EARLY TESTING PROCEDURES APPLIED TO TREE IMPROVEMENT PROGRAMS

W. J. Lowe and J. P. van Buijtenen[1/]

Abstract--Step-wise testing can be used to incorporate early testing procedures into operational tree improvement programs. By rejecting poor performing families after each test, field testing efficiency can be improved. Traits suitable for early testing procedures can be 1) important juvenile traits, 2) mature traits that can be evaluated at juvenile age and 3) a juvenile trait that is correlated with an important mature trait. Complementary mating schemes and positive assortative mating can be incorporated to increase genetic gains. The use of early testing procedures in the Western Gulf Forest Tree Improvement Program's slash pine, loblolly pine and longleaf pine improvement programs is discussed.

Keywords: Step-wise testing, complementary mating, assortative mating, Pinus taeda L., Pinus elliottii Engelm., Pinus palustris Mill.

INTRODUCTION

Tree improvement programs typically have long breeding cycles. The time delay between grafting and flower induction and the time required for progeny testing are the major factors causing the length of the breeding cycle. Research regarding fertilization, drought stress and the use of plant hormones have identified techniques that can be used to reduce the flower induction phase. Lambeth et al. (1983) showed that estimates of rotation age performance for loblolly (Pinus taeda L.) can be obtained at earlier ages than one-half of the projected rotation. Most of the selection work in southern pines is currently done between the ages of five and 10 years.

Selections have been made in the nursery (Zobel et al. 1957, La Farge 1975), and on the basis of greenhouse or growth chamber studies (Cannell et al. 1978, Waxler 1980, Miller 1982 and Davison 1984) in efforts to reduce the selection age. Field performance has not been

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consistently predicted on the basis of these studies.

When early testing procedures are developed, techniques are needed to incorporate them into operational tree improvement programs. A pilot-scale, early-testing study with loblolly pine was described by van Buijtenen et al. (1986). They used a step-wise screening process to evaluate a number of different traits in the laboratory. Lowe and van Buijtenen (1989) describe the procedures incorporated by the Western Gulf Forest Tree Improvement Program (WGFTIP) to utilize early testing techniques for growth in the loblolly pine breeding program.

This paper discusses the opportunities for early testing procedures and the techniques to incorporate these procedures into operational tree improvement programs, using the procedures adapted by WGFTIP as an example.

EARLY TESTING

Early-testing procedures could be used on three major types of traits as follows: 1) a juvenile trait that is important - an example of this is first year survival; 2) a mature trait that can be evaluated at a juvenile age - resistance to fusiform rust is an example of this type of trait and 3) a juvenile trait that is correlated with a desirable mature trait - seed or seedling characteristics that correlate with volume production in the field typify this group of traits.

Early testing procedures can result in two types of errors (Figure 1). A family with good field performance could be rejected on the basis of early test data (type A error). When a family with good early test performance fails to perform well in later field tests, the second type of error occurs (type B error). If the early testing procedure is intended to select a few outstanding families or reject a few poor families, these types of errors assume different levels of importance.

In an effort to reduce the breeding cycle, most early testing studies have concentrated upon selecting a few outstanding families. In this approach, both types of errors are important because families with poor field performance can be selected and families with good field performance can be rejected. Only the type A errors (rejection of families with a good field performance) are important if the objective of the early testing procedure is to reject a few poor families. The families with poor field performance (type B errors) will be identified in later field tests.

To incorporate early testing procedures into operational tree improvement programs, the concepts of step-wise testing and complementary breeding and testing schemes are very useful. Step-wise testing involves subjecting the seedlings to a series of tests. After each test

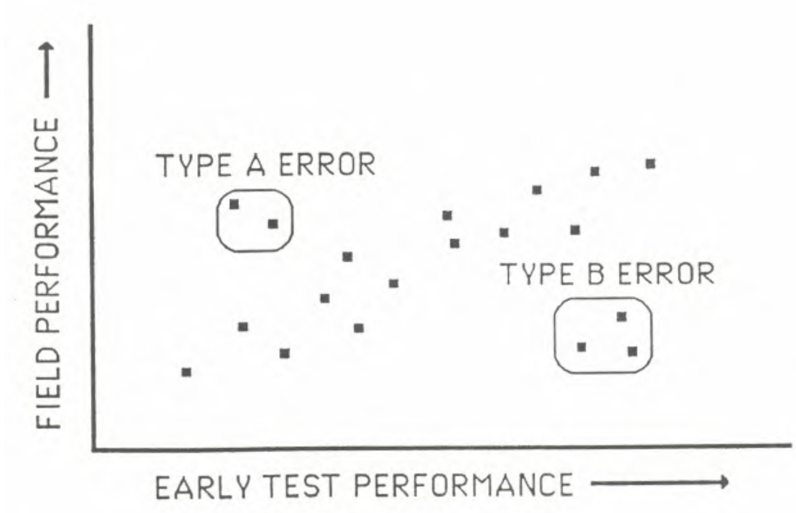

Figure 1. An illustration of type A and type B errors in early testing studies. Type A error involves the rejection of families with good field performance based on early testing data. A type B error occurs when a family with good performance in the early test does not perform well in field tests.

a portion of the families are eliminated prior to the next test. This procedure was first described by Long et al. (1977). Van Buijtenen et al. (1986) described how step-wise testing was applied in a pilot scale breeding project for loblolly pine. It is generally desirable to conduct the least expensive test first on the largest number of seedlings. However, the sequence of tests should consider the genetic relationships among the traits, the precision of the early test, and the relative importance of the individual traits.

Most tree improvement programs have used a single breeding and test design to satisfy the objectives of parental evaluation and advanced generation selection. A complementary system uses a separate mating and field design for each objective. Each design can be more efficient at addressing a specific objective than a single design that addresses both objectives. If the early test for parental evaluation can be completed prior to crossing for development of the selection population, assortative mating can be utilized to increase genetic gains in the selection population. Costs associated with developing the selection population can also be reduced because the rejected families will not be involved in either control-pollination or field testing.

Step-wise testing can be applied to either an open or closed breeding population. Increased genetic gains can be obtained by increasing the selection differential in an open breeding population. If resources restrict field testing activities to 500 families, 665 initial selections could be made if 25 percent of them are rejected on the basis of early testing results. Estimates of genetic gain in this situation would need to consider the effect of indirect selection resulting from the early testing procedure. However, the rejection of poor performing families prior to field testing will allow the use of allocated resources only on better material. In a closed breeding population, the resources required to conduct the field testing can be reduced by the use of an early testing procedure. The savings in cost reduction should be proportional to the rejection rate from early testing. With a complementary mating system, positive assortative mating can be used to obtain increased genetic gains in either an open or closed breeding population.

IMPLEMENTATION IN THE WGFTIP

The WGFTIP is using step-wise testing to implement early testing procedures in its operational tree improvement programs. Complementary mating designs and positive assortative mating will be used when practical. Because different tests are conducted to evaluate different types of traits, separate early testing procedures have been developed for the three major types of traits.

Selection for Juvenile Trait

Survival and grass-stage emergence are important juvenile traits for longleaf pine (*Pinus palustris* Mill.) that can be directly evaluated at an early age. Because phenotypic selection is not effective (Goddard et al. 1973) and the large cost involved with long-term tests (20 years), a two-step testing program was selected.

Initially, open-pollinated seed was collected from 470 selections in the Western Gulf Region and established in a series of short-term tests for three years. Each series contained between 70 and 100 families. These tests were established at close spacing on a wide variety of sites. On the basis of two or three year data, families were selected for inclusion in the long-term tests. Families established in the long-term tests had better than average survival and emergence from the grass stage. For the first series of short-term tests, the selected families averaged a seven percent improvement in survival and a six percent improvement in grass stage emergence (Byram et al. 1986). Approximately 125 families out of 470 original selections will be established in long-term field tests.

Juvenile Evaluation of a Mature Trait

Primary emphasis for genetic improvement in the slash pine (*Pinus elliottii* Engelm.) breeding program of the WGFTIP is to increase resistance to fusiform rust (*Cronartium quercuum* (Berk.) Miyabe ex Shirai R. sp. *fusiforme*). The Resistance Screening Center (RSC) at Asheville, North Carolina, has developed techniques to evaluate the rust resistance of selected material at the seedling stage (Anderson and Powers 1985). This facility can be used as an early testing procedure to improve rust resistance in slash pine.

Open-pollinated seed from 24 slash pine selections belonging to *a* single breeding group has been submitted for testing at the RSC and established in field trials. To consolidate RSC indexes across different tests, each index was expressed as *a* standard deviation compared to the susceptible checklot. Any clone that scored less than 0.5 standard deviations above the susceptible checklot was rogued from *seed* orchards and will be eliminated from the breeding population.

Table 1 presents the results for percent rust infection from the field tests. Rust infection is expressed as standard deviations compared to mean plantation rust infection in progeny tests with at least 30 percent infection and significant effects among genetic entries at the 10 percent level. Families occur in between two and six field tests. A negative score indicates less infection than plantation average. The average rust score for the 24 selections is -0.02. Had the RSC score been used, 10 of the 24 clones would not have been field planted. The average rust score of the remaining 14 selections is -0.32. Even though a type A error (BSS-7) and four type B errors (BSS-14 and BSS-6, K-163 and BSS-9) occurred, use of the RSC would have improved resistance to fusiform rust while reducing field progeny test costs by approximately 40 percent.

Table 1. Standard Field Scores' for percent infection by fusiform classified by Resistance Screening Center (RSC) tests. Scores are the percent infection expressed as standard deviations compared to plantation average.

Resistant at RSC		Susceptible at RSC	
Clone Name	Field Score	Clone Name	Field Score
BSS-14	1.65	01S-3	0.12
K-143	-0.72	OIS-1	0.61
01S-4	-1.02	K-142	1.03
K-211	-0.48	K-2	0.25
BSS-6	0.88	K-149	0.27
01S-2	-0.72	MFCS-1	0.00
BSS-10	-1.34	K-141	1.37
BSS-8	-0.03	BSS-7	-1.14
K-179	-0.25	01S-5	0.79
K-163	0.87	BSS-5	0.73
PE2	-1.60		
BSS-13	-1.08		
BSS-9	1.03		
BSS-11	-1.65		

The WGFTIP uses a partial-diallel for its first generation breeding program (Figure 2). Second generation selections are made from the best 15 percent of the families based on midparent values in field progeny tests. The clones shown in Table 1 were randomly mated in the standard Western Gulf partial-diallel for 50 computer iterations. After each iteration, the best 15 percent (seven) of the full-sib families were selected for rust resistance. Across the 50 iterations, the selected families averaged 1.06 standard deviations less rust infection. To evaluate the effects of both the type A and B errors shown in Table 1 upon second generation selection efficiency, only the 14 parents rated as resistant at the RSC were also randomly mated for 50 computer iterations. The best seven full-sib families selected from each iteration averaged 1.16 standard deviations less rust infection. Assortative mating of the 14 selections rated as resistant at the RSC, resulted in selecting families that averaged 1.07 standard deviations less rust infection. The occurrence of four type B errors in the selected clones was detrimental to the possible benefits of assortative mating.

		MALE								
		A	B	C	D	E	F	G	H	I
	A		X	X						
F	B			X	X					
E	C				X	X				
M	D					X	X			
A	E						X	X		
L	F							X	X	
E	G								X	X
	H	X								X
	I	X	X							

Figure 2. WGFTIP first generation partial-diallel mating scheme.

Rejection of slash pine selections from the breeding population that indicated susceptibility to fusiform rust at the RSC would result in approximately a 40 percent reduction in costs associated with field testing. An opportunity exists to also obtain an increase in genetic gain.

The open-pollinated seed submitted to the RSC was collected at different locations and in different years which resulted in different pollen clouds among the selections. The resistance or susceptibility of the pollen to fusiform rust has been shown to affect family rankings at the RSC (Byram et al. 1987). The use of stable rust susceptible pollen may improve the agreement between RSC and field tests and eliminate a portion of the errors observed among these selections.

Juvenile Trait Correlated with A Mature Trait

Early testing studies with loblolly pine in the Western Gulf region have indicated that shoot dry weight at 6 months was the seedling trait most consistently correlated with field data (Waxier 1980, Miller 1982, Davison 1984 and Strickler 1984). These studies were conducted under greenhouse and/or growth chamber conditions and indicated varying degrees of success in predicting field performance.

A recent review of these and other unpublished studies indicated that type A errors were eliminated when an independent culling level based on germative capacity was used (Figure 3). The deletion of families with less than 85 percent germination eliminated the error of rejecting fast growing families on the basis of shoot dry weight data obtained from greenhouse tests. These studies included approximately 60 different families from different geographic areas within the Western Gulf Region.

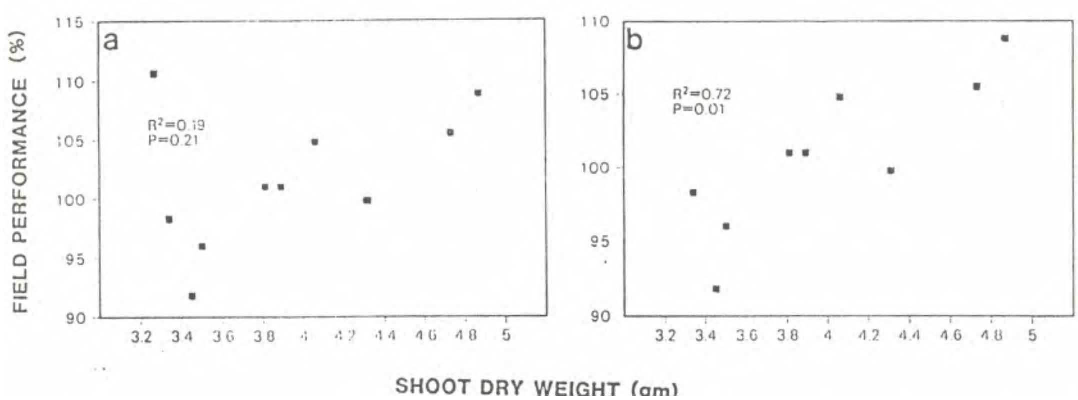

Figure 3. Scatter diagrams, coefficients of determination (R^2) and the occurrence of type A error in Strickler (1984) (a); type A error is eliminated after culling at 85% germinative capacity (b).

Families with small shoot dry weight tend to have average or below average performance in field tests. Step-wise testing can be used to incorporate this early testing procedure into operational breeding programs. Lowe and van Buijtenen (1989) describe how shoot dry weight based on greenhouse data is used in both the first and second generation loblolly pine breeding program of the WGFTIP.

The first-generation breeding program utilizes a partial-diallel mating design (Figure 2) which is planted in three tests. With this design, the crosses containing the parent with the smallest shoot dry weight is not field planted. A composite checklot composed of an equal number of seedlings from each of the rejected families is planted with the test to evaluate the early selection procedure. This results in approximately 17 percent fewer test trees being planted. The replication size is reduced and the costs associated with planting, maintaining and measuring the field test is reduced. Families that were rejected on shoot dry weight data are not planted in later progeny tests.

A complementary mating design will be used in the second generation breeding program. Parental general combining ability estimates will be obtained from polymix crosses and the partial-diallel shown in Figure 2 will be used to develop a population for the third generation selection activities. Greenhouse shoot dry weight data will be used to reject 30 percent of families prior to planting the polymix field tests. The rejected selections will not be crossed in the partial-diallel mating scheme. Shoot dry weight data and mid-parent values will be used to assortative mate the second generation selections in the partial-diallel. Because pollen from the previous generation is used in the polymix, the length of the breeding cycle should not be increased because of the step-wise testing procedure.

Field testing costs will be reduced because approximately 24 percent fewer test seedlings for the polymix crossing scheme will be established. Additional cost savings will result from making and field planting fewer crosses in the partial-diallel portion of the testing program. An evaluation of six studies indicated that assortative mating using shoot weight data resulted in a 1.2 percent increase in genetic gain in the selected breeding population (Lowe and van Buijtenen 1989).

CONCLUSIONS

Early testing procedures can be applied to operational tree breeding programs to increase efficiency by reducing the costs associated with field testing. Step-wise testing is a useful component of an early testing procedure. After each test, a portion of the families are rejected before the next test. This procedure can be applied to either open or closed breeding populations. Complementary mating schemes can be used to incorporate positive assortative mating to increase genetic gains.

Important juvenile traits, mature traits that can be evaluated at a juvenile age and juvenile traits that are correlated with an important mature trait are candidates for early testing procedures. The WGFTIP is applying early testing procedures using step-wise testing techniques to improve field testing efficiency to all three classes of characteristics.

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