Effects of microsite variation on growth and adaptive traits in a beech provenance trial

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ABSTRACT: The effects of the within-trial spatial variation of environmental factors on phenotypic traits were studied in the Slovak plot of the international beech provenance trial coordinated by BFH Grosshansdorf with 32 provenances, established under a randomized complete block design with three adjacent blocks. Five indicators of soil properties (soil moisture, bulk density and pH) and microclimate (average daily temperature and temperature amplitude) were assessed at 96 points distributed over a 10 × 10 m grid and their values for the positions of individual trees were estimated by ordinary point kriging. The evaluation of phenotypic variation (height, diameter, Julian days of spring flushing and autumn leaf discoloration, vegetation period length, late frost damage) using a common two-way analysis of variance showed a significant provenance × block interaction effect indicating the heterogeneity of blocks. Analysis of covariance using single-tree kriging estimates of environmental variables as covariates showed that in addition to provenance, all phenotypic traits were significantly affected by microsite, especially by temperature fluctuation. Employing methods incorporating the spatial component in the evaluation of tree breeding field experiments is advocated.

Keywords: experimental design; Fagus sylvatica; geostatistics; microsite variation; provenance research, spatial variation

In genetic and breeding research on forest trees, homogeneous sites are scarcely available for field trials. Provenance experiments and progeny or clonal tests are usually established on forest land with variable soil conditions, frequently surrounded or bordered by older stands affecting the microclimate of the trial by modifying radiation and air currents. Even in case that abandoned nurseries or similar plots are used, soil properties may vary because of the presence of former roads, spatially variable use of fertilizers and irrigation within the plot etc. All these factors lead to the formation of environmental patches or gradients which may seriously affect the estimation of treatment effects in trials (Ye, Jayawickrama 2008).

Several experimental designs are used to cope with the environmental variation within trials. The most frequently used one in provenance research is the randomized complete block (RCB) design, where the trial area is subdivided into supposedly homogeneous (usually spatially continuous) blocks and each provenance represented by several trees appears once per block. The aim of such subdivision is achieving homogeneous environmental conditions within blocks so that blocking can remove the within-trial environmental variation by using blocks as a source of variation in an analysis of variance or comparable statistical procedures (Sokal, Rohlf 1995).

As forest trees belong to almost undomesticated plants (with very few exceptions), both basic genetic research and practical breeding have to work with large numbers of genetic entries (provenances, progenies, clones), whereby each entry has to be sufficiently represented to receive the reliable estimate of its value. Considering the space required for a tree at the age when the assessment of growth and qualitative traits can reliably be made, the sizes of blocks are usually too large to achieve environmental homogeneity in field trials on forest trees. This results in a significant block × entry interaction, leading to problems in the interpretation of the outcomes of statistical analyses (Potvin 2001; Saenz-Romero et al. 2001). Moreover, microsite

Supported by the Slovak Research and Development Agency, Grant No. APVV-0441-07 and by the COST Action E52.
conditions frequently exhibit spatial continuity at scales larger than the plot size but smaller than the block size, leading to spatial continuity of the measured traits. It has been shown by many studies on forest trees that the observed values on neighbouring plots tend to be more similar than the observations on distant plots (Fu et al. 1999; Joyce et al. 2002; Dutkowski et al. 2006; Zas et al. 2007; Zas 2008). In several cases, direct relationships between the environmental spatial variation and response patterns in genetic tests were observed, such as soil properties and visual Mg-deficiency symptoms (Beets et al. 2004) or wind patterns and Armillaria infection (Metzler et al. 2002; Augustin et al. 2005).

Spatial continuity poses a problem for the use of common statistical methods which are designed for samples drawn from random variables with independent and identically distributed errors (Sokal, Rohlf 1995). Several statistical techniques were proposed to solve this problem, which are generally based on searching for spatial structures in the data themselves incorporating the spatial aspect directly into the statistical treatment (Legendre et al. 1990; Fu et al. 1998; Dutkowski et al. 2002; Costa et al. 2005; Hong et al. 2005; Goncalves et al. 2007). However, the question remains how the variation in traits of interest can be linked with directly measurable environmental indicators. The aim of this study was to clarify to what extent the spatial variation of environment affects growth and adaptive traits in a provenance trial, and whether blocking can efficiently handle this variation.

**MATERIALS AND METHODS**

The study is based on the analysis of the Slovak trial plot of the international European beech (Fagus sylvatica L.) provenance experiment coordinated by the Federal Research Institute for Rural Areas, Forestry, and Fisheries, Institute for Forest Genetics, Grosshansdorf, Germany. The trial was established in 1998 in an abandoned forest nursery of ~ 1 ha at the locality Tále–Jablanka (central Slovakia, 19°02'E, 48°38'N, 810 m a.s.l.) with 2-years-old seedlings of 32 provenances covering practically the whole distribution range of beech in Europe, using the RCB design (three adjacent blocks).

In 2007 (at the age of the trial of 11 years), complex measurements of the trial were performed. Among growth traits, height and diameter (at breast height and at the height of 0.2 m) were recorded. As a strong night frost (up to -8°C) occurred during the night from 30 April to 1 May, frost damage was recorded subsequently using a 5-point scale (0 – less than 5% of leaves damaged, 1 – less than 1/3 of leaves damaged, 2 – less than 2/3 of leaves damaged, 3 – less than 95% leaves damaged, 4 – more than 95% leaves damaged). Spring flushing was scored on 12 days covering the whole flushing season of all trees using a modified scale of von Wuehlisch et al. (1995) (a 7-stage scale: 1 – dormant buds, 2 – buds swollen and elongated, 3 – buds begin to burst, first green is visible, 4 – folded and hairy leaves begin to appear, 5 – individually visible folded and hairy leaves, 6 – leaves unfolded, still fan-shaped, pale scales present, 7 – leaves unfolded, smooth and bright). Autumn discoloration was scored on 6 dates, again dispersed over the whole season, using a 5-stage scale (1 – green leaves, 2 – beginning of autumn colouring of individual leaves, 3 – beginning of autumn leaf colouring on a mass scale, 5–10% of leaves coloured, 4 – mass autumn leaf colouring, ~ 50% of leaves coloured, 5 – completed leaf colouring, 6 – leaves start to turn brown and to dry). The process of flushing represents an irreversible transition between two temporarily steady states: buds are closed for the whole winter, at a certain moment they start to open, develop into green leaves which remain green for the whole summer. Such a process can be best modelled by a sigmoid function:

\[ p = \frac{1 + 2 \tanh \left( \frac{d - c}{w} \right)}{2} \]

where:

- \( p \) – the phenological stage at Julian day \( d \)
- \( c \) – the midpoint of flushing, i.e. the Julian day when the middle stage is achieved (in our case, stage 4),
- \( w \) – the duration of the process,
- \( \tanh \) – the hyperbolic tangent function.

The same approach was used for the modelling of autumn discoloration. The length of the vegetation period was then assessed as the difference between the midpoints of autumn discoloration and spring flushing.

As the measured traits exhibited an obvious spatial continuity not only at the tree level but also at the provenance level (raw data available at the corresponding author), we mapped the variation of selected soil properties and microclimatic variables over the trial plot at 96 points, located in the centre of each provenance plot within each block (i.e. on a 10 x 10 m grid). Soil samples were taken on August 29, 2007, which was a day after a 15-day period of summer drought, from the uppermost soil layer.
(0 to 10 cm) using 100 ml Kopecky sampling cylinders to determine bulk density of soil. Moreover, samples from the 10 to 20 cm depth were used to assess the distribution of soil acidity (pH/H_2O) and soil moisture (gravimetrically, after drying at 105°C for 24 h). Soil temperatures were measured at the 10-cm depth on September 3, 2007 (a day with sunny weather) each hour from 07:00 to 18:00 using 96 Hg-thermometers. From temperature measurements, the average temperature and the amplitude were calculated.

Single-tree estimates of environmental variables were obtained through kriging. Sample omnidirectional variograms with 7.07 m (= ½ diagonal distance between sampling points) distance classes were constructed based on the observed data for all environmental variables and fitted to appropriate models. Ordinary point kriging was then used to estimate the values of environmental variables at the location of each tree. Variowin 2.2 (PANNATIER 1996) was used for all geostatistical analyses.

Two approaches were subsequently used for the statistical treatment of the data. Firstly, we applied a two-way analysis of variance under the classical RCB design. Both provenances and blocks were considered to be random-effect factors. Secondly, we used analysis of covariance with provenance as a categorical predictor and environmental variables as continuous covariates.

RESULTS

As shown in Fig. 1, the distributions of all environmental variables are spatially continuous. In the case of temperature regime descriptors and soil moisture, this is obviously due to the effect of shading. The trial plot is immediately surrounded by an adult beech stand (~ 110 years, mean stand height 31 m). The southern side of the plot receives direct sunlight in the morning, from the noon onwards it is completely shaded. The highest daily temperatures were observed along the SW-NE diagonal, which is the line receiving the highest solar radiation. Daily amplitude of temperatures follows a very similar (although not identical) pattern. The largest temperature fluctuations were observed in the centre of the plot, whereas they decrease towards the margins, influenced microclimatically by the adjacent beech stands. Consequently, soil moisture is high along the southern side of the trial plot, whereas in the centre the soil suffers from water deficit. A close coincidence of temperature and soil moisture patterns indicates that evaporation driven by solar irradiation is a more important determinant of soil water content than water retention capacity resulting from the soil texture and structure on this trial plot. The temperature and moisture pattern is reflected in the ground-layer vegetation at places where the canopy has not been closed yet: the large patch with a high soil moisture and a cold microclimate parallel to the southern side is covered by Tussilago farfara L. and Salix caprea L., whereas the open patches in the centre are overgrown with clonally spreading grasses, mainly Calamagrostis arundinacea (L.) Roth.

As the area had been used as a forest nursery before being converted into a provenance trial, we suspected that there might have been a road along the axis of the plot with compacted soil. However, the assessment of bulk density of soil did not confirm this assumption. There are patches of high and low soil density, maybe resulting from the former use, but they are irregularly distributed over the trial plot.

Soil acidity follows a relatively smooth gradient from the NW to the SE corner of the plot. Local fluctuations may be associated with the former use, but the plot-wide trend itself seems to be caused by changes in the bedrock, as the trial is located in a volcanic area where lava streams and tuff sediments of varying chemical composition may alternate over small distances.

Experimental variograms reflect the observed spatial continuity in environmental data, as the semivariance increases with distance in all variables, at least for distance classes up to 40 m (= one half of the shorter dimension of the rectangular trial plot). Out of the five variograms, 2 were fitted to the classical spherical model, 2 to the exponential model and 1 to the Gaussian model (Fig. 2).

The analysis of data under the classical RCB design brought expected outcomes (Table 1). The effect of provenance proved to be significant for all assessed traits, which is not surprising considering that the set of tested provenances covers almost the whole distribution range, even comprising one population belonging to a different taxon (Gramatikovo, Bulgaria, F. sylvatica L. ssp. orientalis Lipsky). The effect of blocks was non-significant with a single exception of the flushing midpoint date, proving that the spatial arrangement of blocks used was very inefficient in handling the microenvironmental variation within the trial site. All blocks are clearly heterogeneous with respect to all environmental variables that we assessed. On the other hand, the provenance × block interaction was highly significant (P < 0.001) for all traits, indicating that there exists environmental
variation affecting the phenotypic expression on a scale larger than a provenance plot size within a block but smaller than the block size. The percentages of variance explained by the models range from 29% to 46%, indicating that a substantial portion of the trait variation is caused by genetic variation within populations and/or environmental variation on very small scales.

Analysis of covariance confirmed the expected significant provenance effect on all traits (Table 2). Generally, growth traits were significantly influenced by soil properties. Among microclimatic indicators, temperature fluctuation during the day rather than the daily average seems to influence yield and adaptive traits. A significant effect of soil pH on phenology traits might be a statistical artifact resulting from the spatial pattern: soil acidity changes along the NE-SW gradient, which partially coincides with the spatial pattern of the amount of solar radiation. On the other hand, a direct relationship (although

![Fig. 1. Spatial patterns of the assessed environmental variables over the area of the beech provenance trial](image)

**Table 1. Analysis of variance (significance of F-tests) of the beech provenance trial under the RCB design: full set of provenances**

<table>
<thead>
<tr>
<th>Trait</th>
<th>Source of variation</th>
<th>( R^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>provenance</td>
<td>block</td>
</tr>
<tr>
<td>Height</td>
<td>***</td>
<td>NS</td>
</tr>
<tr>
<td>Diameter at 1.3 m</td>
<td>***</td>
<td>NS</td>
</tr>
<tr>
<td>Diameter at 0.2 m</td>
<td>***</td>
<td>NS</td>
</tr>
<tr>
<td>Flushing midpoint</td>
<td>***</td>
<td>**</td>
</tr>
<tr>
<td>Cessation midpoint</td>
<td>***</td>
<td>NS</td>
</tr>
<tr>
<td>Vegetation period</td>
<td>***</td>
<td>NS</td>
</tr>
<tr>
<td>Frost damage</td>
<td>***</td>
<td>NS</td>
</tr>
</tbody>
</table>

*\( P > 0.95, ** P > 0.99, *** P > 0.999, \) NS – not significant
not necessarily causal) between phenology and soil reaction has been found in beech (BEESE 1991).

**DISCUSSION**

Actually, direct inclusion of environmental variables did not increase the predictive power of the models: $R^2$ for ANCOVA models were lower compared to ANOVA under the RCB design for all traits. This was not surprising considering the fact that the phenotypic response to environmental factors need not necessarily be linear (REHFELDT et al. 1999). Moreover, quite rough indicators of microclimate and very incomplete descriptors of soil properties were used. Temperature measurements performed during one day only do not properly characterize the temperature regime of the trial plot. On the other hand, as they were taken on a sunny summer day when the largest temperature differences between irradiated and shaded places can be expected, on a large number of measur-

Table 2. Analysis of covariance (significance of F-tests) of the beech provenance trial considering soil properties and temperature distribution as covariates

<table>
<thead>
<tr>
<th>Trait</th>
<th>Source of variation</th>
<th>soil moisture</th>
<th>soil density</th>
<th>pH/H$_2$O</th>
<th>temperature average</th>
<th>temperature amplitude</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height</td>
<td>***</td>
<td>NS</td>
<td>**</td>
<td>*</td>
<td>NS</td>
<td>***</td>
<td>0.248</td>
</tr>
<tr>
<td>Diameter at 1.3 m</td>
<td>***</td>
<td>***</td>
<td>**</td>
<td>***</td>
<td>NS</td>
<td>***</td>
<td>0.220</td>
</tr>
<tr>
<td>Diameter at 0.2 m</td>
<td>***</td>
<td>**</td>
<td>**</td>
<td>***</td>
<td>NS</td>
<td>NS</td>
<td>0.225</td>
</tr>
<tr>
<td>Flushing midpoint</td>
<td>***</td>
<td>NS</td>
<td>NS</td>
<td>***</td>
<td>NS</td>
<td>NS</td>
<td>0.408</td>
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<tr>
<td>Cessation midpoint</td>
<td>***</td>
<td>NS</td>
<td>NS</td>
<td>*</td>
<td>**</td>
<td>***</td>
<td>0.249</td>
</tr>
<tr>
<td>Vegetation period</td>
<td>***</td>
<td>NS</td>
<td>NS</td>
<td>*</td>
<td>NS</td>
<td>***</td>
<td>0.286</td>
</tr>
<tr>
<td>Frost damage</td>
<td>***</td>
<td>***</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>***</td>
<td>0.317</td>
</tr>
</tbody>
</table>

$^*P > 0.95, ^{**}P > 0.99, ^{***}P > 0.999, NS – not significant$
ing points, they should properly reflect the spatial distribution of heat accumulation within the plot. We preferred to measure soil temperatures as they exhibit less random fluctuations than air temperatures, mainly when the tree distribution over the plot is fairly irregular, and are important for the phenology of hardwoods (Baldočchi et al. 2005; Dittmar, Elling 2006). The same applies to soil properties. Soil moisture is known to affect growth and even phenology in beech (Nielsen, Jørgensen 2003; Schieber 2006; Jazbec et al. 2007). However, the permanent monitoring of soil water content over a large network of points regularly distributed over the trial plot was not feasible technically. Nevertheless, scoring soil moisture after a relatively long drought (15 days) allowed us to distinguish the places with a regular rapid decrease of moisture due to exposure to radiation from places retaining soil water even in the upper densely rooted layers. Similarly, bulk density and acidity are only two examples of physicochemical soil variables, and although they were shown to affect growth in beech (Röhé 1985), by far they do not exhaust all soil properties that may be relevant.

The question remains whether a different arrangement of blocks could efficiently treat the microsite variation within the trial. As the temperature amplitude exerted a highly significant effect on both growth and phenology traits, we used it for a redefinition of replications within the trial. Provenance plots were ranked according to the temperature amplitude and classified into three equal-size classes (blocks): with high, average and low temperature fluctuation, without respect to spatial continuity (Table 3). Provenances were represented in the newly defined classes very irregularly: extreme cases are two provenances placed solely in high-fluctuation patches within all their original blocks. Such patches represent the least suitable environment, resulting in high mortality (data not shown). Actually, a kind of positive feedback may have contributed to this pattern: the plots of mal-adapted provenances with poor growth and high mortality remain open, without closed canopy, and thus exposed to microclimatic extremes, leading to a further drop of survival (Saenz-Romero et al. 2001). The subset of provenances represented in all three “blocks” contained only 10 out of the 32 provenances, and was subsequently subjected to a repeated analysis of variance under the RCB design. As expected, this approach did not help very much. For some traits, $R^2$ slightly improved, but the provenance × block interaction remained significant in all cases. Actually, the “blocking” we used removed only the effects of a single environmental factor (temperature fluctuation). The other ones exhibited different spatial distributions. Moreover, different traits were shown to be differently influenced by individual environmental variables. Naturally, multivariate approaches such as principal components or factor analysis can be used to extract main environmental factors, but such factors typically represent only a minor part of environmental variation and their interpretation is not always straightforward (Gömöryová, Gömöry 1995).

Table 3. Analysis of variance (significance of F-tests) of the beech provenance trial under the RCB design: redefined blocks, subset of 10 provenances

<table>
<thead>
<tr>
<th>Trait</th>
<th>Source of variation</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>provenance block</td>
<td></td>
</tr>
<tr>
<td>Height</td>
<td>*</td>
<td>NS</td>
</tr>
<tr>
<td>Diameter at 1.3 m</td>
<td>*</td>
<td>NS</td>
</tr>
<tr>
<td>Diameter at 0.2 m</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Flushing midpoint</td>
<td>***</td>
<td>*</td>
</tr>
<tr>
<td>Cessation midpoint</td>
<td>*</td>
<td>NS</td>
</tr>
<tr>
<td>Vegetation period</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Frost damage</td>
<td>***</td>
<td>NS</td>
</tr>
</tbody>
</table>

* $P > 0.95$, ** $P > 0.99$, *** $P > 0.999$, NS – not significant.
nance experiments (Zobel, Talbert 1984; König 2005), does not properly handle the spatial variation of site conditions. In our case, blocks were delineated with respect to the shape of the trial. However, any other systematical or random arrangement of blocks would result in a similar heterogeneity. Naturally, if the relevant environmental factors were thoroughly mapped on the plot prior to establishing the experiment, the arrangement of replications could be optimized. However, such an approach could result in spatially discontinuous blocks, potentially leading to problems with the measurement of experiments. Moreover, a direct within-trial mapping of environmental indicators is scarcely done in breeding experiments, as the matter of interest is mostly the composite effect of environmental differences among trials on phenotypic variation rather than its decomposing into the effects of single environmental factors. In any case, the study demonstrates that the within-trial spatial variation cannot be ignored, spatial patterns must be considered when breeding values of parents are assessed in progeny tests or when geographic trends observed in provenance experiments are interpreted in terms of adaptation. Recording tree positions during the measurements of trials is thus indispensable, as it allows for alternative approaches to evaluations (Fu et al. 1999; Saenz-Romero et al. 2001; Costa et al. 2005; Dutkowski et al. 2006; Zas et al. 2007).

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Received for publication July 2, 2010
Accepted after corrections January 5, 2011

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