

## **A Mixed Modelling Approach for Randomized Experiments with Repeated Measures**

H. P. Piepho, A. Büchse, and C. Richter

Authors' addresses: Prof. Dr H. P. Piepho (corresponding author; e-mail: piepho@uni-hohenheim.de) and Dr A. Büchse, Institut für Pflanzenbau und Grünland, Universität Hohenheim, 70599 Stuttgart, Germany; Doz. Dr C. Richter, Institut für Pflanzenbauwissenschaften, Humboldt-Universität zu Berlin, Berlin, Germany

*With 4 figures and 5 tables*

*Received August 1, 2003; accepted November 26, 2003*

### **Abstract**

Repeated measurements on the same experimental unit are common in plant research. Due to lack of randomization and the serial ordering of observations on the same unit, such data give rise to correlations, which need to be accounted for in statistical analysis. Mixed modelling provides a flexible framework for this task. The present paper proposes a general method to formulate mixed models for designed experiments with repeated measurements. The approach is exemplified by way of several examples.

**Key words:** autocorrelation — longitudinal data — mixed model — repeated measurements — serial correlation — spatial statistics

### **Introduction**

Many designed experiments involve measurements repeated in time or space. An analysis of 'repeated measures' (synonymous for 'repeated measurements') needs to account for serial correlation among observations on the same experimental unit. The simplest approach to tackle the problem is to compute an informative summary measure per unit and analyse this by standard analysis of variance (ANOVA) procedures (Rowell and Walters 1976, Mead et al. 1993, Hand and Crowder 1996, Senn et al. 2000). An important limitation of summary measures is that interactions among unrandomized repeated factors and randomized treatment factors are not easily studied and missing data are difficult to cope with in an efficient manner. More elaborate methods use multivariate ANOVA (MANOVA) procedures (Cole and Grizzle 1966) or standard split-plot ANOVA with Box-type corrections to the degrees of freedom (Geisser and Greenhouse 1958). Both are available in linear model packages such as PROC GLM of the SAS System. A severe limitation of both

approaches is that missing values prompt the deletion of each whole record (subject) containing at least one missing value. In addition, only one error term, corresponding to one type of randomization unit, can be accommodated. A more recent development is to analyse repeated measurements within a mixed model framework (Jennrich and Schluchter 1986) using maximum likelihood (ML) or restricted maximum likelihood (REML). Three major advantages are the availability of correlation (variance–covariance) models, which are more parsimonious than the one implied by MANOVA, the facility to accommodate several error terms, and the straightforward analysis of incomplete data, providing the data are missing at random (Verbeke and Molenberghs 2000). Much of the literature in this vein is directed towards applications in the medical and sociological fields, where experimental units correspond to persons. Most of the theory carries over directly to designed experiments in biology and agriculture (Littell et al. 1998, Schabenberger and Pierce 2002), where experimental units may be plots, plants, animals, Petri dishes, or other, but there are some peculiarities which require special attention. Specifically, designed experiments usually involve blocking as well as several nested or crossed levels of randomization, giving rise to multiple block and error effects. It is not obvious how such effects should be treated in a repeated measures set-up. Only few publications explicitly address this problem in the context of randomized block experiments in agriculture and biology (Berzsenyi et al. 2000, Ogliari and Andrade 2001, Singh and Jones 2002). There is an early literature on the design and analysis of long-term experiments (Yates 1954, Patterson 1964, Patterson and Lowe 1970, Preece 1986), which bears much resemblance to more recent developments in

mixed modelling. A consensus as to the most appropriate mixed modelling approach to repeated measures in randomized experiments with blocking is as yet lacking.

In this paper we present an approach for setting up a mixed model for designing experiments involving repeated measures. The main focus will be on block and error effects. We extend on a previous paper (Piepho et al. 2003), which is devoted to randomized experiments without repeated measurements and which integrates rules that have been suggested for such experiments (Nelder 1965, Brien 1983). The procedure we present has a largely heuristic foundation, which we motivate by way of several examples.

### An introductory example (example 1)

Assume that a field experiment comparing three different tillage methods is laid out according to a randomized complete block design (RCBD). The response variable of interest is leaching of nitrogen. Soil samples are taken from four different soil layers. On each plot one water collector is installed per layer. This experiment has one randomized treatment factor (tillage method) and one repeated factor (soil layer). In this paper, the term treatment is restricted to factors indexing the set of different procedures or protocols that are to be applied to different experimental units in randomized fashion in order to compare them (Nelder 1965, Brien 1983). Repeated factors, which cannot be randomized, are to be distinguished from treatment factors. Both factors can be referred to as factors of interest, i.e. there is interest in the comparison among layers (repeated factor) for the different tillage methods (treatment factor). A mixed model for analysing this experiment, which reflects the different status of treatment and repeated factors, should have the following properties:

- (a) The implied model for each layer should be that for an RCBD.
- (b) The model obtained by averaging across layers should be that for an RCBD.
- (c) The model obtained by computing contrasts among layers should be that of an RCBD.
- (d) The model should reflect the fact that the repeated factor cannot be randomized, i.e. it should allow for serial correlation among different layers on the same plot.

The first three properties result from the inherent crossing between experimental units and the repeated factors. The fourth property is required to accommodate correlations arising from the repeated measures design.

The model for an RCBD without repeated measurements is

$$y_{ij} = \mu + b_j + \tau_i + e_{ij} \quad (1)$$

where  $y_{ij}$  is the value of response variable of  $i$ -th treatment (tillage method) in  $j$ -th block,  $\mu$  the general effect (fixed),  $b_j$  the effect of  $j$ -th block (fixed),  $\tau_i$  the effect of  $i$ -th treatment (fixed) and  $e_{ij}$  the error of  $ij$ -th plot (random).

We call this a non-repeated model. To meet requirement (a), we add to each effect in (1) an index for the repeated factor, as depicted in Fig. 1. Thus, the model extended to cover the repeated factor is

$$y_{ijt} = \mu_t + b_{jt} + \tau_{it} + e_{ijt} \quad (2)$$

where  $t$  indexes soil layers. We call this the full repeated model. The effect  $\tau_{it}$  now carries two indices, one for the treatment factor and one for the repeated factor. Effects associated with these two factors may be denoted as repeated-*cum*-treatment model. The repeated-*cum*-treatment structure may be decomposed as  $\mu_t = \mu + \beta_t$  and  $\tau_{it} = \alpha_i + (\alpha\beta)_{it}$  so that

$$\mu_t + \tau_{it} = \mu + \alpha_i + \beta_t + (\alpha\beta)_{it} \quad (3)$$

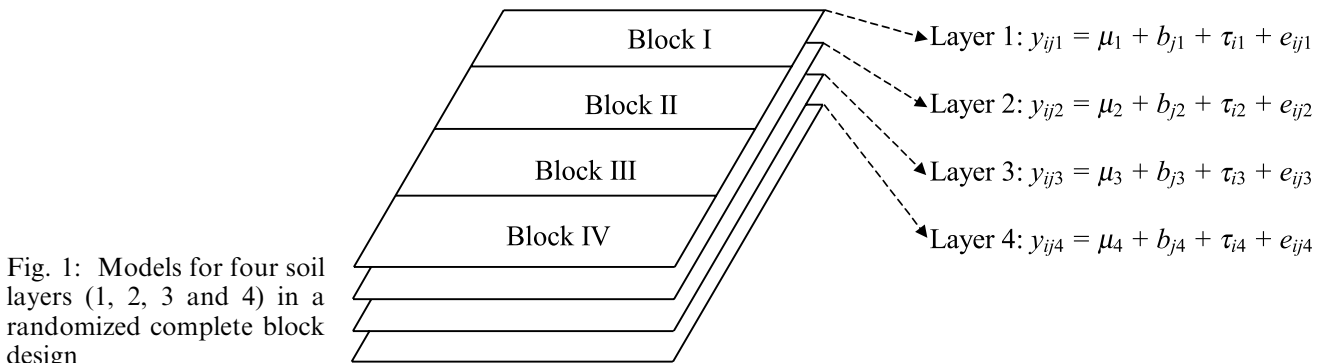


Fig. 1: Models for four soil layers (1, 2, 3 and 4) in a randomized complete block design

where  $\mu$  is the general effect (fixed),  $\alpha_i$  the main effect of  $i$ -th tillage method (fixed),  $\beta_t$  the main effect of  $t$ -th soil layer (fixed) and  $(\alpha\beta)_{it}$  the  $it$ -th interaction effect (fixed).

In addition, the block effect can be decomposed as

$$b_{jt} = c_j + d_{jt} \quad (4)$$

where  $c_j$  is the main effect of  $j$ -th block (fixed) and  $d_{jt}$  is the effect of  $t$ -th layer in  $j$ -th block (fixed).

Thus, the model is

$$y_{ijt} = \mu + \alpha_i + \beta_t + (\alpha\beta)_{it} + c_j + d_{jt} + e_{ijt} \quad (5)$$

An important feature of model (5) is the fixed interaction between the repeated factor and the block factor ( $d_{jt}$ ). In analogy to the usual assumption of block-treatment additivity in randomized experiments (Piepho et al. 2003), the assumption of block-repeated factor additivity has sometimes been put forward (Ogliari and Andrade 2001), but we do not think this is generally tenable. In the case at hand, one can assume that heterogeneity within the soil is such that block differences are not the same in each layer. For example, layers in one block may be relatively similar with respect to mineral nitrogen content, while in another block, layers show a more marked trend, with increasing levels of nitrogen from bottom to top. In this case, one needs to allow for a block-repeated factor interaction. We contend that such interaction should generally be allowed for in repeated measures designs. An important consequence of the block-repeated factor interaction is that the main effect for the repeated factor cannot be tested independently of block effects. One may test contrasts among averages across blocks, i.e. among  $\beta_t + \bar{d}_{\bullet t}$ . These contrasts involve averages across block effects, which restricts the inference space to the blocks used in the particular experiment. Note that the inference space for main effects of the randomized treatment factor is broader because contrasts do not involve block effects.

Model (2) has one random effect, i.e. the layer-specific plot error  $e_{ijt}$ . Due to the repeated measurements, errors of different layers in the same plot (randomized experimental unit) are correlated (see property d). For example, if the nitrogen content is high in the top layer of a plot, then it is likely to be high in the other layers of that plot as well. There are many options to introduce such correlations into the model. Perhaps the simplest is to decompose the error as

$$e_{ijt} = p_{ij} + f_{ijt} \quad (6)$$

where  $p_{ij}$  is a random effect of the  $ij$ -th plot and  $f_{ijt}$  is a layer-specific deviation. Assuming that  $p_{ij}$  and  $f_{ijt}$  are independent normal deviates with zero mean and variances  $\sigma^2\rho$  and  $\sigma^2(1-\rho)$ , respectively, the correlation among errors from layers  $s$  and  $t$  on the same plot is

$$\text{corr}(e_{ijs}, e_{ijt}) = \rho \quad [\text{Compound Symmetry}]$$

This correlation structure is also known as the compound symmetry structure. It implies that the correlation is the same for all pairs of layers. The model is reminiscent of that for a split-plot design with  $p_{ij}$  corresponding to main-plots and  $e_{ijt}$  to subplots (soil layers within a plot). The main difference from a split-plot design is that the repeated factor cannot be randomized. Thus, for a repeated factor, the compound symmetry structure cannot be backed by randomization theory, and the implied homogeneity of the correlation becomes a strong assumption, which cannot usually be justified. Specifically, one would expect the correlation to decay with the distance between layers. Therefore, a more realistic model is the autoregressive [AR(1)] model:

$$\text{corr}(e_{ijs}, e_{ijt}) = \rho^{|s-t|} \quad [\text{AR}(1)]$$

Here, the correlation decreases with the difference in the layer index (assuming layers are indexed consecutively). The rate of decay depends on the autocorrelation parameter  $\rho$ , and layer indices are used to define a distance. Instead, one may use a quantitative measure of spatial (or temporal) distance. One example in kind is the power model,

$$\text{corr}(e_{ijs}, e_{ijt}) = \rho^{|d_{st}|} \quad [\text{Power}]$$

where  $d_{st}$  is the distance in metric units between layers  $s$  and  $t$ . This type of model may be preferable to an AR(1)-model, when layer depths are unequally spaced. There are many alternatives to the AR(1) and power models (Senn et al. 2000, Schabenberger and Pierce 2002), which are not reviewed here for brevity. A common feature of most of these models is a decay of the correlation with distance in space (or time). Many correlation structures can be extended to allow for heterogeneity of variance among levels of the repeated factor. In the case at hand, the variance may differ among layers. Alternatively, if measurements are repeated in time, one frequently observes heterogeneity of variance among dates, especially when the experiment involves different years.

A more general model allows the correlation to vary freely among pairs of layers (apart from the

requirement of positive definiteness of the resulting correlation matrix):

$$\text{corr}(e_{ijs}, e_{ijt}) = \rho_{st} \quad [\text{Unstructured}]$$

This unstructured model is the one employed in the MANOVA approach to repeated measurements (Cole and Grizzle 1966). It is the most flexible model, but also the most costly in terms of the number of parameters. In fact, with many levels of the repeated factors it may easily happen that the unstructured model is not estimable due to lack of data. According to the principle of parsimony (Occam's razor), it is often advantageous to use a simpler model such as AR(1) or compound symmetry. In a mixed model framework, different models, including the unstructured model, can be compared by likelihood ratio tests (Jennrich and Schluchter 1986) or by the Akaike information criterion (AIC) (Wolfinger 1996). Specifically, it can be checked whether parsimonious models give a better fit than complex ones such as the unstructured model.

We conclude this section by taking a look at the requirements (a) to (d). It has already been shown that model (2) is of the same form as (1) (requirement a). When averaging across layers, the model is

$$\bar{y}_{ij\bullet} = \bar{\mu}_{\bullet} + \bar{b}_{j\bullet} + \bar{\tau}_{i\bullet} + \bar{e}_{ij\bullet} \quad (7)$$

which is obviously of the same form as (1) (requirement b). Similarly, it is easily verified that the model for a linear contrast among layers is also of the same form as (1) (requirement c). Finally, model (2) allows for correlation among repeated measurements, provided a correlation structure is fitted to  $e_{ijt}$  (requirement d).

### A simple syntax for mixed models

To develop rules for setting up a model, it is convenient to use Patterson's model syntax, which is reviewed in Piepho et al. (2003). We use operators related to this syntax as summarized in Table 1. The non-repeated model in (1) can be represented as

$$A + \text{BLOCK} : \text{BLOCK} \cdot \text{PLOT} \quad (8)$$

where A and BLOCK code the randomized treatment factor (tillage method) and complete blocks, respectively, while PLOT indexes different plots within a block. For simplicity, we depart from Piepho et al. (2003) in that we do not underline residual error effects. The model falls in two parts (Piepho et al. 2003): the treatment model (A) and the block model (BLOCK : BLOCK · PLOT).

Table 1: Four operators for short-hand notation (Piepho et al. 2003) with illustration of syntax rules

Dot operator (·)
(i) $A \cdot B = B \cdot A$
(ii) $(A \cdot B) \cdot C = A \cdot (B \cdot C)$
(iii) $(A \cdot C) \cdot (B \cdot C) = A \cdot B \cdot C$
Product-term-operator [pt(.)]
(i) $\text{pt}(M) = A \cdot B \cdot C \cdot \dots$ = product term of all factors (A, B, C, ...) appearing in model M
Nesting-operator (/)
(i) $A/B = A + A \cdot B$ (A and B are factors)
(ii) $A/B = A + \text{pt}(A) \cdot B$ (A and B may be models)
(iii) $A/(B/C) = (A/B)/C$
(iv) $A/(B + C) = A/B + A/C$
Crossing-operator (×)
(i) $A \times B = A + B + A \cdot B$
(ii) $A \times (B + C) = A + B + C + A \cdot B + A \cdot C$
(iii) $(A \times B)/C = A \times B + A \cdot B \cdot C$

Fixed effects precede random effects and are separated from these by a colon. Adding the repeated factor 'layer' (LAY), the model equivalent to (5) is

$$A + \text{LAY} + A \cdot \text{LAY} + \text{BLOCK} + \text{BLOCK} \cdot \text{LAY} : \text{BLOCK} \cdot \text{PLOT} \cdot \text{LAY} \quad (9)$$

In this paper we denote randomized treatment factors by single letters, while block and repeated factors are symbolized by full or abridged words. In what follows we show how (9) can be derived in a step-by-step fashion. The repeated-cum-treatment part of this model may be obtained by crossing the randomized treatment factor (A) with the repeated factor (LAY), i.e.

$$A \times \text{LAY} = A + \text{LAY} + A \cdot \text{LAY} \quad (10)$$

where '×' is the crossing operator (Table 1). We note that model (10) has the same structure as the treatment structure for a two-factorial experiment, although it involves both a randomized and an unrandomized, repeated factor. It is also useful to define a repeated factor model comprising only the repeated factors. In the case at hand this is just represented by LAY. The fixed part of the block model in (9), BLOCK and BLOCK · LAY, may be obtained by nesting the repeated factor model (LAY) in the fixed block effect (BLOCK) from (8):

$$\text{BLOCK}/\text{LAY} = \text{BLOCK} + \text{BLOCK} \cdot \text{LAY} \quad (11)$$

The random part is obtained by combining the repeated factor model (LAY) and the random effect in (8) by the dot-operator (·) (Table 1):

$$(\text{BLOCK} \cdot \text{PLOT}) \cdot \text{LAY} = \text{BLOCK} \cdot \text{PLOT} \cdot \text{LAY} \quad (12)$$

As will be shown for a second example, a more generally applicable approach is to combine both effects via the  $\text{pt}(\cdot)$  operator (Table 1) by forming  $\text{BLOCK} \cdot \text{PLOT} \cdot \text{pt}(\text{LAY})$ . In the case at hand, the results are the same with both approaches, as  $\text{BLOCK} \cdot \text{PLOT} \cdot \text{pt}(\text{LAY}) = \text{BLOCK} \cdot \text{PLOT} \cdot \text{LAY}$ . The use of the dot or  $\text{pt}(\cdot)$  operator is a means to derive an error model suitable for accommodating a serial correlation structure (property d in introductory example). Specifically, the resulting random effect  $\text{BLOCK} \cdot \text{PLOT} \cdot \text{LAY}$  would be modelled by fitting some correlation structure using the non-repeated error term  $\text{BLOCK} \cdot \text{PLOT}$  as a subject effect, implying that errors from different plots in a block (experimental units, subjects) are independent, while effects from the same plot may be correlated. For example, the statement to fit an AR(1) model in the MIXED procedure of SAS (SAS Institute, Inc. 1999) is

$$\text{repeated Lay/subject} = \text{Block*Plot type} = \text{AR}(1); \quad (13)$$

To accommodate repeated measures correlation structures for a random effect, we suggest to extend Patterson's notation (Piepho et al. 2003) as follows. The random effect is typed in boldface letters. The subject effect contained in this effect is typed in boldface italics. Thus, the model is expressed as

$$A + \text{LAY} + A \cdot \text{LAY} + \text{BLOCK} + \text{BLOCK} \cdot \text{LAY} : |\text{BLOCK} \cdot \text{PLOT} \cdot \text{LAY} \quad (14)$$

### An example with doubly repeated measures (example 2)

We now extend the first example to allow for several measurements during the growing period, i.e. we introduce the time factor as a second repeated factor. The water collectors installed at the beginning of the growing period remain in place until harvest. Thus, repeated measurements are taken on each collector. By a similar argument as the one elucidated in Fig. 1, we will want the full repeated model to imply the non-repeated model (1) conditional on each point in time and space. Thus, the full repeated model is

$$y_{ijtu} = \mu_{tu} + b_{jtu} + \tau_{itu} + e_{ijtu} \quad (15)$$

where the additional index  $u$  refers to points in time. The treatment part  $\mu_{tu} + \tau_{itu}$  may be decomposed as

$$\mu_{tu} + \tau_{itu} = \mu + \alpha_i + \beta_t + \gamma_u + (\alpha\beta)_{it} + (\alpha\gamma)_{iu} + (\beta\gamma)_{tu} + (\alpha\beta\gamma)_{itu} \quad (16)$$

where  $\alpha$  refers to the randomized treatment factor, while  $\beta$  and  $\gamma$  denote the repeated factors space and time. All main effects and interaction effects are fixed. Similarly, the block effect is decomposed as

$$b_{jtu} = c_j + d_{jt} + g_{ju} + h_{jtu} \quad (17)$$

where  $c_j$  is the main effect of  $j$ -th block (fixed),  $d_{jt}$  the effect of  $t$ -th layer in  $j$ -th block (fixed),  $g_{ju}$  the effect of  $u$ -th point in time in  $j$ -th block (fixed),  $h_{jtu}$  the effect of  $u$ -th point in time, and  $t$ -th layer in  $j$ -th block (fixed).

From (16) and (17), eq. (15) can be re-written as

$$y_{ijtu} = \mu + \alpha_i + \beta_t + \gamma_u + (\alpha\beta)_{it} + (\alpha\gamma)_{iu} + (\beta\gamma)_{tu} + (\alpha\beta\gamma)_{itu} + c_j + d_{jt} + g_{ju} + h_{jtu} + e_{ijtu} \quad (18)$$

In simplified syntax, this can be represented by

$$A \times \text{LAY} \times \text{DATE} + \text{BLOCK} + \text{BLOCK} \cdot \text{LAY} + \text{BLOCK} \cdot \text{DATE} + \text{BLOCK} \cdot \text{LAY} \cdot \text{DATE} : \text{BLOCK} \cdot \text{PLOT} \cdot \text{LAY} \cdot \text{DATE} \quad (19)$$

Again, the repeated-cum-treatment structure ( $A \times \text{LAY} \times \text{DATE}$ ) has the same form as would be used if all three factors were randomized. To derive block effects for the full repeated model, we note that the non-repeated model is the same as in the first example (equations 1 and 8). We may define the repeated factor model as  $\text{LAY} \times \text{DATE}$ . It is seen that the block part in (19) is obtained by nesting  $\text{LAY} \times \text{DATE}$  in the fixed block effect of the non-repeated model (8) as

$$\begin{aligned} &\text{BLOCK}/(\text{LAY} \times \text{DATE}) \\ &= \text{BLOCK} + \text{BLOCK} \cdot \text{LAY} + \text{BLOCK} \cdot \text{DATE} \\ &\quad + \text{BLOCK} \cdot \text{LAY} \cdot \text{DATE} \end{aligned} \quad (20)$$

In analogy to the first example, we might contemplate deriving the random effect  $\text{BLOCK} \cdot \text{PLOT} \cdot \text{LAY} \cdot \text{DATE}$  by using the dot operator to combine the error effects in (8) with the repeated factor model:  $\text{BLOCK} \cdot \text{PLOT} \cdot (\text{LAY} \times \text{DATE})$ . Note, however, that this operation yields three error effects, while there is only one error effect in (19). The desired result is obtained by applying the product term operator  $\text{pt}(\cdot)$  (Table 1) to the repeated factor model prior to combination with the error effects in (8) using the dot operator:

$$\text{BLOCK} \cdot \text{PLOT} \cdot \text{pt}(\text{LAY} \times \text{DATE}) = \text{BLOCK} \cdot \text{PLOT} \cdot \text{LAY} \cdot \text{DATE} \quad (21)$$

One will want to fit a correlation structure to  $\text{BLOCK} \cdot \text{PLOT} \cdot \text{LAY} \cdot \text{DATE}$ , using the non-repeated error term  $\text{BLOCK} \cdot \text{PLOT}$  as the subject effect:

$$\begin{aligned} &A \times \text{LAY} \times \text{DATE} + \text{BLOCK} + \text{BLOCK} \cdot \text{LAY} \\ &+ \text{BLOCK} \cdot \text{DATE} + \text{BLOCK} \cdot \text{LAY} \cdot \text{DATE} : \text{BLOCK} \cdot \text{PLOT} \cdot \text{LAY} \cdot \text{DATE} \end{aligned} \quad (22)$$

This subject effect corresponds to the experimental unit on which repeated measurements are taken in space (LAY) and time (DATE). Fitting an unstructured model, again, corresponds to the MANOVA approach. Alternatively, we may fit more parsimonious models, e.g.

$$\text{corr}(e_{ijtu}, e_{ijsv}) = \rho_1^{|s-t|} \rho_2^{|v-u|} \quad [\text{AR}(1) \times \text{AR}(1)]$$

where  $s$  and  $t$  index two layers, while  $v$  and  $u$  index two points in time, with indices for both dimensions arranged in serial order. This model is known as an  $\text{AR}(1) \times \text{AR}(1)$  model (Gilmour et al. 1997) in spatial statistics, and it is closely related to the anisotropic power model (SAS Institute, Inc. 1999), which uses metric distances  $d_{st}(1)$  and  $d_{vu}(2)$  instead of index differences  $|s-t|$  and  $|v-u|$ , respectively:

$$\text{corr}(e_{ijtu}, e_{ijsv}) = \rho_1^{d_{st}(1)} \rho_2^{d_{vu}(2)} \quad [\text{Anisotropic Power}]$$

The  $\text{AR}(1) \times \text{AR}(1)$  model is seen to be a special case of the anisotropic power model with  $d_{st}(1) = |s-t|$  and  $d_{vu}(2) = |v-u|$ . In a spatial context, the two autocorrelation parameters  $\rho_1$  and  $\rho_2$  pertain to two orthogonal coordinates in a plane, e.g. north-south and east-west directions, and the two distances  $d_{st}(1)$  and  $d_{vu}(2)$  are the distances along these two directions. The model assumes that the decay of the autocorrelation differs among the two coordinates. This property is referred to as anisotropy. The model may also be applied when the two coordinates refer to different dimensions, e.g. space and time, as in the case at hand. Here,  $d_{st}(1)$  is the distance in space, while  $d_{vu}(2)$  is the distance in time.

Instead of the anisotropic power model, an anisotropic exponential model can be fitted, and unequal spacings in time and space are easily accommodated (Schabenberger and Pierce 2002). Finally, a model of the form

$$e_{ijtu} = p_{ij} + f_{ijt} + q_{ijv} + m_{ijtu} \quad (23)$$

may be considered, where all effects are identically distributed independent normal deviates (Steel et al. 1996). The limitations of this model are similar to those of the compound symmetry model (6). For example, correlation is the same for all pairs of layers at the same point in time and for all pairs of points in time in the same layer.

### A step-by-step approach to modelling repeated measurements

From the two preceding examples, the following pattern emerges:

- (i) The repeated-*cum*-treatment model is set up in the usual way, regardless of whether a factor is randomized or repeated.
- (ii) The repeated factor model is nested within all fixed effects of the block part of the non-repeated model.
- (iii) Random effects of the non-repeated model are extended by ‘dotting’ ( $\cdot$ ) with the product term of the repeated factor model. This provides a convenient way to derive a model for random effects, which allows serial correlation structures to be incorporated.

These features are the basis for the following step-by-step approach (also see Fig. 2):

- (1) Set up a treatment model comprising the randomized treatment factors.
- (2) Set up a block model you would be using for a fixed level of the repeated factor or a fixed combination of repeated factors, using the methods outlined in Piepho et al. (2003). This model is denoted as the non-repeated block model. Partition fixed and random effects.
- (3) Check if an interaction among randomized treatment factors and non-repeated block effects is to be expected. An interaction is likely, when the block effect pertains to heterogeneous units, e.g. different trial sites (Piepho et al. 2003). Interactions, if any, are added to the treatment model.
- (4) Set up a repeated factor model, involving only the repeated factors.
- (5) Combine treatment model and repeated factor model, by crossing or nesting as appropriate, into the repeated-*cum*-treatment model. Partition fixed and random effects.
- (6) Nest the repeated factor model in each fixed effect of the non-repeated block model. The resulting effects are denoted as the repeated block effects.

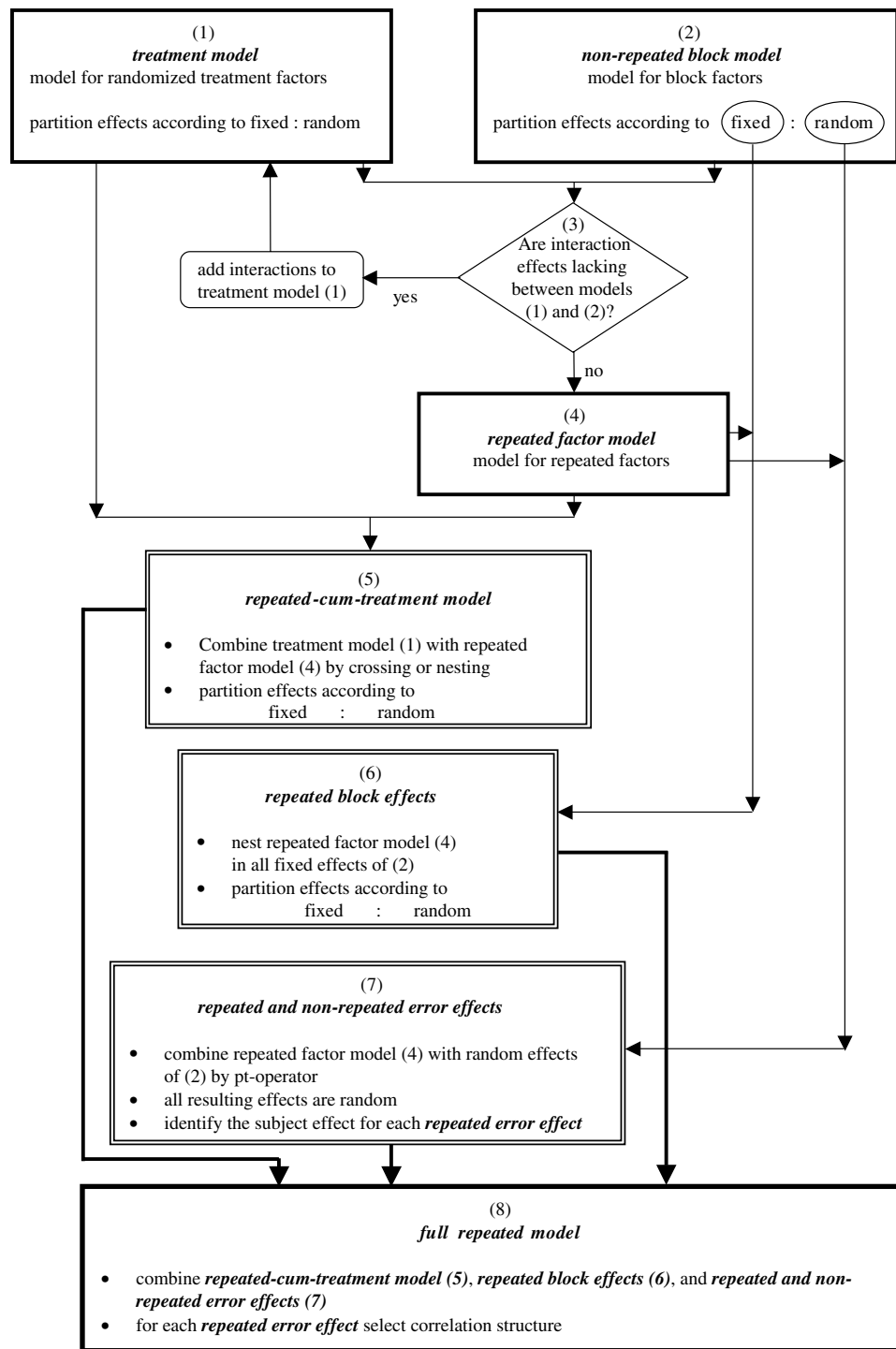


Fig. 2: Diagrammatic representation of approach for setting up a mixed model for designed experiments with repeated measurements

(7) Form (random effects in non-repeated block model) · pt(repeated factor model). For each resulting effect, do the following. The part originating from the non-repeated block model identifies the experimental unit on which repeated measurements are taken. Among the repeated factors contained in the effect, identify those for which repeated measurements are taken on that experimental unit. If at least one such factor is identified, the error effect is a

repeated error effect. Otherwise it is a non-repeated error effect. The subject effect of a repeated error effect is found by deleting from the error effect those factors regarding which repeated measurements are made on the corresponding experimental unit. Generally, the subject effect will contain the non-repeated part of the random effect. In some cases, it also contains one or more repeated factors. For repeated error effects, print the subject effect in

boldface italics and the other terms in boldface. Non-repeated error effects are printed in regular letters (no boldfacing, no italics).

- (8) Combine the repeated-*cum*-treatment model (step 5), the repeated block effects (step 6) and the repeated and non-repeated error effects (step 7) to obtain the full repeated model. Select a repeated measures correlation structure for each repeated error effect from step 7, using the identified subject effect.

Treatment model and non-repeated block model, as obtained in the first three steps, could be combined to form the non-repeated model that would be used without a repeated factor. The two models are kept apart during the step-by-step approach, however, to facilitate integration of the repeated factors in subsequent steps. The step-by-step approach reflects the inherent asymmetry between repeated and treatment factors in that the non-repeated model is formulated first (steps 1–3), at least initially assuming block treatment additivity, while the repeated factors are integrated into the block effects in later steps (steps 6 and 7), generally allowing for an interaction between block and repeated factors. A correlation structure will be fitted to any repeated error effect, thus reflecting the repeated measures set-up. By contrast, serial correlation structures are not fitted to other random effects involving repeated factors, e.g. crossed effects between treatment and repeated factors. As rightly pointed out by a referee, this prescription is somewhat restrictive and may rule out some useful models regarding the factors of interest. Our main objective here is to get the error structure right. The step-by-step approach may be seen as a first step to derive a useful model, in which the error structure appropriately accommodates the repeated measures design. At the same time, an initial repeated-*cum*-treatment model is obtained, which may subsequently be refined, if needed, using more sophisticated models, e.g. a serial correlation structure pertaining to treatment-repeated factor effects. We do not suggest here to generally fit serial correlation structures to all random effects involving repeated factors for two reasons. (i) The resulting models will often be rather complex, often so complex that fitting is difficult if not infeasible. (ii) We think that a serial correlation model is essential for repeated error terms, but not for other random terms. Thus, repeated error effects deserve a special status.

A comment is in order regarding step 8. For some effects the number of realized values will be too small

to go beyond simple correlation structures such as independent or compound symmetry. A model selection criterion such as the AIC helps in deciding on how complex a correlation structure is supported by the data. Nested models can be compared by likelihood ratio tests (Schabenberger and Pierce 2002). Generally, the step-by-step approach will yield a correlation structure for the random effects, which can then be used to test hypotheses regarding the fixed effects by Wald tests (Piepho et al. 2003).

### Some more examples

We now exemplify the procedure by application to examples 1 and 2. We then give a number of additional examples to demonstrate the scope and flexibility of the procedure. An overview of the examples is given in Table 2. In all examples except the last one, we do not analyse real data and so do not exemplify selection of a correlation structure in step 8.

Example 1: RCBD for fixed factor A, repeated over layers (LAY) (fixed). For details see ‘An introductory example’.

- (1) A
- (2) BLOCK/PLOT = BLOCK : BLOCK · PLOT
- (3) -
- (4) LAY
- (5) A × LAY
- (6) BLOCK/LAY = BLOCK + BLOCK · LAY
- (7) (BLOCK · PLOT) · pt(LAY) = BLOCK · PLOT · LAY

Repeated observations are taken per plot, so BLOCK · PLOT is the subject effect.

- (8) A + LAY + A · LAY + BLOCK + BLOCK · LAY : **BLOCK · PLOT · LAY**

Example 2: RCBD for fixed factor A, repeated across layers (LAY) and dates (DATE). Both repeated factors are fixed. For details see ‘An example with doubly repeated measures’.

- (1) A
- (2) BLOCK/PLOT = BLOCK : BLOCK · PLOT
- (3) -
- (4) LAY × DATE
- (5) A × LAY × DATE
- (6) BLOCK/(LAY × DATE) = BLOCK + BLOCK · DATE + BLOCK · LAY + BLOCK · DATE · LAY
- (7) (BLOCK · PLOT) · pt(LAY × DATE) = BLOCK · PLOT · LAY · DATE

Repeated observations on LAY and DATE are taken per plot, so BLOCK · PLOT is the subject effect.



Table 2: Outline of 11 examples considered in this paper

Example	Elements of the repeated- <i>cum</i> -treatment model						Remarks
	Randomized factors		Repeated factors		Combined	Block model	
	A	B	R1	R2			
1	Fixed	–	LAYER fixed	–	$A \times R1$	BLOCK/PLOT	One sample per layer on each plot
2	Fixed	–	LAYER fixed	DATE fixed	$A \times R1 \times R2$	BLOCK/PLOT	One sample point per layer on each plot for all dates
3	Fixed	–	YEAR fixed	–	$A \times R1$	ROW $\times$ COL	One plot per combination of row and column
4	Fixed	–	YEAR fixed	–	$A \times R1$	BLOCK/PLOT/PLANT	Different plants on each plot in each year
5	Fixed	–	LAYER fixed	DATE fixed	$A \times R1 \times R2$	BLOCK/PLOT/SAMPLE	One sample point on each plot per date and stratification per layer
6	Fixed	–	CUT fixed	–	$A/R1$	BLOCK/PLOT	One sample CYCLE random per cutting CYCLE random date on each plot/cutting dates are different for all or some levels of A
7	Fixed	Fixed	CUT fixed	–	$A \times B \times R1$	BLOCK/MAIN/SUB	One sample per cutting date on each subplot/cutting dates are identical for all combinations of A and B/cutting dates need not be equally spaced
8	Random	–	CUT fixed	–	$A \times R1$	BLOCK/PLOT	Similar to example 7 (subplot in place of plot)
9	Fixed	–	YEAR random	–	–	LOC/BLOCK/PLOT ('LOC' is random)	
10	Fixed	Fixed	CYCLE fixed	YEAR in CYCLE random	$A \times B \times (R1/R2)$	FIELD/BLOCK/PLOT	Partial confounding between treatment and block model
11	Fixed	–	IRRIG fixed	DIR fixed	$A \times R1 \times R2$	BLOCK/PLOT	

(8)  $A + LAY + DATE + A \cdot LAY + A \cdot DATE + LAY \cdot DATE + A \cdot LAY \cdot DATE + BLOCK + BLOCK \cdot DATE + BLOCK \cdot LAY + BLOCK \cdot DATE \cdot LAY : BLOCK \cdot PLOT \cdot LAY \cdot DATE$   
 One will want to fit a correlation structure to  $BLOCK \cdot PLOT \cdot LAY \cdot DATE$ , which represents the crossed structure of  $LAY \cdot DATE$ , e.g. an  $AR(1) \times AR(1)$  model.

Example 3: Row-column design for fixed factor A tested on a perennial crop, with one plot per cross of a row and a column, repeated across fixed years (YEAR):

- (1) A
- (2)  $ROW \times COL = ROW + COL : ROW \cdot COL$
- (3) -
- (4) YEAR
- (5)  $A \times YEAR$
- (6)  $(ROW + COL)/YEAR = ROW + ROW \cdot YEAR + COL + COL \cdot YEAR$
- (7)  $(ROW \cdot COL) \cdot pt(YEAR) = ROW \cdot COL \cdot YEAR$   
 ROW  $\cdot$  COL is the plot on which repeated observations are made over years (YEAR). So it is the subject effect.

- (8)  $A + YEAR + A \cdot YEAR + ROW + ROW \cdot YEAR + COL + COL \cdot YEAR : ROW \cdot COL \cdot YEAR$

Example 4: RCBD for fixed factor A tested on a perennial crop, repeated over years (YEAR) (fixed), subsampling of different plants (PLANT) on each plot in each year:

- (1) A
- (2)  $BLOCK/PLOT/PLANT = BLOCK : BLOCK \cdot PLOT + BLOCK \cdot PLOT \cdot PLANT$
- (3) -
- (4) YEAR
- (5)  $A \times YEAR$
- (6)  $BLOCK/YEAR = BLOCK + BLOCK \cdot YEAR$
- (7)  $(BLOCK \cdot PLOT + BLOCK \cdot PLOT \cdot PLANT) \cdot pt(YEAR) = BLOCK \cdot PLOT \cdot YEAR + BLOCK \cdot PLOT \cdot PLANT \cdot YEAR$   
BLOCK · PLOT is the subject effect for BLOCK · PLOT · YEAR. The plant effect BLOCK · PLOT · PLANT · YEAR does not pertain to repeated measurements, since new plants are sampled each year. Thus, it is a non-repeated error effect, to which an independent model should be fitted (no correlation).
- (8)  $A + YEAR + A \cdot YEAR + BLOCK + BLOCK \cdot YEAR : BLOCK \cdot PLOT \cdot YEAR + BLOCK \cdot PLOT \cdot PLANT \cdot YEAR$

A high plot yield in year 1 will bear on the yield in year 2 via crop residues, and this is fully accounted for by a correlation among plot effects (BLOCK · PLOT · YEAR) in different years. While this correlation will affect plot yield, it is appropriate to consider effects of single plants as independent. This is because the correlation occurs at the plot level. A different situation occurs with perennial crops such as strawberries when repeated measurements are taken on the same sample of marked plants per plot. In this case, both plant and plot errors are repeated error effects.

Example 5: An RCBD is laid out for a fixed factor A. On each plot, one random soil column is sampled (SAMPLE) and stratified according to three soil layers (LAY). The measurements are repeated on three dates (DATE), with a new soil sample taken on each plot. Note that this sampling design is different from example 2, where the samples are taken from the same water collector on each date.

- (1) A
- (2)  $BLOCK/PLOT/SAMPLE = BLOCK : BLOCK \cdot PLOT + BLOCK \cdot PLOT \cdot SAMPLE$
- (3) -
- (4)  $LAY \times DATE$
- (5)  $A \times LAY \times DATE$

- (6)  $BLOCK/(LAY \times DATE) = BLOCK + BLOCK \cdot LAY + BLOCK \cdot DATE + BLOCK \cdot LAY \cdot DATE$
- (7)  $(BLOCK \cdot PLOT + BLOCK \cdot PLOT \cdot SAMPLE) \cdot pt(LAY \times DATE) = BLOCK \cdot PLOT \cdot LAY \cdot DATE + BLOCK \cdot PLOT \cdot SAMPLE \cdot LAY \cdot DATE$

For BLOCK · PLOT · LAY · DATE, the subject effect is the plot effect BLOCK · PLOT, because repeated measurements are taken per plot for both LAY and DATE.

For BLOCK · PLOT · SAMPLE · LAY · DATE, we use BLOCK · PLOT · SAMPLE · DATE as a subject effect. Note that the subject effect comprises DATE because at the sample level, repeated observations are made on LAY, but not on DATE.

- (8)  $A + LAY + DATE + A \cdot LAY + A \cdot DATE + LAY \cdot DATE + A \cdot LAY \cdot DATE + BLOCK + BLOCK \cdot LAY + BLOCK \cdot DATE + BLOCK \cdot LAY \cdot DATE : BLOCK \cdot PLOT \cdot LAY \cdot DATE + BLOCK \cdot PLOT \cdot SAMPLE \cdot LAY \cdot DATE$

Contrary to example 2, we now have two repeated error terms, one corresponding to plots (repeated measurements across layers and dates) and one corresponding to samples (repeated measurements across layers). This structure fully reflects the subsampling design. We note for completeness that DATE is confounded with SAMPLE at the level of plots, so one may as well drop one of the two factors from the effect **BLOCK · PLOT · SAMPLE · LAY · DATE** without changing the analysis. Conversely, the analysis will also be correct without this omission.

Example 6: Three different cutting frequencies of grassland (F) are tested in an RCBD. Cutting dates (CUT) constitute a fixed repeated factor, which is nested within F.

- (1) F
- (2)  $BLOCK/PLOT = BLOCK : BLOCK \cdot PLOT$
- (3) -
- (4) CUT
- (5)  $F/CUT$
- (6)  $BLOCK/CUT = BLOCK + BLOCK \cdot CUT$
- (7)  $(BLOCK \cdot PLOT) \cdot pt(CUT) = BLOCK \cdot PLOT \cdot CUT$

A serial correlation structure is fitted, using BLOCK · PLOT as a subject effect.

- (8)  $F + F \cdot CUT + BLOCK + BLOCK \cdot CUT : BLOCK \cdot PLOT \cdot CUT$

Usually, cutting dates will not be equally spaced. Thus, an AR(1) model for **BLOCK · PLOT ·**

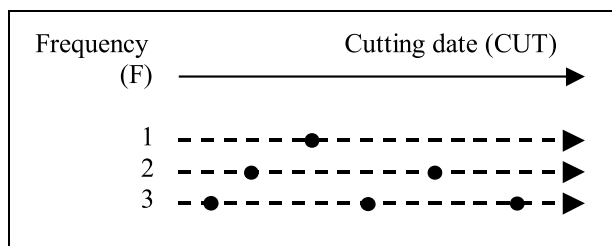


Fig. 3: Three different cutting frequencies (F) with different cutting dates (CUT). Overall there are six cutting dates

CUT is not very plausible, and it may be more appropriate to resort to an unstructured model or to spatial correlation structures.

Cutting dates may be different for each frequency (Fig. 3). Analysis would be difficult with the MANOVA approach using, e.g. the GLM procedure of SAS. To see this, assume that there are three different frequencies (one, two and three cuttings), and that none of the cuttings for different frequencies coincide, i.e. there are six cutting dates overall. If, e.g. CUT is specified as a repeated factor in the GLM procedure, six response variables corresponding to the six cutting dates need to be defined. Thus, there is a six-variate response for each experimental unit (record). The problem is that for each level of F, this six-variate response is necessarily incomplete. In MANOVA mode, GLM will delete any record with incomplete data, so there will be no records left for analysis. By contrast, analysis is possible within a mixed modelling framework by fitting an unstructured correlation model, which is equivalent to the MANOVA model.

Example 7: A split-plot experiment is performed for a grassland crop. The main-plot factor A is laid out in complete blocks (BLOCK), while the subplot factor B is completely randomized among subplots (SUB) within main plots (MAIN). Repeated cuttings are taken on each plot on three dates (CUT).

- (1)  $A \times B$
- (2)  $BLOCK/MAIN/SUB = BLOCK : BLOCK \cdot MAIN + BLOCK \cdot MAIN \cdot SUB$
- (3) -
- (4) CUT
- (5)  $A \times B \times CUT$
- (6)  $BLOCK/CUT = BLOCK + BLOCK \cdot CUT$
- (7)  $(BLOCK \cdot MAIN + BLOCK \cdot MAIN \cdot SUB) \cdot pt(CUT) = BLOCK \cdot MAIN \cdot CUT + BLOCK \cdot MAIN \cdot SUB \cdot CUT$

The non-repeated split-plot model has two error terms, i.e. the main plot error and the subplot

error. For both, a temporal correlation structure needs to be fitted to accommodate the repeated factor 'CUT'. Both main plot and subplot are units on which repeated measurements are made for CUT. Thus,  $BLOCK \cdot MAIN$  and  $BLOCK \cdot MAIN \cdot SUB$  are the subject effects.

- (8)  $A + B + CUT + A \cdot B + A \cdot CUT + B \cdot CUT + A \cdot B \cdot CUT + BLOCK + BLOCK \cdot CUT : BLOCK \cdot MAIN \cdot CUT + BLOCK \cdot MAIN \cdot SUB \cdot CUT$

Example 8: Several randomly selected grass genotypes (G) are tested in an RCBD, repeated across cuttings (CUT) (fixed).

- (1) G
- (2)  $BLOCK/PLOT = BLOCK : BLOCK \cdot PLOT$
- (3) -
- (4) CUT
- (5)  $G \times CUT = CUT : G + G \cdot CUT$
- (6)  $BLOCK/CUT = BLOCK + BLOCK \cdot CUT$
- (7)  $(BLOCK \cdot PLOT) \cdot pt(CUT) = BLOCK \cdot PLOT \cdot CUT$
- (8)  $CUT + BLOCK + BLOCK \cdot CUT : G + G \cdot CUT + BLOCK \cdot PLOT \cdot CUT$

In line with the discussion following the description of the step-by-step approach, we could further refine the repeated-*cum*-treatment part, e.g. by replacing the random effects G and  $G \cdot CUT$  with  $G \cdot CUT$ , using G as a subject effect and fitting a serial correlation model.

Example 9: RCBD for fixed factor A in an experiment with a perennial grass crop, repeated across years (YEAR) on the same plots. Experiments are replicated across randomly selected locations (LOC). Assuming that year effects behave like a random sample, the year factor is regarded as random so as to obtain more broadly valid inferences.

- (1) A
- (2)  $LOC/BLOCK/PLOT = LOC + LOC \cdot BLOCK + LOC \cdot BLOCK \cdot PLOT$  (all effects random)
- (3) Locations may be so heterogeneous that a differential response of treatments (A) is expected. Thus, we add the interaction effect  $LOC \cdot A$  to the treatment model (Piepho et al. 2003):  $A : LOC \cdot A$
- (4) YEAR
- (5)  $(A : LOC \cdot A) \times YEAR = A : LOC \cdot A + YEAR + A \cdot YEAR + A \cdot LOC \cdot YEAR$
- (6) -
- (7)  $(LOC + LOC \cdot BLOCK + LOC \cdot BLOCK \cdot PLOT) \cdot pt(YEAR) = LOC \cdot YEAR + LOC \cdot$

$\text{BLOCK} \cdot \text{YEAR} + \text{LOC} \cdot \text{BLOCK} \cdot \text{PLOT} \cdot \text{YEAR}$

LOC is the subject effect for  $\text{LOC} \cdot \text{YEAR}$ .

$\text{LOC} \cdot \text{BLOCK}$  is the subject effect for  $\text{LOC} \cdot \text{BLOCK} \cdot \text{YEAR}$ .

$\text{LOC} \cdot \text{BLOCK} \cdot \text{PLOT}$  is the subject effect for  $\text{LOC} \cdot \text{BLOCK} \cdot \text{PLOT} \cdot \text{YEAR}$ .

- (8)  $A : \text{YEAR} + A \cdot \text{YEAR} + \text{LOC} \cdot A + \text{LOC} \cdot A \cdot \text{YEAR} + \text{LOC} \cdot \text{YEAR} + \text{LOC} \cdot \text{BLOCK} \cdot \text{YEAR} + \text{LOC} \cdot \text{BLOCK} \cdot \text{PLOT} \cdot \text{YEAR}$

For all random effects of the block model the approach leads to fitting of an autocorrelation structure. By contrast, it yields an independent structure for the random effects of interest, i.e.  $\text{LOC} \cdot A$ ,  $\text{YEAR}$ ,  $A \cdot \text{YEAR}$ , and  $A \cdot \text{LOC} \cdot \text{YEAR}$ . One might consider extending the model to allow for correlations, e.g. among year main effects ( $\text{YEAR}$ ) and among interactions ( $A \cdot \text{YEAR}$ ) at the same level of the non-repeated factor ( $A$ ). For example, an AR(1) model might be considered. Such models, while biologically plausible, require many years of data for reliable estimation.

The year effect was taken as random. Part of the year effect may be due to a systematic trend resulting from carry-over effects, and this should be incorporated into the fixed part of the model. Thus, one might fit a regression on time in the fixed part and regard year-specific departures from the trend as random fluctuations. Further details are not elaborated here, as the main focus of our paper is on the block model.

The final model looks somewhat unfamiliar relative to models for series of experiments replicated across years and locations (Piepho et al. 2003). For example, there is no location main effect LOC. Note, however, that a compound symmetry model can be fitted to  $\text{LOC} \cdot \text{YEAR}$ , which is essentially the same model as one with an independent random main effect LOC and an independent random interaction term  $\text{LOC} \cdot \text{YEAR}$ . Analogous statements hold for  $\text{LOC} \cdot \text{BLOCK} \cdot \text{YEAR}$  and  $\text{LOC} \cdot \text{BLOCK} \cdot \text{PLOT} \cdot \text{YEAR}$ . Thus, when a compound symmetry model is fitted to all repeated random effects, the familiar form of an ANOVA-type mixed model is recovered. The important point to remember here is that the compound symmetry structure is but one candidate model for repeated measures and that often other models provide a more adequate fit.

Example 10: A long-term experiment was conducted to study a crop rotation (Fig. 4). The experiment was laid out on three adjacent fields (FIELD). In a year (YEAR), each of the three

rotation crops (C) was planted to one field. On each field, an RCBD was used to study different management systems (S). A cycle of the rotation (CYC) extends across 3 years, so the year factor is nested within cycles. A joint analysis involving all crops of the rotation is in order, e.g. when a soil nutrient is to be studied or when time trends for different crops are to be compared. The year factor is taken as random in order to broaden the inference space, assuming that year effects behave like a random sample.

- (1)  $C \times S$
- (2)  $\text{FIELD}/\text{BLOCK}/\text{PLOT} = \text{FIELD} + \text{FIELD} \cdot \text{BLOCK} : \text{FIELD} \cdot \text{BLOCK} \cdot \text{PLOT}$
- (3) -
- (4)  $\text{CYC}/\text{YEAR}$
- (5)  $C \times S \times (\text{CYC}/\text{YEAR}) = C + S + \text{CYC} + C \cdot S + C \cdot \text{CYC} + S \cdot \text{CYC} + C \cdot S \cdot \text{CYC} : \text{CYC} \cdot \text{YEAR} + C \cdot \text{CYC} \cdot \text{YEAR} + S \cdot \text{CYC} \cdot \text{YEAR} + C \cdot S \cdot \text{CYC} \cdot \text{YEAR}$
- (6)  $(\text{FIELD} + \text{FIELD} \cdot \text{BLOCK})/(\text{CYC}/\text{YEAR}) = \text{FIELD} + \text{FIELD} \cdot \text{CYC} + \text{FIELD} \cdot \text{BLOCK} + \text{FIELD} \cdot \text{BLOCK} \cdot \text{CYC} : \text{FIELD} \cdot \text{CYC} \cdot \text{YEAR} + \text{FIELD} \cdot \text{BLOCK} \cdot \text{CYC} \cdot \text{YEAR}$
- (7)  $(\text{FIELD} \cdot \text{BLOCK} \cdot \text{PLOT}) \cdot \text{pt}(\text{CYC}/\text{YEAR}) = \text{FIELD} \cdot \text{BLOCK} \cdot \text{PLOT} \cdot \text{CYC} \cdot \text{YEAR}$
- (8)  $C + S + \text{CYC} + C \cdot S + C \cdot \text{CYC} + S \cdot \text{CYC} + C \cdot S \cdot \text{CYC} + \text{FIELD} + \text{FIELD} \cdot \text{CYC} + \text{FIELD} \cdot \text{BLOCK} + \text{FIELD} \cdot \text{BLOCK} \cdot \text{CYC} : \text{FIELD} \cdot \text{CYC} \cdot \text{YEAR} + \text{FIELD} \cdot \text{BLOCK} \cdot \text{CYC} \cdot \text{YEAR} + \text{CYC} \cdot \text{YEAR} + C \cdot \text{CYC} \cdot \text{YEAR} + S \cdot \text{CYC} \cdot \text{YEAR} + C \cdot S \cdot \text{CYC} \cdot \text{YEAR} + \text{FIELD} \cdot \text{BLOCK} \cdot \text{PLOT} \cdot \text{CYC} \cdot \text{YEAR}$

Time trend can be studied by the cycle factor. In addition, one could extend the fixed part of the model by including a regression on time. The random effects  $\text{FIELD} \cdot \text{CYC} \cdot \text{YEAR}$  and  $C \cdot \text{CYC} \cdot \text{YEAR}$  are confounded, so one of the two has to be deleted. Leaving both effects in the model will render the correlation structure overparameterized, which is likely to cause convergence problems, although it does not alter the implied correlation structure for the data.

In step 3, we have not added an interaction. Alternatively, one might want to add interactions of FIELD with treatment effects [ $\text{FIELD} \cdot (C \times S)$ ], providing large differences are expected among fields (Piepho et al. 2003). In this case, fields would be treated in the same way as different environments in a series of trials.

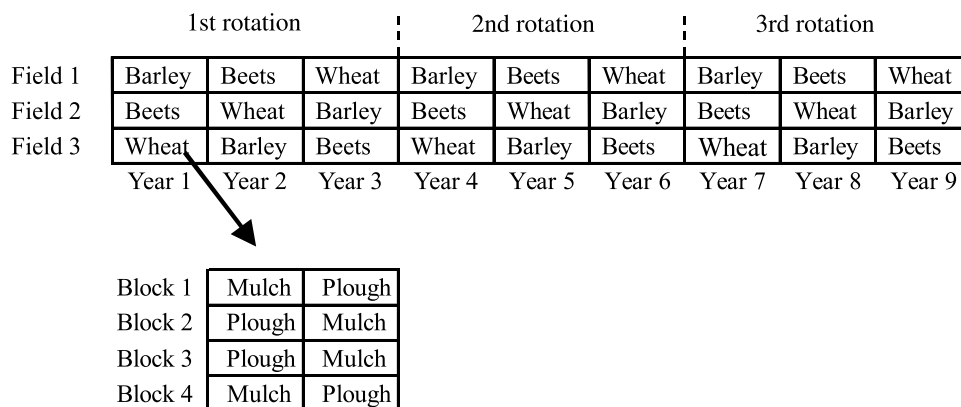


Fig. 4: Schematic representation of experimental design for long-term rotation experiment

In a joint analysis across different crops, variance may differ among crops. One can try to extend the mixed model accordingly, although fitting may turn out to be difficult when the random part is complex and has several random effects. An alternative remedy is a simple data transformation (logarithmic or square root) to remove variance-mean dependencies. A data transformation may also be needed to achieve approximate additivity of effects, when there are gross differences in scale between crops.

We have presented a joint analysis across crops. Alternatively, one can analyse the rotation separately for each crop. In this case, the appropriate model is obtained from the one above by deleting all effects involving C.

The year factor was taken as random to draw more general conclusions. One might also take the year effect as fixed. In this case, the design will be very difficult to analyse, because many of the fixed effects will be confounded. Basically, the confounding problem arises because there are no true replications for the crop factor (C) in a given year. Clearly, a valid analysis of this experiment is not possible for a single year. Replications for the crop factor become available by repeated observations across years, but these replications yield a valid error term for the crop factor only when years are assumed to be random. The confounding problem vanishes if analysis is performed separately for each crop. Moreover, on a crop basis, a valid analysis is forthcoming also with years considered as fixed.

**Example 11:** This example concerns a line-source sprinkler-irrigation experiment, which is described in the SAS/STAT manual (SAS Institute, Inc. 1999, p. 2213): ‘Three cultivars (...) of winter wheat are randomly assigned to rectangular plots within each of three blocks (BLOCK). The nine plots are

located side-by-side, and a line-source sprinkler is placed through the middle [of each plot]. Each plot is subdivided into twelve subplots, six to the north of the line-source, six to the south (DIR). The two plots closest to the line-source represent the maximum irrigation level (IRRIG = 6), the two next-closest plots represent the next-highest level (IRRIG = 5), and so forth.’ Cultivars will be coded by C, while plots are coded by PLOT.

- (1) C
- (2) BLOCK/PLOT = BLOCK : BLOCK · PLOT
- (3) -

- (4) IRRIG × DIR
- (5) C × IRRIG × DIR
- (6) BLOCK/(IRRIG × DIR) = BLOCK + BLOCK · IRRIG + BLOCK · DIR + BLOCK · IRRIG · DIR
- (7) BLOCK · PLOT · pt(IRRIG × DIR) = BLOCK · PLOT · IRRIG · DIR

We fit a spatial correlation structure to BLOCK · PLOT · IRRIG · DIR, using BLOCK · PLOT as a subject effect, because this corresponds to the randomization unit on which repeated measurements (unrandomized) are made.

- (8) C + IRRIG + DIR + C · IRRIG + C · DIR + IRRIG · DIR + C · IRRIG · DIR + BLOCK + BLOCK · IRRIG + BLOCK · DIR + BLOCK · IRRIG · DIR : **BLOCK · PLOT · IRRIG · DIR**

The resulting model is by no means the only possible choice. See Wolfinger (1993) and SAS Institute, Inc. (1999) for further analyses. Our model departs from those in SAS Institute, Inc. (1999) in several respects:

- (i) The repeated-*cum*-treatment structure comprises the three-way interaction C · IRRIG · DIR. We do not think such an effect can be ruled out on *a priori* grounds. For example, a high supply

of irrigation water may favour infection with an airborne disease which spreads predominantly in wind direction. If cultivars show differing degrees of resistance, this will cause a three-way interaction.

- (ii) Block effects are taken as fixed, not random, reflecting the fact that blocks cannot be randomized in this design, nor do they form a random sample from a well-defined universe.
- (iii) The block effects comprise the three-way effect **BLOCK · DIR · IRRIG**, corresponding to 12 rows within a block extending from east to west. We think this effect has a direct physical meaning and so should be included.
- (iv) Spatial correlation is restricted to subplots within a randomization unit (plot), whereas others (SAS Institute, Inc. 1999, and references cited therein) have suggested a spatial correlation over the whole experimental area. Our restriction results from a two-step approach to specifying a repeated block model. We first set up the non-repeated block model based on randomization theory, and then incorporate the repeated structure. We do not mean to say that this is the only option, it is just one convenient way of arriving at a useful model by a unified procedure.

We fitted different models to **BLOCK · PLOT · IRRIG · DIR** (Table 3). An unstructured model could not be fitted due to a lack of observations. A Toeplitz model with heterogeneous variances and a range of three observations (Wolfinger 1996) fitted best according to the AIC (Table 3). This model allows a separate variance for each row of the design. A separate covariance is fitted for contiguous subplots and for subplots separated by one subplot. The covariance for subplots further apart is assumed to be zero. The degrees of freedom for Wald tests were approximated by the method of Kenward and Roger (1997). The SAS code is as follows:

Table 3: Akaike information criterion (AIC) values (in smaller-is-better form) for different correlation structures of effect **BLOCK · PLOT · IRRIG · DIR**

Correlation structure	AIC (smaller-is-better)
Independent	126.3
Compound symmetry	128.2
AR(1)	127.0
Heterogeneous AR(1)	114.1
Toeplitz (5)	119.0
Heterogeneous Toeplitz (3)	107.1

```
proc mixed;
  class Block C Dir Irrig Plot;
  model Y = C Dir Irrig
    C*Dir C*Irrig Dir*Irrig
    C*Dir*Irrig
    Block Block*Dir Block*Irrig
    Block*Dir*Irrig/ddfm = kr;
  repeated/type = toeplitz(3)
  subject = Block*Plot; run;
```

With the Toeplitz model as well as with other time series models, it is important that observations be arranged in serial order within each subject (SAS Institute, Inc. 1999). The resulting F-tests are reported in Table 4. Among the interactions in the repeated-cum-treatment model, only **DIR · IRRIG** is significant. The cultivar main effects were not significant. Thus, we fitted marginal means for **DIR · IRRIG** (Table 5). The mean values indicate that the profiles are different for the two directions, as is expected from the significant interaction. Specifically, in the northern direction, yield decreases more pronouncedly with distance from the source. This interpretation with regard to the repeated factors needs to be taken with a grain of salt, however, because it is restricted to the blocks used in the experiment (see example 1).

## Discussion

Our notion of repeated measures is confined to cases where levels of the repeated factors cannot be randomized, i.e. serial correlation (temporal, spatial or both) results from a lack of randomization and the fixed serial order of levels of the repeated factors. This is in contrast to analyses where a repeated measures correlation structure is assumed with respect to randomized treatment factors. For example, different medical treatments may be administered to the same patients in randomized temporal order. Similarly, different diets may be fed to the same animals in randomized temporal order. Care must be taken to account for carry-over effects, either by leaving enough time between treatments or by using a special design, e.g. a so-called cross-over design (Jones and Kenward 2003), which involves a balancing for sequential treatment order. Clearly, in these types of trial, medical treatment or diet are not repeated factors as defined in this paper, as temporal order is randomized. In these instances, patients or animals serve as blocking units, which may be regarded as random, and treatments are randomized among different points

Table 4: Wald-type F-tests for fixed effects in line-source sprinkler-irrigation experiment computed by Kenward and Roger (1997) method. Heterogeneous Toeplitz (3) structure for error

Effect	Numerator d.f.	Denominator d.f.	F-value	P-value
C (Cultivar)	2	27.8	1.59	0.2227
DIR	1	27.8	3.54	0.0703
C · DIR	2	27.8	1.15	0.3326
IRRIG	5	13.4	184.39	<0.0001
C · IRRIG	10	14.8	2.12	0.0931
DIR · IRRIG	5	15.1	9.74	0.0003
C · DIR · IRRIG	10	16.1	2.25	0.0711
BLOCK	2	27.8	10.11	0.0005
BLOCK · DIR	2	27.8	1.11	0.3438
BLOCK · IRRIG	10	14.8	2.50	0.0541
BLOCK · DIR · IRRIG	10	16.1	1.50	0.2276

Table 5: Marginal yield mean for line-source sprinkler-irrigation experiment. Multiple comparison by Wald-type *t*-tests (Kenward and Roger 1997)

Irrigation level (IRRIG)	Direction (DIR)	
	North	South
1	2.37 <sup>a</sup> S	2.48 <sup>a</sup> S
2	2.57 <sup>a</sup> S	3.80 <sup>b</sup> T
3	4.49 <sup>b</sup> S	5.62 <sup>c</sup> T
4	6.17 <sup>c</sup> S	6.21 <sup>d</sup> S
5	6.60 <sup>c</sup> S	6.20 <sup>d</sup> S
6	6.59 <sup>c</sup> S	6.20 <sup>dc</sup> S

<sup>S</sup>S.E.D. (minimum/mean/maximum) for comparison within a column: 0.123/0.260/0.381; within a row: 0.211/0.290/0.367. S.E.D. is not constant among pairs; thus we report minimum, mean and maximum S.E.D. Mean values in a column followed by the same lower case letter are not significantly different. Mean values in a row followed by the same upper case letter are not significantly different. Letter representation by method of Piepho (2003).

in time. An important difference to the designs considered in this paper is that time is not itself a factor of interest. Instead, replication in time is needed to be able to test several treatments on the same subject (animal, person), thus making the subject a blocking variable. Such designs are less common in plant research, where it is not usually possible to apply different treatments to the same standing crop, except perhaps with perennial species. There are two different options for analysing these types of experiment in a repeated measures framework. One is to fit a serial correlation to observations on the same unit (person, animal,

plot, plant), despite the fact that these involve randomized levels of the treatment factor (Littell et al. 1998). Fitting a repeated measures correlation structure is not strictly necessary because one might as well appeal to randomization theory and fit a standard model, possibly using time as a second blocking variable. Alternatively, one may model the correlation among treatments on the same observational unit. Specifically, observations on some pairs of treatments may be more highly correlated than for other pairs, and variances may differ among treatments (Jennrich and Schluchter 1986). The types of correlation structure suitable for this type of analysis are no longer of the serial type, because ‘distance’ between treatments does not usually have a temporal or spatial dimension. It is perhaps instructive to consider what would be an analogous situation for the two modelling options in a field experiment laid out in randomized complete blocks. An analogy is more easily found with respect to spatial rather than temporal correlation. The first option is analogous to fitting a spatial correlation among plots within the same block. The second option corresponds to fitting a correlation among treatments within the same block. The first option is seldom used in agronomy trials, because randomization theory leads to the more familiar assumption of independent errors. The second option is rarely tenable, mainly because of the small number of observations. Models with correlations among treatments in the same block are more relevant when the blocking variable corresponds to environments, thus having many levels, as, e.g. in the analysis of yield stability (Piepho 1998).

An important feature of our proposed procedure is an asymmetry between the repeated and treatment factors. An interaction with block factors is assumed for the repeated factors, but not for the treatment factors. The main reason for this asymmetry lies in the fact that levels of the repeated factors are 'innate' (Brien 1983) to the experimental units in much the same way as are the block factors. By contrast, treatment factors are randomized and thus not innate to the experimental units. Clearly, if the repeated factor were not innate to experimental units, it could and should be randomized in the first place, thus becoming a randomized factor. The asymmetry leads to a more restricted inference space for the repeated factors. It is instructive to imagine the design for a repeated factor in the case where there are no randomized treatment factors. In example 1 this would mean that the whole experimental area is managed identically and different soil layers are sampled. If the area is divided into plots according to an RCBD and water collectors are placed at a random position within a plot, one may, at least approximately, regard the positions as a random sample from the experimental area. If significant differences are found between layers, this finding will be restricted to the experimental area. We cannot make any inference regarding the immediately neighbouring area. This is in contrast to the treatment factor; providing treatment-block additivity holds on the neighbouring area, inferences from the experiment can be extended to that area. Of course the additivity assumption is a strong one. The more heterogeneous the experimental area, the less realistic is this assumption. In most cases, however, an asymmetry between treatment and repeated factors arises because for the former the additivity assumption is plausible, whereas for the latter additivity must be rejected on *a priori* grounds, as exemplified in example 1; heterogeneity between blocks regarding the soil profiles will imply an interaction between block and repeated factor, whereas block treatment additivity is unaffected.

Our approach is a mix based on randomization theory (non-repeated model) and spatial/temporal correlation modelling (repeated factors). It dictates that an autocorrelation be fitted to repeated error effects, but not to other random effects involving a repeated factor. This approach reflects our focus on an appropriate model for the repeated measures error structure. In principle, modelling of a temporal or spatial correlation can be extended to all random treatment and block

effects involving a repeated factor, and it may be worthwhile to explore this after a model has been obtained by our step-by-step approach. While this extension may yield plausible models, it can dramatically increase the potential number of correlation structures to be considered. Moreover, for at least some of these effects, the number of realized values may be too small to reliably estimate anything beyond an independent model. If the sample size is limiting, it is better to use simpler models with fewer parameters. One has to find the optimal middle ground between very parsimonious but overly simplistic models on the one hand and very realistic but overparameterized models on the other. Model selection using AIC or similar measures may help to identify models which strike the right balance (Burnham and Anderson 1998). Finding a good model depends on many decisions, and the process is usually iterative (Wolfinger 1993, 1996). With repeated measures models, it often happens that variance components have negative estimates or the correlation structure is not positive-definite, and this may cause convergence problems. Usually, this is an indication that the correlation model needs to be simplified.

The approach presented in this paper restricts modelling of spatial correlation to variation within randomization units, while variation between randomization units is modelled in accordance with the underlying randomization procedure. Alternatively, one may extend spatial modelling across randomization units. In the extreme, the spatial correlation structure extends across the whole experimental area (Gilmour et al. 1997, Schabenberger and Pierce 2002). Such models may be viable alternatives to the models generated by our approach. The philosophy of our approach is to restrict spatial modelling to units which cannot serve as randomization units, while appealing to randomization theory wherever possible. This may be seen as a conservative approach, which reduces the need for model selection, and thus the problem of over-fitting, to a minimum. The important point here is that the non-repeated block model is fully specified by the randomization scheme, while there is ample scope for modelling of the repeated part of the block model.

Once a suitable correlation structure has been identified, inference for fixed effects may be based on the Kenward and Roger (1997) method. This has been shown to fare well in a number of settings, but more comprehensive simulation studies on the



performance in small samples with repeated measures correlation structures are still needed before the method can be recommended for routine application (Schaalje et al. 2002).

Our approach is useful mainly for designs where the repeated factors have few levels, and where each level is observed for all or many of the experimental units. The procedure yields essentially the same analysis as a repeated measures MANOVA, providing (i) all levels of the repeated factors are observed for all experimental units, (ii) the model only has one error term, (iii) all treatment and block factors are fixed, and (iv) an unstructured correlation model is fitted. Thus, our procedure subsumes repeated measures MANOVA as a special case. It is a major advantage of mixed modelling that correlation structures simpler than unstructured can be fitted, that more than one random term can be accommodated, and that it is not necessary to observe all levels of the repeated factor on each experimental unit.

In this paper we have focused on the block model and error effects, while mainly ignoring the repeated-cum-treatment part of the model. In fact, our procedure generates a raw model, which gets the block model right and at the same time leaves opportunity for more refined modelling of the repeated-cum-treatment model, e.g. by non-linear regression on covariates varying with levels of the repeated factors (Davidian and Giltinan 1995). For example, to study time trends, a non-linear regression on the time variable is in order. Such models are also known as 'growth curves' in the literature on repeated measures (Krzanowski and Marriott 1995). Regression approaches may be particularly useful when the repeated factors have many levels.

## References

- Berzsenyi, Z., B. Györfy, and D. Lap, 2000: Effects of crop rotation and fertilization on maize and wheat yields and yield stability in a long-term experiment. *Eur. J. Agron.* **13**, 225–244.
- Brien, C. J., 1983: Analysis of variance tables based on experimental structure. *Biometrics* **39**, 53–59.
- Burnham, K. P., and D. R. Anderson, 1998: *Model Selection and Inference*. Springer, New York.
- Cole, J. W. L., and J. E. Grizzle, 1966: Applications of multivariate analysis of variance to repeated measures experiments. *Biometrics* **22**, 810–828.
- Davidian, M., and D. M. Giltinan, 1995: *Nonlinear Models for Repeated Measurement Data*. Chapman & Hall, London.
- Geisser, S., and S. W. Greenhouse, 1958: An extension of Box's results on the use of the F-distribution in multivariate analysis. *Ann. Math. Stat.* **29**, 885–891.
- Gilmour, A. R., B. R. Cullis, and A. P. Verbyla, 1997: Accounting for natural and extraneous variation in the analysis of field experiments. *J. Agric., Biol., Environ. Stat.* **2**, 269–293.
- Hand, D. J., and M. Crowder, 1996: *Practical Longitudinal Data Analysis*. Chapman & Hall, London.
- Jennrich, R. L., and M. D. Schluchter, 1986: Unbalanced repeated-measures models with structured covariance matrices. *Biometrics* **42**, 805–820.
- Jones, B., and M. G. Kenward, 2003: *Design and Analysis of Cross-over Trials*. Chapman & Hall, London.
- Kenward, M. G., and J. H. Roger, 1997: Small sample inference for fixed effects from restricted maximum likelihood. *Biometrics* **53**, 983–997.
- Krzanowski, W. J., and F. H. C. Marriott, 1995: *Multivariate Analysis. Part 2: Classification, Covariance Structures and Repeated Measurements*. Arnold, London.
- Littell, R. C., P. R. Henry, and C. B. Ammerman, 1998: Statistical analysis of repeated measures data using SAS procedures. *J. Anim. Sci.* **76**, 1216–1231.
- Mead, R., R. N. Curnow, and A. M. Hasted, 1993: *Statistical Methods in Agriculture and Experimental Biology*. Chapman & Hall, London.
- Nelder, J. A., 1965: The analysis of randomized experiments with orthogonal block structure. I. Block structure and the null analysis of variance. II. Treatment structure and the general analysis of variance. *Proc. R. Soc. Lond. A* **283**, 147–178.
- Ogliari, P. J., and D. F. Andrade, 2001: Analysing longitudinal data via nonlinear models in randomized complete blocks. *Comput. Stat. Data Anal.* **36**, 319–332.
- Patterson, H. D., 1964: Theory of cyclic rotation experiments. *J. R. Stat. Soc. B* **26**, 1–45.
- Patterson, H. D., and H. I. Lowe, 1970: The errors of long-term experiments. *J. Agric. Sci., Camb.* **74**, 53–60.
- Piepho, H. P., 1998: Methods for comparing the yield stability of cropping systems – a review. *J. Agron. Crop Sci.* **180**, 193–213.
- Piepho, H. P., 2003: An algorithm for a letter-based representation of all-pairwise comparisons. *J. Comput. Graph. Stat.*
- Piepho, H. P., A. Büchse, and K. Emrich, 2003: A hitchhiker's guide to the mixed model analysis of randomized experiments. *J. Agron. Crop Sci.* **189**, 310–322.
- Preece, D. A., 1986: Some general principles of crop rotation experiments. *Exp. Agric.* **22**, 187–198.
- Rowell, J. G., and D. E. Walters, 1976: Analysing data with repeated observations on each experimental unit. *J. Agric. Sci., Camb.* **87**, 423–432.
- SAS Institute, Inc., 1999: *SAS/STAT User's Guide*. SAS Institute, Cary, NC.

- Schaalje, G. B., J. B. McBride, and G. W. Fellingham, 2002: Adequacy of approximations to distributions of test statistics in complex mixed linear models. *J. Agric., Biol., Ecol. Stat.* **7**, 512—524.
- Schabenberger, O., and F. J. Pierce, 2002: *Contemporary Statistical Models for the Plant and Soil Sciences*. CRC Press, Boca Raton, FL.
- Senn, S., L. Stevens, and N. Chaturvedi, 2000: Tutorial in biostatistics – repeated measurements in clinical trials: simple strategies for analysis using summary measures. *Stat. Med.* **19**, 861—877.
- Singh, M., and M. J. Jones, 2002: Modeling yield sustainability for different rotations in long-term barley trials. *J. Agric., Biol., Environ. Stat.* **7**, 525—535.
- Steel, R. G. D., J. H. Torrie, and D. A. Dickey, 1996: *Principles and Procedures of Statistics: A Biometrical Approach*, 3rd edn. McGraw-Hill, New York.
- Verbeke, G., and G. Molenberghs, 2000: *Linear Mixed Models for Longitudinal Data*. Springer, New York.
- Wolfinger, R. D., 1993: Covariance structure selection in general mixed models. *Commun. Stat. – Simulation Comput.* **22**, 1079—1106.
- Wolfinger, R. D., 1996: Heterogeneous variance–covariance structures for repeated measures. *J. Agric., Biol., Environ. Stat.* **1**, 205—230.
- Yates, F., 1954: The analysis of experiments containing different crop rotations. *Biometrics* **10**, 324—346.