A PROPOSAL FOR ADVANCED GENERATION BREEDING AND TESTING

R. J. Weir[1]

INTRODUCTION

The North Carolina State University-Industry Cooperative Pine Tree Improvement Program is an extensive, advanced, applied forest genetics program. Seed orchards developed from the first cycle of mass selection are currently producing seed for 150 million plantation seedlings annually. Ultimately, these orchards will yield seed to regenerate more than 400,000 acres per year with an expected 10 – 20 percent gain in volume over unimproved stock. Improvements in quality traits, adaptability and disease resistance are expected to be of equal or greater magnitude. As with tree breeding the world over, the N. C. State program has captured only a small part of the

---

[1] Liaison Geneticist, School of Forest Resources, N. C. State University, Raleigh, North Carolina.

available improvement through genetics. Ample genetic variation remains within the undomesticated forest populations to allow for continued genetic improvement on a scale not yet realized.

A second cycle of selection is underway, and a number of second-generation orchards have been established (Weir, 1973). Reproductive maturity for the earliest second-generation selections is imminent; thus, it is timely to plan for future breeding and testing. The following proposal is rather specific to the biological and economic constraints encountered with the N. C. State Cooperative Program. While certain aspects may have general application, the apparent attributes and deficiencies must be considered in relation to the resources and constraints of companion programs.

Ideally, decisions among alternative breeding and testing programs should be based on long-range (many generations) economic comparisons of net program returns with respect to breeding and production costs. However, information is only now becoming available to allow meaningful economic evaluations of first-generation improvement cycles (Porterfield, 1973). To detail economic comparisons of future alternatives would be speculative to the extent of doubtful credibility. It seems more useful at this time to consider within the available resources of the current program an improvement schedule which attempts to maximize genetic gain in the next generation without undue restriction of opportunities in subsequent cycles of improvement.

The component parts of the proposed plan are designed with the following objectives:

1. Develop a breeding population suitable for a third cycle of selection and improvement in preparation for third-generation production seed orchards.

2. Establish tests for estimation of clonal worth in second-generation production seed orchards to refine clonal selection, i.e., identify inferior clones so they may be removed from the orchard.

A REGIONAL BASE POPULATION

Breeding and testing of second-generation selections must be planned within the context of available plant resources. The N. C. State Cooperative is comprised of 26 forest industries and three state forestry organizations involved with regeneration activities of loblolly pine (*Pinus taeda* L.) extending throughout the species-range east of the Mississippi River. Each cooperative member works with at least one population of superior trees selected for growth and quality. Many organizations, however, have two or more select populations in production orchards and under test; in the case of several improved populations per organization, each is derived from a distinct physiographic region or set of environmental conditions. Many of these populations will necessarily be useful only within the regional environment of origin because of differential performance.

However, for a given homogeneous environmental area such as the Coastal Plain of the Carolinas, there will be from five to ten breeding programs which can combine resources for second and subsequent cycles of improvement.

The expansive regional first-generation progeny test population serves as the base from which selections are made for second-cycle production seed orchards. Each first-generation improved population consists of approximately 30 genotypes. With minor exception, all have been bred with a factorial mating scheme which utilized four or five male testers. It follows that progeny tests for each regional program are comprised of 20 to 50 completely unrelated half-sib families. Approximately 20 to 30 full-sib families are created within each half-sib family, and each full-sib family contains from 180 to 270 individual trees. Thus, the progeny test population for any single regional program consists of at least 72,000 trees, while a program of maximum size would have as many as 405,000 trees (see Table 1).

Table 1.--Regional base population statistics.

Within a region	Minimum Size	Maximum Size
Improved seed orchard populations	5	10
Clones per orchard	20	30
Half-sib families (testers) per orchard	4	5
Full-sib families per orchard	80	150
Progeny test trees per orchard	14,400	40,500
Region wide totals		
Clones	100	300
Half-sib families (testers)	20	50
Full-sib families	400	1,500
Progeny test trees	72,000	405,000

While of interest the exact selection system by which second-generation orchard trees are obtained is not germane. It is sufficient to recognize that because several economically important traits have generally low-to-moderate heritabilities, some form of family and within-family selection has been employed and will continue to be. The topic of current concern is how to effectively breed and test the selections as they become available and attain reproductive maturity. Because of relatively strong juvenile-mature correlations (Squillace and Gansel, 1972; LaFarge, 1972; Sluder, 1972), selections can be made at an early age and the trees utilized both in breeding and production orchards as soon as reproductive maturity is reached.

The selected breeding population is the key to genetic improvement in future generations. It is within this population that a broad genetic base, i.e., useful genetic variation will be maintained. The breeding population, while intensively selected, will be kept as large as resources can accommodate. New genetic material derived from plantation selections and special studies, e.g., disease-resistant diallel selections and selections from crosses among trees with widely divergent origins, will be infused into the program through the breeding population. New material must be continually added to the breeding population if relatedness is to be prevented from increasing to a level which will cause serious restriction of genetic gain.

Production seed orchards are a subset of the breeding population. The intent for any production orchard is to maximize genetic gains in the short term. Thus, selection intensities are greater for the production orchard and these are considered dead-end with respect to future generations of improvement. At each appropriate opportunity when a need develops for an improved production orchard, a subset of the best available genotypes will be combined to meet the specific requirement (fig. 1).

SEPARATE BREEDING AND PRODUCTION TEST PROGRAMS

Early tree improvement efforts dealt with discrete selected populations obtained within a short time span and the production and breeding orchards were by and large the same populations. It has been argued that testing of parental worth and breeding for a new base population could be accomplished most effectively under these first-cycle conditions with a single-mating scheme (Zobel, et. al., 1972). However, in advanced cycles of improvement, a single-mating scheme will not effectively serve the dual objectives. It is apparent that separation of the breeding and seed production functions will be more efficient in the future and lead to greater gains than would otherwise be possible. Justification for segregation of the breeding and clone evaluation functions is strengthened as the disparity between appropriate investment in each function increases.

A major factor influencing effective early testing is the constrained availability of second-cycle selections over a considerable time span--eight to ten years--due to the nearly constant flow of new selections into the breeding population. For example, Weyerhaeuser Co. and International Paper Co., with breeding programs in the Coastal Plain of the Carolinas, planted their initial first-generation progeny tests in 1964 and their last in 1974. The first second-generation selections were obtained from these tests in 1969, the last are expected in 1979. With this time span and age differential, it is conceivable that breeding and testing of some genotypes selected early could be complete at about the same time that others are just being selected. Since generations can probably be turned over in a 10 to 12 year span and since not all programs within a region began testing at the same time, the flow of new genetic material into the breeding orchard will be nearly perpetual. The breeding scheme designed to maintain the breeding population must be flexible to accommodate this steady stream of newly selected trees. The potential for future improvement will relate directly to the flexibility retained at this stage of the breeding program.

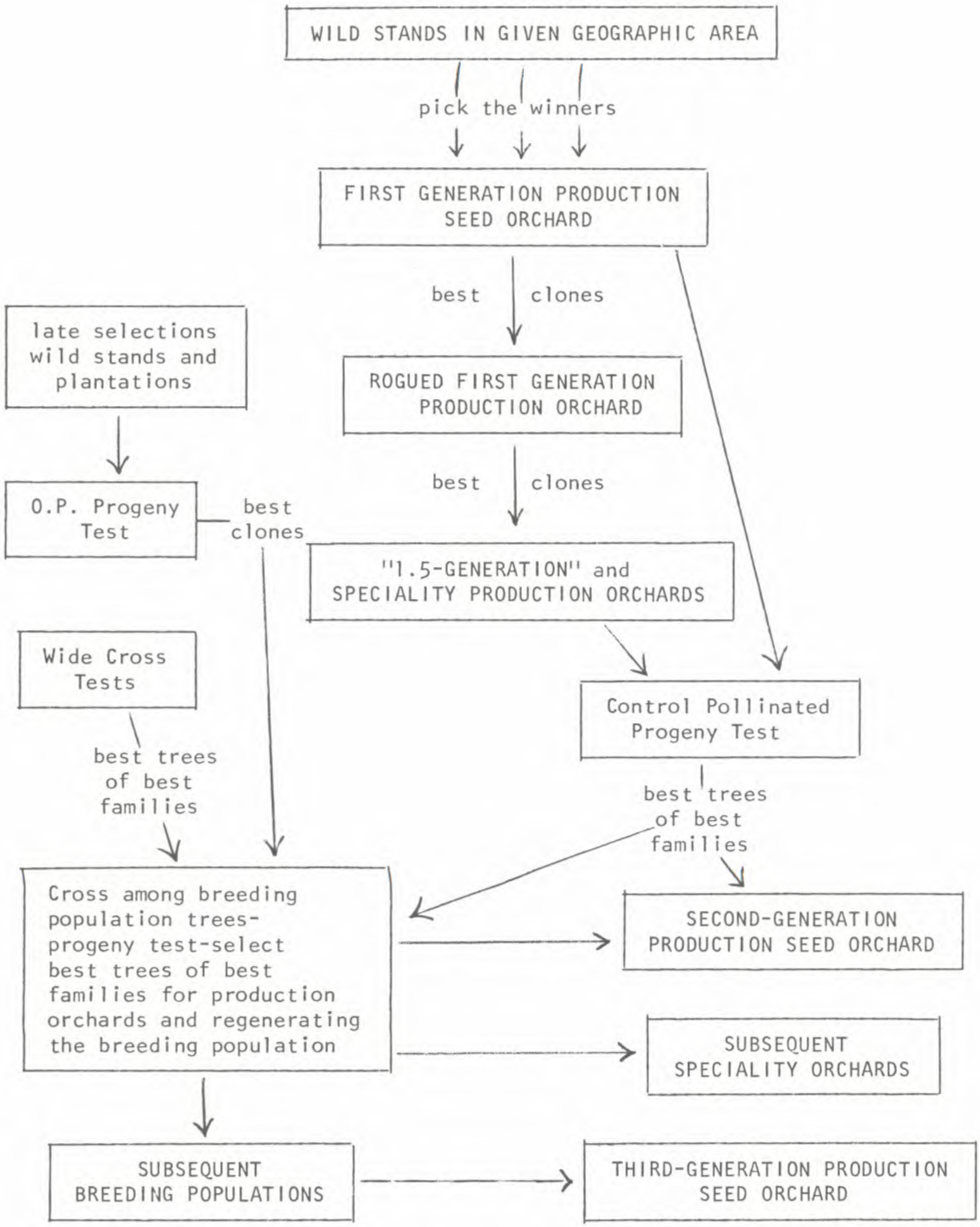

Figure 1.--(Advanced-Generation Seed Orchards) The North Carolina State Cooperative Tree Improvement plan for short-term production and long-term gene base enrichment ("1.5-generation" orchards employ greatly improved first-generation orchards contructed with the best general combiners from a number of orchards).

Second-generation production seed orchards are developed in a deliberate stepwise fashion, with only a portion of the total being grafted in any one year. The stepwise establishment permits utilization of the best set of selections available during each establishment period. Evaluation of parental worth will likewise be accomplished in a stepwise manner as will orchard thinning. Determination of parental worth will be in relative terms to other parents located within an establishment block. It is relative value which determines which clones are left and which are removed during genetic seed orchard thinning, and test results must provide this information for all clones in an orchard block simultaneously. This is a requirement in some conflict with the needed flexibility of a continuous-flow breeding scheme. To resolve this conflict, separate schemes are needed for the testing and breeding objectives.

Time constraints also present a conflict with respect to the dual objectives of breeding for the future and developing clonal-worth information. Expected inefficiencies resulting from selection errors with very young trees (ages 4 and 5) requires second-generation orchards be established at rather close spacing to allow ample orchard rogueing. However, because of the close spacing, thinning to optimize seed production will be needed rather early and the pressure to obtain parental evaluation as soon as possible is intense.

In contrast, the biology of flowering for the young trees presents a complicating condition. During the early developmental stages of the production orchard "female flowers" are expected in significant quantity while pollen production is not. Early estimates of parental worth could be obtained using a pollen source from outside of the second-generation orchard. However, breeding to resynthesize the base population for recurrent selection can only be accomplished properly with pollen from the breeding population. Thus, it is clear that to effectively respond to the pressures of time with respect to rogueing information, separation of the breeding and parental testing functions is required. It is apparent that distinct mating schemes for each of these objectives will increase overall efficiency by a significant amount.

Rogueing a production seed orchard is simply an improvement on initial phenotypic selection. The opportunity for additional improvement within production orchards is restricted due to reduced population size and the correlated fact that additive genetic variance is reduced from that found in the base population (Finney, 1956). While the potential for additional improvement is limited, the culling process is a very sound economic venture (Porterfield, 1973). Under representative first-generation conditions the benefit cost ratio from tree improvement was found in Porterfield's study to increase from 18 to 28 as a result of rogueing. There is no reason to believe that returns from orchard rogueing will be less in advanced generation programs.

The breeding program should receive greatest emphasis in any set of tree improvement activities, This concept was justified in regard to first-generation values (van Buijtenen and Saitta, 1972), and appears that advanced-generation improvement priorities should be weighted even more heavily toward the breeding function. Each successive breeding cycle improves pedigree information and this combined with intensified environmental control in tests serves to increase selection effectiveness. As selection effectiveness increases the proportionate gain now obtained as a result of rogueing must diminish (Franklin and Squillace, 1973).

BREEDING FOR THE THIRD GENERATION

Effective resynthesis of cooperative regional breeding populations is dependent on the mating scheme used. The mating scheme must provide for full-pedigree information and this requires specific controlled crosses. The breeder should strive through the chosen-mating design to maximize the number of unrelated families in order to develop production seed orchards with minimum likelihood for inbreeding depression which seriously limits genetic gains. The family structure derived from the breeding scheme must allow selection on the basis of general combining ability (GCA), which reflects the differences among genotypes associated with additive gene action. Only additive genetic variation can be exploited by selection. Also, the breeding scheme must not restrict the selection differential and thereby place an unnecessary ceiling on possible genetic gains from family and within-family selection (Squillace, 1973), and it should be flexible to accommodate a steady infusion of breeding material over time. It is apparent that the breeding population will be dynamic as it will be continually supplemented with new and better selections and purged of trees which fail to maintain the required measure of superiority. The mating design must regenerate a satisfactory breeding population without excessive costs as measured in terms of both dollars and time. Any significant delays in time resulting from design requirements cannot be tolerated in an applied program because of constant pressure for production of genetically improved seed. Short generation intervals result in greater gain per unit time, and applied programs will generally be more profitable as the interval decreases.

The single-pair mating scheme requires that each selection be mated to only one other parent. Under this scheme all families generated are unrelated, the breeding and testing costs are minimized, and the design is most flexible with respect to infusion of new material into the breeding program. However, the restricted selection differential seriously limits the genetic gains possible with subsequent selection from the resynthesized population (Squillace, 1973). It is also evident that intended selection on the basis of general combining ability is subject to serious bias from specific combining ability, which is not useful with current seed orchard methodology.

Hierarchical mating schemes offer little effective improvement for the resynthesized population because the number of unrelated families is significantly reduced. General combining ability estimates are available on only one side of each pedigree, and the bias of specific combining ability is retained.

Factorial mating schemes such as the tester design are usually the simplest to complete. Few pollens are required; the cost is relatively low. General combining ability estimates are obtained for both the male and female sides of the pedigree but the design is extremely restrictive with respect to maximizing unrelatedness. In the case of distinct regional breeding populations, the level of co-ancestry becomes high in an intolerably rapid fashion.

The full- or half-diallel designs create the ideal resynthesized breeding population. The maximum number of unrelated families is obtained, the largest possible selection differential is realized, and general combining ability estimates are available for all parents. Excessive cost is the overriding limitation to the use of these designs. The number of crosses per parent increases exponentially with each additional selection designated for breeding. These facts in combination with the large size and dynamic nature of the breeding populations maintained in the cooperative preclude the use of full- or half-diallel.

An apparently effective compromise between low cost, seriously deficient single-pair mating and the prohibitively expensive yet functionally ideal half- or full-diallel is the partial-diallel. Some form of the partial-diallel will be utilized by the Cooperative in future breeding efforts. The design offers full-pedigreed, specific-cross parentage and the number of unrelated families is maximized as with the single-pair mating or full-diallel. General combining ability estimates are available for both sides of the pedigree. The number of crosses per parent can be specified to optimize the selection differential within half-sib families. Partial-diallels can be utilized in an open-ended manner, thus accommodating the continuous infusion of new material into the breeding program, and allow opportunity for significant cost reduction. In fact, the design flexibility allows optimization of cost/benefit ratios as determined by the change in genetic gain with change in number of crosses per parent. The exact form of the partial-diallel chosen, whether a series of disconnected half-diallels or the more traditional partial-diallel is the breeder's decision (see examples fig. 2 and fig. 3). Preliminary examination reveals the two configurations to be identical with respect to genetic gains, given that an equal number of crosses per parent are made.

A PRODUCTION ORCHARD TEST PROGRAM

Second-generation production seed orchards are being established as pedigreed second-generation selections become available. Ramets of selected trees are intentionally established at close spacing because of uncertainty with respect to juvenile-mature correlations and the resulting likelihood of selection errors. Early rogueing of genetically inferior parents is essential under these conditions.

Clone evaluation tests for orchard rogueing purposes have traditionally been accomplished in one of two general ways: (1) Open-pollination where wind-pollinated seed are obtained from the ortet in the wild or from ramets in the production seed orchard. (2) Controlled-pollination with pollen mixes or specific crosses. The open-pollination methods are not applicable to second-generation conditions. The ortets of second-generation orchard parents are located in the first-generation progeny tests; because of full stocking and crown competition in these tests, few trees have produced cones or are expected to in the near future. Even if cone production were abundant, the proximity to each selection of full- and half-sibs would promote unknown and variable amounts of inbreeding, a situation not conducive to effective parental evaluation. Controlled pollination methods would provide satisfactory parental evaluations; however, the cost would be considerable. An inexpensive, rapid and effective means of estimating general combining ability of parents is needed.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1		X	X											X	X
2			X	X											X
3				X	X										
4					X	X									
5						X	X								
6							X	X							
7								X	X						
8									X	X					
9										X	X				
10											X	X			
11												X	X		
12													X	X	
13														X	X
14															X
15															

Figure 2.--A partial-diallel mating scheme of the traditional configuration. Each parent is mated with four others.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1		X	X	X	X										
2			X	X	X										
3				X	X										
4					X										
5															
6															
7							X	X	X	X					
8								X	X	X					
9									X	X					
10										X					
11												X	X	X	X
12													X	X	X
13														X	X
14															X
15															

Figure 3.--A partial-diallel mating scheme of the disconnected half-diallel configuration. Group size is five and each parent is mated with four others.

Techniques of mass pollination without "flower" isolation offer a possible means to develop effective, low-cost tests for general combining ability. The major obstacle to obtaining satisfactory combining ability estimates from wind-pollinated seed derived from young orchards is a lack of pollen. In early years, when pollen is available in small quantities, the distribution is often inadequate and certainly unrepresentative. Pollen is abundant, however, in the first-generation orchards; collection of large quantities is neither difficult nor expensive. Methods to optimize application techniques for mass pollination on an operational scale are under evaluation.

A tentative procedure for testing would be: (1) Collection of large quantities (possibly gallons) of pollen from five or six first-generation orchards trees. (2) Mix pollen at the time of application in proportion to its germination capacity. (3) Apply pollen either to individual flowers or dust entire branches; possibly entire crowns of small trees could be dusted. It is likely that effective pollination would require two or three applications of pollen to each tree. No flower isolation would be practiced. (4) Identification of the artificially-pollinated flowers would be appropriately made at the time of pollination and cones would be collected at maturity.

A serious concern with the proposed method is the effect of naturally occurring background pollen. Regardless of the effectiveness of isolation (dilution) zones, there does exist some small influx of pollen from nearby unselected populations. For the purposes of the parental evaluation program, the effects of foreign pollen can be assumed to act in an averaging and random manner. A more serious difficulty would be encountered when one or two parents in the orchard produce quantities of pollen. It is hoped that the supplemental pollination could be complete before any orchard pollen is produced, and by careful timing and observation, this would probably not be a serious situation.

The concept of regional improvement programs is introduced within the context of available plant resources. A proposal is made for breeding and testing schemes which will resynthesize the regional base populations for recurrent selection in the third generation and allow evaluation of the parental worth of second-generation production seed orchard parents. Separation of breeding and parent evaluation functions is advocated for the current and subsequent cycles of improvement. Some configuration of a partial-diallel design is proposed for breeding purposes. To develop general combining ability estimates for second-generation production seed orchard clones, a mass-pollination scheme without flower isolation is suggested.

LITERATURE CITED

Finney, D. J. 1956. The consequences of selection for a variate subject to errors of measurement. Revue Inst, Int. de Stat. 24:1/3.

Franklin, E. C. and Squillace, A. E. 1973. Short-term progeny tests and advanced-generation breeding in slash pine. Can. J. Forest Res. 3(2):165-169.

LaFarge, T. 1972. Relationships among third-, fifth-, and fifteenth-year measurements in a study of stand variation of loblolly pine in Georgia. Proc. Meet. of the Working Party on Progeny testing, IUFRO, Macon, Ga. pp. 2-16.

Porterfield, R. L. 1973. Predicted and potential gains from tree improvement programs--a goal programming analysis of program efficiency. Unpublished Ph.D. dissertation, Yale University. 240 pp.

Studer, E. R. 1972. Open-pollinated progenies of slash pine: their performance at 15 years and the relationships among third-year data and eighth- and fifteenth-year volumes per plot. Proc. Meet. of the Working Party on Progeny Testing, IUFRO, Macon, Ga. pp. 34-47.

Squillace, A. E. 1973. Comparison of some alternative second-generation breeding plans for slash pine, Proc. 12th Conf. on South. Forest Tree Improvement, Baton Rouge, La., pp. 2-9.

Squillace, A. E. and Gansel, C. R. 1972. Juvenile-mature correlation in slash pine. Proc. Meet. of the Working Party on Progeny Testing, IUFRO, Macon, Ga., pp. 17-18.

van Buijtenen, J. P. and Saitta, W. W. 1972. Linear programming applied to the economic analysis of forest tree improvement. J. For. 70(3):164-167.

Weir, R. J. 1973. Realizing genetic gains through second-generation seed orchards. Proc. 12th South. Forest Tree Improvement Conf. pp. 10-23.

Zobel, B. J., Weir, R. J. and Jett, J. B. 1972. Breeding methods to produce progeny for advanced-generation selection and to evaluate parent trees. Can. J. Forest Res 2(3): 339-345.