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Designs for Genetic Field Experiments with Permutated Neighbourhoods for Genotypes and Planned Systematic Thinnings to Eliminate Competition

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Summary

A modified experimental design with single tree plots was elaborated. This design allows the conduct of observations and measurements of trees over a period of 40 to 50 years in Temperate zone conditions. It is possible to perform systematic thinnings, without changing the design orthogonality, but only reducing the number of replications in the experiment. A permutation of genotype neighbourhoods is maintained throughout. A special design with 2 systematic thinnings and 4 complete replications (blocks) is proposed which can be applied for g genotypes where $g = 4p$ and $p \geq 4$. In the paper an experimental design for $g = 20$ genotypes is described as an example.

Key words: Quantitative forest genetics, seed orchard designs, modification single tree plot designs, systematic thinnings, replications, field test design for genotypes.

FDC: 165.3; 165.4.

Introduction

The spatial distribution of trees within seed orchards commonly hinders an appropriate assessment of clones or progenies used. This resulted primarily from the fact that at the moment of orchard establishment the number of trees per clone or progeny differed greatly because of technical difficulties connected with the effectiveness of grafting or poor cropping of seed. Secondly, within seed orchards, is not possible to eliminate soil variability because trees of the same genotypes are not distributed in all blocks. Those difficulties still increased after execution of the 1st systematic thinning. Thirdly systematic seed orchard designs have the same composition of genotypes in neighbourhoods, which may cause bias.

Thus in order to assess the usefulness of clones or progenies used in seed orchards it was necessary to conduct parallel testing experiments. On their basis selection of elite clones for the establishment of 2nd generation orchards is conducted.

There exists a mode of distribution of single-trees, set out by Libby and Cockerham (1980), into random non-contiguous plots in interlocking field layouts. Individual sub-blocks contain trees from all investigated genotypes planned for removal in the 1st and in the 2nd cutting respectively and to be left until the end of the experiment. A testing experiment or seed orchard set out in such a way allows correct evaluation of genotypes during a period longer than that in experiments arranged in a completely randomized block design with single-tree plots, because it is possible to remove competition between trees through systematic thinnings. Sub-blocks, being in fact specifically designed complete blocks distributed over the whole experimental area, do not exclude soil variability because they contain its full range (Libby and Cockerham 1980).

An experimental design for 36 genotypes (progenies) allowing 3 systematic cuttings and soil variability evaluation through replicate blocks was elaborated at the Forest Research Institute (Burzynski, 1992a). It was also presented on 17 th September 1992 at the IUFRO-AFOCEL Congress in Carcans (Burzynski, 1992b). It was used to lay out a Douglas fir seed orchard planted in the spring of 1991. This experiment includes a series of trials with 36 geno-

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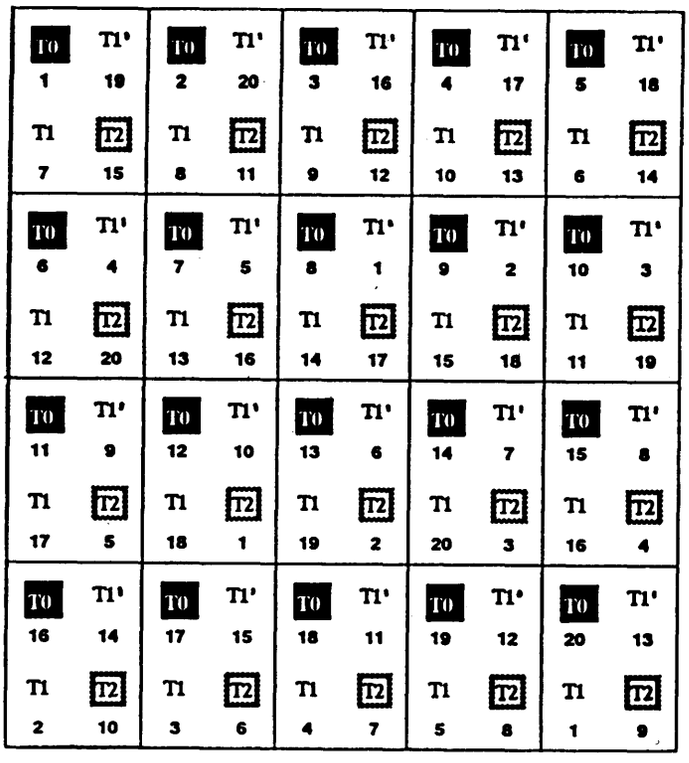


Figure 1a. — A scheme of initial distribution of trees on single-tree plots in the experiment with 20 genotypes with 2 systematics thinnings and 4 replications.

types repeated on several places in our research forest. The proposed experimental design for one such trial contains 8 replications at the onset of the experiment. As a result of the first systematic thinning planned the number of trees will decrease by 50 %, and for every 2 complete replications 1 will remain. Thus the experiment will be reduced to 4 replications. The 2nd systematic thinning will reduce the number of replications to 2, and after a 3rd cutting only 1 replication will remain per trial.

The described design can more efficiently eliminate soil variability than that of LIBBY and COCKERHAM (1980) because it includes the division of all experimental areas into complete blocks. In LIBBY and COCKERHAM's design each complete block includes almost the whole experimental area (the above paper, p. 186, *fig. 1*).

Single-tree-plot designs suffer from intergenotypic competition and therefore are normally usuful only till the first thinning (MAGNUSSEN, 1989, 1993). The proposed design overcomes this problem.

The aim of this paper is to present the mode of constructing such a design for various numbers of genotypes but for 2 systematic thinnings only, because presentation of a general algorithm for 3 thinnings and various numbers of objects is not yet possible.

Basic Assumptions of the Proposed Experimental Design

The discussed spatial design of the genotypes, with 2 systematic thinnings planned can be applied for $g = 4\ p$ genotypes, where $p \geq 4$. So the numbers of possible genotypes can be as follows: 16, 20, 24, 28, etc. The number of complete blocks (replications) on each trial area has to be 4.

Each complete block within a trial is composed of p squares (*Fig. 1a* for $p = 5$), including 2 trees to be cut at the 1st thinning (T1 and T1'), 1 tree to be removed at the 2nd thinning (T2) and 1 tree to be left until the end of the experiment (T0). Neighbourhoods of these trees are permutated. Distribution of trees after the 1st cutting is presented on *figure 1b*, while after the 2nd cutting on *figure 1c*.

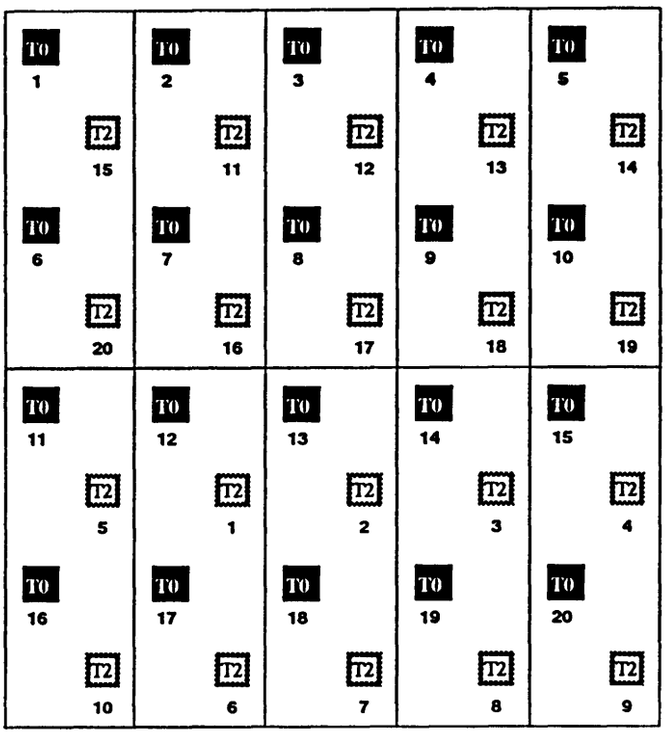
Procedure

Procedure for establishing a complete experimental design of this kind goes through 3 steps.

Step 1

After choosing the number of genotypes to be investigated in the experiment according to the assumptions given above, we make a design of the whole trial within which we put only genotypes in the "T0" position (*Fig. 1c*) i.e. trees to be left until the end of the experiment. The example presented on *figure 1c* is for 20 genotypes ($p = 5$). Assigning genotypes to the "T0'(positions in individual blocks of the experimental design results from the plan of a rectangular lattice replication (COCHRAN and COX, 1957) containing obligatorily 4 incomplete blocks with p genotypes in each.

In the discussed example the 1st replication (the complete block) of the 4 x 5 lattice was used (COCHRAN and COX, 1957, p. 435).

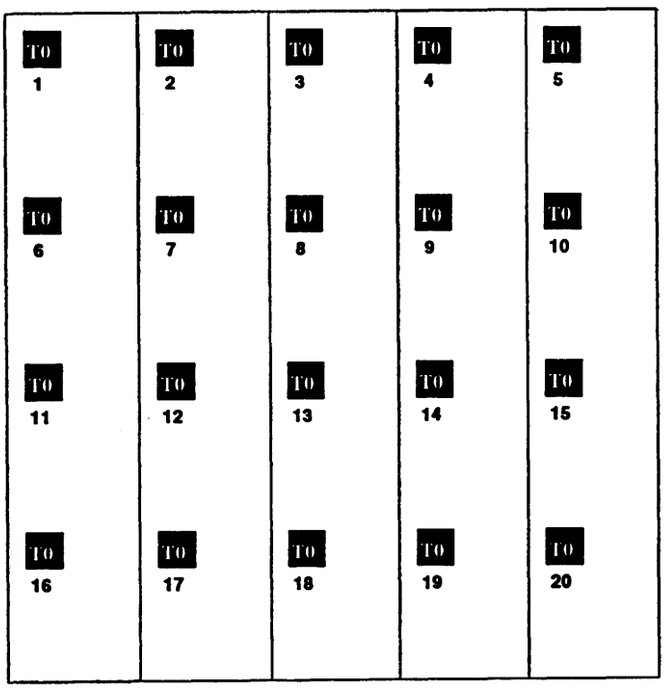


T2 — trees removed at the second thinning

T0 — trees left until the end of experiment

16 — genotype number

Figure 1b. — A scheme of distribution of trees on single-tree plots in the experiment with 20 genotypes after the 1st-systematic thinning with 2 replications.



T0 — trees left until the end of experiment

16 — genotype number

Figure 1c. — A scheme of distribution of trees on single-tree plots in the experiment with 20 genotypes after 2 systematics thinning with 1 replication.

Here it will be treated as the "T0" variant of the experiment destined to survice all thinnings (*Fig. 1c*).

[If such a lattice is not given in the cited handbook it should be set out according to the rules given there. For instance for the lattice with g = 24 genotypes the following parameters should be taken into account: $\lambda = 2$, p = 6 and n = 3, b = 4n = 12, where λ is the number of incomplete blocks in which each 2 genotypes occur together. p is the number of genotypes in incomplete blocks, n is the number of replications and b is the number of incomplete blocks in the lattice design.]

The genotypes from the 1st incomplete block (marked as a) in the lattice mentioned above are put in "T0" position in the 1st replication of the design (*Fig. 1c*). The genotypes from the 2nd incomplete block (marked as b) of this lattice are put in position "T0" in the 2nd replication etc.

Step 2

In this step the 4 x 4 Latin square design is constructed in which as treatments the incomplete blocks a, b, c and d from the rectangular lattice used in step 1 are taken. The rows of the Latin square are the replications (blocks) I, II, III and IV in the established experimental design, while the columns are destinies of trees to be removed in individual cuttings (T1, T1' and T2 or left until the end of the experiment T0). Distribution of treatments in column "T0" should be concordant with the distribution on the experimental plan (*Fig. 1c*), while in the remaining columns the distribution of treatments results from the Latin square rule. Additionally in this Latin square all 2x2 squares (shown on *Fig. 2*) should contain the same treatments a, b, c, d.

Step 3

After preparation of the Latin square as in *figure 2* which assigns incomplete blocks from the rectangular lattice to individual thinning destinies of trees and replications (blocks), we arrange genotypes from the appropriate incomplete blocks to single-tree plot positions (T2, T1 and T1') in the given thinning density in individual replications. For this purpose we take a Latin square for p treatments, 5 in our example, and we mark them with consecutive capitals A, B, C, D, ... Then we take 4 rows of this Latin square (*Fig. 3*), where the 1st

Rows (replication)	Columns (thinning destinies)			
	T0	T2	T1	T1'
I	a	c	b	d
II	b	d	c	a
III	c	a	d	b
IV	d	b	a	c

Figure 2. — Distribution of 4 incomplete blocks from the rectangular lattice in the Latin square 4 x 4.

T0	A	B	C	D	E
T2	E	A	B	C	D
T1	B	C	D	E	A
T1'	D	E	A	B	C

Figure 3. — Four rows of the Latin square with p = 5 treatments in each incomplete block of the rectangular lattice for replicate (block) I.

Replication I

T0	A 1	B2	C 3	D 4	E 5
T2	E	A	B	C	D
T1	B	C	D	E	A
T1'	D	E	A	B	C

a

Replication II

T0	A 6	B 7	C 8	D 9	E 10
T2	E	A	B	C	D
T1	B	C	D	E	A
T1'	D	E	A	B	C

b

Replication III

T0	A 11	B 12	C 13	D 14	E 15
T2	E	A	B	C	D
T1	B	C	D	E	A
T1'	D	E	A	B	C

c

Replication IV

T0	A 16	B 17	C 18	D 19	E 20
T2	E	A	B	C	D
T1	B	C	D	E	A
T1'	D	E	A	B	C

d

Figure 4. — Four times replicated incomplete Latin square —1 per each replication with genotypes inscribed in the rows „T0".

row will contain genotypes placed in thinning destiny "T0", the 2nd row will contain genotypes in thinning destiny "T2" etc. The incomplete Latin square prepared in this way (4 rows of the Latin square p x p) is repeated 4 times, 1 for each replication (block) of the final design.

Then the genotypes placed in step 1 in thinning destiny "TO" in consecutive replications of the experimental design (*Fig. 1c*) are written exactly into the 1st rows (marked T0) of consecutive replications of the design (*Fig. 4*). In this way in each replication in the row marked as „T0" genotypes are placed from the respective incomplete block of the lattice, inscribed beside the p = 5 individual capital letters of the alphabet A, B, C, D, E. This creates 4 incomplete blocks a, b, c, d.

Assignment of genotypes to consecutive thinning destinies T2, T1 and T1' in individual replications of the design proceedes by writing in genotypes belonging to respective incomplete blocks. The decision from which incomplete block the genotypes should be taken and assigned to the given thinning destiny and replication, results from the Latin square constructed in step 2 (*Fig. 2*). Thus following *figure 2* in thinning destiny T2 genotypes from incomplete block c should be placed in the 1st replication, incomplete block d, in the 2nd replication, incomplete block a in the 3rd and incomplete block b in the 4th. The genotypes of the respective incomplete blocks are assigned consecutively to consecutive capitals letters. Each incomplete block is identified with a respective row (thinning density) of each replication in *figure 4*. The sequence of genotypes in each row of *figure 4* is settled in the following way. Considering a given row (from among T2, T1 and T1') in a replication we must find out from which

Replication I

T0	A 1	B2	C 3	D 4	E 5
T2	E 15	A 11	B 12	C 13	D 14
T1	B 7	C 8	D 9	E 10	A 06
T1'	D 19	E 20	A 16	B 17	C 18

Replication II

T0	A 6	B 7	C 8	D 9	E 10
T2	E 20	A 16	B 17	C 18	D 19
T1	B 12	C 13	D 14	E 15	A 11
T1'	D 4	E 5	A 1	B 2	C 3

Replication III

T0	A 11	B 12	C 13	D 14	E 15
T2	E 5	A 1	B 2	C 3	D 4
T1	B 17	C 18	D 19	E 20	A 16
T1'	D 9	E 10	A 6	B 7	C 8

Replication IV

T0	A 16	B 17	C 18	D 19	E 20
T2	E 10	A 6	B 7	C 8	D 9
T1	B 2	C 3	D 4	E 5	A 1
T1'	D 14	E 15	A 11	B 12	C 13

Figure 5. — Distribution of all genotypes in the incomplete Latin square.

incomplete block will originate genotypes, that will be inscribed in it (see *Fig. 2*). Next we recognize at which replication this incomplete block is on thinning destiny "T0". Then we assign the same genotypes to capital letters as in that replication. In this way we obtain assignements as in *figure 5*. The genotypes inscribed in this way for individual thinning destinies and replications are rewritten onto a plan of the experiment presented in *figure 1a*.

Setting the Distribution of Genotypes onto Other Trial Areas

In order to establish a distribution of genotypes in other trial areas we use consecutive replications of the rectangular lattice (step 1) to assign positions of „T0" trees. Basing on this distribution we establish genotype numbers according to the mode given for the first trial area.

In the case of using up all replications of the lattice we may repeat the used replications but before that 1 should randomize rows and columns in the rectangular lattice. The distribution of the incomplete blocks in the 4 x 4 Latin square (step 2) should also be exchanged. Similarly randomization of rows and columns should be made in the 2nd Latin square with "p" treatments and other 4 rows of this square should be chosen (step 3).

Statistical Analysis of the Experimental Results

The experimental design proposed may contain s trials with 4 complete blocks per trial, thus each genotype is repeated s x 4 times.

Each trial may be considered separately. The analysis of the results may be carried out before the 1st thinning is done according to the ANOVA model as follows, ignoring the thinning destinies:

$$y_{jk} = m + a_i + b_k + e_{ik}$$

(i = 1, 2, ..., g = number of genotypes; k = 1, 2, ..., n = 4 = number of replications)

where:

m — general mean,

a_i — the effect of the i^{th} genotype,

b_k — the effect of the k^{th} block,

e_{ik} — random error for the single-tree plot concerning the i^{th} genotype and the k^{th} block.

Additionally one may analyze all results from s trials (assumed to be a rondom sample representing soil variability over a greater forest area) according to the following mixed ANOVA model:

$$y_{ijk} = m + a_i + b_{k(j)} + c_j + ac_{ij} + e_{ijk}$$

(i = 1, 2, ..., g; j = 1, 2, ..., s; k = 1, 2, ..., n = 4)

where:

m — general mean,

a_i — the main effect of the i^{th} genotype,

$b_{k(j)}$ — the effect of the k^{th} block within the j^{th} trial area,

c_j — the main effect of the j^{th} trial area,

ac_j — the interaction effect between the i^{th} genotype and the j^{th} trial area,

e_{ijk} — random error for the single-tree plot concerning the j^{th} genotype and the k^{th} block within the j^{th} trial area.

After execution of the 1 st systematic thinning when 2 complete replications will remain in the design 1 may still analyze results from them according to the above ANOVA models (in this case n = 2).

Conclusions

1. The proposed genetic experimental design can be more efficient in elimination of soil variability than that of Libby and Cockerham (1980) because it includes the division of the whole experimental area into complete blocks. In Libby and Cockerham's design each complete block includes almost the whole experimental area.

2. The use of the proposed design on forest experimental areas with single-tree plots prolongs the period of carrying out observations without mutual competition between trees (beyond the 1st and 2nd thinning).

3. This design may be applied for the establishment of seed orchards where 2 thinnings are planned, which is consistent with the present tendencies in seed orchard management.

4. The use of this design for seed orchards will allow a statistically appropriate evaluation of the clones or progenies used before and after consecutive systematic thinnings.

5. The design combines the advantages of systematic seed orchard designs with those that assure permutated neighbourhoods of genotypes (Giertych, 1975).

6. This design may especially be applied in forest experiments with progenies coming from different mating systems used in quantitative genetics as well as in resistance experiments.

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