottom third based on predicted genetic worth), the 52 crosses per breeding group among main population members are as follows. Each stratum III clone will be mated with one stratum I clone (a total of 13 crosses per breeding group). In this way we hope to make selections of the full-sib progeny which received the best alleles from the stratum III selection, combined with alleles from a selection already in the top third of the breeding population. Thus, we can capture the good alleles from the bottom third of the population, but quickly combine them into good genetic "packages", while broadening the genetic base in the upper portion of the breeding population. Stratum II clones (middle third) will be assortatively mated with one other stratum II clone (6.5 crosses per breeding group; 6 or 7 in reality, but retained as 6.5 for computational purposes) and with one stratum I clone (13 crosses per breeding group). Stratum I clones (top third) will then also be assortatively mated with 2 other stratum I clones (an average of 19.5 crosses per breeding group). Note that the elite clones are also part of the main population, and will be included in crosses made in the main population. In summary, a stratum I clone will be mated with two other stratum I clones, one stratum II and one stratum III clone; a stratum II clone will be mated with one stratum I and one stratum II clone; and a stratum III clone will be mated only with one stratum I clone. While the exact matings will be determined by the cooperators to maximize operational effi-

ciency, we will strive for both complementary crosses (good growth x good rust) and assortative crosses (good growth x good growth, good resistance x good resistance and good both x good both).

In addition to the selfing of the elite population, other types of related matings (among half-sibs, among full-sibs and backcrosses onto backwards selections) will also be allowed among top parents. Current plans call for 25% of the stratum I x stratum I matings (5 out of 19.5) and the stratum I x stratum II matings (3 out of 13) to be among relatives. All other matings will be outcrosses. Thus, intentional inbreeding will account for a relatively small fraction of the total crosses in each breeding group (8 out of 52 = 15%) and will be restricted to the top progenitors (cf "Key Features of the Program").

The field design for all of the above crosses will be unreplicated block plots. Elite full-sib selection plots will contain 75 to 100 trees (approximately 0.06 ha = 0.15 acres) depending on the number of seed available. Main selection plots will contain 40 to 60 trees per cross (approximately 0.04 ha = 0.10 acres). Extra seedlings available from both the elite and main full-sib crosses will be planted in row-plots at sites physically separated from the block-plots to provide some insurance that entire full-sib families will not be lost catastrophically.

All full-sib selection plots will be planted by individual cooperators near their operational breeding facilities, and will be planted as seed from a given cross becomes available (1996 through 1998, *Figure 2*). Selection ages of 8 to 10 years are optimal for slash pine (WHITE and HODGE, 1992). So, in 2004 and 2005 (when both polymix tests and full-sib plots are 8 to 10 years old, *Figure 2*), outstanding full-sib families will be identified on the basis of the polymix tests, and then 5 within-family selections will be made within each of those families in the selection plots. We will also make 5 within-family selections from each full-sib plot of stratum I x stratum III crosses because the stratum III selections will not be tested in the GCA tests. We anticipate that approximately 720 full-sib selection plots (after operational fall down due to crosses not made, mortality etc.) will contribute forward selections to the third-generation breeding population (*Figure 2*).

All other individuals in each full-sib plot will then be removed, and the area will then be managed as a breeding facility. This will avoid the lengthy and costly process of grafting forward selections into a clone bank in order to conduct breeding for the next generation. The 5 selections from a family will be reduced to 1 or 2 on the basis of flower production, wood density, or early testing results for rust resistance or growth potential. Third-generation breeding will begin in 2006 (*Figure 2*). Also at this time, the very best members of the 3.0 breeding population (say the top 20% which is 0.2 x 960 or approximately 100 clones) will be grafted into clone banks both to provide insurance against loss of the most valuable selections and also to multiply scions for cooperators wishing to establish grafted seed orchards.

Tests of Elite Crosses for Full-Sib Family Deployment

As discussed above, there is interest within the our cooperative in the possible use of directed crosses or vegetative propagation as the method of producing propagules for operational deployment. The polymix progeny tests will allow accurate prediction of general combining abilities (GCA), but the unreplicated full-sib selection plots will provide no information on specific combining abilities (SCA). If SCA variance is small, the genetic value of a full-sib family can be predicted accurately from the parental GCA predictions. However, with large SCA variance, there is a possibility to realize additional genetic gain by identifying crosses of 2 outstanding parents which also have positive SCA effects. Although evidence suggests that for loblolly pine the relative size of dominance variance decreases with age (BYRAM and LOWE, 1986; BALOCCHI, 1990), good estimates for slash pine are not currently available. In addition, the slash clones which would be used in the production populations of CFGRP members are at the upper end of the distribution of GCA values, and SCA variance for these clones may be somewhat different than for the population as a whole.

In view of this, we plan to test a sub-set of all possible crosses among the 60 elite parents. Each of the 30 clones in the orange elite will be crossed with one clone in the blue elite (single-pair, disconnected crosses). These full-sib families will then be planted in replicated tests, on the same 14 sites with the polymix families of the elite clones. This will allow estimation of SCA variance by comparison of full-sib family performance to that predicted from parental GCA's predicted from polymix family performance. If SCA variance is significant, some outstanding specific crosses for operational deployment will likely be identified.

Generation Interval and Workload

Regardless of how genetically sound a mating and testing scheme is, if the proposed workload exceeds the economic or logistical abilities of an organization, the expected genetic gain will not be realized. Further, if the strategies take too long to implement, then gain per unit time will not be maximized. Under this strategy, the second generation will take approximately 13 years to complete (1994 to 2006 in *Figure 2* from the beginning of breeding in the second generation to the same point in the 3rd generation) roughly broken down as: 5 years for breeding, cone maturation and test establishment (1994 through 1998), and 8 years waiting for the youngest tests to reach selection age (1998 through 2006). This compares favorably to the 33 years to complete the first generation; one major reduction (by 6 to 8 years) results from breeding directly in the full-sib selection plots (beginning in 2006) instead of establishing clone banks for breeding. It is currently not feasible to reduce the 8-year selection age (although early selection research underway may make that possible in the future), nor is it possible to reduce the 5-year breeding and test establishment period by more than a year.

In terms of workload, this strategy calls for 14 polymix test locations of the 60 elite selections, 14 different polymix test locations for 360 main population selection (7 locations for each of the orange and blue lines), and approximately 1400 full-sib selection plots (150 from crosses among elite selections and 1248 from crosses among main selections which is 52 per breeding group x 24 breeding groups). Including buffers and fillers, each elite polymix test will contain approximately 2000 trees and occupy 1.4 ha (3.4 acres). Each polymix test of main population selections will contain approximately 3300 trees and occupy 2.2 ha (5.4 acres). Finally, each full-sib selection plot occupies 0.04 ha to 0.06 ha (0.10 acres to 0.15 acres).

In summary, each organization in the CFGRP will make approximately 150 crosses: 30 polymix crosses for progeny testing, 120 full-sib crosses for within-family selection,

and 2.5 full-sib crosses for deployment testing. Each organization will establish 2 or 3 replicated polymix tests occupying approximately 4.2 ha (10.4 acres), and establish 4.9 ha (12 acres) of un-replicated full-sib selection plots.

This workload is well within the capabilities of the members of the CFGRP, and it compares very favorably to the first-generation workload. Altogether, the workload for the second generation is 10% to 15% of that done for the first generation (counting breeding, testing and clone banking). In the first-generation, each cooperator established approximately 40 replicated open-pollinated tests and 20 replicated full-sib tests occupying some 98 ha (242 acres). The reduction from 60 to 2 or 3 replicated progeny tests per organization means a huge cost savings to CFGRP cooperators with essentially no loss in genetic gain.

Effective Population Sizes and Future Generations

With rapidly evolving technologies, it is appropriate to plan for one generation at a time (KANG, 1979b), however, the strategy described here could be used for a number of generations. It is then logical to ask what effect the proposed mating design (more matings with superior parents) has on effective population size and how that might influence future generation breeding. The effective population size of the breeding population in any given generation is determined both by the mating design and the intensity of family or index selection. We conducted a simulation to look at two extreme case studies of mating and selection to examine their effects on future effective population sizes.

Both case studies began with the CFGRP breeding population described in *Table 1* (with census number N = 933 and effective population size N_e = 625), then these parents were mated within a breeding group using one of two designs (case 1 = random mating; case 2 = mating design described above for the CFGRP strategy) followed by 2 different types of selection (case 1 = no family selection; case 2 = moderate family selection). We then calculated the effective population size for both cases. For case 1, the 933 members of the breeding population were mated at random, and then each breeding group was reconstituted to its starting size of N = 39 by making one selection from each family created by the mating. Thus, no poor families were discarded and each member of each breeding group contributed equally to the next generation's breeding population.

Case 2 was intended as a realistic simulation of the CFGRP strategy using the mating design described herein and the following selection strategy: 1) 13 selections from the 19.5 stratum I x I families; 2) 6 selections from the 6 II x II crosses; 3) 13 selections from 13 I x II crosses and 4) 7 selections from the 13 I x III crosses. Thus, in both cases the next generation census number is 39 selections x 24 breeding groups = 936. For simplicity of calculation, we assumed that only forward selections were made and that no more than one selection was made from each family. The simulation was run 5 times and the answers were very similar from run to run with the average reported below.

Case 1 (random mating/no family selection) resulted in an effective population size of N_e = 486 which is 79% of the starting size of N_e = 625. For case 2, the next generation breeding population dropped from N_e = 625 to 341 (55% of the starting size). Thus, the increased emphasis on superior material does have a negative impact on effective population that might be difficult to sustain for many generations of this approach. However, even the random mating approach had a substantial impact.

While it is difficult to quantify the positive impact on genetic gain (because of the complexity of the mating and selection strategy), we believe that even small increments of short-term genetic gain arising from placing more emphasis on better material are enough to justify this approach for 1 or 2 generations as long as the original starting population is large. Further, theoretical and simulation studies indicate that effective population sizes on the order of 20 to 100 are large enough to sustain breeding programs for 10 generations or more (WHITE, 1992a). In addition, 14 of the 18 advanced-generation breeding programs reviewed by WHITE (1922a) had *census* numbers of less than 400 selections in the breeding population (with undetermined effective population sizes).

Thus, while we would be satisfied with an effective population size of 341, *figure 2* outlines a possible real scenario for creating the third-generation breeding population that results in a somewhat larger effective population size (probably nearer 400). First, the size of each breeding group is enlarged by 1 to 40 (total census number of N = 960). Next, while the case 2 simulation assumed that only forward selections would be made, it is likely that some fraction of the population will be made up of backward selections (say 20% which is 192 out of N = 936 in *Figure 2*). Backwards selections have less negative impact on effective population size because, in general, they are related to fewer other selections. Next, we plan to add say 2 infusions per breeding group (48 total for 5% of the total population in *Figure 2*) obtained from slash pine tree improvement programs around the world. As these infusions are unrelated to any other selections in the population, their inclusion increases the effective population size by 48.

By subtraction, forward selections make up the remaining 75% of the third-generation breeding population (720 trees), compared to 34% in the second-generation. These 720 forward selections will likely be selected from the 1400 full-sib family selection plots in several ways: 1) there will be operational falldown resulting in fewer than the 1400 anticipated full-sib plots, 2) some full-sib families will not provide any selections because of constraints on relatedness or poor parents, 3) some outstanding full-sib families may provide more than 1 selection to the breeding population because of outstanding parents, 4) early selection techniques for wood properties, growth, and/or rust resistance may be available by 2005 allowing many potential candidates to be screened prior to final selection, and 5) some candidate selections may not flower soon enough to be included in the breeding population. For the purposes of illustration, we have assumed that 3600 individuals are selected from the full-sib selection plots. Then after reselection (based on early screening, wood properties, flowering, etc.), only one fifth of these are retained in the final breeding population.

Other opportunities and technologies will surely arise during the course of the next generation interval, and we hope that these strategies are flexible enough to take advantage of these. In addition, however, all 933 members of the second-generation breeding population are contained in clone banks as an archive population. These will be maintained indefinitely as insurance should the

need arise. In the meantime, this advanced-generation breeding program represents a dynamic "battle plan" based on slash pine biological considerations and CFGRP organizational capabilities. We believe that these strategies will provide for large genetic gains over the next generation at relatively low cost, while allowing substantial flexibility for future generations.

Acknowledgements

We gratefully acknowledge the contributions and support of the member organizations of the Cooperative Forest Genetics Research Program; these organizations have sustained this program for nearly 40 years! We also thank the many colleagues with whom we have had the opportunity to discuss the pros and cons of various breeding strategies. Dr. C. J. A. Shelbourne gave an especially thorough and helpful review of the manuscript. This article is Journal Series No. R-03476 of the Institute of Food and Agricultural Sciences, University of Florida, Gainesville, FL, 32611.

Literature Cited

Allard, R. W. and Bradshaw, A. D.: Implications of genotype-environmental interactions in applied plant breeding. Crop Sci. 4: 503—508 (1964). —— Anderson, R. L., McClure, J. P., Cost, N. and Uhler, R. J.: Estimating fusiform rust losses in five southeast states. South. J. Appl. For. 10: 237—240 (1986). —— Anderson, R. L., Young, C. H. and Triplett, J. D.: Resistance Screening Center Procedures Manual: A step-by-step guide used in operational screening of southern pines to fusiform rust. U. S. Dep. Agric. For. Serv. For. Pest Management Report, 83-1-18, 55 pp. (1983). —— Baker, L. H. and Curnow, R. N.: Choice of population size and use of variation between replicate populations in plant breeding selection programs. Crop Sci. 9: 555—560 (1969). —— Balocchi, C.: Age trends of genetic parameters and selection efficiency for loblolly pine (*Pinus taeda* L.). Ph. D. Dissertation, N. C. State University, Raleigh, NC. 93 pp. (1990). —— Bridgwater, F. E.: Mating designs. In: Handbook of Quantitative Forest Genetics. Eds: Fins, L., Friedman, S. T. and Brotschol, J. V.: Kluwer Academic Pub., Boston, MA. pp. 69—95 (1992). —— Bridgwater, F. E. and Franklin, E. C.: Forest tree breeding: strategies, achievements and constraints. In: Trees as Crop Plants. Eds: Cannell, M. G. R. and Jackson, J. E.: Titus Wilson and Son, Kendal, UK. pp. 36—48 (1985). —— Bulmer, M. G.: The Mathematical Theory of Quantitative Genetics. Clarendon Press, Oxford, GB. 254 pp. (1985). —— Burdon, R. D.: Genetic correlation as a concept for studying genotype-environment interaction in forest tree breeding. Silvae Genet. 26: 168—175 (1977). —— Burdon, R. D. and Namkoong, G.: Multiple populations and sublines. Silvae Genet. 32: 221—222 (1983). —— Burdon, R. D. and Shelbourne, C. J. A.: Breeding populations for recurrent selection: Conflicts and possible solutions. N. Z. J. For. Sci. 1: 174—193 (1971). —— Burdon, R. D., Shelbourne, C. J. A. and Wilcox, M. D: Advanced selection strategies. In: Proc. Third World Conf. on Forest Tree Breeding. March 21—26, Canberra, Australia. pp. 1133—1147 (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). —— Butcher, T. B.: Hapso development in western Australia. In: Proc. Tenth Aust. For. Genet. Res. Group 1. Eds: Dieters, M. J. and Nikles, D. G.. Oct. 30 to Nov. 4, Gympie, Queensland, Australia. pp. 145—148 (1988). —— Byram, T. D. and Lowe, W. J.: General and specific combining ability estimates for growth in loblolly pine. In Proc. IUFRO Conf. on Breeding Theory, Progeny Testing and Seed Orchards. Oct. 13 to 17, Williamsburg, VA. pp. 352—360 (1986). —— Carson, M. J., Burdon, R. D., Carson, S. D., Firth, A., Shelbourne, C. J. A. and Vincent, T. G.: Realizing genetic gains in production forests. In: Proc. IUFRO S2.02-05 Douglas-fir, Lodgepole pine, Sitka spruce and *Abies* spp. August, Olympia, WA. (1990). —— Cotterill, P. P.: Genetic gains expected from alternative breeding strategies including simple low cost options. Silvae Genet. 35: 212—223 (1986). —— Cotterill, P. P.: The nucleus breeding system. In: Proc. Twentieth South. For. Tree Improve. Conf. June 26—30, Charleston, SC. pp. 36—42 (1989). —— Cotterill, P. P. and Dean, C. A.: Successful Tree Breeding with Index Selection. CSIRO Div. Forestry, Canberra, Australia. 80 pp., (1990). —— Cotterill, P., Dean, C., Cameron, J. and Brindbergs, M.: Nucleus breeding: a new structure for rapid improvements under clonal forestry. In: Breeding Tropical Trees: Population structure and genetic improvement strategies in clonal and seedling forestry. Eds: Gibson, G. I., Griffin, A. R. and Matheson, A. C. Oxford For. Inst., Oxford UK. pp. 39—51 (1989). —— Cotterill, P. P. and Jackson, N.: Gains expected from clonal orchards under alternative breeding strategies. Forest Sci. 35: 183—196 (1989). —— Desouza, S. M., Hodge, G. R. and White, T. L.: Indirect prediction of breeding values for fusiform rust resistance of slash pine parents using greenhouse tests. Forest Sci. 38: 45—60 (1992). —— Dickerson, G. E. and Lindhe, N. B. H.: Potential uses of inbreeding to increase selection response. In: Proc. Int. Conf. Quan. Gen. Eds: Pollack, E., Kempthorne, O. and Bailey, T. Iowa St. Univ. Press, pp. 323—342 (1977). —— Falconer, D. S.: Introduction to Quantitative Genetics. Longman and Co., New York, NY. 340 pp., (1981). —— Gensel, C. R.: Effects of several levels of inbreeding on growth and oleoresin yield in slash pine. In: Proc. 11th South. For. Tree Impr. Conf. June 15—16, Atlanta, Ga. pp. 173—177 (1971). —— Goddard, R. E.: The University of Florida Cooperative Genetics Research Program. In: Proc. 15th North. Amer. Quant. For. Genet. Group Workshop. August 6—8, Coeur d'Alene, ID. pp. 31—42 (1980). —— Goddard, R. E. and Schmidt, R. A.: Relative geographic stability of resistance to fusiform rust of selected slash pine families. In: Proc. 15th South. For. Tree Improve. Conf. June 19 to 21, Miss State, MS. pp. 99—107 (1979). —— Griffin, A. R.: Sexual reproduction and tree improvement strategy — with particular reference to eucalyptus. In: Breeding Tropical Trees: Population structure and genetic improvement strategies in clonal and seedling forestry. Eds.: Gibson, G. I., Griffin, A. R. and Matheson, A. C. Oxford For. Inst., Oxford, UK. pp. 52—67 (1989). —— Griggs, M. M. and Schmidt, R. A.: Increase and spread of fusiform rust. In: Management of Fusiform Rust in Southern Pines Sym. Proc. Eds: R. J. Dinus and R. A. Schmidt. Dec. 7 to 8, Gainesville, FL. pp. 32—38 (1977). —— Hallauer, A. R. and Miranda, J. B.: Quantitative Genetics in Maize Breeding. Iowa State Univ. Press, Ames, IO. 468 pp. (1981). —— Henderson, C. R.: Selection index and expected genetic advance. In: Statistical Genetics and Plant Breeding. Eds: W. D. Hanson and H. F. Robinson. NAS-NRC Pub. No. 982, Washington, DC. pp. 141—163 (1963). —— Henderson, C. R.: Applications of Linear Models in Animal Breeding. University of Guelph, Guelph, Ontario, CAN. 462 pp. (1984). —— Hodge, G. R., Powell, G. L. and White, T. L.: Establishment of the second-generation selected population of slash pine in the cooperative forest genetics research program. In: Proc. Twentieth South. For. Tree Improve. Conf. June 26 to 30, Charleston, SC.. pp. 68—74 (1989). —— Hodge, G. R., Powell, G. L. and White, T. L. et al.: Thirty-second Progress Report, Cooperative Forest Genetics Research Program. Dept. For., Univ. FL, Gainesville, FL, 32611. 50 pp. (1990). —— Hodge, G. R., Schmidt, R. A. and White, T. L.: Substantial realized gains from mass selection of fusiform rust-free trees in highly-infected stands of slash pine. South. J. Appl. For. 140: 143—146 (1990). —— Hodge, G. R. and White, T. L.: Genetic parameter estimates for growth traits at different ages in slash pine. Silvae Genet. 41: 252—262 (1992a). —— Hodge, G. R. and White, T. L.: Advanced-generation seed orchard design. New Forests, In Press (1992b). —— Hodge, G. R., White, T. L., de Souza, S. M. and Powell, G. L.: Predicted genetic gains from one generation of slash pine tree improvement. South J. Appl. For. 13: 51—56 (1989). —— Hopkins, I. R.: Some optimum age structures and selection methods in open nuclues breeding schemes with overlapping generations. Anim. Prod. 26: 267—276 (1977). —— James, J. W.: Open nucleus breeding systems. Anim. Prod. 24: 287—305 (1977). —— James, J. W.: Effective population size in open nucleus breeding schemes. Acta Agric. Scand. 28: 387—392 (1978). —— Johnson, G. R. and Burdon, R. D.: Family-site interaction in *Pinus radiata*: Implications for progeny testing strategy and regionalized breeding in New Zealand. Silvae Genet. 39: 55—62 (1990). —— Kang, H.: Designing a tree breeding system. In: Proc. Seventeenth Meeting Canadian Tree Improvement Association. August 27 to 30, Gander, Newfoundland., pp. 51—66 (1979a). —— Kang, H.: Long-term tree breeding. In: Proc. 15th South. For Tree Improve. Conf. June 19 to 21, Miss State, MS. pp. 66—72 (1979b). —— Layton, P. A. and Goddard, R. E.: Low level inbreeding effects on germination, survival, and early height growth of slash pine. In: Proc. 17th South. For. Tree Improve. Conf. June 7 to 9, Athens, GA. pp. 106—115 (1983). —— Lindgren, D.: Genetic gain by progeny testing as a function of mating design and cost. In: Third World Consultation on Forest Tree Breeding. March 21 to 26, Canberra, Australia. pp. 1223—1235 (1977). —— Lindgren, D.: How should breeders respond to breeding values?. In: Proc. IUFRO Conf. Breeding Theory, Progeny Testing and Seed Orchards. October 13 to 17, Williamsburg, VA. pp. 361—372 (1986). —— Lindgren, D. and Gregorius, H. R.: Inbreeding and coancestry. In: Proc. IUFRO Mtg. Adv. Gen. Breeding. June 14 to 18, Bordeaux, France., pp. 49—65 (1976). —— Lindgren, D and Matheson, A. C.: An algorithm for increasing the genetic quality of seed from seed orchards by using the better clones in higher proportions. Silvae Genet. 35: 173—177 (1986). —— Lohrey, R. E.

and KOSSUTH, S. V.: Slash pine. In: Silvics of North America. Vol. 1: Conifers. Agric. Handbook 654. Eds: R. M. BURNS and B. H. HONKALA. USDA For. Serv., Washington, DC. pp. 338—347 (1990). —— LOWE, W. J. and VAN BUIJTENEN, J. P.: The development of a sublining system in an operational tree improvement program. In: Proc. IUFRO Conf. on Breeding Theory, Progeny Testing and Seed Orchards. Oct. 13 to 17, Williamsburg, VA. pp. 98—106 (1986). —— MAHALOVICH, M. F. and BRIDGWATER, F. E.: Modeling elite populations and positive assortative mating in recurrent selection programs for general combining ability. In: Proc. Twentieth South. For. Tree Improve. Conf. June 26 to 30, Charleston, SC.. pp. 43—49 (1989). —— MCKEAND, S. and BEINEKE, F.: Sublining for half-sib breeding populations of forest trees. Silvae Genet. 29: 14—17 (1980). —— NAMKOONG, G.: A multiple-index selection strategy. Silvae Genet. 25: 199—201 (1976). —— NAMKOONG, G., KANG, H. and BROUARD, J. S.: Tree Breeding: Principles and Strategies. Springer-Verlag, New York, NY. 175 pp. (1989). —— NANCE, W. L., FROELICH, R. C., DELL, T. R. and SHOULDERS, E.: A growth and yield model for unthinned slash pine plantations infected with fusiform rust. In: Proc. Second Bieenial South. Silvi. Res. Conf. Nov. 4 to 6, 1982, Atlanta, Georgia. pp. 275—282 (1983). —— NICHOLAS, F. W.: Size of population required for artificial selection. Genet. Res. 35: 85—105 (1980). —— POWELL, G. L. and WHITE, T. L.: Cone and seed yields from slash pine seed orchards. South. J. Appl. For., In Press (1992). —— PRITCHETT, W. L. and COMERFORD, N. B.: Nutrition and fertilization of slash pine. In: The Managed Slash Pine Ecosystem. Ed: STONE, E. L.. Univ. of Florida, Gainesville, FL. pp. 69—90 (1983). —— PURNELL, R. C. and KELLISON, R. C.: A tree improvement program for southern hardwoods. In: Proc. 17th South. For. Tree Improve. Conf. June 7 to 9, Athens, GA. pp. 90—98 (1983). —— SCHMIDT, R. A and GODDARD, R. E.: Preliminary results of fusiform rust resistance from field tests of selected slash pines. In: Proc. Eleventh South. For. Tree Imp. Conf. June 15 to 16, Atlanta, GA. pp. 37—44 (1971). —— SHELBOURNE, C. J. A.: Tree Breeding Methods. Technical Paper No. 55, For. Res. Inst., New Zealand Forest Serv., Rotorua, NZ. 44 p. (1969). —— SHELBOURNE, C. J. A., BURDON, R. D., CARSON, S. D., FIRTH, A. and VINCENT, T. G.: Development plan for radiata pine breeding. New Zealand Forest Serv., Rotorua, NZ. 142 pp. (1986). —— SOHN, S. and GODDARD, R. E.: Influence of infection percent on improvement of fusiform rust resistance in slash pine. Silvae Genet. 28: 173—180 (1979). —— South's Fourth Forest: Alternatives for the Future: USDA For. Serv. Forest Resource Rep. 24. USDA Forest Service, Washington, DC. 512 pp.(1988). —— STRICKBERGER, M. W.: Genetics. The Macmillan Co., London, G. B. 868 pp. (1968). —— VAN BUIJTENEN, J. P.: Mating designs. In: Proc. IUFRO Joint Meeting on Advanced Generation Breeding. June 14 to 18, Bordeaux, France. pp. 11—27 (1976). —— VAN BUIJTENEN, J. P. and BRIDGWATER, F.: Mating and genetic test designs. In: Advanced Generation Breeding of Forest Trees. Southern Coop. Series Bull. 309. Louisiana Ag. Exp. Stn., Baton Rouge, LA. pp. 5—10 (1986). —— VAN BUIJTENEN, J. P. and BURDON, R. D.: Expected efficiencies of mating designs for advanced generation selection. Can. J. For. Res. 20: 1648—1663 (1990). —— VAN BUIJTENEN, J. P. and LOWE, W. J.: The use of breeding groups in advanced generation breeding. In: Proc. 15th South. For. Tree Improve. Conf. June 19 to 21, Miss State, MS. pp. 59—65 (1979). —— VAN BUIJTENEN, J. P. and NAMKOONG, G.: Mating designs. In: Progeny Testing of Forest Trees. South. Coop. Series Bull. No. 275. Texas A. and M. Univ., College Station, TX. pp. 7—13 (1983). —— VAN VLECK, L. D., POLLAK, E. J. and OLTENACU, E. A.: Genetics for the Animal Sciences. W. H. Freemann and Co., New York, NY. 391 pp. (1987). —— WHITE, T. L.: Advanced-generation breeding populations: Size and structure. In: Proc. IUFRO Conf. Breeding Tropical Trees. Oct. 12 to 16, Cali, Colombia. (1992a). —— WHITE, T. L.: Impacts of tree improvement on nursery management. In: Proc. South. For. Nurs. Assoc. July 21 to 23, Calloway Gardens, GA. (1992b). —— WHITE, T. L. and HODGE, G. R.: Best linear prediction of breeding values in a forest tree improvement program. Theor. Appl Genet. 76: 719—727 (1988). —— WHITE, T., FLINCHUM, M., ROCKWOOD, D., KOK, H. and POWELL, G.: Twenty-eight Progress Report, Cooperative Forest Genetics Research Program. Dept. of For., Univ. of Florida, Gainesville, FL. 35 pp. (1986). —— WHITE, T. L. and HODGE, G. R.: Predicting Breeding Values with Applications in Forest Tree Improvement. Kluwer Academic Pub., Dordrecht, The Netherlands. 367 pp. (1989). —— WHITE, T. L. and HODGE, G. R.: Indirect prediction of genetic values. Silvae Genet. 40: 20—28 (1991). —— WHITE, T. L. and HODGE, G. R.: Test designs and optimal age for parental selection in advanced-generation progeny tests of slash pine. Silvae Genet. 41: 293—302 (1992). —— WHITE, T. L., HODGE, G. R., POWELL, G. L., KOK, H. R., DE SOUZA, S. M., BLAKESLEE, G. and ROCKWOOD, D.: Thirtieth Progress Report, Cooperative Forest Genetics Research Program. Dept. For., Univ. Florida, Gainesville, FL. 44 pp. (1988).

Short Note: Genetic and Intra-Tree Variation in the Number of Sapwood Rings in Quercus robur and Q. petraea

By P. S. SAVILL, P. J. KANOWSKI, I. D. GOURLAY and A. R. JARVIS

Oxford Forestry Institute, Department of Plant Sciences, University of Oxford, South Parks Road, Oxford OX1 3RB, United Kingdom

(Received 1st July 1993)

Summary

This study investigates genetic control of the number of sapwood rings in *Quercus robur* and *Q. petraea*, and the variation of this trait with height up the stem and age of the tree. Ramets and open-pollinated progeny of the 2 species were sampled from a German clonal orchard and an unreplicated progeny trial; intra-tree variation was investigated from a sample of English woodland trees of both species. Estimates of the heritability of the number of sapwood rings were high (0.57 ± 0.28 on a narrow-sense basis; 0.83 ± 0.11 on a broad-sense, clonal mean basis), consistent with comparable results for other species. The phenotypic correlation between family means, used here as the best available proxy to a genetic correlation, suggested a moderately negative relationship (r = —0.49) in trees of the same age between the number of sapwood rings and stem diameter at breast height. The number of sapwood rings and the proportion of rings that are sapwood varied with height, following a nonlinear relationship. The number of sapwood rings at breast height increases with the age of the tree, although the proportion of the total number of rings that are sapwood decreases with age. Whilst strong conclusions cannot be drawn from the limited experimental material on which this study is based, our results are generally consistent with those of other studies. They suggest that including the number of sapwood rings as a selection criterion could be beneficial in both breeding and clonal propagation programmes of *Q. robur* and *Q. petraea*.

Key words: Heritability, *Quercus robur*, *Quercus petraea*, sapwood, wood quality.