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Some problems in the design and analysis of field experiments subject to inter-plot interference

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SUMMARY

Results from field experiments with mobile pests and air-borne pathogens are subject to bias as a result of inter-plot interference. Serially balanced designs (SBDs) allow interference to be estimated but other designs may be better for decreasing such effects. To investigate this, systematic replicated designs, comparing sprays applied at different times to control powdery mildew of spring barley, were sited in 2 years alongside SBDs testing similar treatments. Yields of grain and assessments of mildew on the leaves were analysed. Results from the balanced designs provided strong evidence of interference in both years but not in a third (when the systematic design was omitted). Estimates of treatment effects from the systematic designs were often, but not consistently, greater than corresponding estimates from the SBDs. A method of analysis from Draper & Guttman (1980) was adapted to produce estimates of the differences between treatments as if applied to all plots of an experiment; this showed larger differences between treatments than the conventional analysis in 1975 and 1976 (when there was appreciable interference), but failed in 1977 when interference was slight. This method fails when applied to the systematic designs; SBDs (which are a subset of all designs in randomized blocks) are probably optimal for this type of analysis. The difficulties of analysing data in the form of percentages or proportions (with consequent non-orthogonality) are discussed.

INTRODUCTION

Estimating the effect of a treatment applied to a field crop in order to control a mobile organism, whether an insect pest or a fungal pathogen, is beset by difficulties. The true commercial value of the treatment is the difference between the yield of a whole field if treated and its yield if no treatment had been applied to any part of the same field. In some instances this ideal may be approached by using several pairs of fields, each pair as nearly as possible sharing circumstances such as soil type, exposure and cultivar, and applying the treatment to one field of each pair, selected at random, leaving the other untreated. However, this procedure is cumbersome, costly and often impracticable. For comparing larger numbers of treatments, the only possible method seems to involve conventional experiments, each within one field, where all of the treatments are applied to plots that are small relative to the fields in which they are placed, and which they are meant to represent (van der Plank 1963).

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The initial inoculum responsible for causing a disease may arise from sources within the field or outside it, either other fields, near or far, or wild plants or waste dumps. The difficulty that is considered in the present paper is the subsequent spread from point to point within a crop, e.g. by passive transfer of fungus spores by wind or in splash-droplets (or, similarly, the active movement of insects). When inoculum moves between plots of a designed experiment in which amounts of disease vary, and the net transfer of inoculum between plots differs, the resulting effects are called 'inter-plot interference'. This cannot be eliminated by, for example, taking the special precautions that may help to prevent drift of sprayed materials.

Two separate challenges arise: first, to detect and estimate the effects of interference and, second, to design experiments that minimize the bias in estimates of treatment differences and the decreased precision due to interference. Serially balanced designs (SBD; Dyke & Shelley 1976) were used to detect and estimate effects of interference (Jenkyn *et al.* 1979). Using grain yields recorded in three experiments on spring barley in 1975–77 (not the ones reported in the current paper; for details see Jenkyn *et al.* 1983), another possible

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method of detecting interference was tested. This involved covariates representing presence or absence of a fungicide spray applied to adjacent plots (Dyke, unpublished data). In none of these analyses, 12 in all, was there any significant improvement in precision; indeed many of the regression coefficients were in the sense opposite to that expected. Experience suggests that the use of SBDs is much the better method; unfortunately SBDs for more than about four treatments need too many plots to be practicable. Draper & Guttman (1980) suggested models that assume that the yield of a particular plot, though determined largely by its own treatment, is affected to a lesser degree by the treatment(s) applied to neighbouring plots. Methods of analysis that use the yields of adjacent plots, either directly or after adjustment for estimated effects of treatments, cannot be relied upon to mitigate or eliminate the effects of interference; indeed, in certain circumstances, such methods may give a poorer analysis (Ainsley *et al*. 1995).

Many authors have offered methods of analysis that take account of inter-plot interactions, some using as covariates averages of the data from adjacent plots (Kempton 1982). The approach of others is to assume that each plot is affected to some extent by the treatments applied to its neighbours. Pearce (1957) assumed that such an effect was constant for all plots within a block, zero for plots in other blocks; Finney (1962) dealt with an experiment in which each harvested unit included, as a consequence of a mistake, equal areas given two treatments intended for separate plots. The method of Draper & Guttman (1980), which is used in the present paper, develops this method comprehensively. Kempton (1982) considered applying the Draper & Guttman method (DG) in variety trials but concluded that, where competition is the dominant form of inter-plot interaction, effects are likely to be more directly related to the yields of adjacent plots than to their treatments.

The problem of lessening interference by design and layout of experiments is easier if important amounts of inoculum move only small distances in relation to plot dimensions; in such cases the use of larger plots with substantial areas discarded at the edges, is relatively straightforward. However, larger plots imply larger sources, which can be expected to result in extended gradients, and the need for a larger total area, which can create other problems.

The use of 'separators' or 'surrounds' of some standard treatment (e.g. a resistant cultivar) may ensure that every plot has a standard environment, so that replicate plots of the same treatment will experience equal expected transfer of inoculum both inward and outward and may, therefore, be expected to behave similarly. However, it still implies that plots testing at least some of the treatments will have environments which are markedly different from the plots themselves, and thus give results that are different from the results of applying such treatments to a whole field (Jenkyn *et al*. 1996). In the present experiments it was clear that wind was important in generating interference and, in consequence, effects of neighbours on opposite sides are usually different. A similar difference is perhaps also likely in variety trials, since differential shading must sometimes occur in narrow plots running east/west with varieties of differing habits of growth, but such effects have not been detected (Kempton *et al*. 1986). The problem of inter-plot competition when comparing cultivars with different habits of growth (e.g. tall and short cereal cultivars) has been tackled by restricting randomization so that cultivars of greatly different competitive power are not allocated to adjacent plots (David & Kempton 1996; David *et al*. 1996).

Interference will not be a problem if all treatments have identical amounts of disease (e.g. in experiments testing treatments that are unlikely to affect disease), and will tend to become more important as the differences between treatments (e.g. unsprayed *v*. sprayed) become larger. Removing untreated reference plots from an experiment can help but in spray-timing trials, for example, late-sprayed plots are identical to unsprayed until they are sprayed. The use of systematic designs in which treatments are set out in an order chosen to minimize differences between adjacent plots, may help to overcome the biological problems although analysis of the data may not be straightforward (Bainbridge & Jenkyn 1976). Systematic designs have also been used to produce epidemics of differing intensity and timing in studies of crop loss in relation to disease (Shaw & Royle 1987; Thomas *et al*. 1989; Hims & Cook 1992; Hims & Gladders 1994; Cook *et al*. 1999). Gill (1993) discusses optimality and balance of designs in relation to both the linear model and a non-linear model as suggested by Draper & Guttman (1980). The designs of Gill (1993), however, all include treatments applied to pairs of adjacent plots, which the current authors preferred to avoid.

Roberts (1960) used a systematic design in an early attempt to assess interference effects in tests of insecticide sprays applied to cotton in the Sudan Gezira. More complex systematic designs have been used in assessing the optimum rates of application of fertilizers to vegetable crops (Greenwood 1979). It should be noted that none of the papers on systematic designs cited above make any comparison between systematic and randomized designs testing the same treatments; however Phillipeau *et al*. (1996) and David *et al*. (2001) compared various designs and sizes of plots for use in variety trials.

Results from experiments on spring barley in which serially balanced designs were used to detect and estimate interference resulting from the movement between plots of inoculum of powdery mildew (*Erysiphe graminis* f.sp. *hordei*) were reported by Jenkyn *et al*. (1979). Experiments of systematic design were sown

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Table 1. *Dates of applying tridemorph sprays*

(*a*) Systematic designs

Treatment	Code	Date of applying tridemorph 1975	1976
Unsprayed	S0	—	—
Single spray	S1	22 May	18 May
	S2	4 June	21 May
	S3	13 June	27 May
	S4	(not tested)	2 June
Repeated sprays	SR	as S1, S2 and S3	as S1, S3, plus 7 June

The arrangement of the differently treated plots in each of the two years is shown below, omitting the symbol S.
1975: R0R123[R]-gap-R0R321123R0R321R0R
1976: R0R1234R0R43211234R0R4321R0R

(*b*) Serially balanced designs
The SBDs had treatments as follows:

1975	S0, S1, S2, SR (dates as in (*a*) above)
1976	S0, S1, S3, SR (dates as in (*a*) above)
1977	S1, S2, S3, S4 (31 May, 9 June, 20 June, 5 July respectively)

adjacent to the balanced designs in 2 of the 3 years, but results from these have not previously been reported. Although the number of treatments tested in these experiments was limited (mainly because of the practical difficulties of including more in the balanced designs), the contrasting data sets do provide an opportunity to assess the relative merits and drawbacks of each type of design.

METHODS

Source of the data

The data reported in the current paper were obtained from experiments on spring barley, grown at Rothamsted Experimental Station in 1975–77, which tested the effects of applying the fungicide tridemorph at different times to control powdery mildew (*Erysiphe graminis* f.sp. *hordei*).

In the context of the current paper it is the precise arrangement of the differently treated plots that is of most interest. The arrangement of plots, and the treatments compared, are given in Table 1. In 1975 and 1976, the systematic designs were each in one single line of plots, parallel to the line of the SBD. Each SBD occupied 38 plots (including one dummy plot at each end); treatments were arranged in nine 'blocks' of four plots, allocated so that the nine plots of each treatment had as left-hand and right-hand neighbours all combinations of the other three treatments. These designs are thus samples drawn from a subset of all the possible randomizations of four treatments in nine complete blocks. The arrangements of the systematic experiments are based on the assumption that (i) plots receiving a single spray are less likely to interfere with one another if they are sprayed on dates that are close together than if the dates are further apart, (ii) unsprayed plots represent the greatest hazard but will affect repeatedly sprayed plots (where fungicidal cover is maintained) much less than they will once-sprayed plots (where fungicidal residues will diminish over time and where leaves emerging after the application of sprays may be less adequately protected). The 'gap' in the 1975 experiment was necessary to avoid a depression in the field (a 'dell') and as a consequence an additional dummy plot ([R]) was included to provide the appropriate neighbour for the sixth true plot in the sequence.

Individual plots measured 4·3 m wide × 7·6 m long in 1975 and 4·3 m wide × 9·1 m long in 1976 and 1977. A combine harvester was used to determine yield in an area 2·1 m wide × the total length, the outer five rows on each side of every plot being discarded. Plots were separated by fallow paths 0·6 m wide. Tridemorph was applied to the plots at 0·53 kg in 340 litres water/ha. The surrounds of the experiments, to a distance of at least 12 m, were sown with ethirimol-treated barley and sprayed with tridemorph to provide effective mildew control. Other details of the husbandry are given in Jenkyn *et al.* (1979).

Mildew was assessed on the uppermost three or four leaves of 10 shoots taken at random from within the discard areas of plots. This was done in June and July 1975, in June 1976 and in July 1977; on some occasions, the disease was separately assessed on both sides of every plot. The measured grain yield for each plot was adjusted to 850 g dry matter/kg. In the current paper only mildew on leaves 2 and 3 are considered. The 1976 experiment was affected by what appeared to be a Celtic field boundary, revealed by

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aerial photography during the extreme summer drought; the inclusion of an appropriate covariate produced only very small changes in the deviance ratios.

Statistical methods

Mildew data from any one assessment certainly cannot be assumed to have constant variance; repeated sampling on a plot with 0·1% leaf area affected would certainly give a smaller variance than similar sampling of a plot with 50% leaf area affected. Mildew data included many zero values, and in such circumstances the method of Ridout & Demétrio (1992) may be useful, although it was not used. An additional difficulty can arise if the structure of the data is factorial, e.g. all combinations of blocks and treatments. If one treatment has very little mildew, and one block has much less mildew than other blocks, the fitted value for the plot with this combination may well be negative, and the assumption is proved false. A scale ranging from $-\infty$ to $+\infty$ is essential to avoid this contradiction. The angular transformation,

$$s = \sin^{-1}(\sqrt{p}) \text{ where } p = \text{proportion}$$

(which is often used, and has the advantage that the variance of s is independent of the expected value of p), fails in this respect and, with any factorial structure, impossible values may arise. In the analysis of percentage mildew on leaf 2 in the SBD in July 1975, for example, the angular transformation gave negative (and so impossible) fitted values for five of the nine plots of treatment SR.

We chose to use the logit transformation

$$z = (1/2)\log_e(p/q) \text{ where } q = 1-p,$$

though the probit transformation might be equally suitable. The factor 1/2 is often omitted; interpretation is not at all affected.

In much published work presenting analyses of percentages (including several papers by the present authors) iteration has not been applied to logit values; tables of means were presented together with S.E.D.s calculated as if all logits were of equal weight. It is now believed, with computing power vastly increased and, it is hoped, a more statistically erudite readership, that it may be appropriate to consider making iterated (and consequently non-orthogonal) analysis the standard approach. In analysis of non-orthogonal data, estimates of treatment effects will differ with changes in the model fitted, for example, whether block differences are ignored or eliminated.

Where assessments included many records of zero, and occasionally all entries in one cell of a table were zero, the original algorithm failed to converge. This may be dealt with by omitting all data in the relevant cell; since analysis is necessarily non-orthogonal this introduces little difficulty. Tests of significance of differences between the all-zero cell and others are not needed. The original algorithm can easily be modified to deal with this problem without omitting any data; the present algorithm in Genstat does this automatically. This will deal equally well with non-convergence caused by data at the upper limit, 100% or proportions of 1. The presentation of the results based on analysis of transformed data brings difficulties. First, if any transformation (except the angular) is used, in general a different S.E.D. will be appropriate for every difference between treatment means. The same difficulty applies to means back-transformed, in the present instance to percentages. One method is to back-transform the end-points of a confidence interval on the z-scale, mean $\pm t \times$ S.E. (t from Student's distribution with appropriate D.F. and, e.g., $P = 0{\cdot}05$). For example, the back-transformed mean of logit (% mildew) on leaf 3 of SR plots in June 1976 was 0·34%, with confidence limits ($P = 0{\cdot}05$) 0·28%, 0·42%.

Jenkyn *et al.* (1979) found that interference caused substantial decreases in efficiency, as measured by the increase in the S.E.D.s of treatment means. We have chosen to use residual mean square (RMS) and residual mean deviance (RMD) for mildew as the criteria for assessing the efficiency of designs, though some other criterion, e.g. the S.E. of a particular treatment-contrast, might be appropriate in certain circumstances. Since the SBDs used in these experiments are special cases of the wider class of randomized block designs, it seems reasonable to treat them as randomized blocks for comparison with systematic (non-randomized) designs, but the comparison of RMSs between different designs presents some difficulties. The analysis of variance of the systematic designs cannot be interpreted without qualification since the contrasts representing 'residual' are not interchangeable with those representing treatments, as they are under randomization in any design in randomized complete blocks. It is not easy, however, to suggest a better estimate of residual variation. In the systematic designs considered in the current paper, a linear trend across the site would inflate the RMS. If the SBDs are regarded as designs in randomized blocks of four treatments, and the systematic designs as in blocks containing six and seven plots each in 1975 and 1976 respectively, the latter sample a wider range of environmental conditions. In addition, the standard analysis of the systematic designs will include in the estimate of residual variance, four D.F. representing comparisons between duplicate SR plots within each block, and it is at least possible that these should not be considered as homogeneous with the contrasts of the nature of treatment × block interaction. In our data, however, there was no indication of appreciable heterogeneity of variance.

If treatment SR, which had many records of zero mildew (in some assessments all were zero) is included, the fitted value is large and negative, and has very small

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statistical weight. This results in a very large standard error (S.E.D.) for any contrast between SR and another treatment. Since the experiments were planned, not primarily to estimate differences between single sprays and multiple applications, but rather differences between the effects of sprays applied on different dates, we present S.E.D.s derived from analyses omitting SR. The back-transformed means for S1, S2 (or S3) from these analyses (not presented) differ by trivial amounts from those based on analyses including all treatments.

For yields, comparison of the residual mean squares from the SBD (R1) and the systematic design (R2) gives an immediate estimate of the relative precision of the two designs; the usual *F*-test can be applied to the ratio R1 : R2 (or R2 : R1 if R2 > R1). However, in the systematic design, the estimation of residual variance from R2 is logically suspect. For any single treatment-contrast, such as (S1 *v.* SR), it is easy to calculate the difference between the estimates derived from the two designs, and to assign a measure of its variance, and for mildew an iterated, non-orthogonal analysis provides similar information for any single contrast. There is the minor logical difficulty that, if the residual variances estimated by R1 and R2 are believed to be different, the Fisher–Behrens test of significance is required; this is neither easy to apply nor logically easily acceptable.

The combined analysis of both designs by use of Residual Maximum Likelihood (REML; Patterson & Thompson 1971) avoids this difficulty. Components of variance for four strata (B1, B1/P1, B2, B2/P2, where B1, B2 represent differences between blocks and P1, P2 differences between plots within blocks, for the two designs respectively) are estimated simultaneously, and, if the 'fixed' model is D × T (Designs × Treatments), a significance test for the interaction D × T provides evidence whether the two designs are producing consistent or inconsistent estimates of treatment differences. For disease data the REML algorithm was applied first to crude, unweighted logits, and then used in alternation with the recalculation of weights and fitted values by the method of Dyke & Patterson (1952) – a laborious example of generalized linear modelling.

Possible models for interference are now considered. It is possible to construct models allowing for many variants of inter-plot interference; interference to left and right (or in four directions for experiments laid out in several rows of plots) must be expected to be different in many instances; one or more treatments may be little or not at all affected by neighbouring treatments, whereas others may be especially susceptible to interference; finally, interference may not be limited to immediately adjacent plots. It is clear, therefore, that in any experiment with limited replication and more than two or three treatments, the model used may be over-elaborate, and so leave few D.F. for the estimation of residual variation, or none. Rational methods of simplifying the model are now sought and two methods are offered. The first seeks to identify a small number of components of interference (e.g. the effect of treatment A as left-hand neighbour of treatment B); it is then possible to incorporate these in the model while assigning other components to residual variation. For mildew mean percentages of combinations of T (treatment to plot) and LHN (left-hand neighbour), averaging over RHN, were tabulated. To these (transformed to logits) the factor T was fitted; the resulting deviances illustrate the components of interference; the process with LHN, RHN interchanged was repeated.

An alternative method used to simplify the model is that of Draper & Guttman (1980), which provides an elegant and adaptable method of dealing with interference, designated 'overlap'. In the present paper their method is used in a very simple form, slightly different from the one which they used, to reanalyse some of the data considered in the present paper. Draper & Guttman (1980) suggest more complex models, for example one in which plot P is affected by overlap from more distant neighbours:

$$Y_{\mathrm{P}} = t_{(\mathrm{P})} + \alpha t_{(\mathrm{L1})} + \alpha^2 t_{(\mathrm{L2})} + \alpha^3 t_{(\mathrm{L3})} + \cdots + \text{similar terms in } t_{(\mathrm{R1})} \text{ etc.},$$

where $t_{(\mathrm{L1})}$, $t_{(\mathrm{L2})}$ … refer to plots 1, 2, … spaces to the left of plot P.

It is also possible to extend the model to deal with overlap in two dimensions, and to postulate different degrees of overlap for different neighbouring treatments (e.g. one treatment may be supposed to have no overlap effect). The model chosen was

$$Y_{\mathrm{P}} = t_{(\mathrm{P})} + \alpha t_{(\mathrm{L})} + \beta_{(\mathrm{R})}$$

where Y_{P} is the fitted value for plot P, and $t_{(\mathrm{P})}$, $t_{(\mathrm{L})}$, $t_{(\mathrm{R})}$ are constants associated with the treatments applied to plot P, and to adjacent plots to left and right of plot P respectively, and α, β are constants whose values are expected to satisfy

$$-1 < \alpha,\ \beta < +1.$$

Indeed estimates of α, β which do not satisfy

$$-0{\cdot}5 < \alpha,\ \beta < +0{\cdot}5$$

might indicate either a very poor experiment or a wrong model. Positive values of α and β may be expected in experiments with, for example, mobile pathogens, and negative values in experiments where there is competition between adjacent plots.

In the serially balanced designs used in our experiments no two adjacent plots received the same treatment, and dummy plots at each end provided appropriate neighbours for the extreme plots; the plots of treatment t_1 for example had right-hand neighbours given treatment t_2, t_3 and t_4 in equal numbers, and

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the expected value of the mean of all plots of treatment t_1 is

$$E(\bar{y}_1) = t_1 + (\alpha+\beta)(t_2+t_3+t_4)/3.$$

However, in an experiment with k treatments set out in one line, with unrestricted randomization within r blocks (and without dummy plots), t_1 can have t_1 as neighbour if the two plots are in adjacent blocks. It can be shown that the expected value of the mean of all plots of treatment t_1 under the Draper & Guttman (1980) model is

$$E(\bar{y}_1) = t_1 + (\alpha+\beta)[(r-1)t_1 + (rk+r-1)\sum_{i\neq 1}(t_i)]/k^2r.$$

When $r=9$ and $k=4$, as in an experiment with fully randomized blocks, but otherwise similar in layout to the ones considered in this paper,

$$E(\bar{y}_1) = t_1 + (\alpha+\beta)[t_1/18 + 11(t_2+t_3+t_4)/36].$$

Simulations show that the attempt to fit the Draper & Guttman (1980) model fails in certain circumstances. The analysis fails either by not achieving convergence with a reasonable number of iterations, or by apparent convergence with absurdly large estimates of α and β. Using a design of nine blocks of four plots each, either serially balanced or randomized, and with zero treatment effects and block effects included in the fabricated variate X the analysis fails when either

X = a random sample from a normal population;
or X = a simple trend, either linear ($X=1, 2, 3, \ldots, 36$) or $X=\cos(r\times\pi/37)$ or $X=\sin(2r\times\pi/37)$ with $r=1, 2, 3, \ldots, 36$, which both have a strong linear component.

The method of Draper & Guttman (1980) is ineffective when applied to the systematic designs used in these and other experiments; the reason is illustrated by considering plots of treatment S0. These occur only with neighbours on both sides given treatment SR; for these plots therefore the allocation of the resulting measurement between the direct effect t_0 and the overlap effect $\alpha+\beta)t_R$ is completely arbitrary. Each other treatment occurs always with the same pair of neighbours (with order reversed in half the replicates) and so some degree of similar 'confounding' applies to them also.

The current model can be illustrated by supposing that plot P is divided into three sections, or sub-plots, with areas in ratios $1:\alpha:\beta$, which receive respectively $t_{(P)}$, $t_{(L)}$, $t_{(R)}$. This justifies the use by Draper & Guttman (1980) of the term 'overlap' rather than 'interference', though the two terms are interchangeable. It is suggested that the term 'overlap' is appropriate to denote any effect of the treatment of a plot which affects a neighbouring plot; this includes interference by transfer of pathogens, but also spray-drift, accidental mis-application, etc.

The current simple model (and, no doubt, other, more complex models) leads directly to estimates of the yields that would have been recorded if all plots and immediate surrounds had received the same treatment (t_i, say). For the current model these are respectively

$$w_i = t_i(1+\alpha+\beta).$$

The current model may be expressed in a different, but exactly equivalent, form:

$$Y_P = (w_{(P)} + \alpha w_{(L)} + \beta w_{(R)})/(1+\alpha+\beta).$$

In this form the estimates w_i provide 'whole-site' estimates, and routine analysis (e.g. by Genstat) produces variances and covariances etc. of the w_i.

Table 2. *Deviance-ratios† for effects of neighbours in serially balanced designs*

	1975		1976		1977	
	LHN	RHN	LHN	RHN	LHN	RHN
Yield of grain	1·64	2·01	0·57	1·10	1·58	1·13
Mildew (June) on leaf 2	1·97	8·91***	0·56	0·55		
Mildew (June) on leaf 3	1·39	4·62**	0·23	0·91		
Mildew (July) on leaf 2	5·14**	3·59*			0·78	0·42
Mildew (July) on leaf 3	1·99	5·50**			1·29	1·82

LHN, RHN: Treatment to left-hand neighbour, right-hand neighbour respectively; LHN fitted first.
* $P<0·05$; ** $P<0·01$; *** $P<0·001$.
† With 8 and 16 D.F.

RESULTS

Detection of interference

Table 2 presents the deviance ratios for interference effects, separately for effects of left-hand neighbours and right-hand neighbours. For yields these are simply ratios of mean squares from the analysis of variance but for mildew assessments transformed to logits they are ratios of mean deviances; because of non-orthogonality induced by the transformation, the ratios calculated differ a little according to the order of fitting (left followed by right or vice versa). In 1975, deviance ratios for yield were non-significant but for mildew on both leaves in June effects of neighbouring treatments to the right were highly significant, and in July on leaf 2, neighbours on both sides had significant effects; on leaf 3, by contrast, only right-hand neighbours showed significant effects. In 1976 and 1977,

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Table 3. *Means and deviances for treatments with different left-hand and right-hand neighbours in SBDs, for mildew on leaf 2, July 1975*

	LHN/RHN means				
Plot treatment	S0	S1	S2	SR	Total deviance
S0	—	3·39/4·75	5·72/5·51	3·66/2·52	2·30/3·82
S1	3·78/1·97	—	1·36/1·71	1·36/2·82	5·15/0·93
S2	1·27/1·54	1·24/0·77	—	0·54/0·73	1·15/1·18
SR	0·003/0·002	0·025/0·000	0·001/0·027	—	0·13/0·15
Total deviance	3·22/0·81	0·78/0·42	2·56/1·43	2·16/3·43	8·73/6·09

LHN, RHN: treatment to left-hand, right-hand neighbour respectively.
— Indicates combination absent from the design.

however, there was no evidence of neighbour effects on yield nor on mildew.

Components of interference

Treatments that produced very different amounts of mildew, such as unsprayed and repeatedly sprayed, may sometimes be expected to show substantial mutual interference. Also, if plots repeatedly sprayed are effectively protected they may be negligibly affected by unsprayed neighbours, whilst unsprayed plots may benefit from a net loss of inoculum. If the effect of a spray is ephemeral, late-sprayed plots may act as local sources of inoculum and so cause infection of neighbouring early-sprayed plots; indeed the pattern of interference may change during the season. In 1975, for example, interference in June, and on leaf 3 in July was mainly caused by right-hand neighbours, but on leaf 2 in July left-hand neighbours had more effect than right-hand neighbours. Table 3 illustrates a relevant method of examining components of interference; we choose mildew on leaf 2 (July) of the 1975 experiment, in which neighbour effects were clearly significant. The means shown are unweighted means over the three levels of RHN; the implied model has treatment structure

$$\mathrm{T/LHN} = \mathrm{T} + \mathrm{T.LHN}.$$

The deviances result from fitting the main effect of plot treatment to logit values for different neighbours. Marginal totals of deviances are a guide to distinguish between treatments that are more or less effective in interfering with neighbours, and, similarly, to distinguish between treatments that are more or less susceptible to interference. It is clear that plots with treatment S1 were most affected by neighbouring treatments to the left, and that treatment S0 was most effective in influencing plots to the right, though treatments S2 and SR were almost equally effective. Examination of deviances for individual combinations shows that the largest single deviance came from S1 plots with S0 as left-hand neighbours (deviance 3·03, mean of all combinations 0·73). Effects of right-hand neighbours were less clearly detected, but the greatest component of the interaction T.RHN was contributed by S0 plots with SR as right-hand neighbours (deviance 3·82; mean of all 0·51).

Table 4. *Summary of deviances due to effects of neighbours in SBDs, mildew on leaf 2, July 1975*

	Side of plot	
Plot treatment	Left	Right
(*a*) Effects of left-hand neighbours		
S0	3·76	3·25
S1	9·78	1·69
S2	3·61	0·18
SR	0·08	0·17
Totals	17·22	5·29
(*b*) Effects of right-hand neighbours		
S0	1·97	6·05
S1	1·93	0·96
S2	1·16	2·28
SR	0·08	0·24
Totals	5·14	9·54

Each table entry has 2 D.F.; totals have 8 D.F.

The assessments of mildew in July 1975 were recorded separately for the left- and right-hand sides of each plot, and in Table 4 the effects of left- and right-hand neighbours on the mildew on each side are examined. For left-side records left-hand neighbours gave greater mean deviance than right-hand neighbours with ratio 3·25 ($P \approx 0{\cdot}06$); for right-side records the mean deviance due to right-hand neighbours exceeded that due to left-hand neighbours with ratio 1·85 ($P \approx 0{\cdot}2$).

Comparison of designs and methods of analysis

The Wald tests produced by REML analysis of both designs together showed that the interaction

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Table 5. *Comparison of designs and methods of analysis; residual mean squares of yield of grain (and* D.F.*), t/ha at 850 g dry matter/kg*

Year	Systematic	SBD (blocks eliminated)	SBD (blocks ignored)	SBD (DG analysis)	MS for overlap (2 D.F.)
1975	0·14 (16)	0·04 (24)	0·19 (32)	0·20 (30)	0·02
1976	0·04 (19)	0·04 (24)	0·09 (32)	0·07 (30)	0·31*
1977	—	0·02 (24)	0·03 (32)	0·03 (30)	0·07

* $P<0·05$.

Designs × Treatments exceeded its expected value (3·00) in yield in 1976 but not in 1975, and in four of the six analyses of mildew; but in all eight analyses only one value (for leaf 3 in July 1975) achieved significance ($P<0·05$).

The residual variance of yield (Table 5) was greater for the systematic than for the SBD analysed conventionally, with blocks eliminated, in both years, significantly so in 1975 ($P<0·01$). DG analysis in 1975 and 1976, with blocks ignored, gave larger residual mean squares than those of the systematic designs; the overlap effects were less than expectation in 1975, but significantly ($P<0·05$) greater in 1976, when overlap from plots on the right (roughly South-East) was double that from plots on the left ($\alpha=+0·21\pm0·148$, $\beta=+0·41\pm0·149$, deviance ratio for overlap 4·29, with 2, 30 D.F.). This degree of overlap would occur if about one quarter of each plot received the treatment applied to the right-hand neighbour plot.

The ranges of estimated yields of treatments in both 1975 and 1976 were greater for the SBD than for the systematic design (Table 6); in both years, however, the ranges estimated by DG analysis were greater than either. In 1977, when the range was much smaller, DG analysis failed to detect overlap, and lessened the range of treatment estimates.

Residual mean deviance (RMD) of logit (% mildew) was greater for the SBD (blocks eliminated) than for the systematic design in 1975, but smaller in 1976 (Table 7). Analysis ignoring blocks gave larger RMDs than the conventional analysis but the inclusion of overlap effects by DG analysis significantly decreased RMDs for both leaves on both dates in 1975, and for leaf 3 only in 1976. In 1977 DG analysis was ineffective.

Ratios of % mildew (Table 8) based on treatment SR, which had very little mildew, are of less value than ratios of maximum : minimum of other treatments common to both systematic and SBDs (blocks eliminated). For the latter ratio systematic designs gave larger values than SBDs in four of the six assessments, equal values in one, and smaller values in one. S0, not surprisingly, showed less mildew in the systematic designs than in SBDs (blocks either eliminated or ignored) in four of the six sets of data. Estimates of overlap in 1975 and 1976 were all positive, four of the 12 being significant. In 1977 all were negative but only one was greater than its standard error.

Table 6. *Comparison of designs and methods of analysis; ranges of yield of grain, t/ha, at 850 g dry matter/kg*

		Systematic	SBD	SBD (DG analysis)
1975	SR − S0	+0·51	+0·63	+0·68
	S2 − S0	+0·40	+0·78	+0·84
1976	Range	0·60	0·64	1·31
1977	Range	—	0·30	0·09

Table 9 presents a summary of the comparisons of changes of yield of grain and ratios of percentage mildew estimated from the systematic and SBDs analysed conventionally, and the SBDs analysed by the method of Draper & Guttman (1980). For yield there are two sets of data, one for each year, each comprising three differences (S1–S0), (S2–S0) (S3–S0 in 1976) and (SR–S0), each estimated in the three ways listed. For mildew there are six sets of data, each of two values of logit (% mildew). Standard errors are estimated from the variation of the differences of the estimates between sets of data. In the experiments of 1975 and 1976 estimates based on analysis by the method of Draper & Guttman (1980) indicated appreciably greater increases in yield, and significantly greater relative decreases in mildew than estimates based on the simple unadjusted analysis.

DISCUSSION

Interference between adjacent plots of conventional size and shape that received different treatments has been shown to occur in experiments involving airborne (Jenkyn *et al.* 1979) and splash-dispersed (Jenkyn *et al.* 1989) pathogens, and a vapour-active fungicide (Jenkyn *et al.* 1983). Detectable interference is not confined to instances where adjacent plots have extreme treatments such as unsprayed and sprayed repeatedly, and a component of interference that dominates in assessments of disease at an early date is not necessarily

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Table 7. *Comparison of designs and methods of analysis; residual mean deviances of logit (% mildew)*

	Systematic	SBD (blocks eliminated)	SBD (blocks ignored)	SBD (DG analysis)	Mean deviance for overlap
June 1975					
Leaf 2	0·0006	0·0020	0·0023	0·0012	0·0193***
Leaf 3	0·0016	0·0043	0·0064	0·0034	0·0512***
July 1975					
Leaf 2	0·0016	0·0022	0·0057	0·0048	0·0198*
Leaf 3	0·0013	0·0047	0·0110	0·0086	0·0466**
June 1976					
Leaf 2	0·0009	0·0056	0·0064	0·0062	0·0009
Leaf 3	0·0034	0·0229	0·0324	0·0280	0·0987*
July 1977					
Leaf 2	—	0·0016	0·0029	0·0029	0·0027
Leaf 3	—	0·0040	0·0037	0·0036	0·0054

D.F.: Systematic design June 1975: 17; July 1975: 16; July 1976: 19.
SBD eliminating blocks: 24.
SBD ignoring blocks: 32.
SBD Draper & Guttman analysis: 30.
Overlap: 2.
Note: Treatment S3 had not been applied by the sampling date in June 1975.
* $P<0{\cdot}05$; ** $P<0{\cdot}01$; *** $P<0{\cdot}001$.

Table 8. *Comparison of designs and methods of analysis; ratios of % mildew*

	Systematic	SBD (blocks eliminated)	SBD (blocks ignored)	SBD (DG analysis)
June 1975				
Leaf 2 (S0/SR)	1210	1280	*	*
Leaf 2	6·1	6·1	6·1	18·0
Leaf 3 (S0/SR)	5250	2860	5300	*
Leaf 3	85	21	20·5	51
July 1975				
Leaf 2 (S0/SR)	223	460	473	1230
Leaf 2	3·6	4·2	4·2	5·6
Leaf 3 (S0/SR)	18 200	310	304	681
Leaf 3	5·3	3·7	3·7	4·5
June 1976				
Leaf 2 (S0/SR)	254	48	48·2	144
Leaf 2	7·0	7·0	7·05	10·8
Leaf 3 (S0/SR)	1640	88	84·4	270
Leaf 3	6·7	4·9	4·85	6·6
July 1977				
Leaf 2	—	9·5	9·45	3·5
Leaf 3	—	3·1	3·1	2·0

* Fitted value for SR = 0.
—, not determined.
Note: Except as indicated each ratio refers to the maximum and minimum treatment estimates (omitting SR) in each analysis.

dominant in later assessments. Interference effects are necessarily smaller than direct effects, and are difficult to detect if random variability is great; if the intrinsic variability of the site is dominated by smooth changes over long distances, i.e. across large numbers of plots, interference may be relatively easily detectable. Under Rothamsted conditions, where there is substantial short-range variation in soil conditions, it is usually more easily detected in, for example, mildew than in yield. This reflects, in part, the fact that effects of

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Table 9. *Comparison between designs and methods of analysis, mean yield of grain (t/ha), 1975 and 1976, and combined estimates of % mildew*

Design /method	Systematic	SBD (ignoring blocks)	SBD (DG analysis)
Yield (grain, t/ha)*	0·47	0·57	0·87
S.E. of each (10 D.F.)		0·070	
Mildew†	0·271 (3·69)	0·289 (3·46)	0·216 (4·63)
S.E. (approx. 22 D.F.)	0·0191	0·0194	0·0178

* Differences (SR, S1, S2 or S3 minus S0).
† Ratios S1/S0, S2/S0 or S3/S0 (SR omitted); reciprocals in parentheses.

treatments on yield are, typically, proportionately smaller than effects on disease, and that relationships between disease severity and yield loss are often non-linear (Large & Doling 1962). The use of plots much larger than those commonly used, it is believed, will seldom be a practical method to lessen the effects of interference.

Interference, at least in experiments involving pathogens that affect the aerial parts of crop-plants, causes treatment effects, estimated conventionally by differences between means of replicate plots (transformed if appropriate), to be smaller in magnitude than the 'ideal' differences between the values that would be expected if the whole site could be treated with one or other of the respective treatments. Conventional analysis offers no method for obtaining any indication of the sizes of the discrepancies. In particular, when serially balanced and systematic designs give different estimates of the effect of a spray on a particular date, it is not possible to decide which of the two estimates approximates better to the 'ideal' whole-site difference; it cannot even be assumed that the 'ideal' value lies between those of the two designs. The use of systematic designs of the type used in the current experiments offered no clear improvement in precision relative to conventional designs in randomized blocks, and, in particular, these designs are vulnerable to the effects of linear trends; the authors, therefore, have no evidence that any advantage is likely to follow from the use of such designs. The systematic design chosen by one experimenter may differ from that chosen by another whose preconception of the nature and method of interference is different. In some instances, it is suggested, similar differences in preconceptions of the competitive powers of varieties may result in different allocations of varieties to subsets before randomization in designs such as those of David & Kempton (1996); in this sense these also have an element of systematic construction. A randomized design, by contrast, requires no such preconceptions.

The authors believe that the Draper & Guttman estimates are closer approximations to the 'ideal' whole-site differences due to treatments than those of conventional analysis of either type of design that we tested. It seems unlikely that DG analysis involves any tendency to give over-estimates of treatment differences since in 1977, when differences were small and interference was not detected, DG analysis produced no over-estimation (Tables 7 and 9). With $\alpha+\beta=+0{\cdot}62$ (as with yield in 1976) expected values of treatment differences in a randomized block design are about 85% of corresponding DG estimates. For an SBD the bias is a little greater, since no treatment has a like-treated neighbour. The authors therefore suggest that the method of Draper & Guttman (1980) should be considered in the analysis of the results of any experiment in which inter-plot interference is believed to have influenced the data, e.g. experiments on sprays or other treatments to control airborne pests or diseases. A condition for its success is that the arrangement of treatments must have a reasonable degree of neighbour balance between adjacent plots; not surprisingly, it failed completely when it was applied to the systematic designs in which any given treatment had like neighbours in all replicates, and it seems likely that, if interference is expected to be appreciable, RB designs analysed by the DG method offer a better approach than systematic designs. SBDs, however, are appropriate for investigating the nature of interference. In practice such designs are limited to four, or at most five, treatments; no doubt designs with six or more treatments having partial neighbour balance can be found, but the authors have not explored this possibility.

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