Effect of plot size and shape on the efficiency of progeny tests

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TIIVISTELMÄ: KOERUUDUN KOON JA MUODON VAIKUTUS JÄLKELÄISKOKEIDEN TEHOKKUUTEEN

Haapanen, M. 1992. Effect of plot size and shape on the efficiency of progeny tests. Tiivistelmä: Koeruudun koon ja muodon vaikutus jälkeläiskokeiden tehokkuuteen. Silva Fennica 26(4): 201–209.

A simulation approach was applied to study the pattern of environmental variability and the relative statistical efficiency of 14 different plot types. The study material consisted of two nine-year-old field tests of Scots pine (*Pinus sylvestris* L.). The area of the test sites was 1.57 and 0.67 hectares. The efficiency was measured as the error variance attached to the estimate of family mean and the total size of a test needed to detect a given, least significant difference between two family means. The statistical efficiency tended to decline along with increasing plot size. The importance of plot shape was negligible compared to plot size. The highest efficiency was obtained with single-tree plots. Non-contiguous plots appeared to be considerably more efficient than block plots of equal size. The effects of intergenotypic competition on the choice of plot type are discussed.

Tutkimuksessa selvitettiin erilaisten simuloitujen koeruutujen avulla ympäristövaihtelun luonnetta sekä vertailtiin 14 erilaisen ruututyypin suhteellista tilastollista tehokkuutta kahdessa yhdeksän vuoden ikäisessä männyn kenttäkokeessa. Kokeet olivat kooltaan 1,57 ja 0,67 ha. Tehokkuutta mitattiin perhekeskiarvojen vaihteluun liittyvän virheen suuruudella sekä kokeen koolla, joka tarvittiin kutakin ruututyyppiä käyttäen kahden jälkeläistökeskiarvon tietyn suuruisen erotuksen osoittamiseen tilastollisesti merkitseväksi. Tulokset osoittivat tilastollisen tehokkuuden laskevan koeruudun koon kasvaessa. Ruudun muodon merkitys oli vähäinen ruutukokoon verrattuna. Yhden puun ruutujen järjestely oli tutkituista ruututyypeistä tehokkain ja hajaruudut osoittautuivat huomattavasti tehokkaammiksi kuin vastaavan kokoiset yhtenäisruudut. Ruututyypin valintaan vaikuttavista tekijöistä tarkastellaan erikseen koe-erien välistä kilpailua.

1 Introduction

The primary objectives of progeny testing are: to determine the true genetic value of parental trees by comparing the performance of their offspring, to select superior trees within the best families, and to obtain estimates of genetic parameters. The testing is conducted in field experiments that cover large areas and take a considerably length of time (10–30 years) to produce useful information. Since the early 1960's more than 1700 progeny tests have been established with several tree species in Finland (Metsänjalostuksen... 1992). A large number of tests demanding continuous management and measurement represents a considerable expense for a breeding program. This means that much emphasis should be paid to designing efficient tests. To be efficient, a progeny test should contain the minimum number of trees to achieve the test objectives (Lambeth et al. 1983).

The statistical efficiency of a progeny test is usually depicted by the number of individuals needed to achieve a defined precision in the estimate of a family mean (Lambeth et al. 1983), or to show that a given difference between two family means is statistically significant (Wright and Freeland 1960, Correll 1978, Cotterill and James 1984). Correll and Cellier (1987) and Loo-

Dinkins and Tauer (1987) also based their definition of efficiency on the precision of family means. Several studies have shown that in field tests these parameters, and thus, the efficiency, can be affected by modifying the size and shape of the experimental units, i.e. plots (Smith 1938, Wright and Freeland 1960).

So far, there has been no general agreement on the best type of plot to be used in forest genetic testing. The great majority of Finnish progeny tests have been established using square plots of 25 trees (Fig. 1), a practice motivated by the desire to compare stand level growth of families. In the United States, on the other hand, row plots of 4 to 10 trees have been the most frequently used design (Lambeth et al. 1983, Loo-Dinkins and Tauer 1987).

According to statistical theory and several studies, plots of minimum size, i.e. single-tree plots, give the highest experimental precision within the framework of a fixed set of land area or plant material (Conkle 1963, Wright and Freeland 1960, Loo-Dinkins and Tauer 1987). Single-tree plots provide maximum control over environmental variation by minimizing the block size and thus, the soil heterogeneity within blocks. In an alternative design promoted for forest genetic

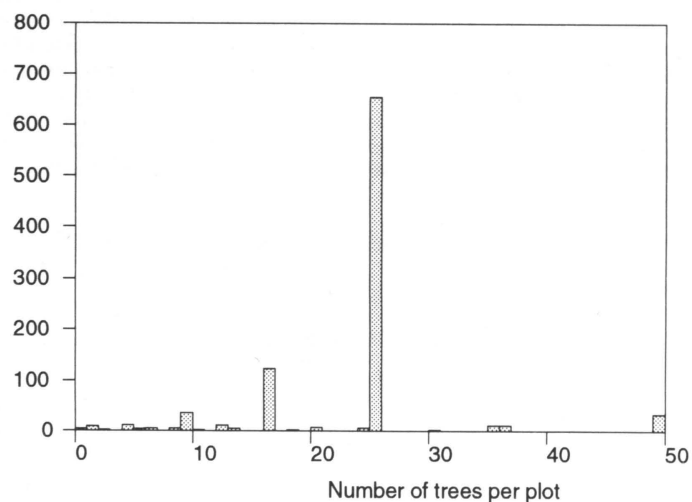testing by Libby and Cockerham (1980), trees belonging to a multiple-tree plot are scattered at random throughout the block. They suggested that the statistical efficiency of the "non-contiguous" plot arrangement is comparable with that of single tree plots, although it avoids some of the major disadvantages of the latter design, including the problem of missing plots arising from natural mortality and artificial thinnings. Despite this, neither of these designs has so far been very popular among Finnish tree breeders.

Two approaches have been commonly used in studies on the plot technique with trees: the analysis of field experiments having sets of blocks with different plot configurations (Johnstone and Samuel 1974, Lambeth et al. 1983, Loo-Dinkins and Tauer 1987), and the simulation of plots in

genetically uniform experiments or artificially regenerated stands (Wright and Freeland 1960, Conkle 1963, Correll and Cellier 1987). The simulation approach has the advantage that different plot configurations can all be evaluated under the same environmental conditions. Furthermore, the number of different types of plot that can be studied is practically unlimited.

The objective of this study was to investigate the relative efficiency of different plot types varying in size and shape, including some non-contiguous alternatives. The efficiency of the plot types currently used in Finnish progeny testing was of particular interest. Estimating the amount and pattern of environmental variability in the test sites was the other subject of investigation.

2 Material and methods

The study material consisted of two field tests of Scots pine, located in Ikaalinen (No. 358/1 – 61°54' N, 23°23' E) and Pylkönmäki (No. 358/2 – 62°41' N, 24°42' E). The tests were established in 1971 with a planting density of 2500 (2×2 m) trees per hectare. The area of the test sites was 1.57 ha (No. 358/1) and 0.67 ha (No. 358/2). The number of trees included in the analysis was 2869 in test No. 358/1 and 1428 in test No. 358/2. The nine-year height of every tree in both tests was measured.

The planting material used in these tests comprised only one entry, a registered stand origin from Lieksa, eastern Finland (63°04' N, 29°49' E). The entry represented a mixture of open-pollinated seeds collected from several trees within the stand. In this sense these tests were analogous to the large number of "uniformity trials" conducted with agricultural crops during the first decades of this century (Cochran 1937). Following the principles of uniformity trial research, any gradual change in height values within the field – indicating correlation between neighbouring trees – was interpreted as environmental variation (Smith 1938). The genetic differences among individual trees, also contributing to the total variance, were ignored since it was assumed that the expected genetic covariances between neighbouring trees to be zero, i.e. that they were unrelated.

The preparatory work consisted of grouping

the data of adjacent trees into plots of varying size and shape. The number of different plot configurations superimposed on the field test data was 14. Plot size ranged from 1 to 49 trees per plot (Table 1). The analysis was based on division of the total variance of the height observations among individual trees into two variance components: 1) variance due to plot effects, $Var_{(plot)}$, and 2) residual variance arising from individual trees within plots, $Var_{(within-plot)}$. These components were solved for each plot type using the restricted maximum likelihood method in the VARCOMP procedure of the Statistical Analysis System (SAS Institute Inc. 1985). Difficulties in completely filling the test site with varying plot types meant that the number of trees analysed in different cases was not equal. However, because of the large number of trees included in the analysis, this was considered to have an insignificant effect on the precision of the variance components.

Two criteria were applied in comparing the statistical efficiency of different plot configurations: 1) the variance of family means attributable to the environment and 2) the size of the test needed to show that a given difference between two family means ($X_1 - X_2$) is statistically significant. The meaningful difference was chosen as 5 % of the overall test mean. Both measures were eventually converted to a percentage scale by relating them to the respective values ob-

Number of tests

Fig. 1. The frequency distribution of plot sizes in Finnish progeny tests of Scots pine.

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tained for single tree plots.

The variance of family means ($Var_{(F)}$), presented as a sum of the variance components, is

$$Var_{(F)} = Var_{(family)} + Var_{(plot)} / r + Var_{(within-plot)} / r\,n, \quad (1)$$

where r is the number of replicates per family, and n the number of trees per plot. Since there were no genetically different entries in the studied experiments, the (irrelevant) between family component of variance was zero. The remaining two terms ($Var_{(plot)} / r + Var_{(within-plot)} / r\,n$) make up *the environmental portion of family variance* (Loo-Dinkins and Tauer 1987).

The size of a test, Z, (see the criterion No. 2) was calculated as:

Table 1. Experiments No. 358/1 (a) and No. 358/2 (b): Plot type and size (n trees), between- and within-plot variance components of height, variance of plot means ($Var_{(P)}$; used to calculate Smith's b-values), relative efficiency (the environmental portion of family mean variance) and relative size of test to detect statistical significance (at 5 % risk level) of differences equal to 5 % of the mean height (when compared to single-tree plots). The symbols of the plot types refer to the number of trees in 'columns' and 'rows', e.g. '7×2' means a rectangular plot with 14 trees arranged in seven columns and two rows; letters 'nc' symbolize a non-contiguous plot.

a) n_{total} = 2869

Plot type	n	$Var_{(plot)}$	$Var_{(within-plot)}$	$Var_{(P)}$	Rel. eff., %	Rel. size, %
1×1	1	18.54	0.00	18.54	100	100
3×1	3	2.47	15.87	9.34	64	151
7×1	7	2.10	15.48	5.11	52	193
8nc	8	0.34	18.20	3.45	67	149
14×1	14	1.63	16.94	3.29	40	249
7×2	14	1.90	16.64	3.53	38	268
16nc	16	0.00	18.54	1.58	73	137
7×3	21	2.02	16.88	3.09	28	353
14×2	28	1.60	17.04	2.43	27	371
6×5	30	1.44	17.39	2.24	28	366
14×3	42	1.53	17.22	2.09	21	481
7×6	42	1.95	17.24	2.43	18	560
7×7	49	1.88	16.69	2.34	16	629
49nc	49	0.06	18.48	0.58	65	156

b) n_{total} = 1428

Plot type	n	$Var_{(plot)}$	$Var_{(within-plot)}$	$Var_{(P)}$	Rel. eff., %	Rel. size, %
1×1	1	29.32	0.00	29.32	100	100
3×1	3	1.39	28.02	11.90	81	122
7×1	7	1.71	27.86	6.25	67	150
8nc	8	0.83	28.54	4.92	75	135
14×1	14	1.43	28.14	3.72	56	180
7×2	14	2.18	27.77	4.44	47	214
16nc	16	0.35	29.03	2.42	76	134
7×3	21	1.90	27.99	3.42	41	248
14×2	28	1.30	28.41	2.46	43	240
6×5	30	1.36	27.90	2.40	40	251
14×3	42	1.07	29.65	1.88	37	277
7×6	42	0.86	28.76	1.64	43	243
7×7	49	1.26	28.12	1.91	31	331
49nc	49	0.45	28.94	1.12	53	195

$$Z = \frac{2\,t^2\,(Var_{(plot)} + \dfrac{Var_{(within-plot)}}{n_H})\,n}{(X_1 - X_2)^2} \quad (2)$$

(adapting White and Freeland 1960)

The term n_H stands for the harmonic mean number of trees per plot. The Student's t-value was computed at the 5 % risk level and $2r - 2$ degrees of freedom.

"The empirical law of Fairfield Smith" (Smith 1938) was applied to determine the degree of environmental heterogeneity. According to the law, there is a linear relationship between the logarithmic variance of plot means and the logarithmic plot size in any field:

$$\log Var_{(P)x} = \log Var_{(P)1} - b \log x, \quad (3)$$

where $Var_{(P)x}$ is the variance of plot means of size x (calculated as $Var_{(plot)} + Var_{(within-plot)} / n_H$), and $Var_{(P)1}$ the variance from plots of unit size (a single-tree plot). The regression coefficient, b, the range of which varies from 0 to 1, indicates the nature of the environmental variability. The smaller b is, the higher is the correlation between neighbouring trees, reflecting a non-randomly patterned environment where the adjacent microsites are more similar than more distant ones. Respectively, values of b approaching 1 are obtained under either very homogeneous or *randomly* heterogeneous site conditions. The coefficient is very useful since it only measures the degree of field heterogeneity and~is completely independent of the amount of environmental variability (Smith 1938). The plot-mean variances of the 11 contiguous plot types provided the data for estimating the value of b.

3 Results

In the present material, the plot size and the statistical efficiency of the respective test designs were strongly negatively correlated (Table 1). The decrease of efficiency was rapid when the number of trees per plot was less than 20, retarding markedly with larger plot sizes (Fig. 2) The curves in Fig. 2 were drawn to demonstrate the difference in the results from the two test sites; their slight upward tendency on the right side arises from the property of the underlying regression model, and should not be interpreted as if the efficiency had reached a minimum value with around 35 to 40 trees per plot.

Of all plot types examined, the single-tree plot design was the most efficient one. Respectively, the largest contiguous plot type (7 × 7 square plots) gave the poorest result, reaching an efficiency of only 16 and 31 % of that of the single-tree plots. Comparison of the single-tree plots and square plots of 25 trees, showed that the efficiency of the single-tree plots was about three times higher than that of the latter plot type.

The three non-contiguous plot types studied represented an exception to the negative trend between plot size and efficiency in that they displayed a considerably similar efficiency independent of the plot size (Table 1). They also appeared to be clearly more efficient than the contiguous plot types of respective size. The relative benefit of non-contiguous plots increased along with the increasing number of trees per plot. When using square plots of 49 trees, the number of replications needs to be approximately two (358/2) to four (358/1) times larger than with non-contiguous plots of 49 trees, if an equal statistical efficiency is aimed at (Table 1). In other words, the information per tree given by contiguous plots is much smaller than that of non-contiguous plots.

The shape of the plots, compared to their size, appeared to be of minor importance for the efficiency (Table 1). The row plots and long rectangular plots, however, controlled the environmental variability slightly better than the more square-like plots with approximately the same number of trees.

Smith's (1938) measure of field heterogeneity, estimated by the slope of the regression between the logarithmic variance of plot means and the logarithmic plot size (b in equation 3), was 0.405 in test no. 358/1 and 0.507 in test no. 358/2 (Fig. 3). The difference between the coefficients was not statistically significant ($p_{(obs)}$ < 0.312). Despite this, the result indicates that the

nature of environmental variability in test No. 358/1 (at Ikaalinen) was slightly more systematic or patterned (smaller b) than in test No. 358/2 (at Pylkönmäki). The relative efficiency differences between the plot types were more emphasized in 358/1 (Fig. 2), which is in accordance with the higher heterogeneity estimated for this test.
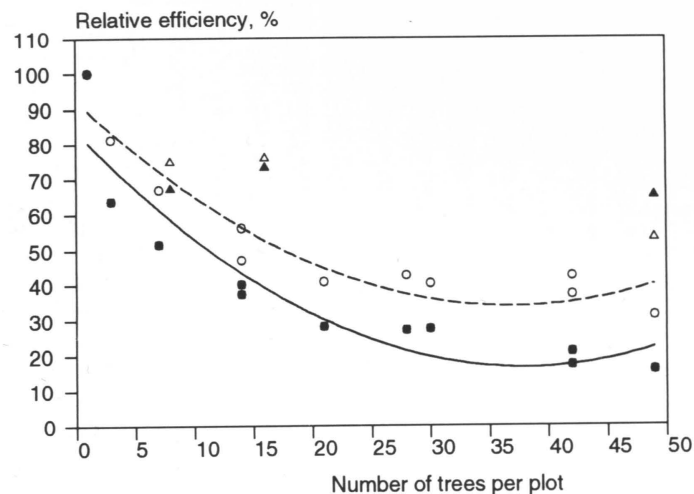

Fig. 2. Relative efficiency of contiguous (circles) and non-contiguous (triangles) plots in relation to plot size in experiments No. 358/1 (filled symbols, straight line) and No. 358/2 (unfilled symbols, dashed line). The regression lines are based on the equation $y = a + b_1x + b_2x^2$ (y representing relative efficiency and x plot size, respectively).
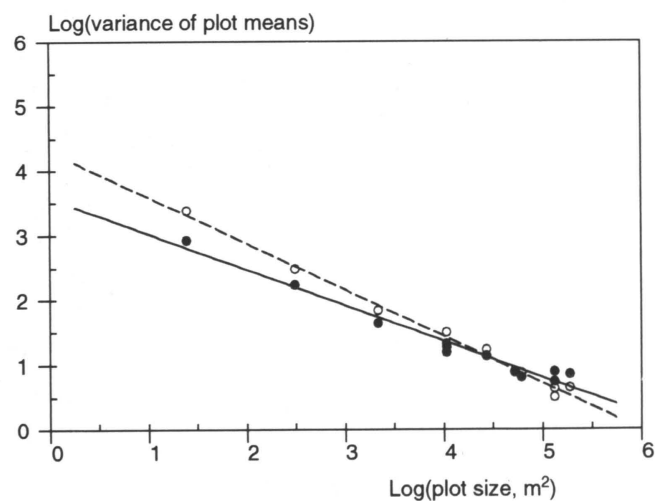

Fig. 3. Comparison of the pattern of environmental variability of the two test sites on the basis of Smith's coefficient of heterogeneity: Logarithmic variance of plot means plotted against logarithmic plot size and the respective regression lines in the experiments No. 358/1 (filled circles, a straight line) and No. 358/2 (circles, a dashed line). For further explanation see the text.

4 Discussion

According to the results, the economics of Finnish progeny testing could be significantly enhanced by decreasing plot size or by introducing non-contiguous plot designs. Even the use of small contiguous plots of, say, 2 to 5 trees would probably result in considerable gains (Fig. 2). The poor statistical efficiency of 5×5 plots found in this study is consistent with the findings of earlier studies: Conkle (1963) reported their relative efficiency to vary between 18 % and 50 % of that of single tree plots, whereas the respective measures obtained by Loo-Dinkins and Tauer (1987) ranged from 7 to 40 %.

Introduction of efficient plot techniques allows one to reduce the total land area assigned for testing, while maintaining the precision at the original level. In Finland, the use of large plots in experiments consisting of 4–6 blocks has obviously resulted in the testing of plus trees with unnecessary many offspring. The average family size in Finnish progeny tests of Scots pine is over 160 trees; moreover, many of the plus trees are being tested at two or more locations. For the sake of comparison, Cotterill and James (1984) suggested that a family size of only 10 to 20 individuals is needed to evaluate parental trees accurately enough, provided that single-tree or two-tree, non-contiguous plots are used.

As regards single-tree and non-contiguous plot systems, the latter appears to be a more attractive alternative for progeny testing. Since a non-contiguous plot consists of several trees, the problem of accidental damage resulting in missing plots is not as acute as with single-tree plots. Furthermore, by applying sophisticated planting designs which involve so-called interlocking replications within blocks, systematic thinnings can be carried out without disturbing the statistical orthogonality of the data (Libby and Cockerham 1980). The laborious establishment stage, which involves tagging all the seedlings and recording their exact location in a field, has usually been considered as an inconvenience common to both non-contiguous and single-tree plot design. However, field electronic data recorders have been developed to facilitate field work. In the opinion of Loo-Dinkins and Tauer (1987), the high statistical efficiency of non-contiguous and single-tree plot designs compared to simpler plot designs is sufficient to outweigh the possible additional work required. This may be, however, too simplistic an assumption. In this study the efficiency of small plots (less than 5–10 trees) appeared to be rather independent of whether they were arranged in non-contiguous or contiguous fashion (Fig. 2). Thus, with small plot sizes the selection of plot type may not always be obvious, but requires pre-evaluation of the expected costs and benefits in each individual case.

The situation is different, as far as large plots are concerned. Large plots, whether contiguous or non-contiguous, unavoidably cover large areas as in randomized block designs, subjecting trees to different levels of environmental variability. The trees arranged throughout a block, however, sample the within-block variability effectively, all the families tested sharing a relatively equal block effect. This is not the case with contiguous plots which, due to the environmental differences within a block, tend to receive a divergent "environmental treatment". This easily causes relative family performances to vary from block to block. For example, Lambeth et al. (1983) found that the family-by-block interaction variance was almost zero in non-contiguous plots, while significantly high in row plots.

The relative statistical efficiency of various plot types is largely determined by the degree of field heterogeneity (Loo-Dinkins and Tauer 1987). The more significant is the systematic component of field variability (with a low Smith's coefficient of heterogeneity), the more the adjacent trees resemble each other as regards their environment and the less the information obtained per tree from plots of a given size, emphasizing the importance of small plots. On the other hand, little is gained by reducing the plot size or introducing a non-contiguous design if the site is relatively uniform or has a fine-grained mosaic structure with a random pattern (Smith's coefficient approaching 1). Unfortunately, accurate visual estimation of the degree of field heterogeneity can be difficult. Anyway, small plots can be expected to be at least as efficient as large ones, regardless of the amount or type of variability. In other words, although the differences between the plot configurations studied may on other, more homogenous sites be less emphasized than here, the general tendency for decreasing efficiency along with increasing plot size is not likely to change.

The trait studied influences, of course, the importance of plot technique as well. Height, as an indicator of growth, is likely to be affected by environment more than many other traits of interest in breeding, e.g. branch angle or stem straightness. This assumption is supported by many heritability studies with several tree species, demonstrating that quality traits generally show higher heritability than growth traits (Pöykkö 1982, Cotterill 1987). Heritability and efficiency are closely related: the family mean heritability, ranging from 0 to 1, is calculated as the ratio of additive genetic variance and the variance of family means (Falconer 1981). Skrøppa (1987) concluded that increasing the number of replications at the expense of plot size is especially profitable when the heritability of a trait is low. It must be recognized, however, that the heritability estimates themselves may be influenced by the plot design; sacrificing sufficient replication in favour of larger plots may decrease heritability because of the poor control over field variability.

Compared to the question of plot size, plot shape has attracted less attention in the literature. Empirical studies with forest trees have indicated that the orientation of the plots in the field is far more important than their shape (Wright and Freeland 1960, Conkle 1963). This especially concerns long row plots. Smith (1938) stated that they can be either more or less effective than square plots, depending on their elongation in the field: plots directed across, rather than parallel to the major environmental gradient may be very inefficient in reducing the variation, contributing rather to the treatment-by-block variance as discussed earlier.

Together with the statistical aspect considered here, other factors influence the decision concerning the plot type. The intensity of inter-genotypic competition after crown closure, for instance, varies considerably by plot size, decreasing as the plot size increases. Whether competition between test entities – or the lack of it – is a disadvantage or not, depends largely on the purpose of the test. This question has been discussed in numerous studies and has not yet been fully resolved. Foster (1989) emphasized that the interactions between trees in the experiment should mimic those in the deployment environment, i.e. in artificially regenerated stands. Considering that production plantations in Finland are established with bulk seed from seed orchards, thus having a random genetic structure, the trees in progeny tests should be subjected to competition with unrelated neighbours. This is an objective that is best achieved with single tree or non-contiguous plots. Under such conditions the correlation between progeny test and stand performance can be expected to be the highest. With large plots the sample of between-family competitive interactions is much smaller than that with small plots (Libby and Cockerham 1980), which may lead to inefficient selection due to biased evaluation of family performances. Large contiguous plots are more justifiable for testing provenances since these are seldom planted in mixtures, and for clonal tests provided that the clones are to utilized as monoclonal plantations. Nevertheless, a single tree design can be useful in the juvenile screening of provenances or clones before the subsequent yield testing with large plots (Shiue and Pauley 1961).

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