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Statistical Efficiency varies with Plot Size, Number of Replications and Seedlots sampled

By C. H. Lee[1])

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Summary

European black pine (*Pinus nigra* Arnold) 2-0 planting stock grown from seed collected from 27 natural stands throughout the species range was used to establish the study plantation in Kellogg Experiment Forest, Augusta, Michigan in 1961. At age 14 from seed, I measured total height to the nearest 3 centimeters on every tree in the plantation in August 1972. All height data were kept separate by individual trees. An analysis of variance was conducted by varying the seedlot size (number of provenances), number of replications and plot size and their sampling efficiency compared. The importance of systematic sampling throughout the species range was discussed and the justification for measuring smaller plot size and partial replications was presented.

Key words: *Pinus nigra*, provenance test, sampling efficiency, height growth.

Zusammenfassung

Im Jahre 1961 wurden 2-0-Sämlinge von *Pinus nigra* Arnold aus einer Samenernte in 27 autochthonen Beständen im natürlichen Verbreitungsgebiet der Art im Kellog-Versuchswald, Augusta, Michigan als Provenienzversuch ausgepflanzt. Im Alter 14 nach der Aussaat im August 1972 wurden die Höhen der Einzelbäume bis auf 3 cm genau gemessen. Alle Daten der Höhenmessung wurden einzelbaumweise getrennt gehalten. Eine Varianzanalyse wurde in der Richtung durchgeführt, daß bei der festgesetzten Samenmenge (Anzahl der Provenienzen), Anzahl der Wiederholungen und Parzellengröße sowie deren Probeneffizienz verglichen werden konnten. Es wurde die Bedeutung einer systematischen Einsammlung der Samenproben im Verbreitungsgebiet der Art diskutiert sowie die Berechtigung der Messung kleinerer Parzellengrößen und partieller Wiederholungen.

Introduction

The purpose of a provenance test is do determine the magnitude of genetic variation patterns and to recommend what seed sources should be planted in the area of concern. The degree of species diversity in growth traits and wood properties and how provenances are sampled systematically in order to give better geographic representation are important factors in the provenance test. The number of seedlots used in the Regional Project (NC-99) varies from 7 in Japanese larch (*Larix leptolepis* Sieb. and Zucc.) to as many as 272 in red pine (*Pinus resinosa* Ait.) improvement program (personal communication with Professor J. W. Wright, Michigan State University).

Seed procurement is immediately followed by the establishment of field test in nursery and subsequently at a permanent planting site. Many adopt a randomized complete block design with 5 to 10 or more replications (blocks) and the number of trees planted per provenance (plot size) per replication also varies from a few to as many as several hundred.

Partial measurements are done for convenience. This paper is designed to analyze what impact the partial measurement has on the efficiency of the assessment of genetic data.

Material and Methods

European black pine (*Pinus nigra* Arnold) is primarily a Mediterranean species extending from 35° to 48° N in latitude and from 6° W to 42° E in longitude. In August 1972 (14 years from seed), I measured total height to the

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nearest 3 centimeters (0.1 foot) on every tree in the test plantation (MSFG 5—61) in Kellogg Experiment Forest, Kalamazoo County, Southwestern Lower Michigan. Height data were kept separate by individual trees.

Plot means were computed in 4 different ways: i.e. based on all 4 trees, based on the 1, 2, or 3 tallest trees in each plot (deliberate sampling). The random number table was used to select 9 different replications (2, 3, 4, 5, 6, 7, 8, 9, and 10 replications) and 4 different provenance sizes (containing 10, 15, 20, or 27 seedlots) following a sampling scheme known as sampling without replacement. Altogether 144 analyses of variance (= 4 plot sizes × 9 different replications × 4 different provenance sizes) were conducted using plot means as items for which the degrees of freedom were (f - 1), (n - 1), and (f - 1) (n - 1) for provenance, blocks and error, respectively. From each analysis of variance, I obtained standard error of plot mean by dividing the error variance by the number of replications (= n), then found the square root. The standard errors were used to determine the sampling efficiency as affected by the provenance sizes (number of seedlots), number of replications and plot sizes (number of trees per plot) sampled.

Results and Discussion

Number of seedlots analyzed: Once the seed procurement is completed, all seedlots received will be used to establish the test plantation. Furthermore, it is a rare practice that part of the genetic material (whether provenances, half-sib or full-sib families, or clones) already planted and available in the permanent test plantation is evaluated.

The success of the seed procurement depends on the degree of cooperation of field foresters throughout the species range and a number of other factors such as the extent of species natural range and the physiology of seed production (whether it is a good seed crop year when the request for seed collection is made). In order to assess the impact of the sampling intensity, I randomly selected (without replacement) fewer seedlots than were readily available in the study plantation and assumed those were the ones available through the seed procurement. Then I proceeded to increase the number of seedlots in the analysis and their results were compared. The original collection of 27 provenances as well as the reduced numbers of provenances analyzed in the present study are summarized in the following tabulation:

Seed Origin	Number of seedlots analyzed			
	10	15	20	27
Spain	—	—	—	2
France	1	1	3	3
Corsica	1	3	3	3
Austria	—	—	—	1
Yugoslavia	—	1	1	1
Greece	6	8	11	13
Turkey	1	1	1	3
Crimea	1	1	1	1

When the purpose of experiment is to discern fine geographic trends, much more intensive sampling throughout the species range may be necessary. However, fewer seedlots may suffice if one's purpose is to obtain improvement and not learn about geographic trends. In that case, tree species must demonstrate significant geographic (genetic) variation. When tested in Pakistan, only 8 seedlots were found sufficient to demonstrate that *Eucalyptus camaldulensis* was geographically variable in height, diameter, volume and wood specific gravity (Siddiqui, *et al.*, 1979). European black pine is no exception. When as few as 10 seedlots were randomly selected and analyzed simulating that those were the ones available for the study, the between-seedlot differences in height growth were found statistically significant (*Table 1*). This implies that improvement of this pine species would be feasible even if there were only 10 seedlots (seed sources) to select from.

Table 1. — F values vary with different number of seedlots, replications and plot sizes analyzed for black pine height growth at age 14.

No. of replications	Plot size	Number of seedlots analyzed			
		10	15	20	27
2	1	2.388	1.999	1.689	2.033*
	2	3.224*	2.393	2.075	2.309*
	3	3.135	2.585*	2.315*	2.459*
	4	5.602**	2.873*	2.676*	2.428*
3	1	2.387	3.064**	2.789**	2.624**
	2	2.776*	3.212**	3.024**	2.906**
	3	2.857*	3.341**	3.100**	3.123**
	4	3.027**	3.230**	2.773**	2.897**
4	1	6.355**	5.346**	4.400**	3.021**
	2	8.510**	5.039**	4.873**	3.485**
	3	8.022**	4.497**	4.709**	3.532**
	4	6.037**	3.565**	3.738**	3.037**
5	1	6.657**	4.838**	4.274**	3.510**
	2	8.180**	4.895**	4.665**	4.145**
	3	7.515**	4.564**	4.554**	4.295**
	4	6.923**	4.115**	3.823**	3.832**
6	1	6.665**	4.303**	4.017**	3.607**
	2	8.958**	5.027**	5.231**	4.936**
	3	9.088**	5.092**	5.455**	5.088**
	4	7.801**	4.723**	4.719**	4.331**
7	1	6.775**	4.151**	5.102**	4.433**
	2	7.376**	4.493**	5.267**	4.992**
	3	6.807**	4.292**	4.892**	4.800**
	4	6.017**	4.021**	4.158**	4.128**
8	1	7.430**	5.969**	5.916**	5.194**
	2	8.457**	6.467**	6.695**	6.332**
	3	8.031**	6.340**	6.574**	6.486**
	4	7.581**	5.901**	5.624**	5.675**
9	1	8.333**	6.673**	6.471**	5.340**
	2	8.363**	6.891**	6.992**	6.150**
	3	7.754**	6.783**	6.921**	6.204**
	4	6.549**	6.086**	5.896**	5.359**
10	1	8.681**	6.871**	6.755**	5.616**
	2	9.414**	7.276**	7.480**	6.639**
	3	8.890**	7.109**	7.385**	6.729**
	4	7.946**	6.502**	6.390**	5.909**

*Significant at the 5 percent level
**Significant at the 1 percent level

Italy was not represented in the original seed collection. The amount of genetic information about black pine should not be severely affected even in the absence of seed sources sampled from Italy. The original collection has a good representation of the less winter hardy western provenances as well as more winter hardy, fast growing and insect resistant eastern provenances. When only 10 seedlots (provenances) were analyzed, we were still able to detect the existence of genetic variation and therefore the possibility of genetic improvement. However, it was not certain whether we would obtain the maximum possible improvement. We learned that seedlots from Austria and Yugoslavia turned out to have satisfactory disease resistant and winter hardy characteristics in Michigan and Nebraska and any design which omits the inclusion of one or the other or both should be considered inadequate.

This situation was not corrected until all the 27 seedlots were thrown into the evaluation. It is obvious that the intensity of sampling throughout the species range has

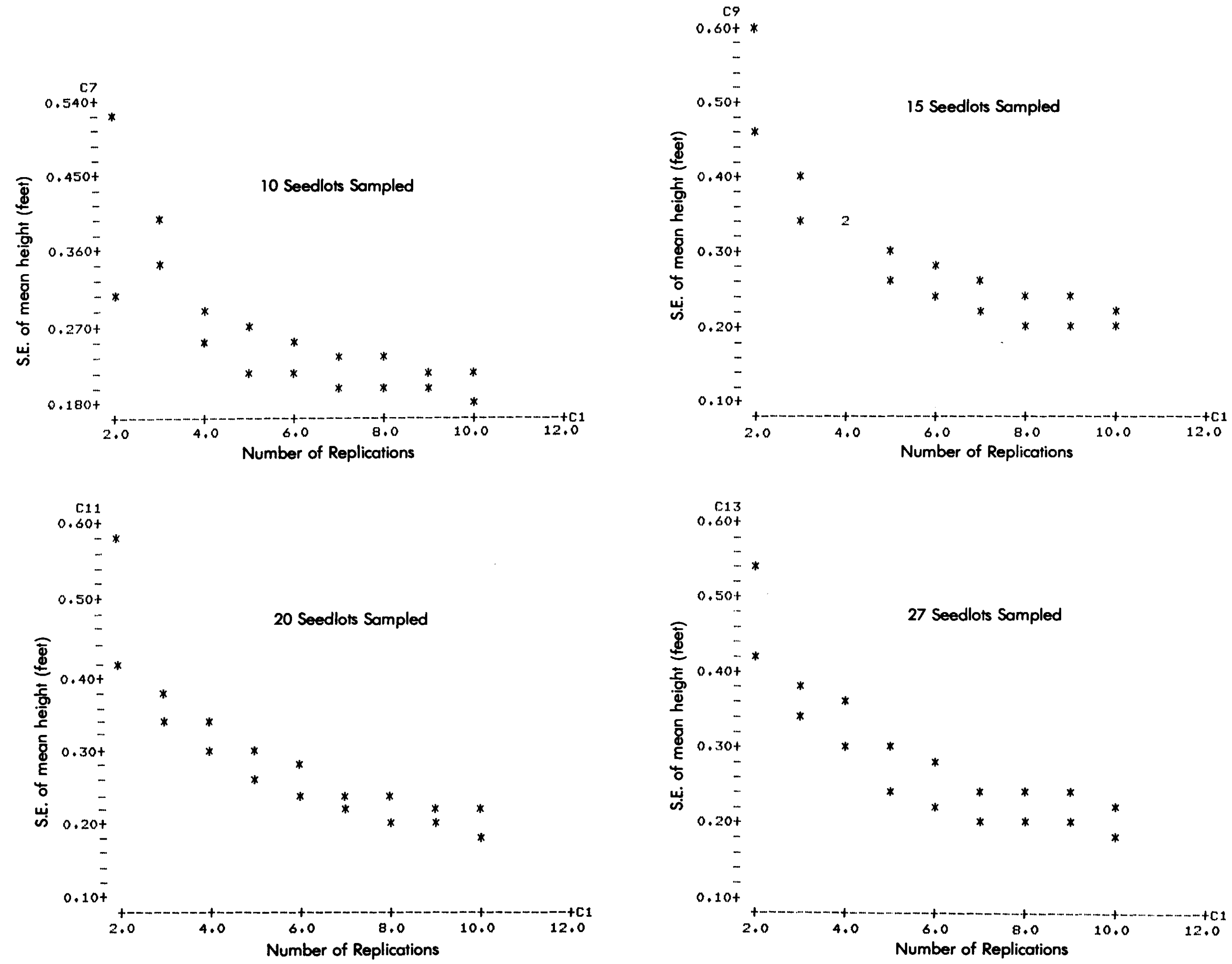

Figure 1. — The magnitude of the standard error of plot mean in height growth varies with plot size, the number of replications and seedlots sampled.

a direct bearing on the amount and quality of genetic information.

The reduced size of seedlots was associated with a sacrifice in the statistical efficiency of estimating about provenance mean *(Figure 1)*. For example, when the number of seedlots was increased from 15 to 27, the size of standard error dropped 0.018 feet from 0.292 to 0.274 feet *(Table 2)*. These were averages based on 36 standard errors (4 plot sizes × 9 different replications). The amount of improvement in the sampling efficiency due to a more intensified sampling was considerably smaller than the other two factors (plot sizes and replication numbers) explored simultaneously in the present study. It was not determined why the sampling of only 10 seedlots gave the smallest standard error.

Plot sizes and number of replications sampled: If the number of seedlots is held constant, then number of trees per plot (plot size) and that of replications sampled become important factors affecting the sampling efficiency in the black pine provenance study. When only the tallest tree per plot and only two replications were sampled, it had to be concluded that this pine species lacked genetic variation *(Table 1)* regardless of the number of seedlots analyzed. Consequently, its potential as a desirable genetic material may be overlooked.

There was a steady decrease in the magnitude of standard error (= improvement in sampling efficiency) when plot size was increased from one (tallest tree) to all 4 trees. The similar effect (improvement in sampling efficiency) was achieved by increasing the number of repli-

Table 2. — Effect of different sampling schemes on black pine height growth at age 14.

Sampling scheme	Standard error	Sampling scheme	Standard error
	--feet--		--feet--
No. of seedlots		No. of replications	
10	0.257	2	0.475
15	0.292	3	0.359
20	0.280	4	0.301
27	0.274	5	0.295
Plot size		6	0.244
tallest	0.325	7	0.223
2 tallest	0.273	8	0.214
3 tallest	0.264	9	0.210
all 4 trees	0.258	10	0.196

Table 3. — The correlation coefficients comparing the effect of different number of seedlots, replications and plot size sampled.

No. of replications and plot size compared	No. of seedlots sampled				Average of 4 different seedlot sizes
	10	15	20	27	
II-1 vs X-4	0.658*	0.632*	0.625**	0.570**	0.621
IV-1 vs X-4	0.624	0.768**	0.708**	0.731**	0.708
VI-1 vs X-4	0.926**	0.925**	0.919**	0.900**	0.918
X-1 vs X-4	0.963**	0.950**	0.934**	0.914**	0.940

*Significant at the 5 percent level

**Significant at the 1 percent level

cations in the sample *(Figure 1)*. The amount of improvement in the sampling efficiency *(Table 2)* was 0.067 feet for plot size (average of 4 seedlot sizes × 9 different replications or 36 standard errors) and 0.279 feet for different replications sampled (average of 4 seedlot sizes × 4 plot sizes or 16 standard errors).

Regardless of the seedlot sizes *(Figure 1)*, measurement of the tallest per plot (top curves) was always less efficient than that of all 4 trees in the plot (bottom curves); similarly, sampling of just 2 replications was less efficient than that of 3 replications and as the number of replications increased, the standard error curves continued to drop and became leveled off when 6 or more replications were included in the sample. The results of the factual black pine data were comparable to those of the computer-simulated fictitious data reported by Lee (1981). Inclusion of 6 or more replications in the sample does not significantly improve the sampling efficiency.

The simple correlation analysis was conducted using seedlot means as items with (f - 2) degrees of freedom, where f is the number of seedlots sampled. The results are shown in *Table 3*. All but one correlation coefficient (= r) were statistically significant at the 5 or 1 percent level (number of replications in Roman numerals and plot size in Arabic figures). The strong correlation means superior seedlots or genetic material can be identified regardless of plot size or number of replications sampled in the present study.

Partial measurement is associated with a weak degree of loss ($= 1 - r^2$) in genetic information. When all four seedlot sizes were pooled together, it was possible to examine the combined plot size-replication effect. Sampling of 4 replications-tallest tree per plot combination (IV-1) resulted in a loss of 50 percent ($r^2 = 0.50$) in genetic information. This figure was reduced to 16 percent ($r^2 = 0.84$) when 6 replications-tallest per plot combination (VI-1) was compared with all 10 replications-all 4 trees per plot (X-4) combination. Sampling of 6 replications instead of all ten will save 40 percent in measurement effort.

When the effect of plot size alone is considered, there is a close similarity in statistical efficiency between black pine and ponderosa pine *(Pinus ponderosa* Dougl. ex Laws.) reported by Lee (1974). The loss in genetic information is under 12 percent (r^2 of X-1 vs. X-4 was 0.88 for black pine; r^2 of tallest vs. all 12 trees was 0.96 for ponderosa pine) in spite of the differences in tree age (14 years from seed for black pine and 2 years from seed for ponderosa pine) and plot size (4 trees were planted per plot for black pine, but 12 trees per plot for ponderosa pine) between the two pine species. Measurement of the tallest (or the best tree) per plot is efficient, time-saving, but accompanied by a small tolerable loss in genetic information.

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Genetic Variation in Needles of Pinus caribaea var. hondurensis Barr. et Golf. from natural Stands[1])

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Abstract

Analysis of the anatomy and morphology of mature needles of nine provenances of *Pinus caribaea* var. *hondurensis* Barr. and Golf. detected that most of the variation is present between and within trees.

Populations in Los Limones and Santa Clara from Honduras showed 80 and 50% respectively of the fascicles with four and five needles, which may be due to gene interchange with *Pinus oocarpa* Schiede. Further work will be necessary to determine if these two populations should be considered as hybrids.

Number of stomata, resin ducts and intrusion of hypoderm cells showed a slight clinal trend with respect to latitude but the provenance Alamicamba (lower value for stomata) and Melinda (higher value) were exceptions to the clinal trend. Also Melinda had shorter needles.

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[1]) The paper is adapted from part of the author's Ph. D. Thesis, Forestry Department, Oxford University, England, 1981.