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Spatial dependence and block designs in spaced plant herbage trials

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SUMMARY

Data from three ryegrass spaced plant variety trials conducted at Crossnacreevy, Co. Down in each of 1989, 1990 and 1991 were investigated for evidence of spatial dependence. Spatial dependence was most evident in variates measuring the overall dimensions of the plants, especially late season variates. However, the 'date of ear emergence' variate, which identifies most of the variety distinctnesses that the trials are intended to detect, was not spatially dependent. On average, 45% of the residual variation in the plot means of the spatially dependent variates was estimated to be of a spatial nature. Consequently, it is likely that the efficiency of analysis of these variates would be improved by using spatial analysis instead of non-spatial analysis or by using alpha (incomplete block) designs instead of complete block designs. The spatial information was used to determine the optimal incomplete block sizes and the likely increase in efficiency due to using alpha designs instead of complete block designs. The optimal incomplete block size for a trial with 10 plants per plot was compared with the optimal incomplete block size for the same trial with single plant plots at the same spacing and was found to be smaller. The gains in efficiency were larger in the trial with 10 plants per plot than in the trial with single plant plots.

INTRODUCTION

Observations made on field trials are prone to spatial dependence between plots. This dependence can be caused by spatial variation in fertility, moisture, intercepted light, and other environmental factors (Grondona *et al*. 1996). Conditions in plots spatially close together are more likely to be similar than in plots further apart, therefore, providing the plants are not in competition, observations made on plants grown on spatially close plots are more likely to be similar in those characteristics that are affected by the environmental conditions than are observations made on plants grown on more distant plots (van Es & van Es 1993).

The main objective of variety trials is to obtain precise estimates of the variety means and variety differences that affect performance under farm conditions (Grondona *et al*. 1996). To estimate the variety effects efficiently, i.e. with small variances, it is necessary to minimize either by design or analysis the residual variation that is not due to variety effects. Design choices, including the block design and the block and plot sizes, configurations and orientations, are all means by which the experimenter can attempt a priori to reduce the residual variation caused by spatial dependence (Cressie 1991; Kristensen & Ersbøll 1992; van Es & van Es 1993; Grondona *et al*. 1996). When the data are spatially dependent, the choice of design also affects the efficiency of estimation of variety effects due to the positions of the varieties within the trial (Martin 1986; van Es & van Es 1993).

Spatial analysis provides the experimenter with an a posteriori modelling method for reducing the variation caused by spatial dependence (Grondona *et al*. 1996). Large scale variation or global trend can be modelled by polynomial regression (Outhwaite & Rutherford 1955), by smoothing methods (Hackett *et al*. 1995), or by modelling row and column effects (Gilmour *et al*. 1997). Small scale variation or local trend can be modelled by one of two basic categories of spatial model: the nearest neighbour type model (Besag & Kempton 1986; Cullis & Gleeson 1991; Kempton *et al*. 1994), and the random field type model (Grondona & Cressie 1991; Zimmerman & Harville 1991; Gilmour *et al*. 1997).

The conventional analysis of variance of a classical randomized design is non-spatial and is based on ordinary least squares (OLS). In the presence of

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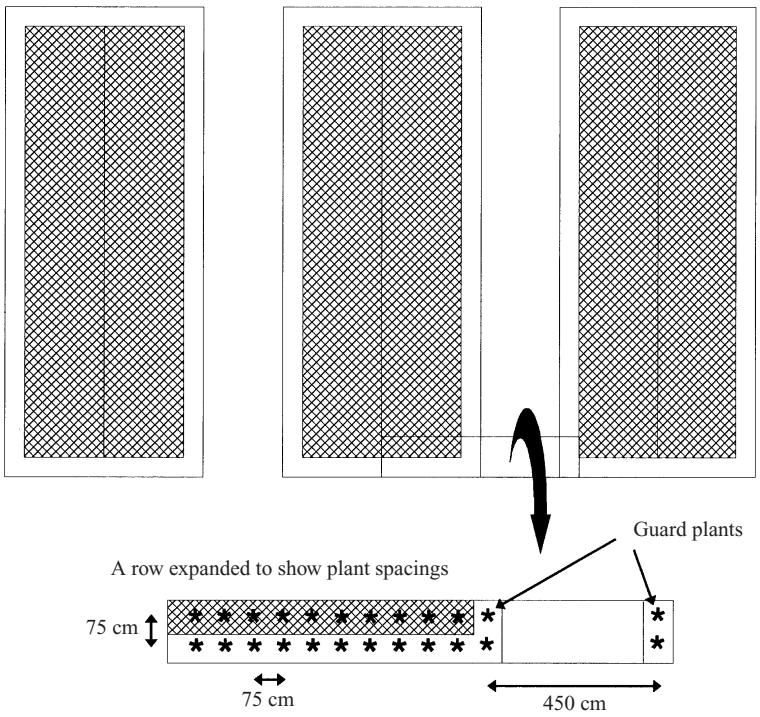


Fig. 1. Field layout of the six blocks in the three tetraploid perennial ryegrass (PRT) trials and the 1989 and 1991 diploid Italian ryegrass (IRD) trials.

spatial dependence, randomization theory (Bailey 1981) predicts that the OLS estimators of variety effects will be unbiased. However, good estimators must also be efficient, i.e. they must minimize the error variances, and OLS estimators are clearly not efficient when there is substantial spatial dependence.

A further problem when the data are spatially dependent is that the OLS estimates of the variances of the variety estimates are inappropriate because they use an average estimate of error based on a single estimate of the residual variation. The OLS analysis ignores the fact that some variety comparisons involve plots that are a short distance apart and, as a result of spatial dependence, have a smaller variance than those with plots that are a greater distance apart (van Es & van Es 1993). In consequence, if variety differences are small and OLS is used, strong spatial dependence may reduce the power of an analysis to correctly estimate and identify variety differences. Tests on near neighbours may underestimate the significance of variety differences, whereas tests on more distant comparisons may falsely identify non-significant variety differences as significant.

Clearly, if spatial dependence is likely, the ideal would be to use a spatial design and a spatial method of analysis. However this has cost implications in terms of more complex analysis and interpretation, and the benefits depend on the strength and range of the spatial dependence. Studies by Zimmerman & Harville (1991) and Grondona & Cressie (1991) show that spatial dependence does occur in field trial yield observations and the purpose of this paper is to investigate the prevalence and extent of spatial dependency in varietal distinctness, uniformity and stability (DUS) data arising from spaced plant herbage trials. (See Weatherup (1980) for a discussion of DUS trials.) Current designs and analyses for these trials ignore any spatial dependence between the observations. This paper assesses the implication of spatial dependence between the observations for the design and analysis of DUS trials.

METHODS

The field trial data

The data came from DUS spaced plant herbage variety trials conducted at Crossnacreevy, Co. Down. In these trials, new and established varieties are grown as spaced plants to determine if the new varieties can be distinguished on the basis of measured characters from the established varieties and also from each other. A new variety must be shown both to be uniform, i.e. to have a spread for each of the important plant characteristics that is similar to

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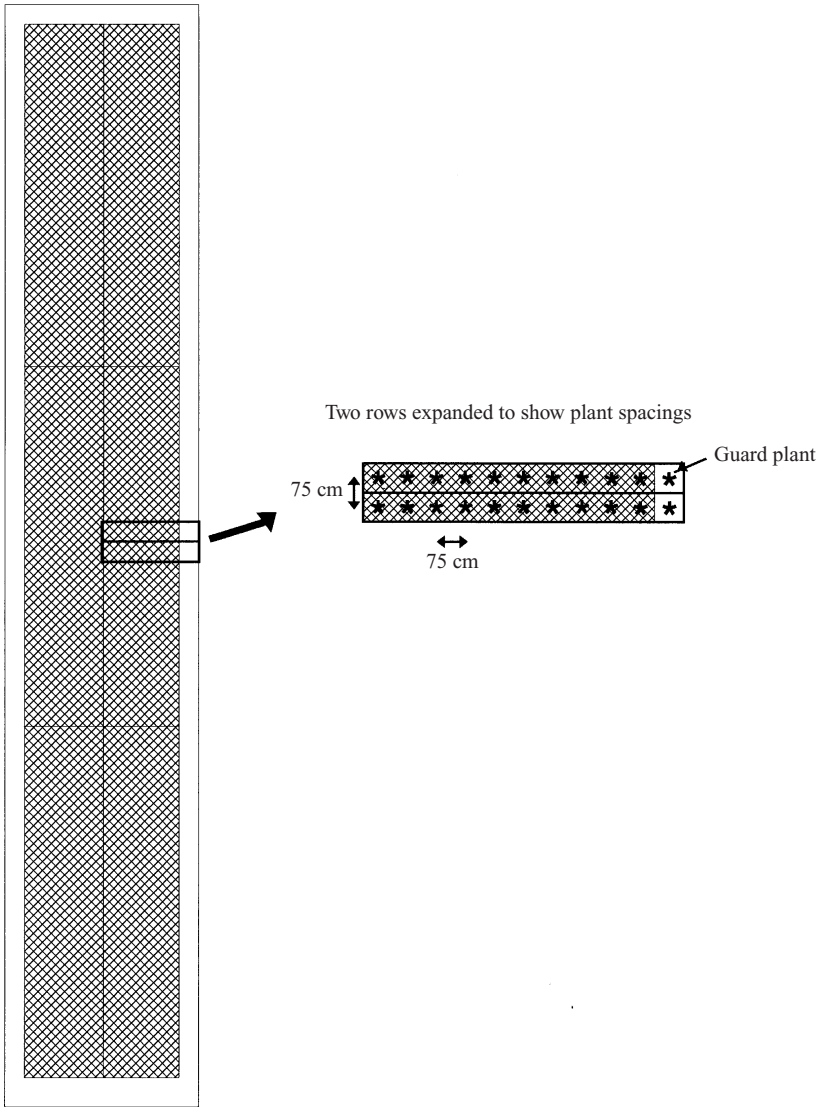


Fig. 2. Field layout of the six blocks in the three tetraploid Italian ryegrass (IRT) trials and the 1990 diploid Italian ryegrass (IRD) trial.

established varieties, and to be stable; i.e. it must remain unchanged after successive reproductive cycles (Weatherup 1980). There were nine trials comprising three series of trials, tetraploid perennial ryegrass (PRT), tetraploid Italian ryegrass (IRT) and diploid Italian ryegrass (IRD), in each of three years, 1989, 1990 and 1991.

The three series of trials were laid out afresh each year in randomized complete block designs with six replicates. Each trial comprised three pairs of blocks with each block subdivided into rows (or plots) of 10 plants. Plants were spaced at 75 cm both within and between rows. The pairs of blocks in the three PRT trials and the 1989 and 1991 IRD trials were laid lengthwise in parallel (Fig. 1), whereas in the other four trials they were laid lengthwise in series (Fig. 2). Because of the wide spacing, the plants were believed not to be in competition. This is supported by studies of the data reported by Watson (1995).

The number of plots in a block was different in each trial. Due to varieties being withdrawn during the trials, not all varieties sown were recorded (Table 1). Together with the presence of guard plants and walkways, this meant that, in any one trial, data were collected from an incomplete grid of plants. Eleven characters or variates were observed on each

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Table 1. *Number of plots per block and* (*in parentheses*) *number of varieties recorded in the tetraploid perennial ryegrass* (*PRT*), *tetraploid Italian ryegrass* (*IRT*) *and diploid Italian ryegrass* (*IRD*) *spaced plant variety trials in 1989, 1990 and 1991*

	Year		
Trial type	1989	1990	1991
PRT	80 (67)	84 (76)	103 (86)
IRD	49 (41)	55 (50)	58 (46)
IRT	28 (24)	37 (35)	47 (45)

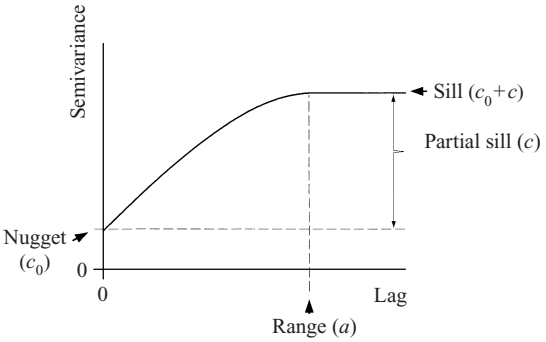


Fig. 3. Features of a typical semivariogram.

individual plant (Table 2). Although DUS tests are based on all variates, 'date of ear emergence' is the most useful because it identifies most of the variety distinctnesses.

The random field model

The spatial dependence of the data was investigated by fitting a random field model:

$$Y_i = \mu_i + \epsilon_i \qquad i = 1, \ldots, n$$

where Y_i is the random variable observed on plant i, and μ_i, the mean structure for plant i, is a composite term comprising an overall mean, variety effects and random effects due to location or block effects. The terms ϵ_i represent the spatially dependent random error variables or error structure. This is a point model with Y_i and ϵ_i regarded as random variables at the precise location of plant i, unlike the regional model in which yields from whole plots are modelled (Zimmerman & Harville 1991).

This random field model makes two assumptions. First, that the ϵ_i have zero expectation so that $E(\varepsilon_i) = 0$ for each $i = 1, \ldots, n$. Second, that the variance of the differences between pairs of error variables, say ϵ_i and ϵ_j, where plants i and j are located distance h apart, has intrinsic stationarity so that $V(\epsilon_i - \epsilon_j) = 2\gamma(h)$. This implies that the variance depends only on the distance or lag, h, between the locations and not on the locations themselves. The function $\gamma(h)$ relating the variance to h is known as the semivariogram. At a given lag h, both the value of the semivariogram and estimates of it are known as semivariances. If the two assumptions hold, the semivariogram contains all the information on the spatial variation of the error structure ϵ_i and, consequently, on the variable Y_i (McBratney & Webster 1986).

The variability of comparisons between observations tends to increase as the separation between plants increases up to, typically, a finite lag a known as the range of influence of the semivariogram. This marks the limit beyond which observations are spatially independent (Fig. 3). For lags greater than

Table 2. *The variates observed on the spaced plant variety trials*

	Description and name of variate	When measured
H_{sp}	Natural average spring height of plant (cm)*	March
W_{sp}	Natural average spring width of plant, calculated as the mean of two widths taken at right angles from above (cm)	March
PH_{sp}	Pulled average spring height of plant (cm)†	March
H_e	Natural height of plant at ear emergence (cm)	May/June
W_e	Width of plant at ear emergence, calculated in same way as W_{sp} variate (cm)	May/June
D_e	Date of ear emergence (days after 1 March)	May/June
L_f	Length of flag leaf of longest stem (cm)	June/July
W_f	Width of flag leaf of longest stem (mm)	June/July
$PLSE_{e+30}$	Pulled length of the longest stem (including the ear) 30 days after the mean date of ear emergence of the within block row (or plot) (cm)	June/July
PLS_{e+30}	Same as $PLSE_{e+30}$ but excluding the ear (cm)	June/July
$PLSN_{e+30}$	Same as $PLSE_{e+30}$ but up to the first node (cm)	June/July

* Where a variate is described as natural, measurements are taken from the plant in its undisturbed state; † where a variate is described as pulled, the plant's leaves or stems are pulled straight before measurement.

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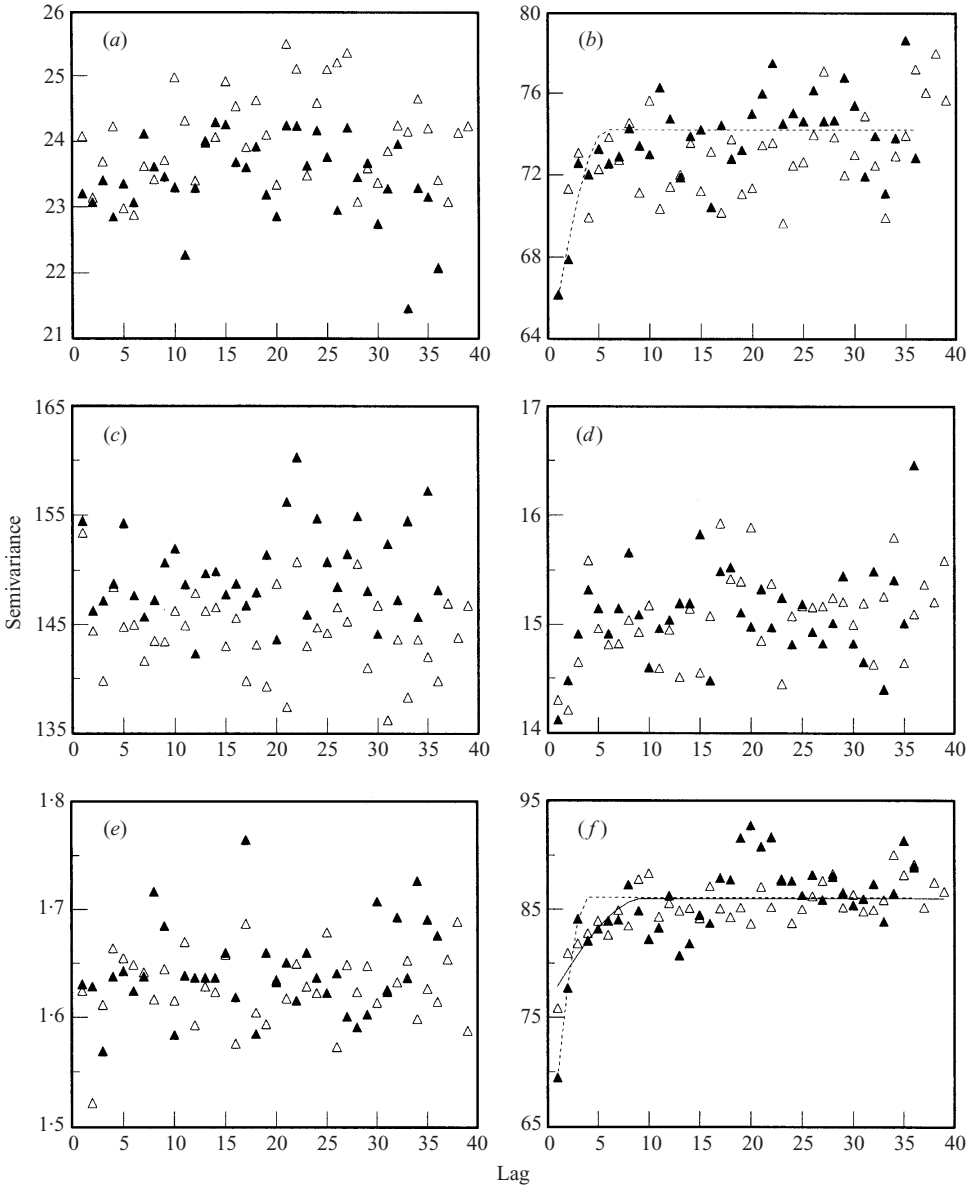


Fig. 4. Row and column semivariances against lag and fitted curves for the tetraploid perennial ryegrass 1989 variates: (*a*) D_e, (*b*) H_e, (*c*) W_e, (*d*) L_f, (*e*) W_f, (*f*) $PLSE_{e+30}$. ▲, row semivariances; △, column semivariances; -----, fitted curve (rows); ————, fitted curve (columns).

the range, the variance between observations remains constant and is called the sill (c_0+c). As the lag decreases towards 0, the semivariance approaches the nugget effect (c_0), which is a measure of the inherent or non-spatial variation (including measurement errors). The partial sill (c) (Cressie 1991) is a measure of the strength of the spatial dependence.

Provided the range of spatial dependence of a variable is finite, corresponding to second-order stationarity, the covariance of two error variables located lag h apart, is related to the semivariogram by the covariance function $C(h)$ (Cressie 1991):

$$C(h) = C(0) - \gamma(h).$$

If, as can usually be assumed for spaced plant experiments, data have second-order stationarity, their spatial dependence may be modelled using either the semivariogram or the covariance function. Here

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the semivariogram was chosen, partly because it is unbiased whereas the classical estimator of the covariance function based on n data points has bias of order $1/n$ (Cressie 1991), and partly because it is better behaved in the presence of trend, particularly at the smaller lags (Cressie 1991).

Estimating semivariances

Random field models are normally fitted to field trial data by least squares using a two stage procedure. The first stage is to fit a semivariogram function to the semivariances estimated from the observed data adjusted for the current estimates of the mean structure effects (Cressie 1991). The second stage is to use the estimated semivariogram to refine the estimates of the mean structure or variety effects. This technique has been used in a field trial context by Brewer & Mead (1986) and by Grondona & Cressie (1991). For a full spatial analysis of the data, the two stages can be cycled or iterated using the estimates from one stage to improve those at the next until successive estimates converge. Here, for simplicity, only the first stage was used to estimate the semivariances using a non-iterative procedure. For a full analysis of treatment means, an iterated analysis would normally be used.

The data were adjusted for the mean structure using analysis of variance appropriate to the randomized complete block design for each trial. For each of the 11 variates in the nine trials, the residuals were placed into an array where the array subscripts were the coded field coordinates of the individual plants, and a unit subscript was equivalent to a physical distance of 75 cm. Some array cells were missing due to unrecorded varieties, missing plants, guard plants and walkways.

Each of the arrays of say m rows by n columns provided a set of semivariances at different lags both from the columns and from the rows. The column semivariance at each lag h was calculated as the mean over columns of the semivariances (i.e. half the squared difference) of all pairs of non-missing residuals within the same column located h rows apart. Thus if $e_{i,j}$ denotes the residual in row i and column j, and setting $\delta_{ihj} = 1$ if both $e_{i,j}$ and $e_{i+h,j}$ are non-missing and $\delta_{ihj} = 0$ otherwise, the column semivariance at lag h or $S(h)_c$ was calculated as:

$$S(h)_c = \frac{1}{2N(h)_c} \sum_{j=1}^{n} \sum_{i=1}^{m-h} (e_{ij} - e_{i+h,j})^2 \delta_{ihj}$$

where

$$N(h)_c = \sum_{j=1}^{n} \sum_{i=1}^{m-h} \delta_{ihj}.$$

The row semivariance $S(h)_r$ at each lag h was calculated in a similar way using residuals located h columns apart within the same rows.

This is the classical unbiased method of moments estimator. However, the resulting semivariance estimates were biased because they were calculated using treatment adjusted residuals (Cressie 1991). Because plants of the same variety are adjacent in rows, adjustment for the bias depends on the lag and is different for the row and column semivariances (Watson 1995). For this reason, no attempt was made to eliminate bias from the semivariance estimates. The robust estimator used by Grondona & Cressie (1991) for a variety trial analysis was not used here because it can have non-negligible bias with normal data (Watson 1997) and this would have further biased the semivariance estimates.

Fitting semivariograms

The spherical function, given by

$$\gamma(h;\theta) = \begin{cases} 0 & h = 0 \\ c_0 + c\left(\dfrac{3h}{2a} - \dfrac{h^3}{2a^3}\right) & 0 < h \leqslant a \\ c_0 + c & a < h \end{cases}$$

where θ is the vector of parameters (a, c_0, c), was chosen to model the underlying semivariograms. It has a nugget effect, c_0, a monotonically increasing section forming a partial sill, c, and a sill, $c_0 + c$, at range a. It also provides positive variances for the error variables and their associated functions.

The spherical function was fitted to the semivariances by a weighted least squares method (WLS). This method was chosen as a practical working compromise between generalized least squares (GLS), which takes full account of the differing variances and covariances of the semivariance estimates at different lags, and ordinary least squares (OLS), which ignores them (McBratney & Webster 1986). WLS aims to weight observations inversely proportional to their variances but makes no allowance for covariances between observations. Hence WLS is likely to be more efficient than OLS but may be less efficient than GLS. However, WLS is considerably simpler to apply than GLS and was preferred for that reason.

The spherical function $\gamma(h;\theta)$ fitted by WLS to the semivariance estimates $S(h_j)$ at lags h_j, $j = 1, \ldots, k$ is defined by the parameters c_0, c and a which minimize:

$$\sum_{j=1}^{k} [S(h_j) - \gamma(h_j;\theta)]^2 / v_j$$

where $\gamma(h_j;\theta)$ is the value of the spherical function at lag h_j and $1/v_j$ is a weighting factor equal to the inverse of the variance of $S(h_j)$.

The variance of a semivariance estimate $S(h)$ depends on the lag, the number of pairs of residuals from which it is calculated, and the underlying spatial

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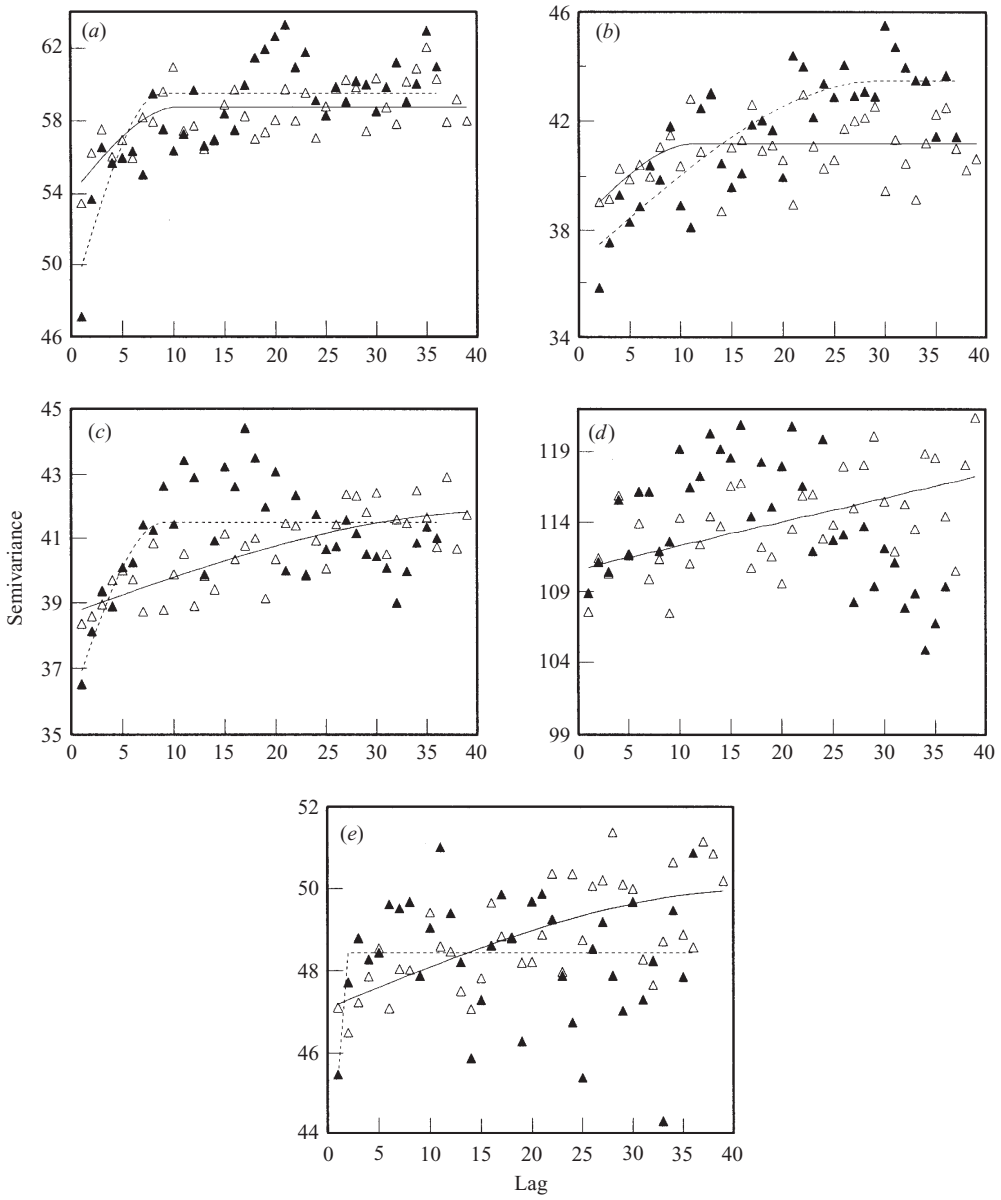


Fig. 5. Row and column semivariances against lag and fitted curves for the tetraploid perennial ryegrass 1989 variates: (*a*) PLS_{e+30}, (*b*) $PLSN_{e+30}$, (*c*) H_{sp}, (*d*) W_{sp}, (*e*) PH_{sp}. ▲, row semivariances; △, column semivariances; -----, fitted curve (rows); ————, fitted curve (columns).

dependence. It is complicated to derive, and too unwieldy for use in WLS. Instead, the term v_j was replaced by $[\gamma(h_j;\theta)]^2/N(h_j)$, where $N(h_j)$ is the number of pairs of residuals from which $S(h_j)$ is calculated. Although Watson (1995) has shown this replacement to be biased at different lags, it has been used elsewhere (McBratney & Webster 1986) and was used here for simplicity of estimation.

Assessing the spatial dependence

Graphical plots of the row and column semivariances, $S(h)_r$ and $S(h)_c$ against lag for the 99 sets of residuals showed that some variates had little or no spatial dependence, whereas others had semivariances that first increased and then levelled off in a way characteristic of spatially dependent variates. Figures

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Table 3. *Mean parameter estimates, R^2_w values and the number out of six (2 directions × 3 years) curves fitted satisfactorily. Predicted optimal block sizes and efficiencies for 96 varieties in six replicates, calculated using the spatial information (see text), are shown for p = 10 and p = 1 plant plots together with estimates of the spatial variation of the means for the two plot sizes*

Variate/ trial type	No satisf. fitted	Mean nugget c_0	Mean partial sill c	Mean range a	Mean sill c_0+c	Mean R^2_w	Predicted block size p = 10	Predicted efficiency p = 10	Predicted block size p = 1	Predicted efficiency p = 1	%† spatial variation p = 10	%* spatial variation p = 1
$PLSE_{e+30}$												
PRT	6	70·3	14·3	11·5	84·5	51·4	5	146·1	8	103·7	55·1	16·9
IRT	5	120·2	18·5	5·5	138·7	46·7	5	109·3	6	100·7	35·5	13·3
PLS_{e+30}												
PRT	6	44·9	8·6	11·4	53·5	46·9	5	142·9	8	103·4	53·4	16·1
IRT	4	89·2	16·3	6·5	105·5	52·3	5	117·0	6	101·5	42·7	15·5
$PLSN_{e+30}$												
PRT	5	33·6	4·2	26·1	37·8	53·7	9	145·8	14	102·7	50·4	11·1
IRT	5	70·2	19·9	5·3	90·1	45·6	4	117·9	5	102·3	49·6	22·1
H_{sp}												
PRT	5	36·0	3·2	20·9	39·2	42·5	9	129·1	12	101·4	40·6	8·2
W_{sp}												
PRT	4	80·4	34·5	277·9	114·9	42·0	14	146·3	>20	102·8	80·8	30·0
IRD	4	66·1	21·8	118·6	87·9	39·4	10	179·5	>20	105·8	76·0	24·8
IRT	5	85·4	6·6	9·9	92·0	38·5	7	112·0	8	100·5	29·8	7·2
PH_{sp}												
PRT	5	41·8	6·7	17·0	48·4	45·7	7	149·2	10	103·3	53·5	13·8
IRT	4	117·0	2·7	18·9	119·6	14·0	11	104·7	16	100·0	14·7	2·3

* Percentage of mean partial sill c to mean sill c_0+c for p = 1; † Percentage of partial sill to sill for p = 10 (see text for details of calculation for tabulated values).

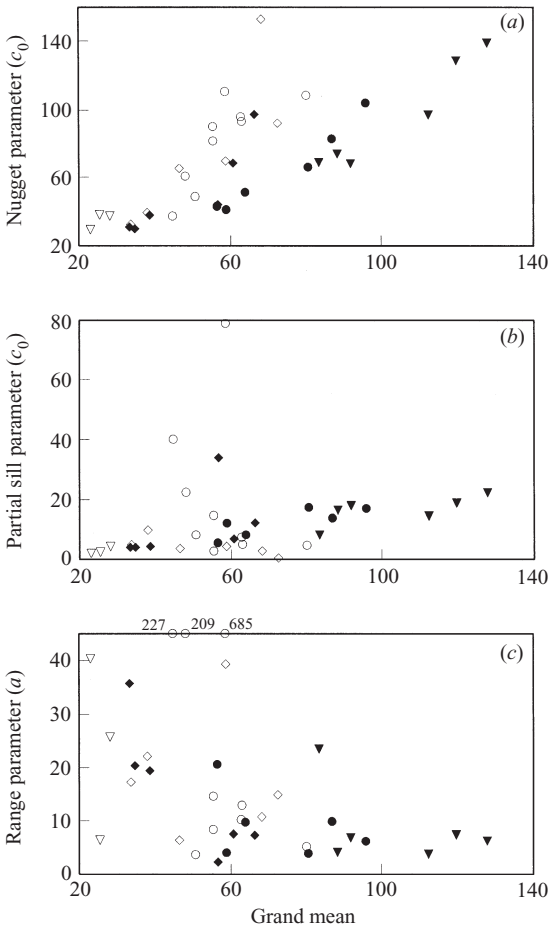


Fig. 6. Nugget (*a*), partial sill (*b*) and range parameters (*c*) plotted against the corresponding trial grand means for the six variates most likely to be spatially dependent. Data points that are off the scale are marked by their y coordinates. Variates: ▼, $PLSE_{e+30}$; ◆, $PLSN_{e+30}$; ○, W_{sp}; ●, PLS_{e+30}; ▽, H_{sp}; ◇, PH_{sp}.

4 and 5 show the row and column semivariances for the PRT 1989 trial.

It was assumed here that any spatial dependence in a particular variate and trial was the same in all directions and, consequently, that both the row and column semivariances estimated the same underlying semivariogram. However, because of their different biases, curves were fitted separately to the row and column semivariances. These curves were only fitted to semivariances at lags of up to half the maximum lag because the sampling variance of the estimates tends to increase excessively at larger lags owing to the decreasing number of observations involved (Watson 1995). Journel & Huijbregts (1978) make a similar recommendation based on the variance caused by local fluctuations in the underlying semivariogram.

Spatial dependence in a variate was assumed if the semivariances were satisfactorily fitted by a spherical function curve, i.e. the iterative fitting procedure converged, the second derivative matrix was non-singular and the parameter estimates were feasible i.e. the intercept and 'slope' parameters c_0 and c positive, and the range parameter $a > 1$. However, in a few cases, e.g. Figs 4*d* and 4*e*, the curve fitting procedure failed to converge despite visual evidence of spatial dependence. A variate susceptible to spatial dependency would be expected to show evidence of spatial dependency in all years of a particular trial type, although not necessarily in all trial types as different genotypes may not be equally susceptible to spatial dependence. Consequently, only those variate-by-trial type combinations that had at least one of their row or column sets of semivariances satisfactorily fitted by a spherical function curve in all three years were considered as potentially spatially dependent (Table 3). Following McBratney & Webster's (1986) recommendation, the goodness of fit of the fitted curves was measured by R^2_w, the percent of the weighted sum of squares explained by the fitted curve.

To determine whether the variation in the shapes of the estimated semivariograms was related to variation in the magnitude of the original observations, the trial average parameter estimates of the spatially dependent variates were plotted against the corresponding trial grand means (Fig. 6).

THE SPATIAL ANALYSIS

Six of the 11 variates were considered spatially dependent in one or more trial types. All measure the early or late season overall dimensions of the plants. The 'date of ear emergence' variate, which is especially important in making DUS decisions about varieties, was considered non-spatially dependent.

Of the six spatially dependent variates, all were spatially dependent in the PRT trials and five were spatially dependent in the IRT trials. Just one variate, (W_{sp}), was spatially dependent in the IRD trials, a finding that may be partly due to genetic reasons. Plants of a diploid variety are likely to be more genetically variable than plants of a tetraploid variety because the chance of phenotypic expression of recessive traits is reduced by the gene dosage in the latter. The mean coefficients of variation of the trial types over the three years (Table 4) show that, relative to plant size, the IRD plants were more variable than the IRT plants in all but one variate although less variable than the PRT plants. If high genetic variability was combined with low sensitivity to spatial factors, spatial dependence could be hard to detect.

The shortness of the rows in trials with pairs of blocks in series did not preclude the row semivariances from being satisfactorily fitted by curves; 88% of the

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Table 4. *Mean coefficient of variation over the three years for the three trial types*

Variate	Trial type		
	PRT	IRD	IRT
$PLSE_{e+30}$	14·6	13·2	11·7
PLS_{e+30}	17·2	15·6	14·6
$PLSN_{e+30}$	24·2	21·5	18·6
H_{sp}	32·2	20·3	21·6
W_{sp}	24·2	19·8	19·1
PH_{sp}	23·4	26·3	18·0

row semivariances for the spatially dependent variates were fitted for blocks in series compared to 85% for blocks in parallel. By contrast, only 63% of the column semivariances were satisfactorily fitted for blocks in series compared to 85% for blocks in parallel.

On average, for the semivariograms fitted to the spatially dependent variates excluding PH_{sp} and W_{sp}, the nugget was 85% of the sill and the range was 12 lags (9 m). The results for the PH_{sp} and W_{sp} variates must be treated with caution, as the curves in the former were poorly fitted in the IRT trials and the ranges in the latter are very large suggesting the possible presence of an underlying trend in the data. The better fit of the curves in the late season $PLSE_{e+30}$, PLS_{e+30}, $PLSN_{e+30}$ variates might indicate that any spatial dependence is shown more clearly in late season variates than in earlier ones, possibly due to the mature plants reflecting more clearly the effects of the spatial dependency factors. Together with the fact that the variates measuring the mid-season overall dimensions of the plants were not considered spatially dependent, these results suggest that, even though there are high correlations between the late season variates, they are more likely to be truly spatially dependent than the early season variates.

There was considerable variation in the shape of the fitted semivariogram for different variates within a trial type. Among the spatially dependent variates, excluding W_{sp} and PH_{sp}, coefficients of variation ranged from 11 to 83% for the nugget and partial sill estimates and from 45 to 90% for the range estimates. For individual variates, differences between trial types and within trial types in the shape of the estimated semivariograms was partly due to differences in the magnitude of the observations. As expected, non-spatial plant variability, as measured by the nugget, was positively related to the magnitude of the observations (Fig. 6*a*). Excluding the W_{sp} and PH_{sp} variates and averaging over the remaining variates, there was a strong overall relationship between the nugget and the grand means of the individual trials. There was little evidence of any relationship between the size of the partial sill and the grand means of the individual trials (Fig. 6*b*). There was some evidence of a weak negative relationship between the size of the range and the grand means of the individual trials (Fig. 6*c*). This could suggest that the range of spatial dependence decreases with increasing plant size, although further investigation is required.

DESIGN CONSIDERATIONS

As the plots of the spaced plant herbage trials were laid out in a one-dimensional arrangement within the blocks, the fitted semivariograms can be used to postblock the trials (Patterson & Hunter 1983). The semivariogram derived from the individual plant observations, $\gamma(h)$, can be used to predict the average within-block error mean square, $MS(k)$, that would have been obtained had each of the blocks of v plots of p plants in the original design been divided into s incomplete blocks of k plots of p plants:

$$MS(k) = \frac{2\sum_{h=1}^{k-1}(k-h)\gamma_p(h)}{k(k-1)}$$

Here $\gamma_p(h)$ is the semivariance of the plot means of observations on two plots of p plants located h lags apart. As the plots of p plants are arranged in parallel, $\gamma_p(h)$ may be shown (see appendix) to be related to $\gamma(h)$ by:

$$\gamma_p(h) = \frac{1}{p^2}\left[p\gamma(h) + 2\sum_{r=1}^{p-1}(p-r)\gamma(\sqrt{(r^2+h^2)}) - 2\sum_{r=1}^{p-1}(p-r)\gamma(r) \right] \quad (1)$$

The efficiency of using such an incomplete block design is the ratio of the average variance of variety differences from the complete block analysis to the average variance from the appropriate incomplete block analysis with recovery of inter-block information. Patterson & Hunter (1983) give the efficiency as approximately

$$\lambda\left[E + \frac{(1-E)(s-1)}{\lambda(v-1)-(v-s)} \right]$$

where E is the efficiency factor of the design (Patterson *et al.* 1978) which adjusts for the increased variances of the variety differences due to the incomplete blocks, and λ is the ratio $MS(v)/MS(k)$.

The efficiencies of alpha (incomplete block) designs (Patterson & Williams, 1976) with blocks of 2, …, 12, 14, 16 and 20 plots in a trial with 96 varieties, 6 replicates and 10 plants per plot were predicted from the average fitted semivariogram of each variate of each trial type. Where designs had unequal block sizes, an average value of λ was used. The efficiencies

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of designs with just one plant per plot were also predicted. The optimum block sizes and corresponding maximum efficiencies are given in Table 3. The percentage of the variation that is likely to be spatial in the plot means of 10 plants was calculated as the percentage of the sill formed by the partial sill in the semivariances predicted using equation (1); these are given for each of the average fitted semivariograms shown in Table 3. Also given are the equivalent percentages for the single plant means, calculated as the percentage of mean partial sill (c) to mean sill (c_0+c).

The optimal block sizes of the alpha design trials with 10 plants per plot were all smaller than the optimal block sizes of the trials with single plant plots. The reason for this is that, although the variation among the plot means of 10 plants is less than the variation among the single plant means, the percentage spatial variation is greater (Table 3). Consequently, compared to the semivariances of the single plant means, the semivariances of the plot means of 10 plants at the lower lags are proportionately smaller than the semivariances at large lags. As the semivariances are cumulatively summed over the lower lags in the equation for $MS(k)$, the error mean squares of the plot means of 10 plants at the lower lags are also proportionately smaller than the error mean squares at large lags. This gives larger values of λ at the lower lags for the plot means of 10 plants than for the single plant means resulting in a smaller optimal block size.

The proportionately greater spatial variation among the plot means of 10 plants than among the single plant means can be explained by considering plots of n plants where the variance of each plant is made up of a spatially dependent component and a non-spatial (independent) component. The variation due to the independent, non-spatial components among the plot means is of order $1/n$, whereas the variation due to the dependent, spatial components is of order $> 1/n$. So the more plants the means are based on, the greater the proportion of the total variation among the plot means that is spatial.

DISCUSSION

Grondona & Cressie (1991) and Patterson & Hunter (1983) recommend that for a full spatial analysis of variety effects, a semivariogram estimated from many trials under similar conditions should be used. The relationships between the semivariogram shape and the mean variate response shown in this study suggest that pooling semivariograms across trials where the mean variate response is different in different trials may not be valid. It cannot be determined how much of the variation in the shape of the fitted semivariograms in this study was due to different underlying semivariograms at the different but not distant trial sites. However, if the site effects were real, this would indicate that the pooled semivariograms should be site specific. Non-stationarity due to trends or systematic effects could have affected the shape of the fitted semivariogram and so contributed to the variation in the shape between trials, although visual inspection of the residuals did not reveal any such trends or patterns. Direct fitting of the random field model by a method such as REML (e.g. Zimmerman & Harville 1991) would have facilitated a more objective examination of residuals and allowed a more explicit model to be fitted to account for any observed effects (e.g. Gilmour *et al.* 1997), but this was not done here.

The efficiency of spatial over non-spatial analysis depends on the strength and extent of the spatial dependency and on the efficiency of the chosen method of non-spatial design and analysis. Grondona & Cressie (1991) found a spatial analysis increased efficiency by 32% when 40% of the variation was spatial (inferred from their graphs) and the range was 2·4 times the average of the plot dimensions. Gilmour *et al.* (1997) found that spatial analysis increased efficiency by 600% in their SA Wheat study. Their analysis allowed spatial dependence to differ with direction and modelled trends and patterns within the data. They found that 65% of the unexplained variation was spatial with a range 1·5 times the average of the plot dimensions.

The extent to which spatial analysis of the spaced plant herbage trial data would increase efficiency for identifying distinctnesses cannot be determined from this study. Although only 12% of the total variation of the spatially dependent variates was spatial on a single plant basis, the range of the spatial dependence was not negligible as 9 m is 2·2 times the average of the plot dimensions. Furthermore, this study has shown that the plot means of 10 plants, which are currently analysed by non-spatial analysis of variance, are likely to be more strongly spatially dependent with on average 45% of the predicted variation of a spatial nature. This suggests that a spatial analysis could increase efficiency by around 20 to 40% relative to a complete blocks analysis.

Other than the need to estimate the spatial dependence over many trials, a second major drawback is the relative complexity of spatial analysis. This applies both to the analysis itself and to the interpretation of the results as the standard errors differ for every pair of varieties compared. However, if a non-spatial analysis is used in the presence of spatial dependence, the different standard errors will still exist even though they are ignored. The greater the spatial dependency, the more potentially misleading the standard errors for decision making concerning variety distinctnesses.

One solution is to improve the trial design. It is not practicable to space the plots to ensure spatial

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independence, as the plot spacing would have to exceed the minimum range of spatial dependency, about 3 m, and the trials are already extensive. The nearest neighbour balanced designs suggested by Martin (1986) for efficient estimation of variety effects are of limited relevance to the spaced plant herbage trials due to the large number of replicates required to ensure neighbour balance of the large numbers of varieties involved. Prohibitive replication also precludes the use of balanced incomplete block designs as suggested by van Es & van Es (1993). However, the use of incomplete block designs such as alpha designs chosen to equalize, as far as possible, the average distance of each variety comparison, could both improve variety effect estimation and reduce the residual variation.

In planning a future experiment, a pooled semivariogram may be used to compare the effect of the variety positions on the efficiency of variety effect estimation of a design (see Cressie (1991) for details). It may also, as seen in this study, be used to compare the efficiencies of different incomplete block designs. Assuming 10 plants per plot, of all the possible alpha designs for a trial with 96 varieties in 6 replicates, those with incomplete blocks of between 4 and 9 plots gave the maximum predicted efficiencies for the spatially dependent variates of the spaced plant herbage trials. These block sizes are similar to the optimal block size of 8 plots for the same size trial that Ainsley *et al*. (1987) report based on the results of 29 ryegrass spaced plant variety trials grown between 1974 and 1983 at Crossnacreevy. The predicted increases in efficiency of between 9 and 46% in this study are comparable with the 16% reported by Ainsley *et al*. (1987) for the variate H_e, although H_e was not classed as spatially dependent in this study.

A pooled semivariogram may also be used to assess the effects of altering the plot dimensions and block configurations on the residual variances (Kristensen & Ersbøll 1992). Such changes would only be worthwhile if they improved the efficiency of estimation of the variety effects of the spatially dependent variates sufficiently to maintain the effectiveness of the DUS decision making process while allowing a reduction in trial costs. By contrast, the use of alpha designs would entail no extra costs and would allow the spatially dependent variates to be analysed more efficiently, possibly by using spatial methods. However, it is questionable whether the increase in efficiency would be sufficient to make the spatially dependent variates as useful for DUS decision making as the non-spatially dependent ones.

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APPENDIX

Let $\gamma_p(h)$ denote the semivariance of the plot-means of observations on two plots, say plot 1 and plot 2, arranged in parallel h lags apart and each containing a row of p plants spaced 1 lag apart. Let $\gamma(h)$ denote the semivariance of observations made on two plants lag h apart. Then

$$\gamma_p(h) = \frac{1}{p^2}\left[p\gamma(h) + 2\sum_{r=1}^{p-1}(p-r)\gamma\sqrt{(r^2+h^2)} - 2\sum_{r-1}^{p-1}(p-r)\gamma(r) \right].$$

Proof: $\gamma_p(h)$ may be written as $\frac{1}{2}\mathrm{V}(\bar{x}_1-\bar{x}_2)$ where $\bar{x}_1 = 1/p\ \Sigma_{i-1}^{p} x_{1i}$ and x_{1i}, $i = 1, \ldots, p$ are the observations on the p plants in plot 1. Likewise, $\bar{x}_2$ is the mean of the observations on the p plants in plot 2.

$$\begin{aligned}
\gamma_p(h) &= \tfrac{1}{2}V(\bar{x}_1-\bar{x}_2) = \tfrac{1}{2}V\left(\frac{1}{p}\sum_{i-1}^{p} x_{1i} - \frac{1}{p}\sum_{j-1}^{p} x_{2j} \right). \\
&= \frac{1}{2p^2}\mathrm{V}\left(\sum_{i-1}^{p} x_{1i} - x_{2i} \right) \\
&= \frac{1}{2p^2}\left[\sum_{i=1}^{p}\mathrm{V}(x_{1i}-x_{2i}) + \sum_{i=1}^{p-1}\sum_{j=i+1}^{p} 2\mathrm{Cov}((x_{1i}-x_{2i}),(x_{1j}-x_{2j})) \right] \\
&= \frac{1}{2p^2}\left[\sum_{i=1}^{p}\mathrm{V}(x_{1i}-x_{2i}) + \sum_{i=1}^{p-1}\sum_{j=i+1}^{p} \begin{pmatrix} 2\mathrm{Cov}(x_{1i},x_{1j}) + 2\mathrm{Cov}(x_{2i},x_{2j}) \\ -2\mathrm{Cov}(x_{1i},x_{2j}) - 2\mathrm{Cov}(x_{2i},x_{1j}) \end{pmatrix} \right]
\end{aligned}$$

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$$= \frac{1}{2p^2}\left[\sum_{i=1}^{p} \mathrm{V}(x_{1i}-x_{2i}) + \sum_{i=1}^{p-1}\sum_{j=i+1}^{p} \begin{pmatrix} \mathrm{V}(x_{1i})+\mathrm{V}(x_{2j})-2\mathrm{Cov}(X_{1i},x_{2j}) \\ +\mathrm{V}(x_{2i})+\mathrm{V}(x_{1j})-2\mathrm{Cov}(X_{2i},x_{1j}) \\ +2\mathrm{Cov}(x_{1i},x_{1j})-\mathrm{V}(x_{1i})-\mathrm{V}(x_{1j}) \\ +2\mathrm{Cov}(x_{2i},x_{2j})-\mathrm{V}(x_{2i})-\mathrm{V}(x_{2j}) \end{pmatrix} \right]$$

$$= \frac{1}{2p^2}\left[\sum_{i=1}^{p} \mathrm{V}(x_{1i}-x_{2i}) + \sum_{i=1}^{p-1}\sum_{j=i+1}^{p} \begin{pmatrix} \mathrm{V}(x_{1i}-x_{2j}) \\ +\mathrm{V}(x_{2i}-x_{1j}) \\ -\mathrm{V}(x_{1i}-x_{1j}) \\ -\mathrm{V}(x_{2i}-x_{2j}) \end{pmatrix} \right]$$

$$= \frac{1}{p^2}\left[\sum_{i=1}^{p} \gamma(h) + \sum_{i=1}^{p-1}\sum_{j=i+1}^{p} (2\gamma(\sqrt{(j-1)^2+h^2}) - 2\gamma(\sqrt{(j-1)^2})) \right]$$

$$= \frac{1}{p^2}\left[p\gamma(h) + 2\sum_{r=1}^{p-1}(p-r)\gamma(\sqrt{(r^2+h^2)}) - 2\sum_{r=1}^{p-1}(p-r)\gamma(r) \right]$$

since the observations x_{1i} and x_{2i} are made on plants h lags apart, observations x_{1i} and x_{1j} are made on plants $\sqrt{(j-i)^2}$ lags apart, and observations x_{1i} and x_{2j} are made on plants $\sqrt{(j-i)^2+h^2}$ lags apart.

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