ESTABLISHMENT OF THE SECOND-GENERATION SELECTED POPULATION OF SLASH FINE IN THE COOPERATIVE FOREST GENETICS RESEARCH PROGRAM

G.R. Hodge, G.L. Powell, and T.L. White[1]

Abstract.--A total of 886 individuals have been selected to comprise the second generation of improved slash pine in the Cooperative Forest Genetics Research Program (CFGRP). The bulk of the second-generation selections were made from a base population which included a total of 2373 original first-generation selections and the best offspring from each of 2700 full-sib crosses among first-generation parents. Best linear prediction was used to predict breeding values for volume and fusiform rust resistance, and the 5073 candidates were ranked according to their predicted breeding values. The primary objectives in the second-generation selection were: 1) to maximize genetic gain for the economically important traits of volume growth and rust resistance, and 2) to maintain a broad genetic base. Of the 886 second-generation selections, 304 were selections of the best offspring from the best full-sib families (forward selections). Within family selection in the field was aided by screening progeny test data by computer. A total of 404 selections were the best original first-generation parents (backward selections) which were retained for the second generation. In addition a total of 178 selections which generally had not been tested in the field were infused into the second-generation population. These infusions were generally superior for traits of special interest such as a high degree of rust or pitch canker resistance, or gum production. The 886 selections have been assigned to 24 breeding groups with approximately 35-40 selections in each group. All relatedness is confined within breeding groups. Each member of the CFGRP is responsible for clone banking, breeding and testing one or two breeding groups. To date 90% of the 886 second-generation selections have been grafted into clone banks where breeding will be conducted.

Keywords: *Pinus elliottii,* breeding strategy, forward selection, backward selection.

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[1] Research Scientist, Biological Scientist III, and Assoc. Prof., Dept. of Forestry, University of Florida, Gainesville, Florida, 32611. This manuscript is Florida Agricultural Experiment Stations Journal Series No. 10046. The authors wish to acknowledge the assistance provided by CFGRP memebers, and the tremendous contributions of Ray Goddard and Harm Kok in laying the foundation for the continued genetic improvement of slash pine.

INTRODUCTION

The Cooperative Forest Genetics Research Program (CFGRP) at the University of Florida began mass selection of slash pine *(Pinus elliottii* Engelm. var *elliottii)* from unimproved natural stands in the mid-1950's. These selections were grafted into first-generation seed orchards, and progeny tests of these selections were established using wind-pollinated orchard seed. Numerous full-sib crosses between first-generation parents were made and full-sib progeny tests established for the purpose of selecting the best individuals within the best full-sib families. Over the past three years, the CFGRP has created the second-generation selected population for slash pine and is nearly finished grafting these selections into clone banks. This paper describes the process which proceeded as follows: 1) formation of the second-generation population on paper, 2) delineating breeding groups, 3) making forward selections in the field, and 4) establishment of clone banks.

FORMATION OF THE SECOND-GENERATION SELECTED POPULATION

Objectives

The main objectives guiding the formation of the selected population were to 1) maximize genetic gain, and 2) maintain a broad genetic base for continued improvement in future generations. This meant selecting the best clones in the program for inclusion in the population with some constraints on relatedness of the selections. Material potentially available for inclusion in this second-generation selected population consisted of: 1) 2373 original first-generation selections, called backward selections, 2) forward selections of the best individuals made from 2700 different crosses in full-sib tests, and 3) a number of promising (but basically untested) clones which could be included as infusions into the population.

Ranking of Candidates

The first step in forming the population was to rank all possible candidates in the CFGRP according to their predicted genetic value. Based on progeny test data, breeding values for volume and rust resistance of original first-generation parents were predicted using best linear prediction (BLP) (White and Hodge 1988). We also wished to make forward selections of the best individuals from the best of some 2700 different full-sib families which have been established in cross tests over the last 15 years. Breeding values for forward selections were estimated as the mean of the parental breeding values plus an incremental increase in breeding value from within family selection (BV_w). Only one selection was made from a full-sib family, and BV_w was calculated assuming selection of the tallest rust-free individual in a single progeny test with 30 trees per full-sib cross. Breeding values of forward selections calculated in this manner were directly comparable to breeding values of the first-generation parents, thus first-generation parents and forward selections could be ranked together.

Using a growth and yield program that quantifies the effect of rust infection on yield (Nance et al. 1983), economic weights for volume breeding value (BVVOL) and rust breeding value (BVR50) were estimated. Since the economic weight estimates are rather imprecise, and the importance of the two traits may differ among cooperative members (and over time), three indices were developed that have different relative weights on growth and rust:

$$IB = 0.5\ BVVOL + 0.5\ (50 - BVR50)/100,$$
$$I_G = 0.9\ BVVOL + 0.1\ (50 - BVR50)/100, \text{ and}$$
$$IR = 0.1\ BVVOL + 0.9\ (50 - BVR50)/100$$

where IB = aggregate genotypic value when rust and growth are given their estimated economic weights,
IG = aggregate genotypic value with primary emphasis on growth, and
IR = aggregate genotypic value with primary emphasis on growth.

All 5073 candidates for the second-generation population (2373 original first-generation selections and a forward selection from each of 2700 full-sib crosses) were ranked according to these three indices.

Forward versus Backward Selections

The 2700 full-sib crosses made by the CFGRP were formed more or less at random (from a genetic quality standpoint). Thus the best of the 5073 candidates (ranked by any of the three indices) which were available included a mixture of forward and backward selections. In forming the second-generation selected population, there was no inherent advantage in choosing forward selections versus backward selections. In some cases, it may be better (from the standpoint of making genetic gain) to include an outstanding backward selection, say parent A, in the population as opposed to an average, or even above average offspring of A crossed with another parent, say B, that is inferior to A. The objectives were to maximize genetic gain, while maintaining sufficient genetic diversity. If parent A is the best single genotype currently available, it makes sense to carry that genotype on into advanced generation breeding.

In the absence of positive assortative mating, the best original first-generation parents in a breeding program are invariably mated only with other parents which are substantially inferior. This is simply a result of sampling: outstanding parents are rare, so if parents are mated at random it is unlikely that two outstanding parents will be mated. This generally results in the scenario described above: the expected breeding value of the best forward selection from a full-sib family of an outstanding parent crossed with an average parent is lower than the breeding value of the outstanding parent. This underscores the importance of assortative mating for future generations (Lindgren 1986).

Constraints on Relatedness

Generally, if a candidate was outstanding for any of the three indices, it was included in the selected population. However, 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 5, and this number decreased with decreasing predicted genetic value. 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 one forward selection from each of two to four full-sib crosses of A with other parents (i.e., up to 5 relatives). In contrast, an above average but not outstanding parent (say B), might be included in forward selections from two full-sib crosses of B with other parents, or a selection from one full-sib cross and the original selection (i.e., two relatives). Finally, an average parent (C), was usually included as only one forward selection from a full-sib cross of C with a better parent.

Final Composition of the Selected Population

The second-generation selected population for slash pine currently has 886 members (Table 1). Note that 404 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. Similarly, 304 members of the population are forward selections from the better of the 2700 full-sib families. The 178 infusions come from a variety of sources and, for the most part, were not ranked according to IB, I_G and I_R. Their purpose is to broaden the genetic base of the selected population. For example, pitch-canker-free selections from highly infected stands were included in this selected population. The only trees listed as "infusions" were those that were not included on the basis of IB, IG or IR. Overall, a total of 826 unrelated individuals contributed genes to the second-generation selected population, thus giving this population a broad genetic base (objective 2). However, substantial genetic improvement (objective 1) was also made as a result of the second-generation selection. After first-generation mass selection, there was a 10% gain in volume and no gain in rust resistance over unimproved checks (Hodge et al. 1989). Average predicted breeding values for the second-generation selected population indicated a 20% gain in volume over unimproved (volume per tree at 15 years in row-plot tests), and substantially increased rust resistance (e.g. 34% rust infection expected when unimproved material would incur 50% infection). Use of the best available clones in a production seed orchard would result in significantly higher genetic gain in both traits.

Table 1.--Sources of selections in the CFGRP second-generation slash pine selected population.

Backward selections (first-generation selections)		404
Forward selections from cross tests		304
Infusions		178
Rust-free selections	127	
High-gum selections	33	
Pitch-canker-free selections	8	
Rhodesian slash pine program	10	
TOTAL		886

DELINEATING BREEDING GROUPS

Genotypes (clones) in the selected population were divided into 24 different breeding groups of approximately 37 clones each. Breeding groups allow the management of inbreeding while still allowing establishment of outcrossing production seed orchards of unrelated genotypes (van Buijtenen and Lowe 1979, McKeand and Beineke 1980). Each cooperator is responsible for the breeding and testing of all genotypes in either 1 or 2 different breeding groups. Genotypes <u>within</u> a breeding group will be bred and tested together as a unit. There will be no breeding amorg genotypes in different breeding groups. All related selections (e.g., two selections with a common parent) were <u>always</u> assigned to the same group. For the most part, the 24 groups have similar average breeding values for rust resistance and volume growth. The infusions (e.g., pitch canker free selections, high gum selections and rust free selections) were distributed across groups.

FIELD SELECTION OF FORWARD SELECTIONS

<u>Computerized Selection of Individuals</u>

Much preparatory work was done by computer in order to maximize efficiency of selection and minimize the time spent in the field. Once it had been determined that forward selections from particular full-sib families would be included in the selected population, the CFGRP data management system was used to generate a second-generation selection form for each forward selection. First, all tests that the particular full-sib family occurred in were identified. The test in which to make the forward selection was then chosen to 1) maximize the effectiveness of selection, and 2) minimize the number of different tests to be visited during the selection process. Selection for growth was most efficient in the oldest tests, while efficiency of selection for rust resistance was maximized in tests with high infection. Using the most recent field measurements of a particular test, the five tallest rust-free trees (all heights standardized by block) were chosen as possible candidates for each full-sib family. These candidates were identified on the selection form by their block and position location in the progeny test.

There was some concern that the computer selection approach might tend to identify trees with missing neighbors thus decreasing the effectiveness of selection. Height growth is generally considered relatively independent of spacing (hence it is used as a measure of site index), but a small study was conducted to examine the effects of missing neighbors on height growth in 5 to 12-year-old progeny tests of slash pine. No significant effect was found, and therefore there is no reason to suspect that trees with missing neighbors were preferentially selected as candidates (White et al. 1988).

<u>Final Field Screening</u>

The second-generation selection forms were distributed to the CFGRP member who maintained the progeny test where a particular selection would be made. Candidate trees were ordered by standardized height and examined sequentially for 'acceptability' for three characteristics: 1) <u>freedom from fusiform rust</u>

infection (selection was often made one to three years after last measurement, and there was a possibility of subsequent rust infection), 2) straightness, 3) crown, primarily forking or ramicorn branching. Using a low selection intensity (i.e., acceptability) for straightness and crown form allowed for a much higher selection intensity for the more important trait of height growth.

Field selection using this technique was quite efficient. It was rarely necessary to examine more than the first three candidate trees to find a selection.

ESTABLISHMENT IN CLONE BANKS

In early 1988 the CFGRP began grafting the second-generation slash pine selected population into clone banks. These clone banks will serve to: 1) provide scions to establish production seed orchards with elite clones, 2) provide a location to make crosses for second-generation breeding and testing, and 3) preserve all genotypes of the population.

Once breeding groups were established on paper, it was clear that a great deal of scion exchange among cooperators would be necessary to get a selection from one cooperator's test or orchard to the cooperator responsible for its clone banking, breeding and testing. In order to facilitate future breeding and progeny testing of this selected population, an effort was made to complete the clone establishment in a two-year period (1988 and 1989). The following procedures were used in both years.

Prior to scion collection and grafting, the CFGRP data management system was used to generate lists tailored to each cooperator's needs. First, each cooperator was provided with the list of forward, backward and infusion selections from which to collect scions to be distributed to other cooperators. Also, each cooperator was provided with a list of all clones from which they were to receive scions collected by other cooperators. Standardized scion collection procedures were used, and all cooperators collected scions the last week in January in both years. On an assigned day, each cooperator then brought their scions to Tallahassee, FL, and exchanged them with other cooperators for scions of clones to be grafted into their clone banks. These scions were grafted during the first week of February.

At completion of grafting in 1989, CFGRP cooperators had made 15,052 grafts of 834 second-generation selections. The original target called for six ramets of each selected clone to be planted in a clone bank; a selection is considered "established" if at least four ramets survive after one year in the field. By the end of the summer of 1989, approximately 90% of the second-generation selections will be successfully established in clone banks. By the end of 1990, we anticipate that all 886 second-generation selections will be established in clone banks. At completion, each clone in the selected population will be preserved is two different locations: a primary location (clone bank or seed orchard) owned by the cooperator responsible for breeding that genotype, and a secondary location maintained by a different cooperator to ensure against loss.

SUMMARY

The 886 member second-generation selected population will serve as the basis for continued improvement of slash pine (through breeding and testing) and will also provide material for 2.0 and 2.5-generation seed orchards. The population includes backward selections (original first-generation parents), forward selections (offspring from full-sib crosses of first-generation parents), and infusion selections (promising but untested clones, or clones with special traits). The population has a broad genetic base, and substantial genetic gain resulted from selection. For example, average offspring from the second-generation selected population would incur 34% rust in areas where unimproved material would incur 50% infection, and yield approximately a 20% gain in volume over the unimproved check. Substantially more gain is possible by taking the best members of the population for use in a production seed orchard.

The second-generation formation and clone bank establishment was a major undertaking by the CFGRP. In two years, the cooperative identified 886 second-generation selections and then collected scion, grafted, and established 834 of these in clone banks. By the end of 1989, approximately 90% of these second-generation selections will be established in clone banks. In the winter of 1990, the cooperative will graft the final 52 selections and re-graft those selections with poor clone bank survival.

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