Efficiency of 3^5 factorial design determined using additional information on the spatial variability of the experimental field

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SUMMARY

The paper presents the results from two methodological 3^5 factorial experiments with pea conducted on heterogeneous soils in north-eastern Poland in 2001 and 2002. Before beginning the experiments soil samples were taken and traits were measured on the control plots to evaluate the spatial variability of experimental fields. The results from experimental plots were analyzed by the classical ANOVA, as well as ANCOVA with spatial characteristics as covariates. Finally, indices of relative efficiency (*RE*) of factorial designs and alternative analyses with the use of additional information on spatial variation were calculated. The relative efficiency of factorial designs with incomplete blocks in relation to a hypothetical RBD was in the range 124-162% and 102-117% for plot yield and biometric traits per plant, respectively. At the same time the increase of relative efficiency of ANCOVA of seed yield with soil properties and control plot traits as covariates was within a range of 48-60%, 37-49% and 0-5% in relation to a completely randomized design, a randomized block design and a factorial design with incomplete blocks, respectively. All covariance analyses with a single covariate can be an alternative to analysis of variance in a factorial design with blocking, but the only increase in efficiency repeatable in both experiments was recorded when all soil properties, pH, P, K and Mg, were used as covariates (increase 4-7%). It was stated that when there is a problem with confounding of important agricultural main and/or interaction effects with blocks, soil variation can be eliminated through a combination of statistical and geostatistical methods.

Key words: experimental design, factorial design, spatial variation, geostatistics, kriging.

1. Introduction

The theoretical basis for factorial designs with the same number of levels for each factor were stated in the first half of the 20th century (Fisher 1926, 1942a; Yates 1935; Snedecor 1937). These designs were applied mainly in industrial research, but recently agronomists too have tried to use them in their studies. Factorial designs seem to be especially valuable at the initial stage of a study, or when a complex of agricultural factors have to be tested, e.g. in the context of a new plant production technology. The main barriers to the broad application of these designs in agricultural experimentation are the high costs connected with the need to test a great number of treatments, and problems with soil variability control. Despite the fact that many methodological problems of factorial designs in agricultural experimentation still need to be solved, there is an increasing interest in their application. This is confirmed by a number of field experiments conducted at many Polish research institutions (Kuś et al. 1992; Mądry et al. 1995; Kukuła and Pecio 1998; Wyszyński 2004). The main characteristic of these experiments is two levels for each factor. This approach assumes linearity of treatment effects even if the factors are quantitative, while it is commonly known that effects of agricultural factors e.g. date of sowing, sowing density, fertilization, etc. are usually curvilinear.

Thus it is obvious that, in agricultural practice, a design including three levels of each factor should be developed. Until now there have been no reports on the application of factorial designs with three levels in which the number of factors exceeded four.

In agricultural field factorial experiments an increase in the number of factors causes an exponential increase in the number of treatments to be tested, and in experimental area. For instance, when four factors are tested it is necessary to establish 81 plots for 81 treatments; when there are five factors, 243 plots and treatments have to be tested. This large number of treatments is the main reason for maximal reduction of replications (to one), and for soil variation control (to some extent) by grouping plots into incomplete blocks. When 3^k designs are applied in practice it is assumed that three-factor and higher interactions are negligible, so usually one or more of them are sacrificed as the so-called generator(s) of blocking, and the others are included in the experimental error. With the above assumption, the degrees of freedom for the experimental error are sufficient, so it seems rational to use one replication in 3^k experiments, although it should be remembered that, in an agricultural field study, plot(s) may be destroyed (Cochran and Cox 1957; Mądry et al. 1995; Gołaszewski and Szempliński 1998). The effective control of soil variability in the experimental field by blocking may also pose a problem. An increase in the number of blocks results in an increase in the confounding (aliasing) of treatment effects with soil variability, which leads to identical estimates of soil variation and treatment effects (Barnard 1936; Yates 1937; Bose and Kishen 1940; Fisher 1942b; Finney 1947; Oktaba 1956; Cochran and Cox 1957).

Efficient estimation of treatment effects from factorial experiments conducted in natural field conditions is guaranteed only when the spatial variability of the field is as uniform as possible, and the experimental error is inflated by soil variation to a small extent only. However, it is difficult to proceed on this assumption in practice. To overcome the problem, an alternative methodological approach to factorial experimentation might be taken. Numerous papers have recently been published on the application of geostatistics to spatial variability estimation in agricultural experiments (Bhatti et al. 1991; Van Es and Van Es 1993; Gołaszewski 1997). The pioneers of geostatistics were Kriege (1966), who used an extrapolation method to predict gold deposits, and Matheron (1971), who used this method to develop a theory of regionalized variables. Many geostatistical terms have a geological origin, e.g. nugget effect, sill and kriging (from Kriege). Among a variety of geostatistical procedures, two methods seem to have great practical importance: semivariance and kriging. The former is a measure of spatial autocorrelation of observations at distance *h*, the latter enables prediction of points, which were not sampled (Cressie 1993).

In field experimentation the process of spatial variability estimation with geostatistical methods can be summarized as follows: (i) estimation of semivariances for the distance *h*, (ii) analysis of a semivariogram and fitting of a mathematical model, and (iii) kriging with the use of model parameters to predict plot values (Gołaszewski 1997, 2000).

The application of spatial variability to statistical analysis changes the efficiency of experiments. The purpose of many recently published methodological papers on field experiments was to assess relative efficiency when information about spatial variability estimated by different methods (geostatistics, nearest neighbor analysis, trend analysis, etc.) was included (Bhatti et al. 1991; Brownie et al. 1993; van Es and van Es 1993; Gołaszewski 1999; Qiao et al. 2000). However, the use of spatial information to improve experiment efficiency is still at the stage of comparison between methods .

The objective of the study was to present a two-stage process of analyzing the results from factorial experiments, and to estimate the relative efficiency of the experiments. In the first stage the spatial variability of the experimental field was estimated by geostatistical methods, and then information about spatial variation, in the form of concomitant variables, was included in the analysis of covariance.

2. Material and methods

The basis of the study was the results from two one-replicated 3^5 field experiments with pea (*Pisum sativum* L.), conducted in north-eastern Poland (Experimental Station – Tomaszkowo) in 2001 (D_01) and 2002 (D_02). The soils of the region are very heterogeneous, so the main problem for experimenters is effective control of soil variability. In the experiments there were 9 blocks in 5 strips with 27 treatments per block (2 blocks per each strip in four strips, and 1 block in the last strip). Each of five factors, i.e. A – cultivars, B – sowing date, C – sowing density, D – fertilization and E – plant protection, had three levels, denoted 0, 1, 2. The plot size was 9 m^2 with six rows, 6 m long, 0.3 m apart.

For the sake of geostatistical analysis, before starting the experiments a total of 170 soil samples were collected to determine pH and available macronutrients: phosphorus (P), potassium (K), and magnesium (Mg). The nodes were taken at the nodes of a regular grid with 4 x 6 m meshsize. The alleys between strips were sown with one cultivar, and measurements of plants (plant height and seed weight) were taken on the control plots, on both sides of the experimental plot. Soil properties at the sampling points and plant characteristics in the control plots provided the basis for calculating semivariances (Eq.1), and for fitting a semivariogram model. Finally kriging was applied to the predict values of all soil chemical properties for each plot.

$$\gamma(h) = \frac{1}{2 \cdot N(h)} \sum_{i=1}^{N(h)} [z(i) - z(i+h)]^2 \qquad (1)$$

for i = 1,2,3, ..., $N(h)$, where: $N(h)$ - number of observation pairs $\{z(i), z(i+h)\}$ distant by h.

At harvest, 10 plants from each plot were sampled to measure morphological and yield component traits (plant height, height to the first pod, no. of nodes to the first pod, no. of nodes with pods, no. of pods, no. of seeds, seed weight). The other plants from each plot were threshed, and the seeds were weighed.

A linear model with main effects and two-factor interactions was used in the statistical analysis, while all higher-order interaction effects were incorporated into the experimental error. ANOVAs of a completely randomized design (Eq.2) and a design with incomplete blocks (Eq.3) were found:

$$y_{ijklm}=\mu+A_i+B_j+AB_{ij}+C_k+AC_{ik}+BC_{jk}+D_l+AD_{il}+BD_{jl}+CD_{kl}+E_m+AE_{im}+BE_{jm}+CE_{km}+DE_{lm}+\varepsilon_{ijklmn} \qquad (2)$$

$$y_{ijklmn}=\mu+\pi_n+A_i+B_j+AB_{ij}+C_k+AC_{ik}+BC_{jk}+D_l+AD_{il}+BD_{jl}+CD_{kl}+E_m+AE_{im}+BE_{jm}+CE_{km}+DE_{lm}+\varepsilon_{ijklmn} \qquad (3)$$

where i, j, k, l, m denotes the level combinations (0, 1, 2) for five factor A, B, C, D, E, y_{ijklm} is the plot value, π_n n = 1, 2, ..., p denotes the n^{th} block, A_i, B_j, C_k, D_l, E_m are main effects, AB_{ij}, AC_{ik}, AD_{il}, AE_{im}, BC_{jk}, BD_{jl}, BE_{jm}, CD_{kl}, CE_{km}, DE_{lm} two-factor interaction effects and $\varepsilon_{ijklm(n)}$ is the random error, where the error terms are assumed to be iid $N(0, \sigma^2)$.

ANCOVA of a completely randomized design with predicted values as concomitant variables was performed. The ANCOVA model relating the dependent variable Y_i to its mean and the single covariate X_i, is (Milliken and Johnson 2002)

$$y_i = \mu_i + \beta x_i + \varepsilon_i, \quad i = 1, 2, \ldots, n, \tag{4}$$

where i denotes the i^{th} treatment combination, μ_i the mean response of the i^{th} treatment combination when the value of x is zero, β denotes the slope of the regression lines, and ε_i denotes the random error, where the error terms are assumed to be iid $N(0, \sigma^2)$;
and ANCOVA with multiple covariates is

$$y_i = \mu_i + \beta_1 x_{i1} + \beta_2 x_{i2} + \ldots + \beta_h x_{ih} + \varepsilon_i, \quad i = 1, 2, \ldots, n, \tag{5}$$

where h is the number of covariates, i denotes the i^{th} treatment combination, μ_i the mean response of the i^{th} treatment combination when the value of all of the x_{ik}'s are zero, β_k (k = 1, 2, …, h) are the slopes of regression planes in the direction of the respective covariates, and ε_i denotes the random error, where the error terms are assumed to be iid $N(0, \sigma^2)$.

In order to compare different approaches to data analysis, relative efficiency (RE%) was calculated. Because of the confounded effects of soil variability with treatment effects due to blocking, and the lack of comparable experiments in randomized block designs (RBD), the theoretical mean square error for RBD (MSE_{RBD}) was approximated with the formula suggested by Yates (1935) and Oktaba et al. (1956)

$$MSE_{RBD} = \frac{df_B \times MSB + df_T \times MSE}{df_B + df_T} \tag{6}$$

where df_B, df_T are degrees of freedom for blocks and treatments respectively, and MSB, MSE are the mean squares for blocks and error respectively.

The relative efficiency of a factorial design (FD) in relation to a RBD was calculated acc. to Eq.7,

$$RE_{1/2} = \frac{(df_1 + 1)(df_2 + 3)MSE_2}{(df_2 + 1)(df_1 + 3)MSE_1} \cdot 100\% \tag{7}$$

where df_1, df_2 are the degrees of freedom of the factorial design and RBD respectively, and MSE_1, MSE_2 are the mean squares of FD and RBD respectively.

The relative efficiency of ANCOVA to ANOVA was calculated acc. to Eq.8 (Steel and Torrie 1980). According to Steel and Torrie, to test the effectiveness of covariance as means of error control, a comparison of the variance of a treatment mean before and after adjustment for the independent variables X_i should be made.

$$RE = \frac{100[MSE.Y]}{MSE.Y(adj.)\left[1+\frac{MST.X_i}{SSE.X_i}\right]} \quad (8)$$

where MSE.Y is the mean square error of Y before adjustment, MSE.Y(adj.) is the mean square error of Y after adjustment, $MST.X_i$ is the mean square for treatments of X where i denotes the number of variables, $SSE.X_i$ is the sum of squares for error of X.

3. Results

The results of geostatistical analysis showed that all chemical soil properties were spatially correlated. The spatial variability of pH and phosphorus content in D_01 and pH and magnesium content in D_02 was described with a spherical model, while that of the other properties was described with a linear one (Tab. 1)[1]. The proportion of structural variance in the sill for all spherical models was relatively high, and spatial dependence of observations was within a range of 15-24 m in D_01 and 11-18 m in D_02.

Among the plant characteristics determined on the control plots, only observations of plant height in the second experiment had a slight tendency to spatial dependence with smaller semivariance for lag 1 (1.5 m) and a similar level of semivariance for longer distances. Generally, it can be assumed that variation of plant height and seed yield measured on the control plots across the experimental field was approximately random and equal to the sample variance.

The results concerning the efficiency of factorial designs with incomplete blocks in relation to RBD for traits per plant and seed yield per plot are presented in Table 2.

High relative efficiency was obtained for traits showing high variation and largely dependent on environmental conditions. Generally, in comparison with a randomized block design (RBD), a factorial design with incomplete blocks (FD) was more effective in 2002 (D_02) when climate conditions during the season and soil fertility of the experimental field were more differentiated than in 2001 (data not presented). In estimation of seed weight per plot the efficiency of FD to RBD was 124% and 162%. As regards biometric plant traits, efficiency was relatively low, in a range of 102-117%. Such results suggest that potential efficiency is likely to be higher for agronomic utilitarian characteristics measured per plot, such as yield, than for biometric traits measured per plant.

The analysis of the relative efficiency of 3^5 factorial design with additional information on spatial variation, compared with CRD and RBD, showed that the mean increment in efficiency was 14-16% and 11-13% respectively when plant height was analyzed, and 48-60% and 37-49% when seed weight was analyzed (Table 3).

[1]range – distance where the points of observation are spatially dependent; nugget effect is the effect of measurement errors and limits the precision of interpolation; structural variance represents the range of variance due to spatial dependence in the data; sill denotes the value at which the variance stabilises.

Table 1. Variogram parameters in estimation of the spatial variability of chemical soil properties.

Variogram parameters(*)	**D_01**				**D_02**			
	PH	P_2O_5	K_2O	Mg	pH	P_2O_5	K_2O	Mg
Model (S – spherical, L – linear)	S	S	L	L	S	L	L	S
Range *(a) (in m)*	24	15	-	-	11	-	-	18
Nugget effect (C_0)	0.010	6.150	8.620	0.374	0.080	19.304	7.196	0.095
Structural variance (C)(**)	0.073	11.535	0.222	0.023	0.135	0.253	0.253	1.125
Sill $(C+C_0)$	0.083	17.685	-	-	0.215	-	-	1.220

Note:* for linear model – regression coefficient

Table 2. Efficiency of factorial designs 3^5 with incomplete blocks to randomized block designs in estimation of pea traits (in %).

Experiment	Plant height	Height to first pod	No of nodes to first pod	No of nodes with pods	No of pods	No of seed	Seed weight from one plant	Seed weight
D_01	109	104	114	122	107	110	117	124
D_02	117	102	103	114	108	111	115	162

The above estimates were similar to those calculated in relation to FD with blocks. It should be noted that in the case of to FD with blocks, including additional information on spatial variation in the seed yield analysis resulted in a higher increase in efficiency, by about 2% when seed yield on the control plots was taken as a covariate, and by 5% when all soil properties, pH, P, K, Mg, were included as concomitant variables.

4. Discussion and summary

The results of spatial analysis showed that experimental fields were heterogeneous in all soil properties determined in the study, while the variation of traits recorded on the control plots sown with the same cultivar was random. Fitted linear and spherical models of semivariograms are typical of agricultural studies on spatial variation in field experiments (Kristensen and Ersbol 1992; Gołaszewski 1996, 1997, 1999). The relative efficiency of 3^5 factorial design with blocks in relation to a hypothetical randomized block design was higher for highly variable traits, largely dependent on environmental conditions. For such a trait – plot yield – relative efficiency was in the range 124-162%, and for biometric traits measured per plant it lay in the range 102-117%. Oktaba et al. (1956) reported similar relations concerning relative efficiency for plot and plant traits in 4^3 factorial experiments with spring wheat.

The increase in relative efficiency (RE) of 3^5 factorial experiment with additional information on spatial variability depended on the plant trait or soil property included in the study. It was significantly higher in relation to a completely randomized design (CRD) and a randomized block design (RBD) than in relation to a factorial design with blocks (FD). In assessment of plant height RE was in the ranges 14-16%, 11-13% and 0-1%, and in assessment of seed weight it was in the ranges 48-60%, 37-49% and 0-5%, for CRD, RBD and FD respectively.

In the present study considering all soil properties in spatial variation estimation, and adopting them as concomitant variables in only covariance analysis, generated the highest repeatable efficiency of experiments, this being in the range 4-7%. It is noticeable that all covariance analyses with a single covariate can be an alternative to analysis of the variance of factorial designs with blocking. The above conclusion is of practical significance because if there are any problems with the confounding of important agricultural main or interaction effects with soil variation at the stage of planning, some additional measurements to describe spatial variation may be an alternative to blocking. Besides, the conclusion may also be considered in the context of maximal reduction in treatments to be tested in fractional designs where the problem of confounding is of primary importance, and a rational way to eliminate spatial variation is a combination of geostatistical and statistical analyses.

Table 3. Relative efficiency of 3^5 factorial designs determined by ANOVA and ANCOVA with additional information on spatial variability.

Analysis methods	CRD			RBD			FD with blocks		
	D_01	D_02	average	D_01	D_02	Average	D_01	D_02	average
PLANT HEIGHT									
$ANOVA_{(FD)}$ with blocks	112	120	116	109	117	113	-	-	-
ANCOVA, covariates:									
PH	111	119	115	108	116	112	99	100	100
P_2O_5	110	118	114	108	115	111	99	99	99
K_2O	111	119	115	109	116	112	100	99	100
Mg	111	120	115	109	117	113	99	100	100
pH, P_2O_5, K_2O, Mg	111	118	115	109	116	112	99	99	99
plant height $_{(control\ plots)}$	111	120	116	109	118	113	100	101	100
seed weight $_{(control\ plots)}$	110	119	114	108	116	112	99	99	99
SEED WEIGHT									
$ANOVA_{(FD)}$ with blocks	130	178	154	124	162	143	-	-	-
ANCOVA, covariates:									
PH	124	177	151	118	161	140	96	99	98
P_2O_5	123	181	152	117	165	141	94	102	98
K_2O	134	177	156	128	161	145	103	99	101
Mg	126	170	148	120	155	137	97	95	96
pH, P_2O_5, K_2O, Mg	134	186	160	128	169	149	104	107	105
plant height $_{(control\ plots)}$	125	172	148	119	156	138	96	96	96
seed weight $_{(control\ plots)}$	131	182	156	125	165	145	101	102	102

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