Sublining for Half-Sib Breeding Populations of Forest Trees

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Summary

Advanced generation breeding in forest trees is difficult when control pollinations are not made. Coancestry among offspring cannot be identified without a fully pedigreed breeding population, and related individuals may be mated in advanced generation production seed orchards. The potential loss of genetic gains from inbreeding depression necessitates the use of a mating design which allows some inbreeding in the breeding population but none in the production seed orchard.

Sublining of the breeding population offers an efficient and practical solution to the problem. The breeding population is randomly divided into several sublines or mating groups. Trees within a group mate at random, and the best individual within each subline is included in a seed orchard. No mating between sublines in the breeding orchards assures that individuals in the seed orchards will be unrelated, and their progenies will be totally outcrossed.

Key words: Black walnut *(Juglans nigra)*, inbreeding, mating design.

Zusammenfassung

Advanced-generation-Züchtung bei Waldbäumen ist schwierig, wenn Kontroll-Bestäubungen unterbleiben. Eine gemeinsame Abstammung von Nachkommenschaften kann ohne eine vollständig nach Pedigree-Züchtung entstandene Züchtungspopulation nicht identifiziert werden; verwandte Individuen können in Produktionssamenplantagen fortgezüchteter Generationen gepaart werden. Der mögliche Verlust genetischen Gewinns durch Inzuchtdepressionen erfordert die Anwendung eines Kreuzungsplanes, der zwar einen geringen Anteil von Inzucht innerhalb der Züchtungspopulation duldet, nicht aber in der Produktionssamenplantage.

Eine Untergliederung der Züchtungspopulation bietet sich als wirksame und praktikable Lösung des Problems an. Die Züchtungspopulation wird zufällig in einzelne Unterlinien oder Kreuzungsgruppen geteilt. Bäume innerhalb einer Gruppe werden zufällig gekreuzt, und das beste Individuum in jeder Untergruppe wird in eine Samenplantage einbezogen. Keine Kreuzung zwischen Untergruppen in den Züchtungsplantagen stellt sicher, daß die Individuen in den Samenplantagen nicht verwandt sind und daß ihre Nachkommenschaften ausschließlich nach Fremdung entstehen.

Introduction

Tree improvement has progressed rapidly in the past few decades with the establishment of several very succesful breeding programs throughout the world. First generation breeding has produced encouraging gains in both yield and quality traits, especially in several coniferous species, the *Eucalyptus* species, and several poplars *(Populus* species). Progeny tests of initially mass selected parents have indicated gains generally around ten to twenty percent.

Forest geneticists have realized that much more improvement is possible from future generations of breeding. Many organizations such as the *Pinus radiata* program in New Zealand and the *Pinus taeda* programs of the North Carolina State and Western Gulf Cooperatives in the Southern United States have progressed to second generation seed orchards.

Progeny tests, established primarily for estimating the breeding values of the phenotypically selected clones, have been the primary source of second generation selections. Unfortunately, mating designs which give an efficient estimate of breeding values, such as open-pollinated tests, polycrosses, or a tester design (N.C. State Design II) are often poor sources for selections (van Buijtenen, 1976). Pedigrees are unknown in open-pollinated and polycross tests, and in the tester design the number of unrelated individuals available for selection is limited by the number of pollen parents used. In each design, related individuals and small population size could become serious problems for future breeding.

Several mating designs have been proposed and used in recent years for selection of offspring for advanced generation breeding. Single pair matings (Libby, 1968), polycross and hierarchical designs (N.C. State Design I) (Burdon and Shelbourne, 1971), and variations of the diallel cross (Zobel, Weir, and Jett, 1972) each allow for reasonably high selection intensities while maintaining a population size large enough to prevent high levels of inbreeding in future generations.

There is a trade-off between intensive selection and maintaining a large effective population size. Namkoong (1974) equates a breeding population to a renewable resource which will yield continued returns as long as the resource is not depleted. With very intensive selection, initial gains will be large but later gains could be reduced because of reduced genetic variation and loss of low frequency favorable alleles. Trade-offs between intensive selection and large population size are inevitable no matter which mating design is used, but preventing related matings in a seed orchard should be a high priority if the presumed deleterious effects of inbreeding depression are significant. There are strong indications that this may be so, but definitive studies have not yet matured to yield results.

Each of the designs mentioned above is predicated on the ability to obtain significant numbers of control pollinated progenies. Unfortunately, there are many commercially important tree species which are difficult to control pollinate because of biological limitations. In the oaks *(Quercus species)* and black walnut *(Juglans nigra)* the flowers are borne singularly and contain only one ovule, so at most one seed per flower is obtained. Producing enough pedigreed offspring is very difficult with these species.

Another limitation to using complex mating designs may be economic. Most tree improvement organizations have concentrated their efforts on one or at most a few

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species. Programs may be established for species of secondary importance, but, because of limited resources, time, and manpower, intensive breeding methods usually are not applied to the secondary species. Obtaining fully pedigreed offspring in large enough numbers to develop a full scale, balanced program requires a substantial investment. The amount of time and money needed to develop complex mating designs is usually considered unwarranted for the minor or secondary species or those difficult to work with such as the oaks, walnut and some tropical species.

This paper suggests a mating design to overcome these problems. It will specifically use black walnut and the improvement program conducted by Purdue University and Indiana Division of Forestry as a case example. Both biological and economic constraints are encountered in a program with walnut. Control pollinations are very difficult. The amount of labor and capital involved in the operation of the program is considerable, but it does not have the backing which large breeding programs for many coniferous species have. The mating design developed here will be applicable to any species in which control pollinated progenies are difficult to make or where economic limitations make a full scale program impractical.

Status of Program

At present, approximately two hundred parent trees have been initially screened for inclusion in the black walnut program at Purdue. Fifty-five of these selections have been included in progeny and/or clonal tests since 1969. Emphasis is being placed on grading potential candidates for the program, both from natural stands and non-selected plantations. Additional selections are necessary to increase the genetic variation and the size of the foundation population.

Virtually all of the progeny tests established through 1977 were with open-pollinated progenies from the parent trees. Only since 1975 have clones in the breeding orchard produced enough progenies for inclusion in the tests. Many attempts to control-pollinate enough clones for establishing a full-sib progeny test have met with failure.

The consequences of not having a fully pedigreed population on which to base an improvement program are troublesome. Estimates of general combining ability (gca) should be reasonably good, but problems arise when attempting to make selections for the next generation of breeding. WEIR (1974) reports that in progeny tests of *Pinus taeda* from seed orchards, mass selection results in the same male parents generally being chosen, because of the high gca of several of the clones which supply the pollen. A similar superiority of good general combiners could be expected with black walnut, since there are several clones that produce outstanding progeny. If these clones serve as the pollen parents in the breeding orchard, then some maternally unrelated offspring will in fact be paternal half-sibs. If included in a second generation orchard, expected gains may be diminished because of inbreeding depression from related matings.

No data exist which confirm that inbreeding depression is serious in black walnut. However, since black walnut is a predominantly outcrossed species, we assume that inbreeding does reduce progeny vigor as is true for most outcrossed agronomic and forest species. The ability to

mate unrelated individuals for commercial seed production is a high priority of the Purdue program.

Fortunately, most of the progeny tests were established with wind-pollinated offspring from the parent trees. If one selection is made from each family in these half-sib tests, there is little chance for relatedness among individuals, since the maternal parents are so separated that the pollen parents will be different. Selection among such open-pollinated progenies is not very efficient, because the pollen parents are not selected (VAN BUIJTENEN, 1976). It is, however, a better alternative than selecting related individuals for advanced generation breeding.

The problem of how to efficiently select among progenies produced in the breeding population still remains. Since inbreeding in closed populations is ultimately inevitable, a system is necessary which will accomodate some inbreeding but, at the same time, prevent coancestry among individuals in the production seed orchard (LINDGREN and GREGORIUS, 1976).

Proposed Mating Design

In recent years the concept of sublining has received much attention (VAN BUIJTENEN, 1976; BURDON, SHELBOURNE, and WILCOX, 1977). Sublining is accomplished by establishing small breeding groups and mating trees only within a group. The best progenies from each group are selected as parents for the next generation of breeding and each group is maintained for as many generations as desired. Sublining allows for some inbreeding within each breeding group, but, since only one clone per subline is included in the production orchard, inbreeding levels will not increase above those currently suffered in wind-pollinated seed orchards.

There are many advantages of sublining cited by BURDON et al. (1977). It is a very flexible system of mating, with almost any mating design within the sublines being suitable. It also allows for enriching the population by adding new material to existing sublines or by creating new sublines with the new material. Sublining can be abandoned at any time simply by intermating the different groups. Finally, with the acceptance of inbreeding within the groups, full pedigrees are not necessarily required.

This final consideration of not having a fully pedigreed population is a primary factor for utilizing sublining for tree species which are difficult to control-pollinate. Within each spatially separated subline, clones are allowed to mate at random and selection is among and within maternal half-sib families. Inbreeding will occur within the groups, but the seed for commercial plantations will be totally outcrossed.

A possible drawback to sublining could be reduced seed production in the seed orchards by using partially inbred clones. Inbreeding will increase faster in smaller subline populations than in a single breeding population. We feel, however that some potential loss of seed production can be offset by increased gains made by sublining.

The sublining method proposed here is very similar to the nested polycross design proposed by BURDON and SHELBOURNE (1971). The only difference is that in their scheme random mating is assured by crossing all clones within a nest with a pollen mix from the clones within that same nest (complete polycross nest). The authors further state that if nests (sublines) are of reasonable size, the sampling error variance between nests should

be small and the assumption that nests are all of equivalent genotypic value should be reasonably reliable. They suggest the use of twenty to thirty clones per nest.

The decision of how to divide the population into sublines is critical. The number of clones within a subline must be large enough to insure genotypic equality among sublines. If the genetic variation within the population is very large, the number of clones per sublines should be large. Initial phenotypic selection reduces the variation, thus the potential for a large sampling variance among groups is reduced. A population size of twenty-five clones in each subline should be sufficient to eliminate inequality among groups and keep inbreeding levels acceptable.

The number of sublines required will equal the desired number of clones in the seed orchard. The number of clones to be included in a seed orchard depends upon the design of the orchard, spacing between trees, and the number of clones needed to maintain a specified amount of variation in the offspring. If, for example, repeating blocks of clones are used, spacing is ten by ten meters, and a distance of forty meters between ramets of the same clone is required, then sixteen clones will be used in the orchard (*Figure 1*). Sixteen sublines with twenty-five clones each would require 400 trees for a breeding region. If fewer trees are available for breeding, the number of sublines should be reduced, not the number of clones per subline. With as few as five or six unrelated clones in an orchard, the progeny will still be genetically diverse.

The testing and breeding phases of the sublining method are shown in *figure 2*. The clones used in the first generation of sublining will be randomly assigned to different sublines to inhibit genotypic inequality among groups. The clones will be either mass selected trees from natural stands or offspring from natural stands which were included in early progeny tests.

To verify genotypic equality among sublines, the clones from each group will be progeny tested at several common locations. If significant differences exist between sublines, the poorer groups can be enriched with new material.

Seed orchards are established from the best clone from each subline. Orchard establishment must be delayed until initial progeny test data are available. It would be impractical to include each clone from a subline in the orchard. A few clones (three to five) could be selected based on early progeny performance, seed production, and grafting ability. These clones would be planted in close groups in the seed orchard, then the inferior clones would be rogued, leaving the best clone per subline. The final roguing is based on later progeny test data which are considered reliable for final selection.

Selection of the best clones for inclusion in seed orchards could be a problem, especially after the first generation. Selection of the best progenies is based solely on family and within family performance of maternal half-sibs. Some the twenty-five progenies selected for second generation sublines will undoubtedly be paternally related. When these twenty-five trees mate at random, the level of inbreeding among the third generation families will not be uniform. Some families will show more inbreeding depression than others because of the nonuniform levels of relatedness among the second generation parents. The clone with the highest gca may not be selected simply because it is mated with more relatives than a poorer clone.

One must remember, however, that the inability to adjust for different levels of inbreeding during selection is present in any open-pollinated breeding method. With or without sublines, selection in open-pollinated populations is never as efficient as in fully pedigreed populations. When faced with the problem of selection among half-sib progenies, one should keep in mind the limitations involved.
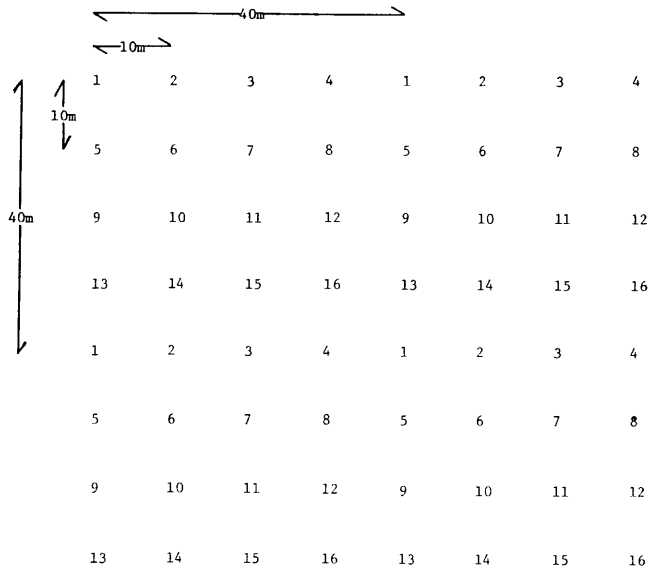

Figure 1. — Repeating block seed orchard design; sixteen clones with spacing at ten by ten meters[1].

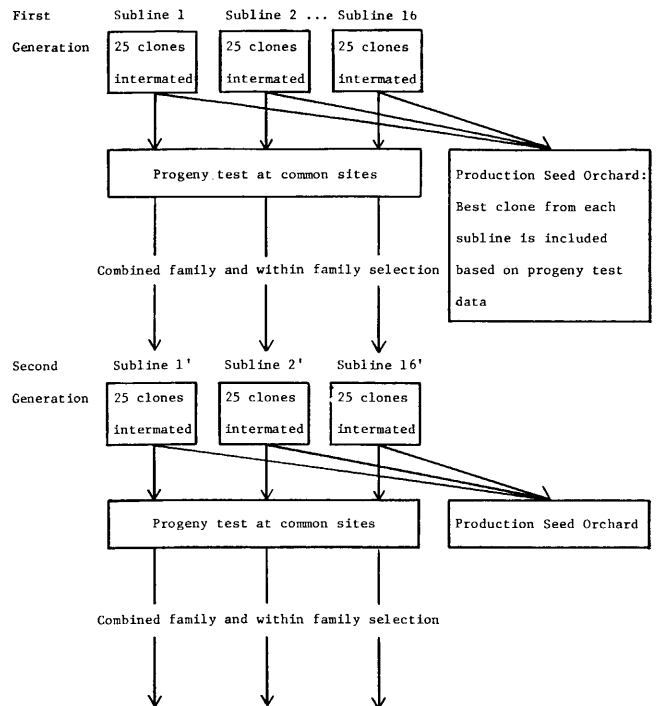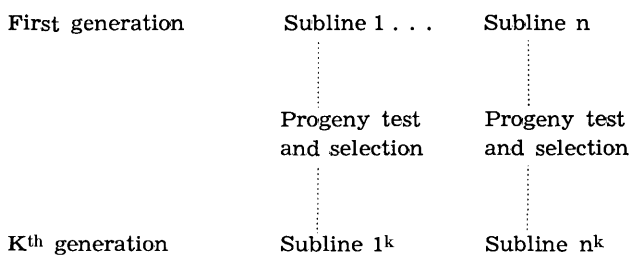[1]Distance between ramets of the same clone is maintained at forty meters.

Figure 2. -- Proposed Method of Sublining

Selection of individuals for inclusion in the second generation breeding population is seperate from selection for production seed orchards. Twenty-five individuals are selected based on family and within family performance. Selection differentials are not as high as with selection for the seed orchards, and the probability of losing favorable alleles at low frequencies will be diminished. Kang (1977) states that alleles should be fixed more quickly in subpopulations than in a single large population. He shows that alleles which occur at low frequencies in a large population will increase in frequency in some smaller subpopulations. For example, if a favorable allele occurs in a heterozygous state in one individual in 250, its frequency is 1/500. If the population is broken into ten sublines each with twenty-five trees, the allele will be at a frequency of 1/50 in one of the groups and absent in the other nine. Since the probability of fixation or loss of an allele is dependent upon initial frequency, sublining should increase the chances of maintaining low frequency favorable alleles.

The integrity of each subline is maintained each generation by selecting offspring only from the same groups each generation. For example:

First generation	Subline 1 . . .	Subline n
	Progeny test and selection	Progeny test and selection
Kth generation	Subline 1^k	Subline n^k

The progenies selected for the next generation of breeding are grafted into archives for second generation sublines. Subsequent mating and progeny testing is performed as in the first generation. The best clone from each subline is included in second generation seed orchards. Combined family and within family selection for third generation breeding populations is performed as before.

An assumption inherent in sublining is that trees with high gca within groups will also have a high gca when mated to trees in other sublines. Since control-pollinations are not performed, test crosses between sublines to verify this assumption cannot be made. However, lack of agreement for within and among group combining abilities is not foreseen as a problem as long as sublines have equivalent genotypic values and reasonable population sizes.

A final consideration when utilizing sublines is the probability of capturing heterosis in the commercial seedlings. With selection occurring within sublines, favorable alleles will be fixed in the progenies. Presumably, different favorable alleles at different loci will be fixed in different groups. When the unlike genotypes are intercrossed in the seed orchards, new combinations of favorable alleles can be produced resulting in hybrid vigor in the progeny trees (Allard, 1960).

It is unfortunate that in many important tree species mating designs which have proved very succesful in coniferous and other species cannot be used. When, and if, control-pollination techniques become feasible with these species, more elaborate mating designs can be utilized within the sublines. Until that time, the use of open-pollinated sublines seems to be the most practical system to use in the genetic improvement of tree species which are difficult or overly costly to control-pollinate.

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