

# Within-Plot Subsampling of Trees for Assessment in Progeny Trials of Scots Pine

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Tree height data from 33 progeny trials of Scots pine (*Pinus sylvestris* L.) were used to determine the effect of within-plot subsampling on the magnitude of statistically detectable differences between families, family heritability and correlation of family means based on different sample sizes. The results indicated that, in trials established with a standard plot configuration of 25 trees per plot, measuring only 10–15 trees gives nearly the same precision as with assessment of all the plot trees. Even as few as 4–6 trees assessed per plot may constitute a sufficient sample if families or parental trees of extreme performance are being selected. Trials established with non-contiguous plots were found to be more efficient than those established using multiple-tree contiguous plots.

**Keywords** non-contiguous plots, plot size, progeny testing, sampling, statistical analysis, efficiency, *Pinus sylvestris*.

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## 1 Introduction

The genetic testing of forest trees is normally carried out in a large number of field experiments, the establishment, maintenance and measurement of which all represent a considerable financial burden. To reduce the costs incurred in such operations, the experiments should be as small as possible with regard to the desired precision (Lambeth et al. 1983). In practice, how-

ever, the ideal size of an experiment is difficult to achieve. It is, of course, important to ensure that the number of replicates is sufficient to reveal meaningful differences between genetic entries. On the other hand, abundant replication, possibly accompanied by inefficiently large plots, easily results in the excessive use of plant material and land. This, in turn, inflates the management and measurement costs.

A part of the extraneous costs arising from

poor experimental design can be eliminated by careful planning of the measurement strategy. Sampling within experimental units is an option worth considering, especially when the units are contiguous (trees belonging to the same unit are planted adjacent to each other) and relatively large multiple-tree plots. This applies to Finland, where most of the progeny trials have been established using square-shaped plots of 25 trees (Mikola 1985, Haapanen 1992). Trees within contiguous plots are generally positively correlated as a result of the partially common micro-environment (e.g. Smith 1938, Hühn 1970). Environmental correlation causes the relative information obtained per tree to decrease along with increasing plot size (Conkle 1963). Consequently, only a subset of all the surviving trees in a large plot needs to be assessed in order to obtain a reasonable precision. This will naturally reduce the total number of trees that have to be measured.

The obvious question that arises here is, how far can one go in reducing the sample size without raising the experimental error above the acceptable limit? To study this, one approach is to generate within-plot samples of the tree records of various size from existing data sets, perform series of analysis of variance, and observe how the residual variance changes (Lee 1974 and 1983, Stevenson and Savill 1976, Correll 1978). This is referred to as a subsampling investigation (Correll 1978, Snowdon and Waring 1982). The size of a subsample is considered representative at the point where an increase in the number of trees sampled within plots no longer provides a significant reduction in the residual variance and consequently, in the chosen measure of efficiency. Such often used statistics include e.g., the magnitude of the difference between two family means found to be statistically significant (*least significant difference*) (Cochran and Cox 1957) and correlation between entry (family, provenance etc.) means based on samples of different size (Lee 1974, Lee 1983, Kung 1977, Cotterill and James 1984).

The efficiency of genetic testing can be further improved by selecting among the trials to be measured. The precision of genetic field trials generally varies considerably due to differences in microsite variability, site preparation, efficien-

cy of blocking etc. Thus, it would be important for a tree breeder to be able to identify and reject those trials that have the least value as a source of genetic information. Estimates on the amount of residual variation from earlier data, if available, undoubtedly provide the most reliable basis for this sort of screening. In addition, information on the lay-out of experiments might also be used (see e.g. Lee 1983, McCutchan et al. 1989).

The objective of this study was to determine the effect of within-plot subsampling on the statistical efficiency in a representative set of progeny trials, as regards the measurement age, plot configuration and number of replication in Finnish conditions. Another object of interest was to study to which degree the efficiency can be predicted by factors related to experimental design, such as block size and number of blocks.

## 2 Material and Methods

The study material consisted of 33 sets of tree height data from the same number of Scots pine (*Pinus sylvestris* L.) progeny trials (Fig. 1). The most recent data available from every trial, the age of which ranged from 5 to 20 years (median 10 years), was used in the analyses. All surviving trees were measured for total height in each trial.

The main body of the data, 30 trials, represented a random sample of the total number of 784 Scots pine progeny trials established by Finnish Forest Research Institute since the 50's. The experimental design of these trials typically involved 4–6 randomized complete blocks. Trees were most commonly planted at 5 × 5 position in 25-tree block plots (Fig. 2). The standard planting distance was 2.0 m × 2.5 m. Most entries were open-pollinated progeny of selected plus-trees, usually accompanied by 4–8 control seedlots per trial. The remaining three trials were established using non-contiguous plots (Libby and Cockerham 1980), and included subjectively to study the effect of plot configuration on the experimental efficiency. For more detailed information on the trials (Fig. 1) see Pajamäki and Karvinen (1991).

Randomized within-plot subsampling was sim-

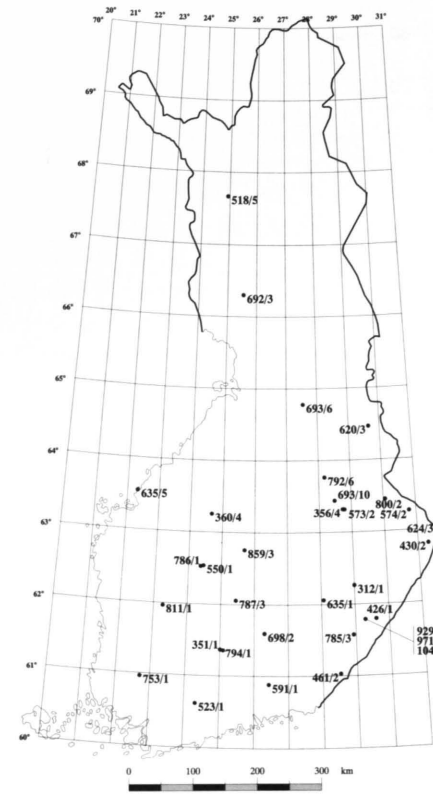


Fig. 1. Location of the progeny trials in Finland.

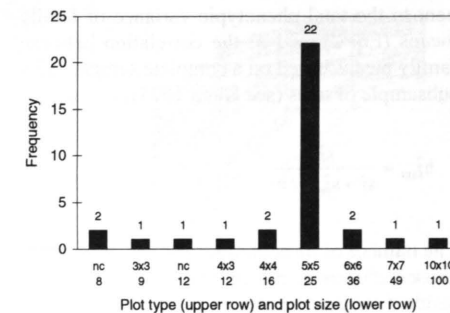


Fig. 2. Frequency of different plot types and plot sizes (number of trees planted per plot) in the studied data set consisting of 33 progeny trials of Scots pine ('nc' = non-contiguous plot design).

contiguous plot, which violates the assumption of the independence of the within-plot error terms.

The effect of subsampling on statistical efficiency was determined by using three criteria. The least significant difference (LSD, Eq. 1) measures the smallest detectable difference between any two treatment (here family) means at a chosen level of significance (Cochran and Cox 1957):

$$LSD = \sqrt{\frac{2(t_a + t_b) s_{resid}^2}{b}} \quad (1)$$

where  $b$  in denotes the number of blocks. The  $s_{resid}^2$  is the residual variance, obtained from an analysis in which variance components due to family and block effects were subtracted from the total variance of plot means. The MIXED procedure (method REML) of the SAS statistical package (SAS Technical Report 1992) was used in the estimation of the variance components. The values of Student's  $t$ -distribution were calculated using  $2(b-1)$  degrees of freedom and 0.05 ( $t_a$ ) and 0.20 ( $t_b$ ) probability levels for errors of type I and II, respectively. To permit comparison of the LSD values between different trials, they were converted to a percentage scale (% of the experiment mean).

The two other measures studied were: 2) family heritability  $h_{fam}^2$  (Falconer 1981), which is the ratio of the among-family ( $s_f^2$ ) variance compo-

ment to the total phenotypic variance of family means (Eq. 2), and 3) the correlation between family means based on a complete sample and a subsample of trees (see Kung 1977).

$$h_{fam}^2 = \frac{s_f^2}{s_f^2 + s_{resid}^2 / b} \quad (2)$$

The number of trees sampled per plot affects the chosen efficiency criteria in two ways: At first, residual variance increases along with decreasing sample size, since  $s_{resid}^2$  is actually the sum of the between-plot variance component and the  $n^{\text{th}}$  part of the within-plot variance (Eq. 3) (where  $n$  is the average number of trees measured per plot). Secondly, reducing sample size increases the sampling variance of the variance component estimates.

$$s_{resid}^2 = s_{plot}^2 + s_{within-plot}^2 / n \quad (3)$$

The variability of the estimates of efficiency at different sample sizes was measured by the coefficient of among-sample variation (standard deviation in a set of 10 or 15 replicated samples divided by the mean of the sample estimates), which was averaged across the 33 trials.

The relationship between block size and number of blocks, and the estimated statistical efficiency (the LSD and  $h_{fam}^2$ ) was studied using Pearson's correlation analysis.

### 3 Results

The efficiency parameters responded to subsampling most significantly when the sample size was increased from 2 to 6 trees. When the number of trees sampled per plot exceeded 10, the average LSD values, for example, improved by 1–3 % (Fig. 3). The curves for family heritability showed a similar, although opposite trend (Fig. 4). The heritability and LSD values showed higher variability among trials than the correlations. The correlations between the family means based on all the plot trees and subsamples of different size were high in all trials ( $r = 0.80$ ), even with sample sizes as small as 4–6 trees per plot (Fig. 5).

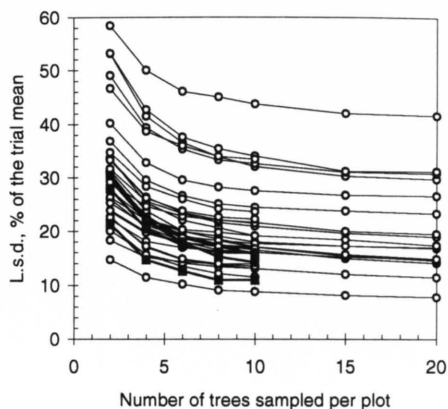


Fig. 3. Average least significant differences (LSD) at different sample sizes in 33 progeny trials. The probabilities of making an error of type I (false rejection) and II (false acceptance) were set to 0.05 and 0.20. The non-contiguous plot trials are denoted by the filled squares, and the others by open circles.

The coefficient of among-sample (residual) variation decreased along with increasing sample size. Family heritability was associated with a high coefficient of variation, especially at small sample sizes (Fig. 6). This was also reflected in the mean heritability values, which did not increase regularly as a function of the sample size in a few cases (Fig. 4).

Independently of the measure of efficiency, the three non-contiguous plot trials showed as high efficiency as the best contiguous plot trials, although the number of trees per family planted in these trials was considerably smaller.

The number of blocks established per trial was associated with lower LSD values, and thus, with higher efficiency ( $r = -0.43$ ,  $p < 0.02$ ). The block size, on the other hand, was unfavourably related with the LSD ( $r = 0.32$ ,  $p < 0.18$ ). The trials with the largest blocks showed the poorest efficiency, especially when the number of blocks was small (less than 5). The respective correlations with family heritability values were non-significant (No. of blocks:  $r = 0.06$ ,  $p < 0.74$ ; Block size:  $r = -0.13$ ,  $p < 0.46$ ).

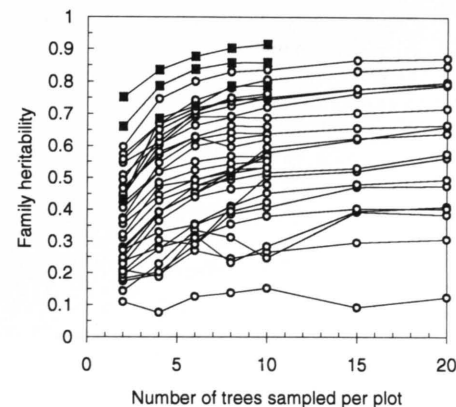


Fig. 4. Average family heritability estimates at different sample sizes in 33 progeny trials. The non-contiguous plot trials are denoted by the filled squares, and the others by open circles.

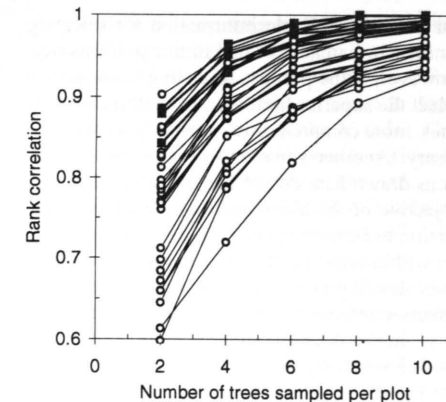


Fig. 5. Average correlations between family means based on complete assessment of all plot trees and a subsample of different size.

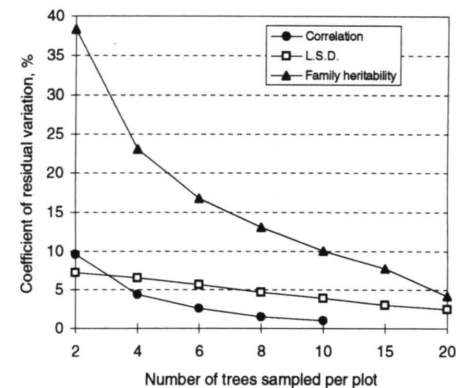


Fig. 6. Average coefficients of among-sample (residual) variation for 1) least significant difference, 2) family heritability and 3) correlation between family means based on complete plot assessment and a subsample.

### 4 Discussion

The partial assessment of plots seems to be worthwhile especially in the trials established with large multiple-tree plots, as they have a low effi-

ciency on a per tree basis. The results obtained here indicate that measuring only 10–15 trees in the 25-tree block plots is likely to give statistical precision (LSD) that is not significantly different from the costly alternative of complete plot assessment. This is in accordance with the findings of e.g., Stevenson and Savill (1976), who sampled trees from 36-tree plots in two Sitka spruce (*Picea sitchensis* (Bong.) Carriere) experiments. They found that little additional information was achieved by measuring height and girth at breast height on more than 20 trees per plot, 12–16 trees per plot giving results with sufficient precision in most cases. The total savings in costs resulting from reducing the number of measured trees are, of course, primarily dependent on the extent of the testing programme. Taking as an example the number of offspring trees planted in Scots pine progeny trials in Finland since the early 50's, i.e. nearly six million, the use of complete plot assessment as a standard method is likely to lead to substantial accumulated losses in terms of information obtained per time and labour spent.

The high correlations between pairs of family means based on complete samples and subsamples of different size as obtained in this study (Fig. 5), indicate that even as few as 4–6 trees

per plot give reliable information for selecting families or parent trees of extreme performance. However, if the purpose of testing is not just to select the superior families or to cull the poorest ones, more comprehensive sampling will be necessary. Another situation to which the conclusions drawn here do not apply is when the main objective of the assessment is to provide information to be used in selecting the best individuals within families. In that case all the available trees should preferably be measured to make the selection intensity as high as possible. One way to avoid this dilemma is to design different trials for different selection objectives: single-tree plots for family selection and large block plots for selecting sibs in a common environment. (e.g. Lambeth 1986, Loo-Dinkins and Tauer 1990).

The coefficients of residual variation (Fig. 6) indicate the degree of variation among estimates computed from replicated samples (Figs. 3, 4, and 5). In large plots the subsamples of 10–15 trees seem to give both sufficient precision (on the average) and adequately good protection against strikingly poor estimates obtained by chance. It should be noted, however, that all the CV's are more or less underestimated since the individual samples were not mutually independent (due to the infinite number of trees per plot, the different samples were, in part, composed of the same trees).

The reliability of the family heritability estimates was significantly poorer than that of the family mean correlations and LSDs, particularly when the sample size was small. This was obviously due to the fact that the residual variance of heritability (see Eq. 2) is increased by the sampling error of both the residual variance component and the family variance component.

The efficiency of the sampled trials measured in terms of absolute LSD values was rather low. This was the case even with the results from the largest samples. It can be asked whether the ability to detect height differences of 15 to 30 % of the general mean is satisfactory for a tree breeder, if we consider that true genetic differences between families are often much smaller. To conclude, the experiments analysed in this study would have needed greatly increased replication to be truly effective at the chosen levels of error. The increased replication as such will

not, however, solve the problem of the inefficiency of large contiguous plots. On the contrary, the efficiency in terms of information per total number of planted trees may even decrease. The results indicate that the non-contiguous arrangement of plot trees which eliminates a large proportion of the residual variance due to spatial correlation of related individuals, (Lambeth et al. 1983), can be recommended for future trials. The results of this study strongly suggest that non-contiguous plot trials need a considerably smaller number of trees per family to yield a statistical precision equal to that of the ordinary trials established using large contiguous plots. More data are, however, needed to give well-founded numerical guidelines for the appropriate family size, number of replication etc. for the former type of trial.

Block size and number of blocks together appeared to indicate the statistical efficiency (LSD) of a trial to some extent, if height is taken as the target trait. Even though the association was not strong, there was a clearly detectable trend: those trials having both the smallest number of blocks and the largest block size proved to be of the least value. Since there is no sense in measuring trials that will probably never give sufficient information, these two variables might be used as a rough tool in differentiating among the existing trials and culling the poorest ones from measurement programs. Further savings might also be attained by omitting some of the families from the measurement on the basis of *a priori* information.

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