A Computer-Aided Test Layout for Open-Pollinated Breeding Populations Of Insect-Pollinated Tree Species

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Summary

In the genetic improvement of an insect-pollinated tree species using open-pollinated progeny, there are opportunities and restrictions which need to be taken into account in the design of progeny tests so that these tests rank families meaningfully, allow selection of individual trees, and at the same time minimise chances of inbreeding. This article describes an efficient progeny test design for achieving each of these objectives. Through its use, the best families and the best individuals within families can be selected. Selection can be made to provide seed for the next generation's breeding population, and to provide improved seed for commercial plantings; scion material can be collected from the very best trees for grafting and planting in clonal seed orchards. A computer program called "NO INCEST" has been developed to assign families to plots in each block of the progeny test. Families are assigned to rows at random, subject to the constraint that plots of the same family in adjacent blocks are not located within the "effective pollination range" of the species.

Key words: computer layout, breeding population, insect-pollinated trees.

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Background

Many commercially important tree species are pollinated almost exclusively by insects[3]); these include members of the following genera: *Acacia* (I, B), *Albizia* (L), *Cassia* (B), *Cedrela* (L), *Ceiba* (I), *Cordia* (B, D, L), *Dipterocarpus* (C), *Eucalyptus* (H, D, C), *Gmelina* (B), *Melia* (B, L, D), *Robinia* (B), *Tectona* (B) and *Triplochiton* (H, D, C) (Sedgley and Griffin, 1989).

The distances that an insect is likely to disperse pollen from a given tree vary for different insect species. Some pollinating insects can fly great distances in a relatively short periods. Generally, however, when such long-range flight takes place, it is for migratory purposes only. When insects are feeding, for example on the nectaries of a flower, they tend to exhaust one flower's supply and then fly (or crawl) away, only to be distracted and detained by the next available food source. The distance insects travel between feeding stops (flowers) tends to be relatively short (Johnson, 1969) and the exact distance that a pollen grain is likely to be carried from one flower to another depends on the type of insect vector, the insect's fitness and appetite, the temperature, wind velocity, presence and magnitude of rain, and various aspects of flower morphology. There are some studies which have measured the collective effect of these factors. For example, Pryor (1976) found that when *Eucalyptus fastigata* trees were 10 m, 20 m, 30 m, 40 m and 50 m apart from *Eucalyptus robertsonii*, the number of F_1 hybrids which formed from a fixed number of capsules was 35, 18, 9, 3 and 1, respectively. This pattern indicates that as distance between 2 trees in a stand increases, the probability of the pollen of one fertilising the flowers of another progressively decreases.

The term "effective pollination range" (EPR) is described here as the range within which 90 % of the pollen causing natural fertilisation of a designated tree must originate. The EPR varies with species. In the case of the Eucalypt hybrid mentioned above, the EPR would be approximately 40 metres. The concept of EPR is an integral part of our proposed test design.

All tree improvement programmes use progeny tests as breeding populations. These provide information that will allow selection between and within families of the parent trees of the next generation of improvement (i.e. the next breeding population) (Zobel and Talbert, 1984). The tests can be converted to seed sources by thinning, and scions from the best selections can be grafted for clonal orchards.

Usually 2 types of progeny tests with complementary test designs are desirable to provide all the information needed to rank families and within-family selections. Furthermore, establishment of grafted or seedling seed orchards is also needed so that the best genotypes can cross with each other and produce seed. Establishment of tests and seed orchards is expensive, and in many situations cheaper alternatives would be welcome. In this paper we have developed an inexpensive yet efficient design for a combined breeding population progeny trial and seedling seed orchard to achieve all these objectives. This design has been dubbed the "Forward Selection Plot" and is described in detail by Cannon and Shelbourne (1993). It should work well for most insect-pollinated tree species.

Development of the Experimental Design

Randomisation of families within each replication is necessary for valid progeny testing whether the families

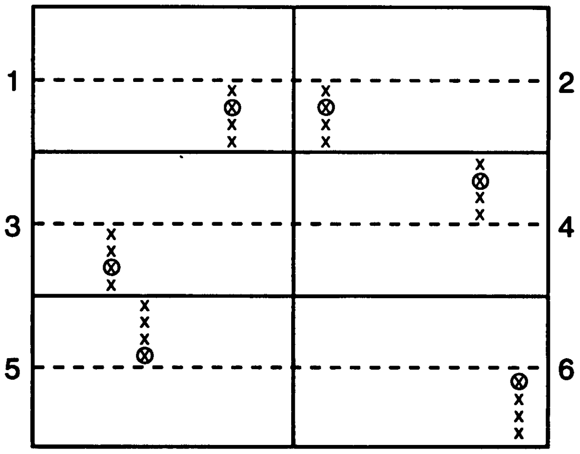

Figure 1. — Randomised allocation of families. Family "X" was allocated at random in a completely randomised progeny test. However, when this plot is thinned to leave only the best tree in each row plot (the circled x's) there is a chance that mating between half-sibs could take place; in this example between members of family x in blocks 1 and 2 and in blocks 3 and 5.

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[3]) I = insects, B = bees, L = Lepidoptera, D = Diptera, C = Coleoptera, H = Hymenoptera

are represented by single-tree or multi-tree plots. The overall shape of the test should be roughly square to increase chances of selected trees mating with each other rather than with trees outside the experiment. However, with a randomised layout and multi-tree plots there could be appreciable mating between half-sib members of each family even after removal of all but the best tree in every row plot (see *Figure 1*). Inbreeding would result where sibs are juxtaposed and is undesirable as it reduces growth rate and disease resistance in the offspring.

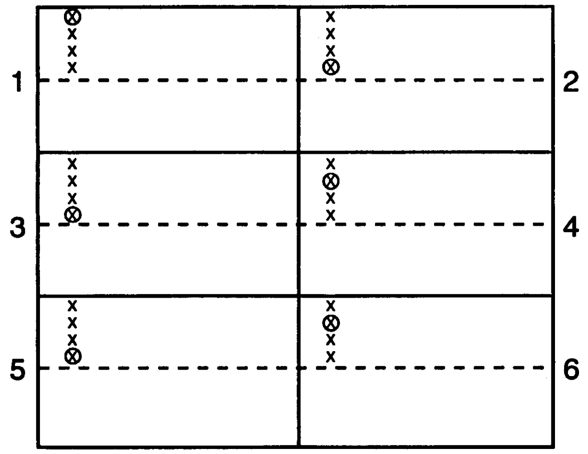

Figure 2. — Systematic allocation of families. Where blocks are square in shape, the assignment of family x to the same position in each black guarantees a maximum distance between the remaining tree of family x in each block following thinning. However, this layout is non-random, so rankings of family performances would be uncertain statistically.

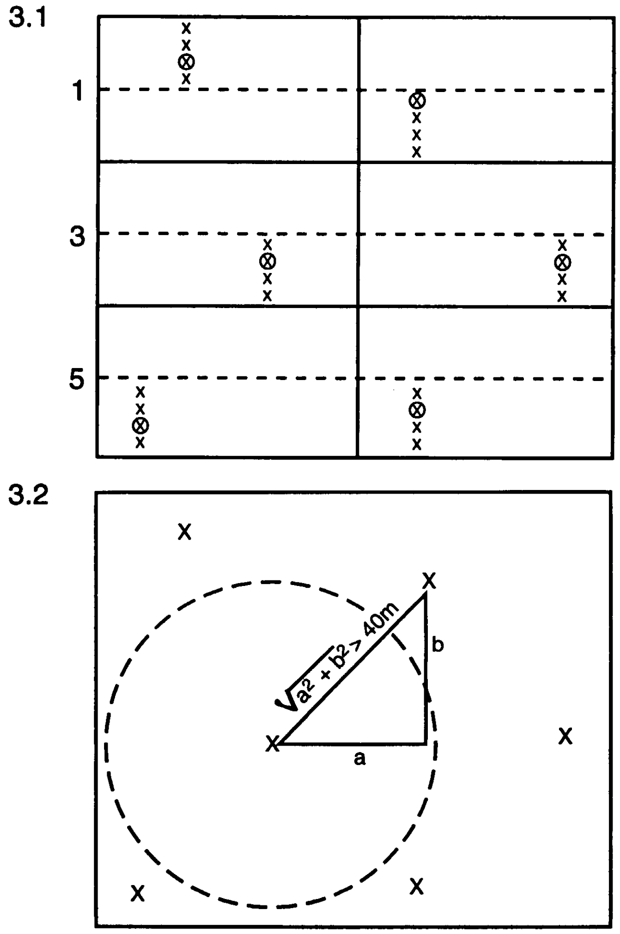

Figure 3. — Diagram showing a design with partial randomisation for a species with an effective pollination range (EPR) of 40 m. In this example, the square root of a^2 (the distance between rows times the number of rows between circled x's in adjacent blocks) plus b^2 (the length of a row plot times the number of row plots between circled x's in adjacent blocks) must exceed 40 m.

A systematic layout, such as the one shown in *figure 2*, would lead to minimal inbreeding but its non-random quality makes it impossible to state how accurate it is for ranking families.

In order to compromise between the statistical desirability for randomness and the need for a maximum reproductive isolation between related family row plots in the test, some departure from randomness is necessary. Provided more than 30 families are being tested and the species has a relatively short effective pollination range (EPR), this is acceptable. Many insect-pollinated species have EPR's of 30 m to 70 m (Adams et al., 1992). With a test design as shown in *figure 3*, it is expected that the departure from randomness will only slightly inflate the error term for testing differences between families while the reduction in potential inbreeding in seedling offspring of trees in the test area should be substantial.

Within blocks, a distribution of families in row plots is convenient. It is relatively easy to lay out, peg, plant, map, measure and assess family row plots in contrast to single-tree plots. It is also easy to make within-plot selections because this can be done visually. Other shapes of multi-tree plots could also be used, but we have found that these are more difficult to lay out and that they are not as useful for making visual comparisons whithin families.

Single-tree-plot and 5-tree non-contiguous plots have been found to be more efficient than row-plot tests for discriminating between family means and calculating heritabilities (Loo-Dinkins and Tauer, 1987), but visual phenotypic comparisons between family members are not possible with these designs and block and neighbour adjustment procedures may be needed before the best trees in each family can be identified.

Furthermore, an additional time-consuming stage in the field would be necessary in which every tree to be culled or saved would have to be found, assessed, and marked. Finally, inbreeding would be a problem where such tests were thinned to leave just the parents for the next breeding population because of the inherently short distances between trees of the same family in adjacent replications.

We have found that by using a single-tree-plot test on one site and a row-plot test on a second site we can obtain the best features of both tests and get a measurement of family by site interaction. Single-tree-plot tests are described in detail elsewhere (e.g. Loo-Dinkins and Tauer, 1987); we will confine our approach in this paper to that of a row-plot-test.

If the number of families being tested in each block of the row plot test is large (e.g. over 60) and if the EPR of the species being studied is short (less than 30 m) then assigning family plots so that their positioning fulfills the objectives shown in *figure 3* could perhaps be done without a computer (although this could be cumbersome). However, in the situation where the number of families to be tested in adjacent blocks is less than 60 or where the EPR is greater than 50 m, a computer is essential to optimise family positions in adjacent blocks to satisfy the

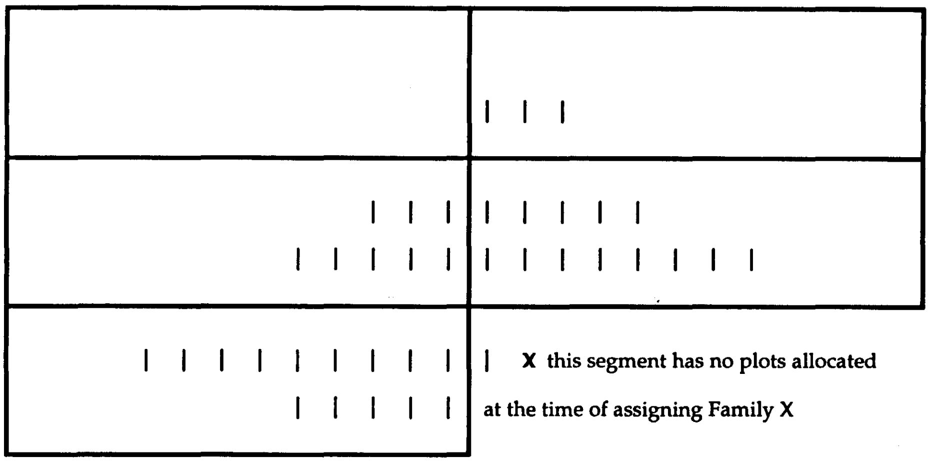

Figure 4. — Schematic diagram of the area the NO INCEST programme checks to make sure that family X will not have any half-sib relatives within its effective pollination range. To determine if family X can be assigned to the position shown above, the programme checks all the plot positions (|) to see if any of these have already been assigned family X.

conditions indicated in *figure 3* as literally hundreds of thousands of possible positions may have to be considered before the task is complete.

Until now, there has been no software which could perform this task. There are several programmes that can be used to generate random layouts for progeny tests (but a simple randomised progeny test is unsatisfactory for reasons already demonstrated in *Figure 1*). There is also a programme called COOL (Bell and Fletcher, 1978) which is useful for minimising chances of related matings in seed orchards, but layouts resulting from this programme have no replication of families within blocks and so the accuracy of family rankings would be unknown. A programme called SOD (Vanclay, 1991) has similar limitations. Therefore, in order to assist the layout of this type of progeny test a new computer programme has been developed.

This programme, written in Turbo Pascal and dubbed "NO INCEST" requires input of the following parameters: effective pollination range (EPR), number of families number of trees per row-plot, number of row-plots across a block, number of columns of row-plots per block, number and location of blocks relative to each other, and spacing of trees within and between family row-plots.

The programme considers each plot of each family to have a position in a 2-dimensional array. In the first block, the position of family row-plots is totally at random, but in the second and all other blocks an intermediate step is taken to ensure that the distance between a family's position in the block being built is at least that of the EPR of the species concerned from that family in adjacent blocks. Then the programme assigns the family at random to one of the as yet unfilled positions that remain. The programme works position by position, from left to right. The number of positions checked is dependent on 6 things:

- the number of trees in a plot or row;
- the spacing between rows;
- the spacing between trees within a row;
- the EPR;
- the number of blocks; and
- the layout of the blocks.

The net result of this approach is that the programme will end up checking a lens-shaped array of positions as depicted in *figure 4*.

With a large number of families (e.g. 30) it may be possible to ensure that the EPR is exceeded in all cases. However, although the programme begins by rejecting candidate positions which are within the EPR, if no satisfactory position can be found, the programme will automatically reduce the stringency of checking by one row and several columns after attempting for the present number of rows for a given position. For example, in *Figure 4* if the level of checking was 3 rows by 11 columns, this would be reduced to 3 rows by 10 columns then 3 rows by 9 columns. After successful placement of 1 family in a block, the checking stringency immediately reverts to the original level for the positioning of the next family.

Since 1992, the NO INCEST programme has been used to layout several row plot progeny tests, including sublined breeding population tests and seedling seed orchards. The resulting layouts have been well-spaced, and the only obvious departure from randomisation is the absence of adjacent plots of the same family.

Readers interested in obtaining a copy of the computer programme "NO INCEST", including more detailed explanations about how the programme works, should contact the authors.

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