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The Breeding Seedling Orchard in the Multiple Population Breeding Strategy

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Summary

Genetic improvement includes selection, testing and breeding from the species down to the clonal level. Tree breeding programmes in tropical countries are often required to work with many provenances of many species for many sites. A Multiple Population Breeding Strategy (MPBS) was proposed in response to this need and the breeding seedling orchard (BSO) devised to combine the conventional hierarchy of sequential testing, selection and seed production populations in a single planting.

In the MPBS the breeding population is divided into subpopulations which are kept separate so as to produce trees with different gene complexes. These can then be used as replicate populations (to avoid inbreeding effects in the operational seed), diversified populations (to exploit genotype-environment interaction), heterotic populations (to exploit heterosis) or structured populations (to place more emphasis on the elite elements).

The BSO lies between a Seedling Seed Orchard (SSO) and a progeny test (PT). The functions of the SSO or PT are inevitably compromised in the BSO; the nearer it lies to the SSO the higher the selection intensity and seed production whereas the nearer it is to the PT the better the genetic information and the selection precision. Objectives should be clearly stated before the BSO is designed if these conflicts are to be resolved.

Breeding intensity within the BSO can be simple (mass selection), intermediate (half-sib pedigree control) or intensive (full-sib pedigree control). In determining the genetic constitution of the BSO the crucial issues are: sampling the base population effectively, avoiding inbreeding and selecting the genetic checks. The elements of environmental design, plot size, shape and spacing, replication and sub-blocking, and siting can all be crucial in achieving the objectives of the BSO. Genetic gain is dependent on the effectiveness of selection which in turn is dependent on its intensity, precision and the number of criteria.

The conclusion is that this strategy is flexible, provides the potential to respond to new materials and new demands and allows the breeder freedom to be adventurous without taking unacceptable risks. At the same time it accommodates the need to work with many populations of many species at different levels; and it conserves variation.

Key words: multiple population breeding strategy, breeding seedling orchard, progeny test, selection, experimental design, inbreeding, seed orchard, genetic checks, juvenile-mature correlations, genotype-environment interaction.

FDC: 232.311.3; 232.11; 165.4; 165.62.

Introduction

A genetic improvement strategy in its broadest sense includes the whole process of selection and testing from the species level, through the population and family, down to the clonal level, and the conservation of variation within them. Most tree improvement programmes in tropical countries are concerned with many provenances of many species for many sites. This has been especially true in the last 30 years when there has been an expansion of species and provenance testing which has brought about a need for a strategy with which to manage this proliferation of potentially valuable material. A Multiple Population Breeding Strategy (MPBS) was proposed in response to this need (NAMKOONG *et al.,* 1980).

When adopting the MPBS, it is usually impracticable to maintain the full hierarchy of the sequential testing, selection and seed production plantings of a conventional programme for every population of every species. These functions may have to be combined in various degrees, depending on the structure of the multiple populations, in a single planting which is now formally known as the Breeding Seedling Orchard or BSO (BARNES, 1981), although such plantings have been used in tree breeding programmes since the early 1960s (*e.g.* MULLIN *et al.,* 1981; REDDY *et al.,* 1986). Since the concept of the BSO in the

MPBS was formally proposed, it has been put into practice in many programmes, notably in the Forestry Commission's tree breeding programme in Zimbabwe where the design has undergone considerable evolution over the past decade (BARNES, 1984a and b, 1987; BARNES and MULLIN, 1989). In this paper, the current status of the concept, design of and selection in the BSO and the resolution of the conflicts that arise within the MPBS are discussed.

The Multiple Population Breeding Strategy

In the MPBS, the breeding population for a single species is divided into a number of sub-populations which are kept separate so as to produce trees with different gene complexes. The objective is to maintain or create differences between the sub-populations while practising various intensities of breeding within them. Diversity will occur between the sub-populations within a species even if the selection criteria, selection pressures and the environments are the same because most traits are under multiple gene control and different sets of genes will be brought together in different populations and currently neutral alleles will not be lost in all populations (NAMKOONG, 1984b). At any time, variation can be re-instated, inbreeding depression overcome, inter-population heterosis exploited and new populations with specific attributes created by crossing between populations (NAMKOONG *et al.*, 1980).

There are 4 types of multiple population breeding:

1) Replicate populations (BAKER and CURNOW, 1969; BURDON and NAMKOONG, 1983) in which independent sub-populations (sometimes called sub-lines) are bred for the same trait and adaptability objectives; elements from each are brought together for commercial seed production to ensure out-crossing.

This strategy is most appropriate where genotype-environment interaction (*gei*) is not large enough to justify maintaining separate breeding populations and where the requirements in the nature of the raw material are the same for all end products. At least half the benefit available from what *gei* might exist can be used by establishing the replicate populations in different environments and keeping the open-pollinated seed from the composite orchard identified by groups of seed bearers from the individual sub-populations; the seed can then be returned to that particular environment for commercial use. The view has been expressed that the existence of broadly-adapted genotypes has often been based on belief rather than on hard evidence (BURDON, 1992) and the above strategy may go some way to safeguarding against that misconception.

This variant of the MPBS has been used in breeding some of the southern pine species in the Western Gulf Forest Tree Improvement Program (LOWE and VAN BUIJTENEN, 1986).

2) *Diversified populations* (NAMKOONG, 1976; BURDON and NAMKOONG, 1983) in which existing differences are used or new differences created by selecting for different traits and/or adaptability to different environments while retaining the option of crossing between them to escape from inbreeding and to re-create variation; commercial seed production occurs within sub-populations for specific products and/or environments.

The potential of a forest area to produce wood is exploited by careful matching of species to site, *i.e.* by using *gei* at the species level. There is now increasingly substantial evidence of *gei* at the sub-specific level. The differences in environment that cause these interactions, however, are more subtle and difficult to identify and measure. Nevertheless, not to use them is to ignore adaptation, one of the principal mechanisms of natural speciation and a significant, probably the major, source of genetic potential to increase productivity in a species through genetic manipulation (BARNES, 1984a).

The strategy of diversified populations can be used to make rapid gains in breeding for specific traits in different populations; it can be used to keep distinct the identities of naturally-occurring gene complexes and genotypes specifically adapted to particular environments so as to retain the option of using them to maximize productivity over environments; and it can be used to maintain genetic diversity within the breeding population (NAMKOONG, 1984b).

The MPBS in this sense has been in operation for a number of pine and eucalypt species in Zimbabwe since 1981 (BARNES and MULLIN, 1989).

3) *Heterotic populations* (NAMKOONG, 1984a) in which both additive and heterotic gene effects are used through reciprocal recurrent selection in parallel sub-populations with parallel test-crossing of selected individuals in each sub-population to a select sample from the other population; this allows cumulative improvement and moulding of two sub-populations so that they have increasingly divergent gene frequencies and thus increase heterosis on crossing (SHELBOURNE, 1969). Commercial seed is produced by crossing between sub-populations to exploit the heterosis.

The most spectacular increases in productivity in plantation forestry have been achieved through the use of species hybrids; provenance hybrids may also yield gains. Plantations can be hybrid habitats (ANDERSON, 1949) both because a new environment is created by the cultural practices and because the plantations are often established outside the species' native range. Heterosis therefore can be expected but there is also evidence that high general combining ability (*gca*) in the individual parents is reflected in their hybrid progeny. Heterotic populations can be used to breed successive generations of hybrid parents of high *gca* and to preserve the option of testing new hybrid combinations in future, as yet unknown, hybrid habitats.

The Queensland hybrid breeding programme with *Pinus elliottii* ENGELM. and *P. caribaea* var. *hondurensis* BARR. & GOLF. illustrates the practical evolution of this strategy in forestry (NIKLES, 1992).

4. *Structured breeding populations* (WHITE, 1992) in which the breeding population is divided into sub-groups, consisting of *e.g.* a nucleus population, which contains the highest ranking individuals of the breeding population, and one or more larger main populations (COTTERILL *et al.*, 1989).

The nucleus population provides each generation's propagation population; the main population ensures the potential to maintain diversity and avoid inbreeding by infusing genes into the nucleus; these will be incorporated provided they outperform current members of the nucleus. This type of multiple population is taken to include any variant on this scheme that facilitates placing more emphasis on members with higher genetic quality.

The breeding progamme for *Eucalyptus grandis* HILL ex MAIDEN in Florida is an example of the MPBS strategy in this sense (REDDY *et al.*, 1986).

The Breeding Seedling Orchard

If the test, breeding and seed production populations are combined, the planting may be expected to provide, to various degrees, estimates of genetic variances, a comparison of parental genetic values, a demonstration of realized gain, material for selection of the founders for the next generation of breeding and seed for commercial planting. Conflicts can, therefore, be expected in planning to achieve these objectives (BURLEY, 1972). These conflicts must be resolved before the BSO is planted and in doing this the objectives must be clearly defined, the breeding intensity set, the genetic constitution decided, a precise environmental design drawn up to fit the site and, finally, a management procedure adopted that will ensure maximum production of information, materials and gains from the planting.

Concept and definition

It is helpful to imagine the BSO as lying somewhere between the 2 extremes of a dedicated Seedling Seed Orchard (SSO) on the one hand and a dedicated progeny test (PT) on the other. No compromises need be made in design if the purpose of the planting is solely for seed production or if it is solely to test the progeny; but anything between does require compromises in design and these are most pronounced where the 2 functions are equally weighted. For example, the line in *figure 1* below represents the continuum between a SSO (point 1) and a PT (point 5) with points 2, 3 and 4 representing intermediacy between the 2.

high plant density	more accurate genetic information --> <-- greater seed production	low plant density
-		-
early, selective thinning	SSO<-----------BSO----------->PT 1 2 3 4 5	late, systematic thinning
-	<-- higher selection intensity	-
forward selection	increased selection precision -->	backward selection

Figure 1. – Diagrammatic representation of how the Breeding Seedling Orchard (BSO) can vary (positions 2 to 4) between the pure Seedling Seed Orchard (SSO) at the one extreme (position 1) and the pure Progeny Test (PT) at the other (position 5) in its design and management and yield of information and materials. Low plant density is equivalent to routine plantation spacing.

Plantings at points 1 to 5 might then be described as follows:

1. The dedicated SSO would be planted at close initial spacing and thinned selectively, before any competition set in, to a final spacing that was optimum for seed production. There would be no family structure and therefore no potential to estimate genetic variances. Its function would be to produce seed and, if required, to provide material for mass selection of founders of the next generation. Forward selection would be obligatory; it is conducive to a faster turnover of generations, one of the principal aims of using the BSO approach, and should increase the cumulative genetic gain per year (COTTERILL, 1986; BURDON, 1992).

2. This would be a compromise that would include testing and seed production functions but would favour seed production at the expense of precision of selection, estimation of genetic parameters and ranking of families. Close spacing between trees would allow for selective thinning which would be carried out early to avoid prejudicing seed production. The principal difference between this and the SSO would be the maintenance of family identities to ensure that a broad and balanced genetic base was kept in the next generation.

3. The balanced BSO would be designed to give the best compromise for producing seed, estimating genetic parameters, ranking families and selecting the founders for the next generation. Seed production would be compromised in that collection would have to wait until thinning had brought numbers down to one tree per plot and thinning would have to be delayed to some extent to improve precision of selection and this would affect crown development and prejudice seed production. Selection would be prejudiced in that thinning would have to take place too early for it to be fully efficient. Estimation of genetic parameters and ranking of families would be compromised in that they would only be accurate up to the age of the first selective thinning.

4. This would be a compromise that would favour estimation of genetic parameters and ranking of families at the expense of intensity of selection and seed production. There would be wider initial spacing and later thinning.

5. The dedicated PT would be designed to give the most reliable estimates of genetic parameters and family rank. It might be at wide spacing so that there was no thinning to disrupt the genetic constitution of the trial or, if thinning were to take place, it would be systematic. Its sole function would be to estimate genetic parameters and rank families. Backward selection (using the parents retained in a clonal archive as the seed production population) is obligatory unless the PT is large enough to permit an acceptable selection intensity and controlled pollinations are made in their crowns to produce seed for the next generation.

The compromises that have to be made in a BSO arise as a result of expecting all three functions to be fulfilled together at the end of the experimental period. Theoretically, a BSO could be designed and managed with little or no compromise if it were accepted that the experiment acted in the early stages as a progeny test, then as a selection base and finally as a seed orchard, although if non-operational spacing is used there is the risk of genotype-management interaction prejudicing gain.

It is of crucial importance to recognize that what is meant by compromise is a reduction in the inherent potential of the experiment to yield data for precise estimates or to yield the quality or quantity of seed that would be possible without compromise. A compromise does not imply that the environmental design or the conduct of the experiment can be any less precise; if statistically significant differences between entries in the traits of interest cannot be shown, the compromises cannot be made.

Setting objectives

Before deriving a constitution and design for a BSO, the objectives must be defined. The weighting set on the various functions will depend on the importance attached to producing the genetic information needed to ensure that acceptable levels of short and long term gain can be achieved in the seed produced from it. For example, in the absence of existing knowledge, it may be necessary to demonstrate the extent to which the traits of interest are under *gca* control, how susceptible they are to inbreeding depression, whether there are usable juvenile-mature correlations, whether flowering is synchronous and whether seed production would be seriously delayed by late thinning. At this stage of the planning, the genetic structure and origin of the material that is to be used in the BSO must also be taken into account. For example, where the base population is not panmictic, where sampling of it is non-random, where inbreeding is suspected or where the sample is too small, there is a risk of setting objectives that are too ambitious in terms of the precision of estimation of genetic parameters for the inherent capacity of the material.

Once the objectives of the BSO have been set there must be a process of resolution of the conflicts met in designing and managing it for various combinations of the functions of:

i. giving unbiased estimates of genetic parameters;
ii. giving a genetically meaningful ranking of families;
iii. providing a selection base;
iv. preserving the best founders for the next generation;
v. ensuring that the best genotypes produce seed; and
vi. maximizing seed production.

Breeding intensity

The level of breeding within the BSO can be simple (mass selection), intermediate (half-sib pedigree control) or intensive (full-sib pedigree control) (NAMKOONG *et al.,* 1980). There are many mating designs to choose from if there is full-sib pedigree control and yet another compromise must be made between those that give most genetic information, the best material for selection of the next generation and the lowest rate of build-up of coancestry. The breeder's aim is usually to find a resolution that will result in the highest rate of gain (per unit of time) obtainable with the resources available. This is a separate issue in itself but not one that need confuse that of the function, design and management of the BSO which is the subject here.

Constitution

Critical considerations in determining the genetic constitution of the BSO are how effectively it samples the base population, how well it avoids inbreeding and what genetic checks are included to permit estimation of genetic gain and a comparison with other potential seed sources.

Sampling of the base population

The base population for the BSO may be very large, as it would be, for example, for a widely distributed naturally-occurring or exotic industrial species such as *Pinus taeda* L. or *P. radiata* D. DON, or very small, as it would be if the initial base population for the BSO were a single entry in a provenance trial. Whatever the size of the base population, if genetic parameters are to be estimated from the BSO, the underlying assumptions are that the parents of the families in it are a valid sample of the population about which inferences are to be made, that there are no inbreeding effects, that there has been no selection among progeny in the nursery and that nursery effects are fully confounded with field environmental effects. If seed production is the only objective, then it is only the best genotypes that are required and these should be unrelated if there is a risk of inbreeding depression in the important traits.

Avoidance of inbreeding

Inbreeding effects (including selfing as the extreme form) can arise in the breeding and/or production population as a result of neighbourhood inbreeding in the base population or as a result of family coancestry, within-family crossing or selfing in the BSO.

Although the initial selection of trees from the base population is usually made with the aim of minimizing co-ancestry between them, this does not preclude the chances of inbred individuals occurring in their naturally-pollinated progeny. The level of inbreeding will vary from family to family depending upon the relatedness of the parent tree's neighbours, seed and pollen dispersal distances and the degree of isolation of it from unrelated mates. Provided that the chances of within-family crossing are minimized in the BSO, there should be a release from inbreeding in the operational seed collected from it and in the founders of the next generation selected within it. The inbreeding effects in the first BSO will, however, be confounded with other genetic effects and affect both the precision of their estimation and the ranking of families. There is a case, therefore, for not estimating genetic parameters, not ranking families, making no between family selections in the F1 generation and designing the BSO specifically for maximum efficiency of within-family selection and maximum chance of out-crossing among the final seed producers. Estimation of genetic parameters, ranking of families and between-family selection will be more precise in the F_2 BSO. It is possible that the gain achieved from this release from inbreeding could be the most significant step in the breeding process and the sole objective of the BSO might be to make this gain.

There may be a rapid build-up of family coancestry if the genetic base is too small initially or if selection intensity for families is high with a consequent rapid reduction between generations. Even though the MPBS allows for an escape from this by crossing between populations, it is desirable to delay inbreeding in individual sub-populations for as long as possible without unduly prejudicing the potential of making large gains through heavy selection. In this connection, it is encouraging that there are instances where less inbreeding depression has been found among families produced from parents with high *gca* for growth (LINDGREN, 1986; MULLIN *et al.,* 1978). NAMKOONG (pers. comm.)[1]) believes also that, provided there are as many families to select from as there were parents in the previous generation (say 20) and that each of those parents is equally represented among the founders of the next generation, inbreeding depression can be avoided for many cycles of selection. High selection pressure can continue to be applied among individuals within families and among families raised from those individuals in the next generation. In the intensively bred populations, the circular mating designs (BRAY, 1971; HUBER *et al.,* 1992) can be used to produce as many families as there are parents. This should contribute towards achieving a high rate of gain over a number of generations particularly in a population that started with a small number of parents with proven high *gca*.

If an environmental design other than single tree plots is used for the BSO, within-family crossing can be a danger until plots are reduced to a single tree. Seed production may start before this stage is reached and there could be a high proportion of related crosses. Therefore, if the species is precocious and seed collection is to be made early, plot size should be reduced so that the point of commencement of seed production, and reduction by selective thinning to one tree per plot, coincide.

Genetic checks

There is a need to include genetic checks in the BSOs to monitor the performance of entries so as to:

i. assess the rate of gain;
ii. detect the onset of inbreeding effects;
iii. test the assumptions of genetic theory that are made;
iv. compare performances between sub-populations or between the sub-population and other potential sources of seed either for operational use or for infusion into the breeding programme;
v. show that the best genetic material is being passed on to the operational clients through the seed.

The use of genetic checks for achieving gain, detecting inbreeding depression and testing genetic assumptions are not strictly necessary although research on the alternative gain to be achieved without genetic checks has yet to be done.

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However, as the sub-populations of a species are, by design, not planted on the same site, it is necessary to include the same standard genetic checks in every BSO for a species to make comparisons between entries in different BSOs and to demonstrate practically that superior material is being produced for operational planting.

To ensure that their performance is as consistent as possible over time and site, genetic checks should ideally be full- or half-sib families selected for their stability over environments. If this is not possible, they should consist of a number of full- or half-sib families that, between them, perform well over the range of environments in which the species is grown. Failing this, they should consist of a controlled mix of many genotypes to ensure that the effects of *gei* are buffered. Controlled crosses between full-sibs in the best families in the current generation could be included in the BSO as genetic checks to monitor incipient inbreeding depression before it occurs in the production population. The seedlot(s) should be repeatable over many years.

Genetic checks ideally should be planted as additional treatments in the BSO and replicated like all the other families in that BSO. However, by the time seed is produced, it will be desirable to have removed all representative trees of the genetic checks although consideration could be given to leaving them especially if they were crosses between outstanding general combiners that could contribute to the quality of the operational seed but could be prevented from passing their genes on through the founders of the next generation, *e.g.* through the intensive breeding route.

Environmental design

As the pure progeny test end is approached in the continuum described above, the more critical it is that the environmental design of the BSO should be such as to reduce experimental error if the objectives are to be met. Plot size, shape and spacing, and replication and sub-blocking can all be critical in achieving one or more of the objectives of the BSO. The argument is often advanced that there is the danger of selecting sites with atypical uniformity; but unless that typical variability can be removed in the environmental design, the lack of precision in comparing entries can easily make the whole experiment an expensive waste of time.

Plot size, shape and spacing

It might appear that, ideally, the BSO would have a replicated design with single tree plots. However, this or any other form of single tree plot design (*e.g.* non-contiguous plots), would be costly to lay out particularly at the high plant populations used and, once thinning started, the difficulties in keeping track of identities of the remaining trees at irregular spacing and of thinning families down progressively to equal numbers of trees would be unmanageable. There is also the argument that within-family selection is less precise in single tree or non-contiguous plots. If within-family selection is more important than between-family selection, multi-tree plots, with as many trees in them as other constraints of the design will allow, should be used. This would apply, for example, where half-sib rather than full-sib families are being used or if there were to be one generation without family selection to release inbreeding.

There is also the option of using square, rectangular or line plots. Greater within-plot environmental variation can generally be expected in line plots and therefore within-plot selection may not be as precise. On the other hand, they sample environmental variation in the block more efficiently and could result in greater precision of ranking of families. Square plots have not been found easy to manage in the field; labelling is difficult and once the third or fourth thinning has been carried out, plot boundaries are difficult to discern. One option is to leave an unplanted line between plots, but this wastes space that might otherwise carry trees for selection and it increases block size. Line plots have been found to be much easier to handle. They also make it more acceptable to collect seed before the final reduction to one tree per plot because each tree's nearest neighbour is less likely to be a relative than it would in a square plot (A. J. DUNSDON, pers. comm.)[2]), especially if the lines are long. The compromise of 2-line plots has been tried. The advantages are said to be that comparison for selection is easier but as the 2 lines are treated separately in the selective thinning operation, this does not give any gain over the single long line (except at the last thinnings when selection is for one of the final trees in each group) as trees in a line plot can be divided up into groups of convenient numbers for making comparisons within the group and giving better control over spacing after thinning.

The choice of spacing in plots will depend on the selection intensity required at an early age which itself will be dependent on the amount of tree to tree variation expected and the heritability of juvenile traits and their correlation with the mature traits of interest. Plot size and spacing should be designed so that after thinning and roguing individuals and families, the spacing is optimum for seed production for that species.

Replication and blocking

The number of replications and therefore the precision that can be expected in comparing the entries in a BSO is a function of its size and this is determined by the quantity of seed that is required from it. There is an advantage in having enough replications (a minimum of 6) to superimpose 2 different treatments on the BSO either by the initial design or because unpredicted circumstances demand it. For example, there may be the option to manage some replications to provide better estimates of genetic parameters by delaying, or adopting systematic thinning in a sub-set selected for its potential to give precision.

It is almost inevitable that when the stock for a BSO is raised in the nursery, there will be unequal numbers of plants per entry when the time comes for planting out. Because of the selection advantage of including as large as possible a number of families, tree breeders are loathe to leave any out even if it means not having all entries in all replications. These unequal numbers can be accommodated in the design either by having replications of diminishing size or by distributing the available material in equal sized, but differently constituted replications; the latter is not quite as flexible in accommodating unequal numbers. Either way there are statistical objections and there is also the sacrifice of the option of being able to use sub-blocking to remove more of the environmental variation; although the availability now of computer programmes such as REML (ROBINSON *et al.*, 1982) makes it possible to deal statistically much more efficiently with such non-orthogonal designs. If precise estimation of genetic parameters and family ranking are crucial for making genetic gain (where heritabilities are low) and if the material is inherently capable of giving these, consideration should be given to establishing an orthogonal experiment in the core of the BSO and planting the excess families as a surround to be included in later selection if

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heritabilities are high. On the other hand, if the BSO is to be used principally for seed production with most gain expected from phenotypic selection within families and perhaps some from culling the worst families, then whether to use equal- or unequal-sized non-orthogonal replications will depend on how best the material can be distributed in the BSO to maximize outcrossing after the final thinning.

Replications will often be too large to be effective in removing the environmental effects within the BSO and some form of incomplete block design may be appropriate. There is much more scope for this now that such designs can be generated for almost any number of entries (PATTERSON and WILLIAMS, 1976). As the superimposition of these designs does not preclude the experiment's analysis as a simple randomized complete block, it is almost always worth using incomplete blocks; they have been found to increase precision in progeny tests by up to 200% for traits of low heritability (BARNES and SCHWEPPENHAUSER, 1979). Incomplete block designs also contribute towards maximizing panmixis in the ultimate arrangement of the seed producers.

The use of unbalanced designs has been suggested (MCCUTCHAN *et al.,* 1985) to make more efficient use of the resources available but research on their relative efficiency is yet to be done.

Siting of the BSO

By definition, the BSOs have to be established far enough apart to avoid gene transfer between them. This is not so critical for BSOs of different generations in a given sub-population as it is for different sub-populations themselves. For some species, there is sufficient difference in periodicity of flowering between provenances to be able to site sub-populations next to each other without risk of interchange of genes.

There is also a need for the BSOs to be as free as possible from contamination from an unimproved source of pollen. This should obviously be kept to a minimum from the point of view of the commercial seed produced from the BSO concerned. However, a moderate amount of contamination can be accepted in the breeding populations themselves. In the case of the simple and intermediate levels of breeding, in which there is no control of the male parent of the founders of each generation, there is a chance that some of the half-sib relatives may have stray male parents; but as selection at an early stage is intense in the BSO, progeny of a poor stray parent has a high chance of being thinned out. If the stray parent were good, it could contribute to the sub-population to advantage. At the intensive breeding level where full-sib families are the founders of the next generation, the progeny are produced by controlled pollination between selected trees and therefore contamination is of no consequence in the breeding process although it remains a problem in the operational seed produced.

BSOs containing successive generations of a sub-population should be planted in the same environment. They could be adjacent to each other or could even occupy the same site if there is no need to keep the previous generation for seed production. If successive generations occupy the same site, the unthinned plot performance of the previous crop can be used as a covariate for the succeeding one in order to increase precision by adjusting each tree or plot mean for environmental variations in the site.

Selection

Genetic gains from the BSO are dependent upon the efficacy of selection; this in turn depends upon the selection differential that can be imposed, the efficiency with which superiority is recognized and the number of traits that are included as criteria. The selective practice adopted in the thinning regimes used in the BSO impinge on all the objectives.

Selection intensity

If all selection is to be carried out in the BSOs, a large number of close-spaced seedlings is required at establishment to achieve a satisfactory selection differential on the size of area required for testing and seed production. This applies particularly in the early cycles of selection from a wild population when there is likely to be a high proportion of inferior individuals. Nevertheless, selection must not start until the breeder has confidence that it is effective. This depends on juvenile-mature correlations and the ability to recognize variation in the trait of interest. BURDON (1982) cautioned that plantation forestry imposes very different growing conditions at different stages of the rotation and that different crop ideotypes may be optimal for productivity at the different stages; therefore it is important to establish the correlations before using high selection intensity on the juvenile trees other than for removing silviculturally unacceptable individuals from progeny of wild parents. In advanced generations, there will be less variation, the curve of gain against selection intensity (FALCONER, 1981) will flatten off sooner and there will not be the need for the very high numbers of plants because advance will be more dependent on efficient family selection. To some extent, the requirement of seed to be produced from the BSO will determine its size and therefore the potential to practice a high selection intensity.

Selection efficiency

Maximizing the response from tree breeding is principally a matter of efficient selection (COTTERILL, 1986). To be effective, selection must be carried out on time and with precision at each thinning and there must be good genetic, particularly juvenile-mature, correlations.

It is surprising how easy it is for selection in the juvenile trees to be dysgenic. Where there is a high tree density, for practical reasons initial selection often must be by personal judgement without measurement and not by index; it is difficult to hold a number of traits in mind and at the same time consider a projection of the development of the juvenile tree. Experience has shown that it is critical to limit the number of selection traits and to use experienced staff at this crucial stage; poor selection practice at this time can jeopardize all advance. At later selective thinnings, fewer trees are involved and it might be practicable to use a combined selection index. The genetic parameters used in the construction of the index should include those estimated from the last measurements made in full plots, *i.e.* before any selective thinning had been done that might have biased the genetic constitution of the experiment. The effects of uneven spacing on the performance of the individual trees could be taken into account by correcting the individual tree value for growing space as well as for replication and incomplete block effects.

With advancing generations, inferior individuals that were present in the unimproved populations will have been removed and there will be less tree to tree variation in the selected traits. Genetic advance will then be more dependent on family selection, the need for very close initial spacing will decline and the use of a combined index (see *e.g.* COTTERILL and DEAN, 1990) at this stage would be a practical option to improve the precision of selection. The efficacy of tandem selection and individual culling levels (HAZEL and LUSH, 1942) may prove to be greater in MPBS systems than in single population breeding.

Selection criteria

Selection criteria will vary for each species. For the newly domesticated non-industrial species, they may not yet be known. For some species the criteria may be measured at a very young age but for others it may be necessary to wait until the tree is mature before the most important criterion can be assessed. Spacing and thinning in the BSO will be dictated to some extent by the importance of the criteria exhibited at various ages and whether or not juvenile-mature correlations have been shown to exist. The fewer the criteria used, the greater will be the advance in those selected. There could be very appreciable advantages in using a single criterion, particularly in early thinnings where selection is subjective.

Thinning practice

When fast growing tropical trees are planted at close spacing with the aim of reducing the stocking to produce seed from selected trees in five to ten years, thinnings must be carried out precisely on time or the crowns will be ruined for seed production (although this does need to be quantified for example by comparing production from a progressively thinned BSO and one that has a single late thinning after it has served as a progeny test) and even the potential for selection will be reduced through the difficulty of working in, and of recognizing individual superiority in an under-thinned stand. The timing of thinnings is crucial and cannot be delayed. This has been found to be one of the most onerous and demanding tasks in the tree breeding programmes in which BSOs have been used. The situation with eucalypts is not as difficult as it is with pines; thinning in the former can be delayed because, although seed production can be prejudiced in the seedling rotation, when the BSO is coppiced only the selected seed producers are allowed to re-grow and they will then have ample space for crown development and seed production. However, there would be some years delay before maximum seed production is reached compared with a BSO thinned early.

Conclusions

The implementation of the MPBS has overcome most of the objections to conventional breeding strategies. In particular:

i. it is compatible with practising selection and breeding in many species within a single programme;

ii. long-term pedigree control by population as well as by individual tree can simplify record keeping and increase security of control;

iii. the breeder can maximize short-term advance within a population with the knowledge that he can overcome inbreeding or regenerate variation at any time;

iv. new material can be brought in as additional sub-populations and improved by simple breeding methods until it can contribute to commercial seed production;

v. genetic variation can be preserved and enhanced in the sub-populations indefinitely and this variation can be passed on to the forest both in space and time;

vi. *gei* can be used and developed to increase production by planting diverse populations on diverse sites instead of planting all genotypes over all sites to test for stability;

vii. heterosis can be exploited through hybridization both at the specific and at the sub-specific levels.

In other words, this strategy is flexible, provides the potential to respond to new materials and new demands and allows the breeder freedom to be adventurous without taking unacceptable risks. At the same time it accommodates the need to work with many populations of many species at different levels at the same time; and it conserves variation.

The BSO has been a central mechanism for the practical implementation of the MPBS particularly where the brief of the genetic improvement programme covers the whole spectrum of selection, conservation and testing at the species, population, family and clonal levels. It has made it possible to retain the potential to use populations at the sub-specific level in the replicate, diversified, heterotic or structured mode to conserve variation, use gei and exploit heterosis depending on the emerging characteristics of the particular species. However, ideas on its concept, design and management have been evolving rapidly. For it to be fully effective it is crucial to make a careful assessment of the potential of the constituent material to produce seed and to yield genetic information and gain before the objectives are defined and the design and management procedures set.

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Provenance Variation of *Pinus radiata* Grown in Greece

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Summary

Analyses of a *Pinus radiata* experimental plantings, including 18 provenances from the 1978 International Collection (4 from Ano Nuevo, 6 from Monterey, 3 from Cambria and 1 each from Cedros and Guadalupe islands, plus 3 controls), grown at 2 locations in Greece, gave the following results:

There are significant differences between provenances in total tree height, diameter at breast height, bark thickness, stem straightness, crown form, number of whorls, number of branches per whorl, branch diameter and resistance to frost.

The fastest growing populations were Ano Nuevo and Monterey while Cedros island was the slowest and is completely unadapted to Greek conditions.

There is a highly significant correlation between frost resistance and latitude of the provenances and populations (r= 0.80**). An exception is the Guadalupe island population, which although it originates from a low latitude was the most frost resistant in both locations.

The natural populations of radiata pine are suffering from inbreeding depression, however, heterosis is released in inter-population hybrids. The "Guadalupe ex Camberra" provenance (control) which is a hybrid between Guadalupe and Monterey at the age of 12 years had a mean height at Raches 12.19 m, while the original Guadalupe island population was only 9.83 m. The Guadalupe ex Camberra was also the best at Granitsa planting with mean height at the age of 9 years of 6.56 m, followed by Talaganda seed orchard (control) at 6.27 m.

The differences between provenances within populations were insignificant for all characteristics studied, indicating that selection within the best populations (Ano Nuevo and Monterey) can be practiced without a concern at the provenance level.

Key words: Pinus radiata, Monterey pine, population, provenance, variation, correlation.

FDC: 165.52; 232.12; 174.7 *Pinus radiata;* (495).

Introduction

Pinus radiata D. DON also called radiata, Monterey or insignis pine is a well known and widely grown species. Its natural

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