

Analysis of unreplicated Scots pine (*Pinus sylvestris* L.) provenance trials

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Three *Pinus sylvestris* L. provenance trials in Lithuania belonging to the so called Prokazin series of trials without replications were assessed at ages 19-23 with respect to three growth traits and four quality traits. There were large among-population differences in all trials. Since they were unreplicated any significant difference could not be proven. ANOVAs were run based on the trials as replications, in pairs and in combination of three, and after grouping of populations into 14 and 6 regions, respectively. For tree height, pooling of populations into six regions in some cases resulted in significant differences among regions. The effects on differences were illustrated as deviations from the trial means in units of the standard deviation. When the ranking of the populations differs among trials as in our case such a pooling leads inevitably to considerable reduction of the estimated differences. However, pooling is a necessary sacrifice to be able to prove any significant differences. To be of use, unreplicated trials in one experimental series must have homogeneous conditions both within the trial and among the trials. In future the main use of the Lithuanian trials may be as gene resources, in which various types of crosses can be carried out.

Key words: *Pinus sylvestris* L., population, tree growth, quality traits, analysis of unreplicated trials

Introduction

The original intention of the establishment of provenance trials was in most cases to investigate whether seed transfer would result in a higher production than the local seed sources. Provenance trials have also been used for delineation of breeding zones and to get a better understanding of the evolution of the species under study. In recent years they have been used to obtain estimates of the effects of a climatic change. The country-wide series of provenance trials of *Pinus sylvestris* in Sweden established by Vilhelms Eiche during the fifties has served all four purposes (Eiche 1966, Raymond and Lindgren 1986, Eriksson et al. 1980, Persson and Beuker 1997, respectively).

The so-called Prokazin series of provenance trials established during the mid seventies and distributed over a large part of the European part of former Soviet Union could serve the same purposes for that part of Europe. This series, although, has a serious drawback since it consists of unreplicated trials with the trees of each population growing in one large block in each trial. Assessments have been made in three Lithuanian field trials belonging to this series of trials. The data as regards stem volume in each of the trials are given in

Figures. 1a-c. There is a considerable variation between the poorest and best population in each of the three trials. We assume that there is a considerable variation among populations but this cannot be proven for any of the trials individually since there are no replications. To clarify our position, we do not believe that the conspicuous differences should be attributed to random events alone. To verify if the observations are real or owing to random events, two approaches may be used. The first is to regard each trial as one out of three replications. Another possibility is to pool populations into regions as was done by Abraitis and Eriksson (1996) for one of the Lithuanian trials.

When the plots are as large as they are for some populations in the Prokazin series of experiments it would be tempting to utilize random subplots within an unreplicated large plot to obtain replications of each population in each trial. Mead and Curnow (1987) treated this in their book on Statistical Methods in Agriculture and Experimental Biology. We prefer to quote the following from page 288 of this book to exemplify why such an approach is not statistically sound. "Within-plot variation of plant yields may give an overestimate of plot-to-plot variation, because of interplant competition, or it may give an underestimate of plot-to-plot variation,

because of a very homogeneous within-plot environment. The crucial point is that we have no way of telling whether the within-plot variation is the same as between-plot variation and therefore we cannot use within-plot replications to compare treatments applied to different plots". In our case population is equal to treatment in their text.

As far as we know there are many unreplicated forest genetics trials in the former Soviet Union. Therefore, it might be of interest to find the means to make statistical calculations in such experiments. The purpose of our paper is to demonstrate the means for analysing unreplicated trials and to illustrate the effects it may have on the proven differences. The purpose is not to advise on best seed sources for cultivation in Lithuania.

Material and methods

The provenance test plantations concerned were established in 1975 following the instructions by E. Prokazin and unified for the territory of the former Soviet Union. Dr. E. Barniškis had the local responsibility for the establishment of the 3 Lithuanian trials. 1+0 year old seedlings were used with spacing of 1.5x1.5 metres, planting 4.400 seedlings per ha. In 1976, the dead seedlings were substituted by 2+0 year old plants. Multitree plot design without replications within the trial was used since all three trials were regarded as replications of one big experiment. The number of trees per plot in our analyses varied considerably. Population 20 at Plungė with only 3 trees constituted one extreme. The other extreme was population 69 at Plungė with 129 trees. Since the seed lots for establishment of these trials were harvested in just one stand each, we prefer to refer to populations rather than provenances in the text.

Only populations occurring in all three field trials (Jūrė, Plungė and Venta) were analysed in this study. In Table 1 geographic data of the populations are given together with the pooling of populations into 6 and 14 regions. Pooling of trials was done in pairs or comprising all three trials included in this investigation to see if any trial deviated from the others. The pooling of populations was mainly based on their geographic origins which means that not too large differentiation should be expected among neighbouring populations of a wind-pollinated species like *Pinus sylvestris* (Eriksson 1998).

The field assessments have been made in 1993-1997. The traits evaluated are presented in Table 2. Stem volume was computed according to Brandel (1990):

Table 1. Geographic data of the studied populations and the pooling of populations into 6 and 14 geographical regions, respectively

6 regions	14 regions	Population	N. lat.	E. long.
6	1	16 Karelia, Russia	61°50'	30°28'
6	2	19 S.-Petersburg, Russia	60°00'	30°25'
6	2	21 Pskov, Russia	57°43'	30°31'
1	3	24 Elvassk, Estonia	58°10'	26°28'
1	4	25 Jaunjelgava, Latvia	56°42'	25°10'
1	5	20 Mažeikiai, Lithuania	57°15'	22°40'
1	5	26 Prienai, Lithuania	54°38'	23°58'
2	7	27 Mogiliov, Byelorussia	53°18'	28°40'
2	7	30 Gardin, Byelorussia	53°25'	25°15'
2	7	33 Rovno, Byelorussia	51°30'	27°40'
4	10	42 Kalinin, Russia	57°45'	36°40'
4	10	43 Moscow, Russia	55°40'	37°10'
3	11	41 Smolensk, Russia	54°00'	33°00'
3	11	49 Kaluga, Russia	54°25'	36°16'
3	11	50 Ryazan, Russia	54°40'	39°45'
2	12	29 Gomel, Byelorussia	52°14'	31°43'
3	13	52 Orlov, Russia	52°50'	36°00'
4	14	45 Gorkiy, Russia	56°40'	43°28'
4	14	47 Kostroma, Russia	58°00'	40°50'
5	16	66 Tartary, Russia	55°40'	51°26'
5	17	69 Baskiria, Russia	55°30'	54°40'
5	18	83 Orienburg, Russia	52°47'	52°15'

Table 2. Assessed and calculated traits included in the analyses

Traits	Evaluation unit	Comments
Breast height diameter, D	cm	
Tree height, H	m	
Stem volume, V	dm ³	
Height to dead branch	cm	
Height to first green branch, K	m	
Stem straightness	points	Visually evaluated straightness
	1 - very crooked,	
	2 - moderate,	
	3 - straight.	
Tree status	points	Visually evaluated tree status
	1 - vital,	
	2 - injured,	
	3 - suppressed,	
	4 - dead.	
Branch angle	points	Branch angle evaluated visually at the 5-th whorl above ground
	1 - very acute,	
	2 - moderate,	
	3 - right.	

$$V=0.17144 \cdot D^{1.87368} \cdot (D+20.0)^{0.01317} \cdot H^{1.99652} \cdot (H-1.3)^{-1.02070} \cdot K^{0.02882} \tag{1}$$

where: D, H, K - are explained in Table 2.

Only superior trees (cf. Table 2, tree status=1) were included into analyses. To illustrate the effects on differences it was necessary to standardize data to avoid scale effects owing to the large growth differences among the trials. This was done by using logarithmically transform-

ed data and expressing the population deviation from the trial means in units of the standard deviation according to the formula:

$$(x_i - \bar{x}) / \sigma \quad (2)$$

where: x_i - population mean value of a certain trait, \bar{x} - overall mean of the same trait, s - standard deviation of this trait.

When pooling of trials and/or populations had taken place, the mean values of the pooled entries were calculated. The full statistical model was used:

$$y_{ijk} = \mu + t_i + r_j + p_{j(k)} + [t*r]_{ij} + [t*p]_{ij(k)} + e_{ijk} \quad (3)$$

where: μ - overall mean, t_i - trial effect, r_j - regional effect, $p_{j(k)}$ - effect of population within region, $[t*r]_{ij}$ - effect of trial x region interaction, $[t*p]_{ij(k)}$ - effect of trial x population within region interaction, e_{ijk} - error term.

Reduced models were used when pooling was less comprehensive.

SAS procedure GLM was used in calculations (SAS 1988).

Results and discussion

Trials used as replications without pooling of populations into regions

When studying individual populations with each trial used as a replication, there was only one significant difference for growth traits, as seen from Table 3. A possible

reason for this is the many changes in rank between the three trials as is illustrated in Figures. 1a-c.

Among the quality traits, the height to the first green branch showed significance in three cases. It should be noted that there was a considerable variation among populations also for the quality traits.

For the three trials included in our study it must be concluded that using them as replications was mostly not sufficient to reveal significant differences with one exception, height to first green branch.

Pooling of populations into regions

Pooling of the populations into 14 regions did not increase the number of significant differences for growth traits. Not until pooling into six regions was carried out, were significancies obtained in several cases for tree height, while for DBH and stem volume significance was obtained in one case only; stem volume after pooling Jürè and Venta data. It may also be seen from Table 3 that individual analysis of data from each trial resulted in fewer significances than when data from two trials were pooled. Noteworthy is that inclusion of data from Plungè in most cases resulted in non-significant differences. This is somewhat surprising since the growth at Plungè was intermediate to that in the other two trials (cf. Figure 1b). As seen from Table 4, the absence of significant population differences must be attributed to the significant region x trial interaction.

Table 3. Significant differences among populations and geographical regions for three growth and four quality traits based on ANOVA, in which trials were used as replications with and without pooling of populations into 6 and 14 regions. * and ** show significance of differences at 5% level and at 1% level, respectively. Empty cell means non-significant difference.

Traits	Individual populations				14 regions				6 regions							
	Individual trials	Pool.	Pool.	Pool.	Pool.	Pool.	Pool.	Pool.	Pool.	Pool.	Pool.	Pool.				
	J+P	J+V	P+V	J+P+V	J+P	J+V	P+V	J+P+V	J	P	V	J+P	J+V	P+V	J+P+V	
	no regional pooling				pooling into 14 regions				pooling into 6 regions							
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Stem volume	Not testable													**		
Tree height	..**..		**					*		**		*	*	*	**	
Diameter	..**..													*		
Height to dead branch	..**..		**			*		*	**							
Height to green branch	..**..		*	*	**					*						
Branch angle	..**..															
Stem straightness	..**..								*					**		*

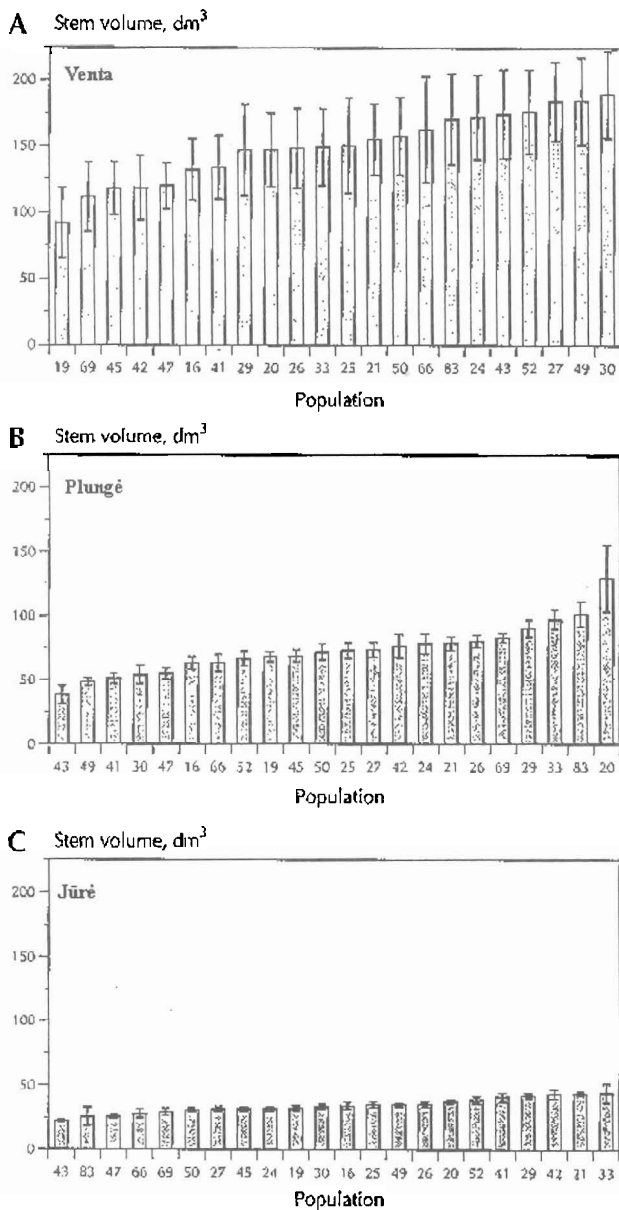


Fig. 1. Stem volume ± standard error, expressed in dm³, for individual populations in three field trials of *Pinus sylvestris* in Lithuania

Since stem volume is one of the economically most important traits, we shall use this trait to graphically illustrate the effects of pooling data in different ways. The standardized values of the individual trials indicate that the distribution of values is rather similar in the three trials in spite of the large growth differences among them (Figures 2a-c). This means that the standardization was successful by eliminating the scale effects. The amplitude of the standard deviation approaches 4 units in Jūrė and Venta and exceeds this value in the Plungė trial. A pooling of the standard deviation units for the Jūrė

Table 4. Significant region x trial interaction for three growth and four quality traits based on ANOVA, studying trials as replications, pairwise (Jūrė + Plungė, Jūrė + Venta, Plungė + Venta) and in combination of three (Jūrė + Plungė + Venta). *, **, and *** show significance of interactions at 5%, 1% and 0.1% level, respectively. Empty cell means non-significant interaction.

Traits	J+P	J+V	P+V	J+P+V
Stem volume	**		**	**
Tree height	***	**	***	***
Diameter	*		**	*
Height to dead branch			***	**
Height to green branch	***	***		***
Branch angle	*	*	*	**
Stem straightness				

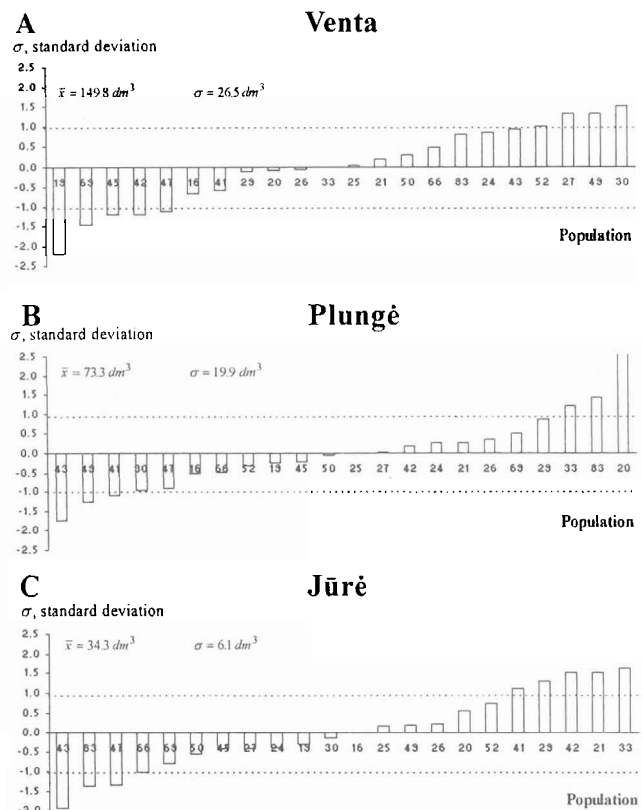


Fig. 2. Stem volume, expressed in standard deviation, for individual populations in three field trials of *Pinus sylvestris* in Lithuania

and Venta trials resulted in a drastic reduction of the amplitude to slightly above 2 units (Figure 3). Still lower amplitudes were obtained for the pooling into six regions of individual trials, Jūrė and Venta (Figures 4a-b, corresponding to columns 11 and 13 in Table 3). Finally, the smallest amplitude was obtained for column 15, six regions and pooling of the data from Jūrė and Venta, with an amplitude of 1.2 units (Figure 5). To obtain significant

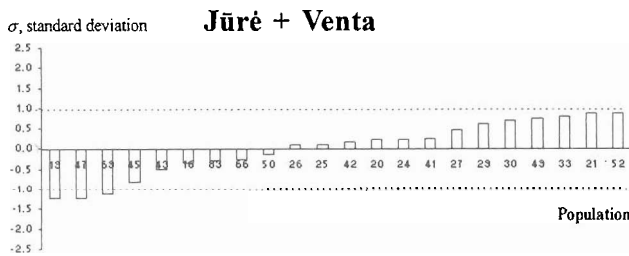


Fig. 3. Stem volume, expressed in standard deviation, for individual populations based on pooled data from two field trials of *Pinus sylvestris* in Lithuania

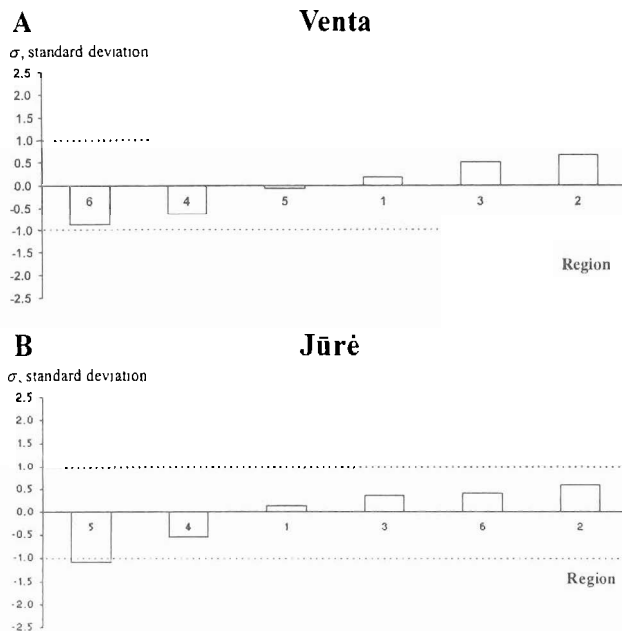


Fig. 4. Stem volume, expressed in standard deviation, for 6 geographical regions in two field trials of *Pinus sylvestris* in Lithuania

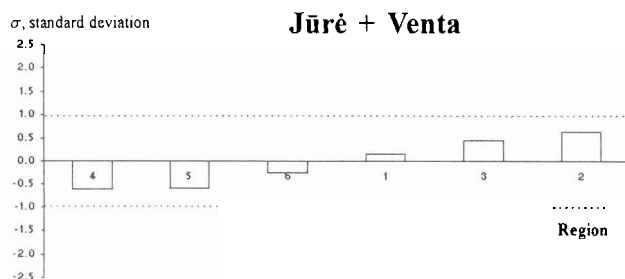


Fig. 5. Stem volume, expressed in standard deviation, for 6 geographical regions based on pooled data from two trials of *Pinus sylvestris* in Lithuania

differences for stem volume, a geographically wide pooling of the data was required. If the ranking of the populations differs considerably among trials, such a pooling leads inevitably to a strong reduction of the estimated differences. However, pooling is a necessary sacrifice to be able to prove any significant differences.

Among quality traits only stem straightness had a tendency to increase the number of significant differences with increased pooling of data (Table 3). Noteworthy is the opposite trend with disappearance of significance with increased pooling for height to first green branch. A similar disappearance of significance when extending the pooling from 14 to 6 regions was noted for height to dead branch. The possible explanation for these observations is that this trait is more specific for each population and pooling increases heterogeneity.

General remarks

It might be questioned whether results of the kind obtained in our investigation are of any use for tree breeding or expressed in another way: Is it worthwhile to invest in measurements in a series of trials leading to such a meagre resolution? The answer depends largely on the homogeneity within and among trials. If the homogeneity is good in both these respects investment would be useful. This is supported by our attempt to analyse the consequences of having the same ranking order in all trials. ANOVA was run based on stem volume in each trial under the assumption of identical ranking in all trials and using each trial as one replication. This analysis resulted in a strongly significant difference between the populations. With huge trials, the environmental heterogeneity mostly becomes a problem. We do not know if the variation in site conditions which we faced in our study is typical of other trials in the Prokazins series. If the variation is typical, the series will have limited implications for applied forestry.

Unreplicated trials of the kind studied in this investigation still have a value since they constitute one type of gene resource population (cf. Varela and Eriksson 1995) and as such may be useful for tree breeders and forest geneticists as archives in which various types of crosses can be carried out.

In conclusion, the value of unreplicated provenance trials is probably limited since the objective to identify populations performing well in the tested environment will rarely if ever be fulfilled. Neither will the objective of getting better understanding of the previous evolution of the species be obtained.

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АНАЛИЗ БЕЗ ПОВТОРНОСТЕЙ ЗАЛОЖЕННЫХ ГЕОГРАФИЧЕСКИХ КУЛЬТУР СОСНЫ ОБЫКНОВЕННОЙ (*PINUS SYLVESTRIS* L.).

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Резюме

В Литве, в трех районах в 1975 г. заложено географические культуры сосны обыкновенной (*Pinus sylvestris* L.), принадлежащие к так называемой серии культур Проказина. Всего использовано 44 популяции. В каждом районе заложены культуры из этих же самых популяции без повторных вариантов. Культуры изучались в возрасте 19-23 лет и оценены по трём признакам роста и четырём признакам качества. Были установлены статистические меж-популяционные различия во всех культурах. В связи с тем, что культуры были заложены в одном опыте без повторностей, никаких статистически достоверных различий доказать не удалось. ANOVA анализы были основаны на двух принципах: как повторности культуры были взяты из двух районов и всех трех районов вместе, а также произведена группировка популяции 14 и 6 регионов. Группировка популяции в шести регионах в некоторых случаях показали статистически достоверные различия между регионами по высоте деревьев. Эффекты в различиях были показаны как отклонение от среднего показателя по единицам стандартного отклонения. Когда ранги популяции отличались от среднего географических культур, как в нашем случае, такая группировка популяции неизбежно ведёт к существенной редукции оцененных различий. Всё-таки группировка популяции есть обязательное пожертвование отдельных популяций для доказательства статистических различий. Для использования без повторностей в одном опыте заложённых культур, они должны иметь гомогенные условия как внутри культур, так и между ними. В будущем такие географические культуры могут быть использованы как генетические ресурсы для различных типов размножения.

Ключевые слова: *Pinus sylvestris* L., популяция, рост деревьев, признаки качества, анализ без повторностей заложённых географических культур.