Statistical modelling of a split-block agricultural field experiment.

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ABSTRACT

This is a statistical review of a split-block experiment used to evaluate the effect of fungicides on modern and old spring wheat varieties historically grown in North Dakota. A split-block experiment with random blocks has implications regarding the correlation structures between plot yields in the field. These correlation structures are often unreasonable for agricultural field trials. Considerations in the design and analysis of such an experiment are discussed and an alternative approach to traditional analysis of variance (ANOVA) is presented. A Linear Mixed Model (with uses a residual maximum likelihood algorithm) is used to fit correlation structures to a row x column analysis and provide an improved statistical model. REML provides a flexible and powerful analytical tool for fitting complexities not handling by traditional ANOVA techniques.

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KEY WORDS. Split-block, correlation structures, linear mixed models, residual maximum likelihood, deviance, wheat, fungicide, row x column, agriculture, and field experiment.

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1. Background

An experiment was conducted as a preliminary evaluation of how spring wheat (*Triticum aestivum*) varieties respond differently to the use of modern fungicide treatments, and to determine if older wheat varieties would yield equally or better than currently grown lines with the use of fungicides. Interest in old wheat varieties (sometimes referred to as heritage wheats) has increased with a conservation movement to preserve wheat germplasm and cultural cuisine. Heritage wheat line popularity has increased as entrepreneurs attempt to develop niche markets in the wheat industry. Studies have claimed some possible advantages to growing old wheats. This includes higher nutrient content, superior weed-crop competition, and less need for inputs in sustainable type farming systems.

Hard red spring wheat varieties were planted near Velva, North Dakota, on April 28, 2007 and harvested on August 20, 2007. Wheat varieties were chosen based on historical significance to the regional wheat industry and seed availability. The 16 varieties evaluated are presented in Table 1. As the data set is only from one year and one environment, its usefulness is limited to it original purpose.

Table 1. Spring wheat variety numbers (#), names and years of release.

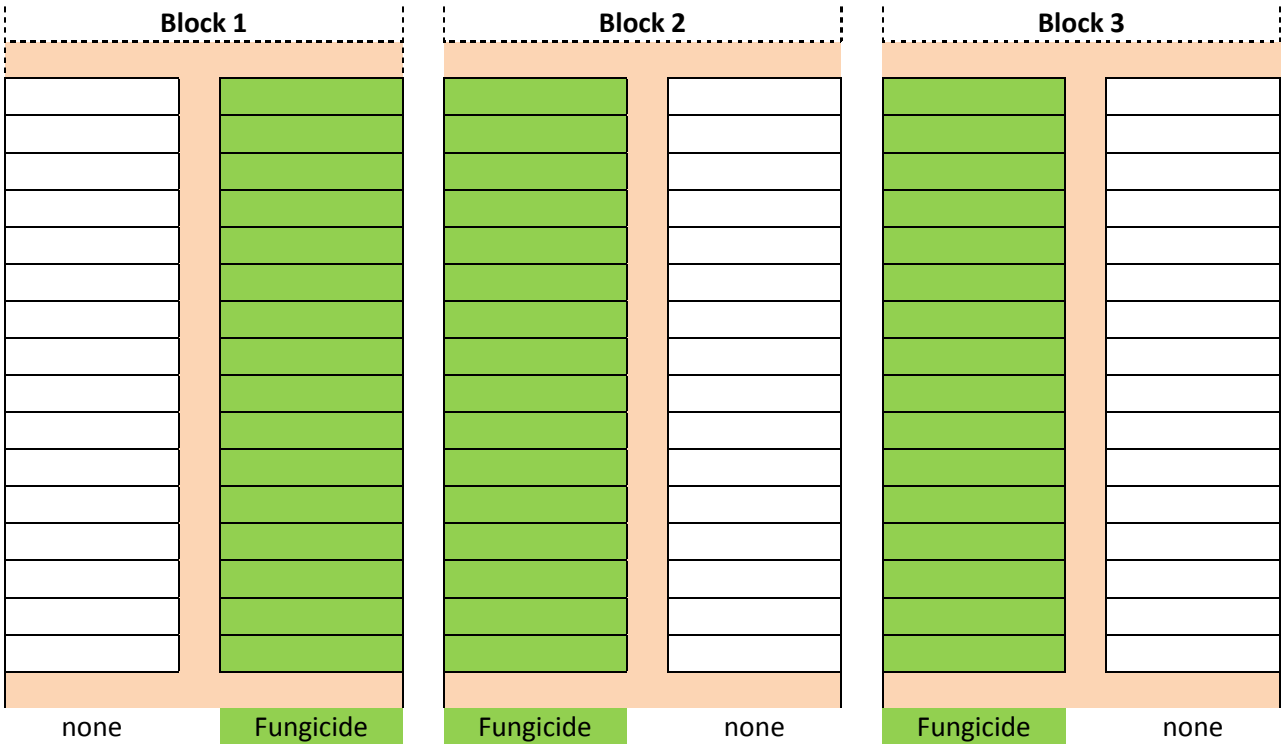
#	*Name*	*Year*	#	*Name*	*Year*	#	*Name*	*Year*	#	*Name*	*Year*
1	Red Fife†	1841	5	Len§	1979	9	Grandin§	1989	13	Alsen§	2000
2	Marquis‡	1901	6	Stoa§	1984	10	2375#	1990	14	Steele-ND§	2004
3	Waldron§	1969	7	Butte 86§	1986	11	Parshall§	1999	15	Glenn§	2005
4	Era	1970	8	Amidon§	1988	12	Reeder§	1999	16	Howard§	2006

† = Canada
‡ = Dominion Department of Agriculture, Ottawa, Canada.
§ = North Dakota Agricultural Experiment Station
¶ = Minnesota Agricultural Experiment Stations
# = Pioneer Hi-Bred

Wheat has been selected for improved disease resistance throughout history. Consequently, older varieties are normally more susceptible to fungal diseases as they have been replaced as new strains occur and resistance is lost. New varieties are bred for increased disease resistance to specific pathogens and are often adapted to specific production areas (environments). So differences were expected as to (i) the diseases varieties are resistant to and (ii) the level of resistance they have.

The varieties were randomized into three blocks using a randomized complete block design (RCBD) for varieties. Plots in a block were contiguous, however to anticipate the application of a fungicide treatment, each variety was sown in two sub-plots, each sub-plot being 5 ft wide by 30 ft long with a 5 ft alley between them. Blocks were also contiguous with a 5 ft alley between them. Plots were sprayed with Headline (pyraclostrobin, 3 oz/acre) at the 5 leaf stage and with Folicur (tebuconazole, 4 oz/acre) at flowering. This fungicide treatment was applied at random into half of each block, i.e. into the left or right sub-plot of each variety but consistent (stripped) across the whole block – see Figure 1.

Figure 1 Site of field trial, with the randomized fungicide treatment indicated



Plots were trimmed to an equal length before harvest and grain yield (t/ha) was calculated based on 13% moisture content. The yield in t/ha is given in Table 2, along with the randomization of varieties in the blocks. We have labelled the rows from 16 down to 1 to allow residuals to be plotted in field order in subsequent analyses – one needs to imagine an X-Y grid placed over the plots in the field, with a mathematical origin (0, 0) placed at the bottom left corner plot. Then as one moves to the right from the origin towards the bottom right corner plot the X coordinates on this grid system will be 1, 2, ..., 6, while moving up from the origin towards the top left corner plot the Y coordinates will be 1, 2, ..., 16.

Table 2. Allocation of varieties to plots, with the yield (t/ha)

Block 1	Block 2	Block 3
Era	2375	Marquis
Amidon	Steele-ND	Reeder
Butte 86	Grandin	Grandin
Reeder	Stoa	Stoa
Waldron	Marquis	Butte 86
Stoa	Era	Steele-ND
Howard	Red Fife	2375
Parshall	Amidon	Era
Glenn	Parshall	Red Fife
Alsen	Howard	Amidon
2375	Len	Len
Marquis	Alsen	Waldron
Steele-ND	Butte 86	Parshall
Grandin	Glenn	Glenn
Red Fife	Waldron	Howard
Len	Reeder	Alsen

Y	Block 1		Block 2		Block 3	
16	2.641	**3.190**	**3.121**	2.861	**2.047**	1.871
15	2.310	**3.021**	**3.020**	2.452	**2.928**	2.344
14	1.827	**2.684**	**2.535**	1.961	**1.748**	1.768
13	2.471	**3.390**	**3.484**	2.653	**3.079**	2.433
12	1.649	**2.639**	**2.201**	1.840	**2.675**	2.476
11	1.954	**2.969**	**2.902**	2.435	**2.616**	2.569
10	2.172	**3.087**	**2.539**	2.394	**2.801**	2.687
9	2.770	**3.132**	**3.186**	3.215	**2.946**	2.550
8	2.712	**2.983**	**3.047**	2.953	**2.379**	1.906
7	2.450	**2.839**	**3.465**	3.107	**2.936**	2.414
6	2.380	**2.974**	**2.512**	2.265	**2.354**	2.196
5	1.558	**1.941**	**2.534**	2.363	**2.742**	2.231
4	2.380	**3.247**	**2.925**	2.622	**3.076**	2.786
3	1.847	**3.088**	**3.149**	2.818	**3.092**	2.921
2	1.468	**2.480**	**2.598**	2.294	**2.916**	2.678
1	1.894	**2.457**	**3.294**	2.937	**2.649**	2.576
Origin	1	2	3	4	5	6
			X			

2. Blocking issues

This trial was set up as a demonstration and poses some interesting questions, such as

- The direction the soil variation was unknown, so was the experiment blocked correctly?
- Was the correct design used, or should the trial have been laid out differently?

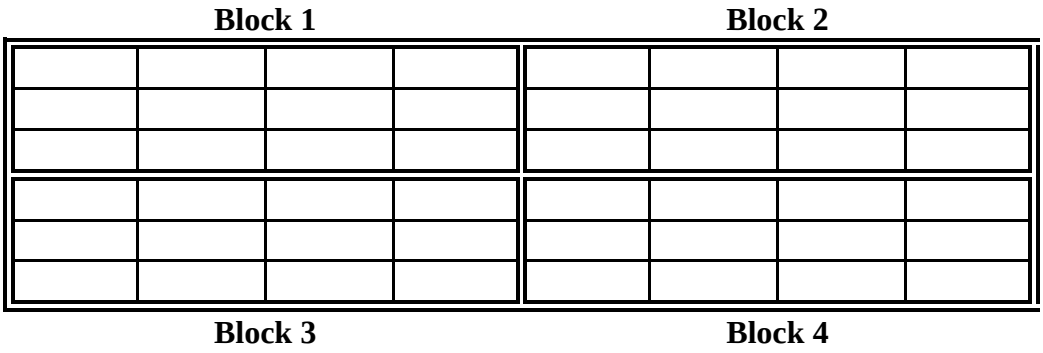
The design was chosen for one reason - time and labor were short, so under the circumstances it seemed the most efficient. Also it made a nice demonstration site for growers as they could walk down the alley between the fungicide and no fungicide treatments and make direct comparisons for each variety. Using a block design allowed all the varieties to be assembled in the one block rather than scattered randomly across the field.

Notice that if there *is* a block effect from left to right in the field, is it realistic to assume that the trend jumps from one block to the next, but is not present within the block? Blocks were contiguous, so a trend in the field is also likely to manifest itself *within* the 65 ft width of each block. That being the case, randomizing the fungicide treatment to the left half or right

half of each block is fraught with danger. Suppose that by chance the yield increases from left to right of the field simply because of a change in fertility. Suppose also that each randomization of the fungicide is to the right half of each plot. Then will any difference between the fungicide-treated plot and the fungicide-control plot be due to the extra fertility or the application of the fungicide? We say the two effects are *confounded*.

So in cases where a second treatment is to be stripped across a block where blocks are arranged left to right, it is better to apply the treatment to the top half or bottom half of each block at random. Alternatively, if there were an even number of blocks, then pairs of left/right randomizations would go some way towards evening out any trend within a block. This is like a 2 × 2 Latin Square arrangement, with either F/C in block 1 and C/F in block 2, or vice versa (here C is the control-fungicide, F the fungicide-treated plot).

Experiments have been published with randomized blocks in two directions, for example:



Again, blocks are contiguous in both directions. If there really is a trend left to right and top to bottom, then the trend is likely to be reflected within the blocks in both directions. This is why it is important to examine residuals in field position, to ensure that no extraneous source of variation remains. Modern analyses allow plots to be correlated in both directions. Linear mixed models with a residual maximum likelihood algorithm are now used to measure the variance and correlation parameters. It is instructive to see how this proceeds.

3. The assumptions underlying the statistical model of an RCBD

Let us first assume that the 16 varieties by 2 fungicides were arranged at random in blocks. We will label the t=32 treatment combinations simply as *Treatment*. The RCBD model and ANOVA (with b blocks) are as follows.

Model	ANOVA component	
Yield = overall mean		
+ Block effect	Block	(*b*-1) df
+Treatment effect	Treatment	(*t*-1) df
+ Error	Residual	(*b*-1)(*t*-1) df df

Notice that for each effect in the model, there is a corresponding component in the ANOVA; the Residual component is based on the sum of squares of the observed errors.

For this model the errors are all assumed to be independent, with constant variance. The Error is simply the Block.Treatment interaction - that is, it measures the failure of the treatments to respond alike in every block. Assuming there are no other possible sources of variation in the experiment, and if there is no reason why the treatments should not respond alike in all blocks, then the Residual term is used to form F statistics (variance ratios, v.r. in GenStat's terminology) in the ANOVA.

The assumption concerning the Block effect has very interesting implications in the field.

In older text books, blocks are assumed to be fixed effects, so that the only random term in the model is the error term. The errors for the plots in the field are assumed to be uncorrelated, which implies that the plot yields are all independent of each other.

Fixed effects, like the varieties and fungicides chosen in this experiment, force us to make conclusions from the analysis *only for those varieties and fungicides used in the experiment*. So if blocks are really fixed, you would technically be able to extend any differences in the varieties of with the fungicide treatment only at the site used in the experiment.

A random effect on the other hand assumes that the levels taken were taken from a larger possible set, and that any conclusions from the randomly chosen set apply to the wider set – the only condition being that the levels used are typical of the wider set (and hence the importance of randomization). Varieties could well have been a random effect, had the 24 varieties chosen come from a much larger set. In this case they were of fixed interest.

On the other hand, one would hope that the blocks used in an experiment were a random choice from many other sites that could have been chosen, so that the conclusions about the

treatments applied to sites of a similar kind to the experimental site. Hence, it is more likely that blocks are random in an RCBD.

GenStat always assumes blocks are random: no P value is calculated for the Block F statistic. This is also partly due to the fact that (i) random effects cannot be tested using an F statistic, and (ii) blocks are not replicated: Block 1 has some fertility factor which is different from Block 2 and so on; there is no replicate of Block 1.

Table 3 presents GenStat's RCBD ANOVA of the yield data. Here, the F statistic for blocks is 7.88, however no P value (labelled F pr. In the ANOVA) is calculated. The blocks are placed in a stratum of their own to reflect stage 1 of setting up the experiment: blocks are formed in the field, each block being 16×5 = 80 ft by 65 ft. They form the first "layer" or "stratum" in the experiment. Individual plots are 5 ft by 30 ft (although treatments are not randomized in each block, there is a two-stage randomization to be discussed later).

Table 3. GenStat's RCBD ANOVA of yield

Variate: Yield

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	0.94068	0.47034	7.88	
Block.*Units* stratum					
Variety	15	9.62752	0.64183	10.75	<.001
Fungicide	1	4.84112	4.84112	81.08	<.001
Variety.Fungicide	15	0.63413	0.04228	0.71	0.767
Residual	62	3.70186	0.05971		
Total	95	19.74531			

Notice that, against our expectations, there was no significant interaction between varieties and the fungicide treatment (P=0.767). However, since the model is not correct we will defer discussion of this problem.

In the ANOVA options we also requested GenStat to print **Estimated Stratum Variances**:

Stratum	variance	effective d.f.	variance component
Block	0.4703	2.000	0.0128
Block.*Units*	0.0597	62.000	0.0597

This gives rise to the next point of discussion, namely that *a random effect is associated with a separate variance*. Specifically, with blocks random in an RCB model we assume:

- Blocks are distributed normally and independently of each other with zero means and variances σ^2_{Block} . They are also uncorrelated with the
- Errors, which are distributed normally and independently of each other with zero means and variances σ^2.

In the analysis above, the estimate of σ^2 is 0.0597, while the estimate of σ^2_{Block} is 0.0128.

The assumption that blocks are random, however, has another implication: *every plot within a block is uniformly correlated with every other plot*. This comes from the model. Ignoring the fixed effects (which play no part in evaluating plot variances and covariances or correlations), the model for (say) plot 1 and plot 2 in block 1 is

Yield in block 1, plot 1 = fixed effects + Block_1 random effect + Error_{11} random effect
Yield in block 1, plot 2 = fixed effects + Block_1 random effect + Error_{12} random effect

This means that the variance of a plot yield is $\sigma^2_{Block} + \sigma^2$ and the covariance between any two plot yields is σ^2_{Block} , since for the two (typical) plot 1 and plot 2 models above the Block_1 random effect is common to both. Furthermore, since a correlation is the covariance divided by the two standard deviations (which are equal in this case), the correlation between any two plots in a block is $\sigma^2_{Block}/(\sigma^2_{Block} + \sigma^2)$. For the example at hand, this gives the uniform correlation of 0.0128/(0.0128+0.0597) = 0.177. We will see that this is so later.

The RCB model,

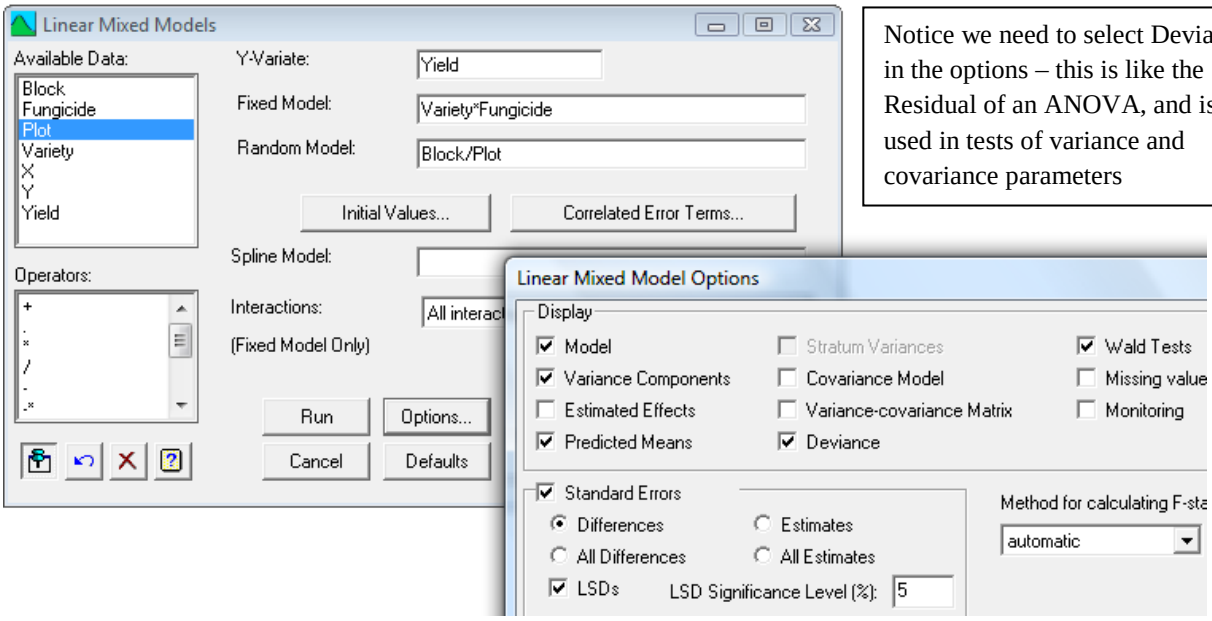
Yield = fixed effects + Block random effect + Error random effect

is an example of a *linear mixed model* (LMM)*; linear* because the effects are additive, and *mixed* because the model involves fixed and random terms. Even if blocks were assumed fixed, the RCB model would be a special case of a LMM. The beauty about a LMM with a residual maximum likelihood algorithm is that no assumption is made in general about the variance structure of the random terms: correlated random terms are permissible, as well as changing variances. Consequently, for the RCB with no missing values, the tests from any ANOVA should be the same as those produced by LMM methods in which constant variance is assumed as well as uncorrelated errors.

The output from a LMM (REML) analysis of the yield data follows. In the following menu we entered a Plot factor which had values 1, 2, ..., 48 for the 48 plots in each block.

Remember that we need to apply the 48 treatments at random to the plots in each block for a randomized block, so for the RCBD the random model technically is Block/Plot to reflect this. This is GenStat's shortcut for Block+Block.Plot. The final stratum Block.Plot can be omitted, and GenStat will always add it for you. However, if you need to set up a changing variance or a correlation structure, you will need to enter an appropriate structure to set up the appropriate covariance model.

Figure 2. GenStat's LMM for a randomized block analysis



Notice we need to select Deviance in the options – this is like the Residual of an ANOVA, and is used in tests of variance and covariance parameters

REML variance components analysis

Response variate: Yield
Fixed model: Constant + Variety + Fungicide + Variety.Fungicide
Random model: Block + Block.Plot
Number of units: 96

Block.Plot used as residual term

Note. If you omit Block.Plot the message instead is:
Residual term has been added to model ANOVA

Estimated variance components

Random term	component	s.e.
Block	**0.01283**	0.01470

Same as σ^2_{Block} from Stratum Variances in the ANOVA

Residual variance model

Term	Factor	Model(order)	Parameter	Estimate	s.e.
Block.Plot		Identity	Sigma2	**0.0597**	0.01072

Same as σ^2 from Stratum Variances in the ANOVA

Deviance: -2*Log-Likelihood

Deviance	d.f.
-77.09	62

Note: deviance omits constants which depend on fixed model fitted.

Tests for fixed effects

Same as the F statistics in the ANOVA

Fixed term	Wald statistic	n.d.f.	**F statistic**	d.d.f.	F pr
Variety	161.24	15	**10.75**	62.0	<0.001
Fungicide	81.08	1	**81.08**	62.0	<0.001
Variety.Fungicide	10.62	15	**0.71**	62.0	0.767

The Wald statistics are sometimes used when F statistics are unavailable; P values are then based on χ^2 distributions.

- To demonstrate how to test whether blocks effects are zero, we need to re-run the LMM without blocks, and use a χ^2 distribution for the change in deviance. The actual assumption here is that the block variance is zero (i.e. σ^2_{Block} =0). We mentioned that when blocks are considered fixed it is not possible to test for blocks since there is no replication. On the other hand, when blocks are considered random, each block is a random choice (i.e. a replicate) from a large potential population of blocks; if the variance of this distribution is constant, all blocks must be alike.

Model	deviance	d.f.	P value
With blocks	-77.09	62	
Without blocks	-68.76	63	
Change	8.33	1	0.004

- It turns out that as far as testing fixed effects is concerned, it makes no difference whether blocks are assumed fixed or random for an RCBD. Here is the output with the fixed model being Block+Variety*Fungicide. The only difference in the F statistics is the presence of a test of the fixed blocks again, we would ignore this P value for the reasons given above):

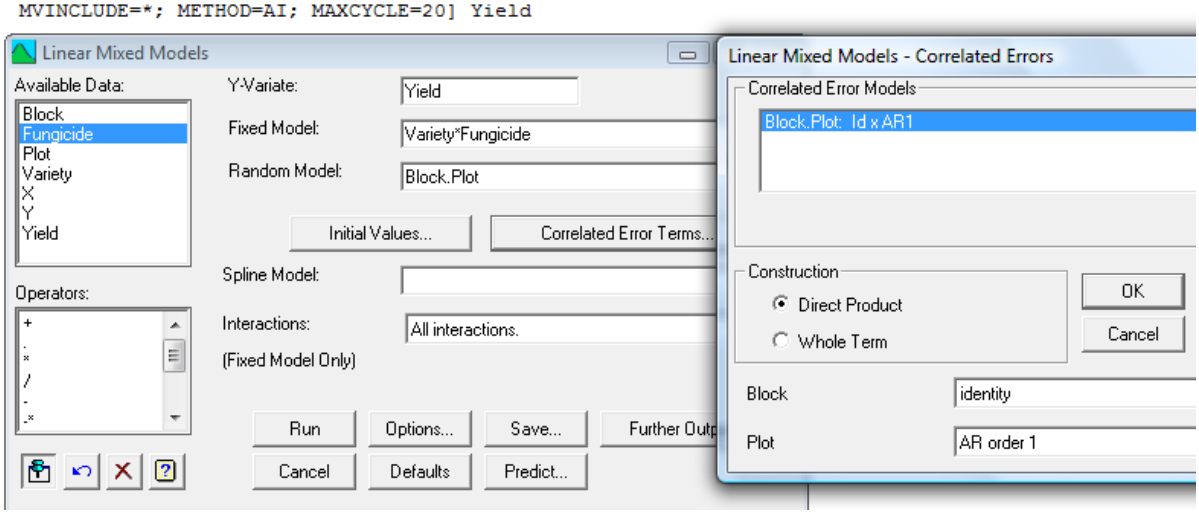
Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Block	**15.75**	**2**	**7.88**	**62.0**	**<0.001**
Variety	161.24	15	10.75	62.0	<0.001
Fungicide	81.08	1	81.08	62.0	<0.001
Variety.Fungicide	10.62	15	0.71	62.0	0.767

(Note that treatment means are shrunk slightly towards the grand mean when blocks are assumed random. As a consequence, standard errors of treatment means will be

slightly smaller when blocks are assumed random. Standard errors of treatment mean differences, however, are unaffected by the assumption made about blocks.)

- To demonstrate that a uniform correlation model is assumed when blocks are assumed fixed, we need to place a uniform correlation model on the Block part of Block.Plot in a random model that consists only of Block.Plot (that is, we need to remove the Block+ part of the previous random model). Unfortunately a uniform correlation model is not one of the models available in the drop down dialogue box when Correlated Error Terms is selected in LMM. We suggest you select say an AR1 model (to be discussed later) and run this model, then copy the appropriate three lines from GenStat's input window, paste them in a new input window, change AR1 to uniform and submit the window or lines (in the Run menu):

```
VCOMPONENTS [FIXED=Variety*Fungicide; FACTORIAL=9] RANDOM=Block.Plot; INITIAL=1; CONSTRAINTS=none
VSTRUCTURE [TERMS=Block.Plot; FORMATION=direct] MODEL=identity,uniform; ORDER=*,1; FACTOR=Block,\
Plot
REML [PRINT=model,components,deviance,waldTests; PSE=differences; FMETHOD=automatic;\
 MVINCLUDE=*; METHOD=AI; MAXCYCLE=20] Yield
```



In this screen capture, we ran the AR1 model and have changed AR1 to uniform to produce:

REML variance components analysis

Response variate: Yield
Fixed model: Constant + Variety + Fungicide + Variety.Fungicide
Random model: Block.Plot
Number of units: 96

Block.Plot used as residual term with covariance structure as below

Covariance structures defined for random model

Covariance structures defined within terms:

Term	Factor	Model	Order	No. rows
Block.Plot	Block	Identity	1	3
	Plot	Uniform	1	32

Residual variance model

Term	Factor	Model(order)	Parameter	**Estimate**	s.e.
Block.Plot			Sigma2	**0.0725**	0.01800
	Block	Identity	-	**-**	-
	Plot	Uniform	theta1	**0.1769**	0.1694

Deviance: -2*Log-Likelihood

Deviance	d.f.
-77.09	62

Tests for fixed effects

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Variety	161.24	15	10.75	62.0	<0.001
Fungicide	81.08	1	81.08	62.0	<0.001
Variety.Fungicide	10.62	15	0.71	62.0	0.767

The F statistics, means, sed and lsd values are all unchanged. The estimate of the uniform correlation among plots in a block is labelled theta and is estimated as 0.1769 as we saw before as $\sigma^2_{Block}/(\sigma^2_{Block}+\sigma^2)$. In this case, GenStat has estimated the total $(\sigma^2_{Block}+\sigma^2)$ as 0.0725. Hence we can conclude that the block variance is $0.177 \times (\sigma^2_{Block}+\sigma^2) = 0.1769 \times 0.0725 = 0.01283$ as was obtained in the first LMM analysis. By subtraction, the estimate of the error variance is 0.0725-0.01283 = 0.05967, again as was obtained in the first LMM analysis.

To summarise,

- ANOVA and LMM (REML) analyses give the same information when the assumptions are the same, however LMM (REML) is far more flexible in that correlated errors and changing variances are possible.

- Blocks are generally assumed random. However this implies that plots in a block are uniformly correlated. It is unlikely in practice that plots close together are correlated in the same way as plots further apart. Rather, it is much more likely the plots close together are more strongly correlated than plots far apart. Some of these models will be demonstrated later.

4. Examining residuals from an analysis

Again, we stick to the RCBD for demonstration purposes. There are two ways that residuals from field trials should be examined. Residuals should be completely random across the data. So:

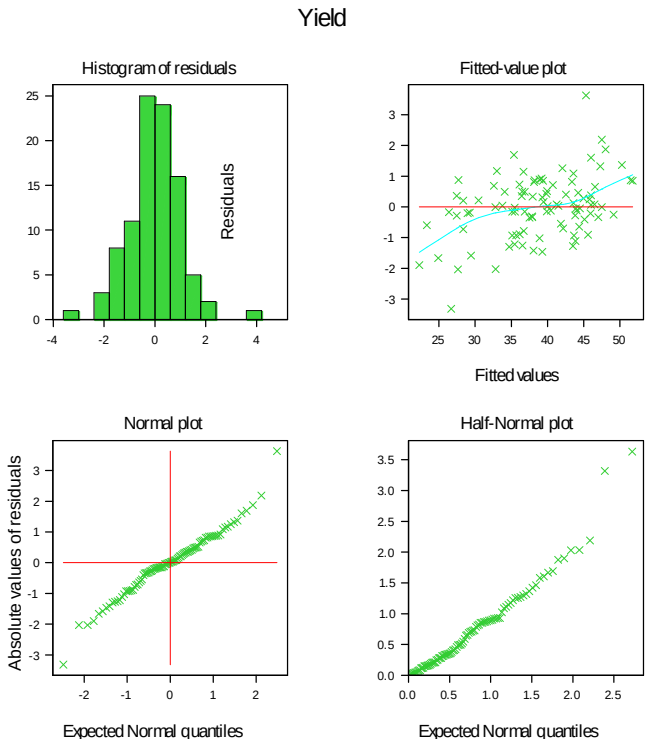
- Residuals should be plotted against fitted values to ensure that there is no trend. A fanning in residuals with increasing fitted value indicates that the variance is not constant. Often log-transforming the data removes this fanning. When a log-transform is used, back-transformed means are the geometric means of the original data; back-transformed differences of two means are the ratios of the two geometric means of the original data. You can back-transform the end points of confidences intervals of differences on the log-scale: these are then confidence intervals of the ratio of the two geometric means.

- Residuals should be plotted in field order to ensure there is no residual trend in the field. This either indicates a badly selected model (and hence analysis), or assumptions that do not hold for the analysis selected.

The General Analysis of Variance option of GenStat's ANOVA menu allows either ordinary or standardised residuals to be plotted against fitted values. Where possible, standardised residuals should be selected, as it is easier see visually what values are outside the (-2, 2) range which applies approximately to 95% of standardised residuals when sampled from a standardized normal distribution. It is especially important to choose standardised residuals when a changing variance model is used in LMM, although unfortunately the current version of GenStat does not produce these values.

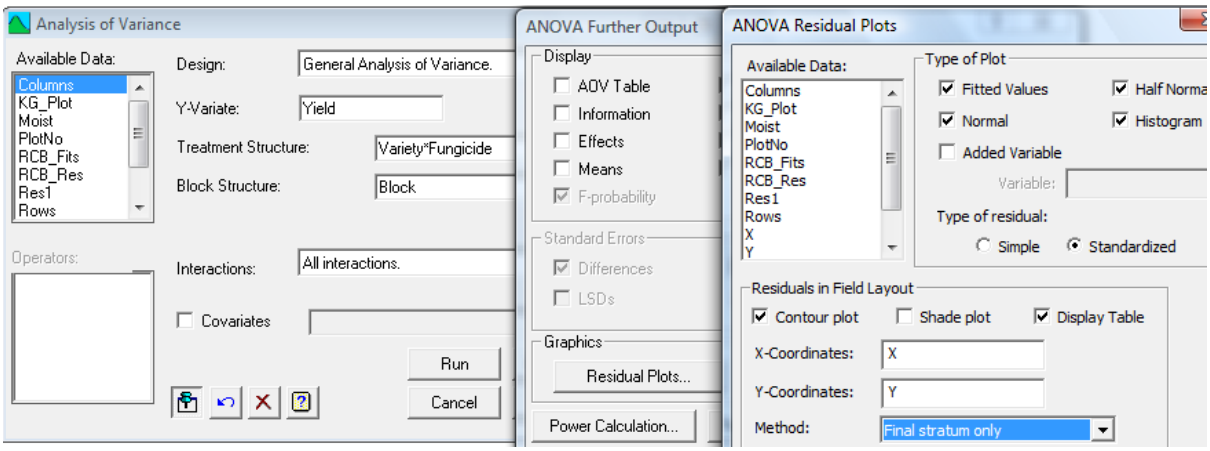
A Normal plot of residuals is also useful – this is a Q-Q plot, in which the residuals are plotted against the quantiles of a normal distribution. The resultant plot should be a straight line if normality holds. Histograms are a visual indication of normality, although one needs a large number of residuals to gain an accurate picture.

Here is the standardised residual plot with all options selected:



The graph in the top right hand corner has a trend superimposed on the residuals as a visual assistance. It appears that smaller fitted values are associated with negative residuals, and vice versa for larger fitted values. This suggests a poorly specified model (which we know to be the case as the design was not simply RCB).

The General Analysis of Variance option of GenStat's ANOVA menu also allows the residuals to be printed out in field order and optionally a contour plot. This is one use of the X-Y grid system discussed earlier. For this application, both X and Y need to be variates, not factors:



Notice there are two methods here, Final stratum only and Combine all strata. With blocks random, there are two strata and two error terms:

- Combine all strata for a randomized block means that the residuals will be calculated for (Block effect + Error) which, from the RCBD model, leads to residuals whose values are (Yield – estimated fixed treatment effect).

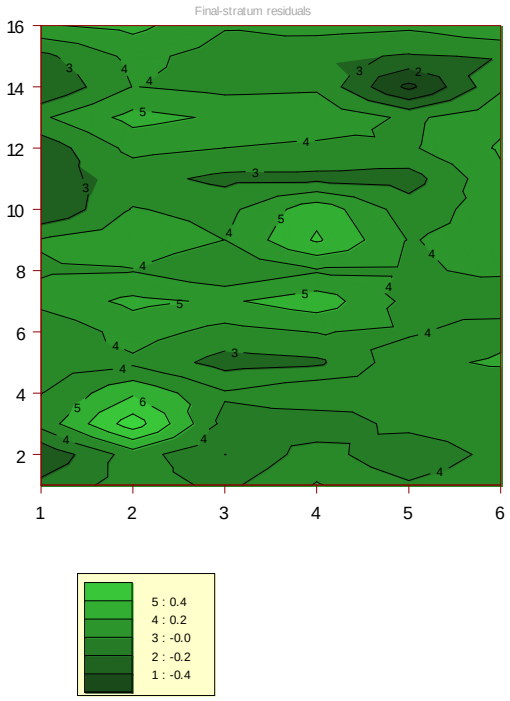
- Final stratum only for a randomized block means that the residuals will be calculated for Error only which, from the RCBD model, leads to residuals whose values are (Yield - estimated fixed treatment effect – best estimated block effect) for the blocks chosen in the experiment. (These are what are saved in the Save menu.)

You will see that the two sets of residuals differ by -2.62 for plots in block 1; by 4.46 for plots in block 2; and by -1.84 for plots in block 3. Each of these is simply the block mean minus the overall mean. That is, they are the three residuals for the block stratum. We took the Final stratum only residuals into Excel and set a conditional format to reveal negative residuals.

Row	Block 1		Block 2		Block 3	
16	0.923	1.323	0.084	0.404	0.211	0.878
15	-1.298	0.282	-0.410	-0.787	-1.115	-0.932
14	-2.038	0.023	-0.313	-0.188	-3.324	-0.170
13	-0.159	1.367	0.854	0.853	-0.209	0.733
12	-1.669	0.313	-0.007	-0.284	-0.148	1.148
11	-1.585	-0.645	-1.275	-1.257	-1.467	0.813
10	-2.033	0.066	-0.338	1.694	-0.546	0.519
9	0.080	0.658	-0.005	2.191	-0.047	0.334
8	-0.118	-0.049	-0.905	-0.115	-0.151	0.208
7	0.352	1.260	0.866	1.609	-0.277	-0.893
6	-0.922	0.462	-0.348	0.038	-0.151	0.689
5	-0.594	-0.205	-1.425	-1.221	0.713	1.176
4	-0.026	1.877	0.124	0.890	0.247	0.036
3	0.359	3.637	-0.331	-0.706	0.381	0.823
2	-1.902	0.489	-1.025	0.493	-0.932	0.423
1	-0.727	0.499	-0.252	1.090	0.165	0.869

It is apparent that the residuals are not particularly randomly +/- throughout the field. In each block, the negative residuals appear mainly in the left hand half-block. This indicates a badly specified model. We will suspend further discussion until we have reanalysed the data.

One could, of course, have used several rules to pick up residuals in different bands, e.g. in the Excel file we have used different shading to indicate residuals that are <-2, within (-2, -1) and within (-1, 0). However, this is basically what the contour plot does, though in a smoother way:



Notice that even with a badly specified model, the contour plot appears to detect a trend top to bottom. The contour ellipses appear elongated left to right. Again, we will re-examine this plot later with a more appropriate set of residuals.

5. Analysis of the data as a split-block or strip-plot experiment

In practice, there were four strata in this experiment. Stratum 1 relates to the formation of blocks, as has been discussed. Then:

Stratum 2. **Plot units within blocks for randomising varieties**. In each block, individual plots are formed to accommodate the varieties. Technically these are 1/16 block shapes of dimension 5ft by 65 ft – we will call these plots $Plot_{Var}$ for simplicity. Normally, a 65 ft drill pass would be planted and the desired plot alley (if needed) would be cut out with a tiller or mower as needed to accommodated plot maintenance, application of treatments, and harvest. In this trial, a 5 ft alley was left in the middle anticipating the fungicide application, and allowing farmers an area to walk in the middle of each block and make comparisons of fungicide treatments on each variety. This is just an RCBD for varieties, so to test for varieties, the ***Block.Variety*** interaction is used.

Block 1

1	5ft by 65 ft
2	
3	
4	
5	
6	
7	
8	
9	
10	
11	
12	
13	
14	
15	
16	

Stratum 3. **Plot units within blocks for randomising fungicides**.

In each block, the fungicide treatment described earlier was applied to the left or right half at random. Hence for testing the fungicide treatment we use half-block plots – we will call these plots $Plot_{Fung}$ for simplicity. This is just an RCBD for fungicide, so to test for the fungicide, the ***Block.Fungicide*** interaction is used.

Stratum 4. **Plot units within blocks for comparing fungicides across varieties**.

Individual plot yields are for one variety with either a fungicide applied or not. Hence the Variety.Fungicide interaction is tested using a residual based on individual plots that are 5 ft by 30 ft. This unit is simply the ***Block.Variety.Fungicide*** interaction.

The random model is therefore

$Block+Block.Plot_{Var}+Block.Plot_{Fung}+Block.Plot_{Var}.Plot_{Fung}$

which can be simplified to

$Block/(Plot_{Var}+Plot_{Fung})$

Notice this is not a split-plot design. That design would have the allocation of the fungicide at random in every variety-plot. The randomizations of the fungicide would not all be to the left half or the right half of the block.

The ANOVA for this split-block model is as follows.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	0.94068	0.47034		
$Block.Plot_{Var}$ stratum					
Variety	15	9.62752	0.64183	9.07	<.001
Residual	30	2.12226	0.07074	3.31	
$Block.Plot_{Fung}$ stratum					
Fungicide	1	4.84112	4.84112	10.32	0.085
Residual	2	0.93820	0.46910	21.94	
$Block.Plot_{Var}.Plot_{Fung}$ stratum					
Variety.Fungicide	15	0.63413	0.04228	1.98	0.055
Residual	30	0.64141	0.02138		
Total	95	19.74531			

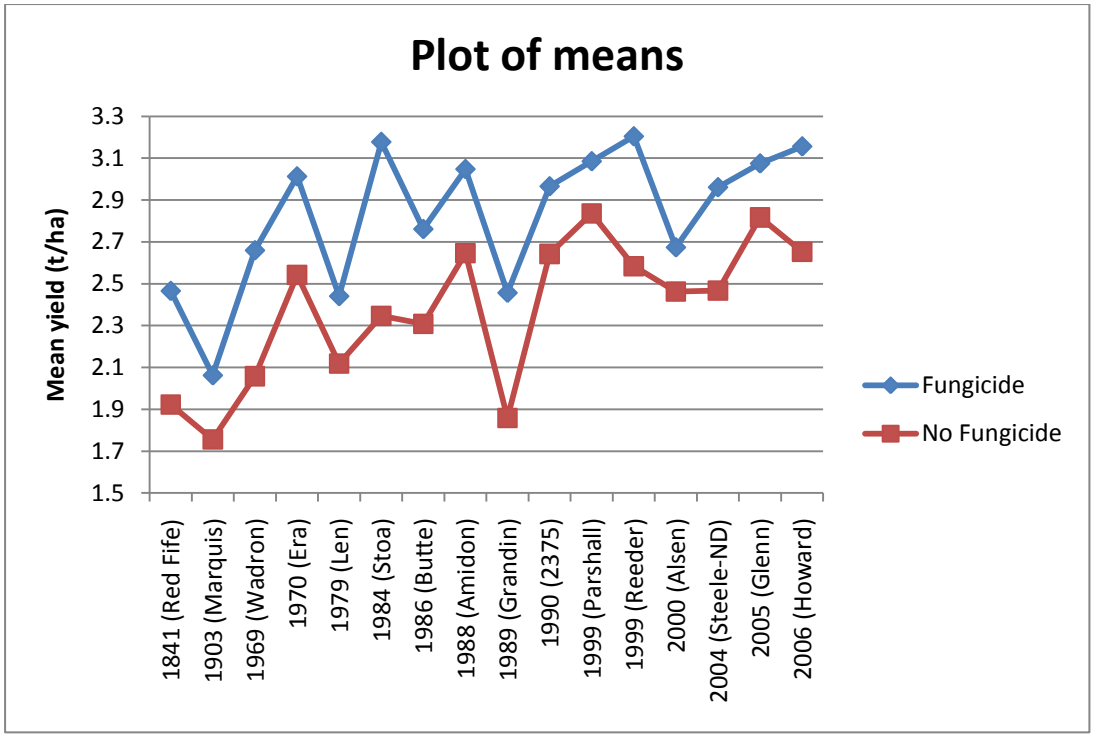
Notice that each F statistic is formed using the Residual from the same stratum. These residuals are just the interactions described in the discussion above of the four strata. The stratum variances are estimated to be:

Stratum	variance	effective d.f.	variance component
Block	104.349	2.000	-0.329
Block.Variety	15.692	30.000	5.474
Block.Fungicide	103.919	2.000	6.198
Block.Variety.Fungicide	4.744	30.000	4.744

- What was a significant block effect when analysed as an RCBD has been eradicated when analysed as a split-block design.

- The previous P value for the Variety.Fungicide interaction (0.767) has collapsed to 0.055, just failing to reach 5% significance. The difference is that the appropriate denominator MS is now less than half what it was (0.02138 compared to 0.05971) when an RCB analysis was used, and hence the F statistic is more than double the previous value (0.71, now 1.98). However, is the statistical evidence in line with our expectations? If not, are the differences we are trying to detect too small for the number of replicates used in the experiment? Or is there a problem with our assumptions? A plot of varietal means suggest there should be a detectable interaction, with the effect of applying the fungicide greater for some varieties than for others.

Notice that the residuals now appear in each variety-plot as +value, -value. This follows from the model: for a balanced split-block design, it can be shown that the residuals sum to zero over each factor combination. A contour plot of these residuals would therefore be quite misleading. It would be better to temporarily restrict the plot only to (say) the left hand set of residuals in each block.



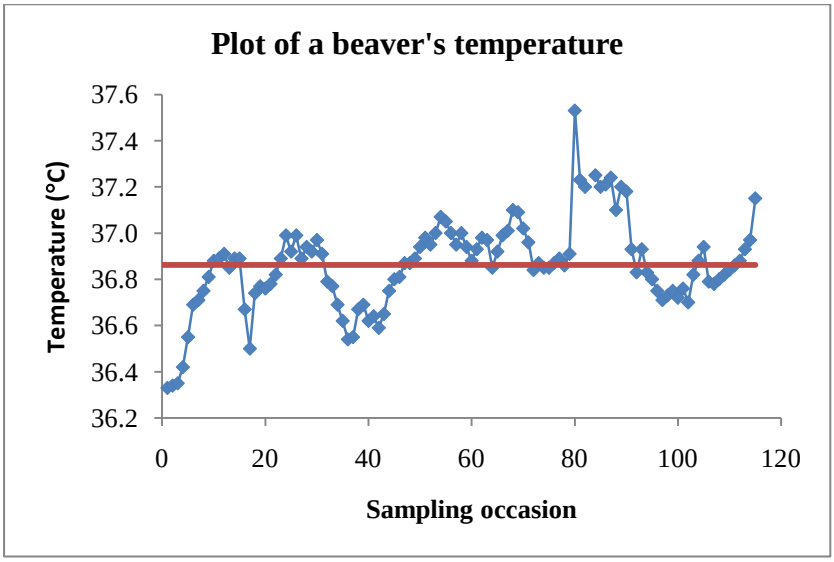
To check our assumptions an inspection of the residuals is necessary. Part of the output is a list of any residual whose standardized value is outside (-2, +2). What is given is the raw (Final stratum only) residual as well as its standard error:

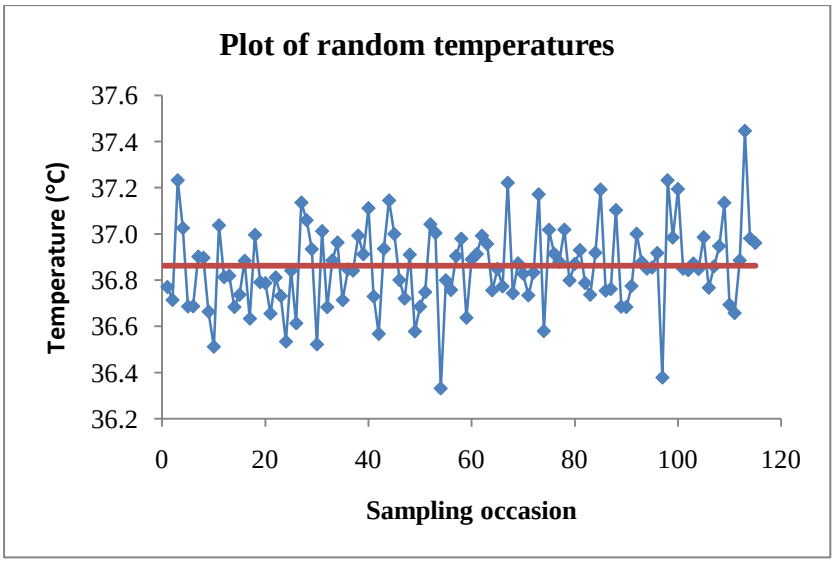
Block 1 Variety 1989 (Grandin) Fungicide Fungicide 0.182 s.e. 0.082

Hence, once the residuals are saved, the standardized values can be calculated.

	Block 1		Block 2		Block 3	
16	1.219	-1.219	0.321	-0.321	0.197	-0.197
15	-0.192	0.192	1.154	-1.154	0.774	-0.774
14	-0.767	0.767	0.554	-0.554	-2.776	2.776
13	-0.127	0.127	0.705	-0.705	-0.132	0.132
12	-0.671	0.671	1.034	-1.034	-0.555	0.555
11	0.573	-0.573	0.680	-0.680	-1.732	1.732
10	-0.811	0.811	-1.726	1.726	-0.278	0.278
9	1.006	-1.006	-1.921	1.921	0.539	-0.539
8	1.615	-1.615	-0.240	0.240	0.565	-0.565
7	0.611	-0.611	-0.185	0.185	1.730	-1.730
6	0.042	-0.042	0.241	-0.241	-0.010	0.010
5	1.231	-1.231	0.459	-0.459	0.441	-0.441
4	-0.578	0.578	-0.212	0.212	1.246	-1.246
3	-2.222	2.222	1.150	-1.150	0.465	-0.465
2	-1.161	1.161	-1.112	1.112	-0.626	0.626
1	0.231	-0.231	-0.901	0.901	0.152	-0.152

However, a visual check on the standardised residuals suggests that there are too many runs of residuals of the same size to be comfortable with their randomness in field position. The picture is more like one would find with errors which are autoregressive-correlated. There is a well known data set that can be used to demonstrate this. Temperatures were taken on a single beaver every 10 minutes. Compare the plot of temperature versus time for this beaver, and compare that with a plot of random normal data whose mean and standard deviation are exactly the same as the beaver's.





The horizontal line is the mean of the beaver's temperatures. In the top plot, you can see that the residuals (temperature minus mean temperature) have long runs of the same sign; in the bottom plot, they are noisily +/- with no apparent long runs of the same sign. For the beaver temperatures, a time series analysis indicates that the animal's temperature at any given teime

depends in a linear fashion directly on its temperature only at the previous time. This is called an autoregressive model of order 1, or an AR1 process. Of course the temperature will depend *indirectly* on the earlier temperatures as well. There are some applications where the process at time *t* depends directly on the two previous times - this is known as an AR2 process. We don't go beyond AR2 processes when modelling in field trials, as AR1 and AR2 processes generally prove adequate.

6. A row × column analysis of the data

The split-block analysis with random blocks implies several things about the correlation between plot yields in the field:

- Yields from plots in one block are uncorrelated with those from plots in another block.

- Yields from two varieties to which a fungicide has been applied are uniformly correlated, i.e. they have the same correlation irrespective of whether they come from plots close together or far apart. This correlation is the same as the uniform correlation among plots in a block which had no fungicide applied.

- Yields from plots in a block that contain the same variety but different strip-plot treatment (the fungicide) are uniformly correlated, but with a different correlation than that above.

- Yields from different varieties and different fungicide treatments are also uniformly correlated, again with a different correlation than the two structures above, and again irrespective of whether they come from plots close together or far apart

These are fairly unreasonable structures for field trials. As mentioned already, plots closer together are likely to be more highly correlated than plots far apart. Moreover, if two plots at the end of one block are correlated, and two plots at the start of the next block are also correlated, it is more likely that the plot at the end of one block is also correlated with that at the start of the next contiguous block.

Consequently, the six row-plots across the field in this trial are likely to be all correlated, with a correlation structure that declines with distance apart.

Similarly, the sixteen plots in a column down each block are also likely to be correlated, in general with a different correlation than for the row plots, but also with a correlation structure that declines with distance apart. We might expect this correlation to be the larger, because the plots are each only 5 ft wide and share a 30 ft side.

Models that allow this kind of structure are AR1 and AR2 processes for rows and columns. We generally make an assumption that the two-dimensional correlation structure is *multiplicative*. The alternative is that it is unstructured, and this gives rise to a inordinate number of parameters to estimate.

How is this achieved?

We have already shown how a uniform correlation structure is built into a model: move the random block effect into the error term, defining the error so that all plots in the field are indexed (e.g. Block.Plot), then setting a uniform correlation structure among the plots with an independent structure among the blocks.

In the field plan now under consideration, rather than thinking of the experiment as 3 contiguous blocks, each having 16 contiguous row-plots and two contiguous column-plots, we think of it as having 16 contiguous row-plots (Y) and 6 contiguous column-plots (X). We then explore AR1 and AR2 structures for both rows and columns for the random model X.Y, using change in deviance to detect significantly better structures. Here X and Y need to be declared factors.

Another variant is to allow for a fixed trend in the rows or columns. Here an examination of the row-yield averages suggests that no such trend exists. An examination of the column-yield averages is dangerous since the fungicide treatment is confounded with any detected trend. Furthermore, the trend detected when the data were analysed as an RCBD indicated the trend across columns changed within blocks for the two fungicide treatments, which is just a Block.Fungicide interaction; hence using a split-block analysis, which incorporates this interaction, should effectively remove this trend.

Since we have detected too many runs of positive and negative residuals in the field when the yields are analysed as a strip-block design, the possibility of correlated plots in the row (Y)

and column (X) directions can now be assessed. We therefore fitted the following models for X.Y as the random model:

1. AR2 for X and AR2 for Y
2. AR2 for X and AR1 for Y
3. AR1 for X and AR2 for Y
4. AR1 for X and AR1 for Y
5. Id for X and AR1 for Y (Id, shortcut for Identity, represents uncorrelated plots)
6. AR1 for X and Id for Y

The deviances and the changes in deviance as you compare models are given in the following table. For example, if the deviance for model (2) is not significantly different to that for model (1), then the more simple model (2) – it has one fewer correlation parameter - is judged to be adequate. Judgment is based on the change in deviance using a χ^2 distribution with change in degrees of freedom to assess the P value.

X	Y	deviance	d.f.	Change in deviance	Change in d.f.	P value
AR2	AR2	-102.06	59			
AR2	AR1	-101.86	60	0.20	1	0.655
AR2	AR2	-102.06	59			
AR1	AR2	-100.39	60	1.67	1	0.196
AR1	AR1	-100.29	61	0.10	1	0.752
id	**AR1**	**-81.98**	**62**	**18.31**	**1**	**<0.001**
AR1	AR1	-100.29	61			
AR1	**id**	**-70.86**	**62**	**29.43**	**1**	**<0.001**

It is clear that the AR2×AR1 model is just as good as the AR2×AR2 model (P = 0.655). We could have explored the simpler model in the X direction instead: the AR1×AR2 model is also just as good as the AR2×AR2 model (P = 0.196).

Next, we chose to check whether an AR1×AR1 model is just as adequate as an AR2×AR1 model. Again, the simpler AR1×AR1 model is adequate (P = 0.752).

Finally, we found that the id×AR1 model is statistically worse than the AR1×AR1 model ($P < 0.001$), as is the AR1×id model ($P < 0.001$). This means that the yield in any plot depends directly on the neighbouring plots *in both directions*. Ticking the Covariance Model option of LMM allows a visual explanation of the plot structure. The full analysis is given in the Appendix.

Firstly, the correlation between neighbouring yields from plots immediately above or below each other is 0.699. The first 10 rows and columns of the correlation matrix for plots *vertically aligned* is:

1	1.000									
2	0.699	1.000								
3	0.489	0.699	1.000							
4	0.342	0.489	0.699	1.000						
5	0.239	0.342	0.489	0.699	1.000					
6	0.167	0.239	0.342	0.489	0.699	1.000				
7	0.117	0.167	0.239	0.342	0.489	0.699	1.000			
8	0.082	0.117	0.167	0.239	0.342	0.489	0.699	1.000		
9	0.057	0.082	0.117	0.167	0.239	0.342	0.489	0.699	1.000	
10	0.040	0.057	0.082	0.117	0.167	0.239	0.342	0.489	0.699	1.000
	1	2	3	4	5	6	7	8	9	10

The correlation between neighbouring yields from plots immediately to the left or right of each other is 0.648. There are only six columns in the field, so the 6×6 correlation matrix for plots *horizontally aligned* is:

1	1.000					
2	0.648	1.000				
3	0.420	0.648	1.000			
4	0.272	0.420	0.648	1.000		
5	0.176	0.272	0.420	0.648	1.000	
6	0.114	0.176	0.272	0.420	0.648	1.000
	1	2	3	4	5	6

For plots in different rows and columns, simply multiply the correlations from these two tables for the number of rows and number of columns apart. For example, the two plots *diagonally* alongside each other (and hence down one row and across one column) will be correlated as $0.699 \times 0.648 = 0.453$ under this model.

Using this more sensitive analysis, there is now a strong interaction (P=0.009) between varieties and fungicide. We are generally interested in comparing the fungicide effect for each of the varieties. Notice that because plots are correlated, the standard error of a mean difference will change (in this case only slightly) depending on the random allocation of varieties to plots - i.e. to their distance apart. For this discussion we have extracted the standard errors of those differences only.

	Mean		Mean	
Variety	Fungicide	No fungicide	Difference	s.e.d.
1841 (Red Fife)	2.359	1.987	0.372	0.128
1903 (Marquis)	1.967	1.813	0.154	0.124
1969 (Wadron)	2.734	2.198	0.536	0.125
1970 (Era)	2.841	2.465	0.376	0.128
1979 (Len)	2.356	2.160	0.196	0.120
1984 (Stoa)	3.219	2.504	0.715	0.119
1986 (Butte)	2.785	2.374	0.411	0.121
1988 (Amidon)	2.858	2.551	0.307	0.126
1989 (Grandin)	2.495	2.158	0.337	0.122
1990 (2375)	2.827	2.560	0.267	0.127
1999 (Parshall)	2.897	2.607	0.290	0.129
1999 (Reeder)	3.354	2.752	0.603	0.126
2000 (Alsen)	2.604	2.368	0.237	0.124
2004 (Steele-ND)	2.871	2.529	0.341	0.128
2005 (Glenn)	2.934	2.614	0.320	0.124
2006 (Howard)	3.085	2.593	0.492	0.127

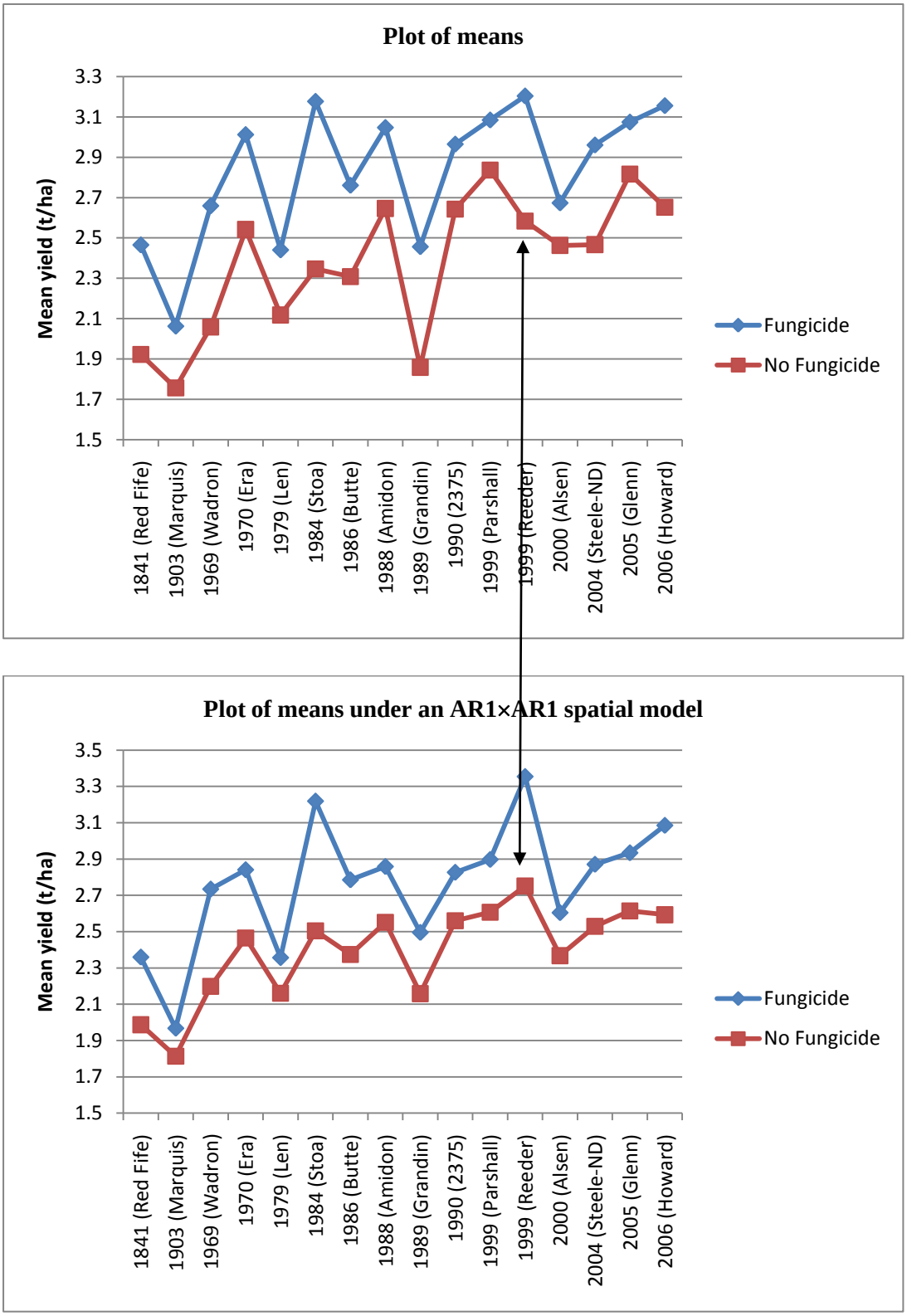
In fact, these means are slightly modified from those from the split-block analysis (as a more complex spatial model has been fitted). The means from the two analyses are compared in the following tables and plots.

Variety	Fungicide	No Fungicide	Fungicide	No Fungicide
	Means from split-block		**Means** from AR1×AR1	
1841 (Red Fife)	2.47	1.92	2.36	1.99
1903 (Marquis)	2.06	1.76	1.97	1.81
1969 (Wadron)	2.66	2.06	2.73	2.20
1970 (Era)	3.01	2.54	2.84	2.47
1979 (Len)	2.44	2.12	2.36	2.16
1984 (Stoa)	3.18	2.35	3.22	2.50
1986 (Butte)	2.76	2.31	2.79	2.37
1988 (Amidon)	3.05	2.65	2.86	2.55
1989 (Grandin)	2.46	1.86	2.50	2.16
1990 (2375)	2.97	2.64	2.83	2.56
1999 (Parshall)	3.09	2.84	2.90	2.61
1999 (Reeder)	3.20	2.58	3.35	2.75
2000 (Alsen)	2.67	2.46	2.60	2.37
2004 (Steele-ND)	2.96	2.47	2.87	2.53
2005 (Glenn)	3.07	2.82	2.93	2.61
2006 (Howard)	3.16	2.65	3.09	2.59

Variety	Fungicide	No Fungicide	Fungicide	No Fungicide
	Ranks from split-block		**Ranks** from AR1×AR1	
1841 (Red Fife)	13	14	14	15
1903 (Marquis)	16	16	16	16
1969 (Wadron)	12	13	11	12
1970 (Era)	7	7	8	9
1979 (Len)	15	12	15	13
1984 (Stoa)	2	10	2	8
1986 (Butte)	10	11	10	10
1988 (Amidon)	6	4	7	6
1989 (Grandin)	14	15	13	14
1990 (2375)	8	5	9	5
1999 (Parshall)	4	1	5	3
1999 (Reeder)	1	6	1	1
2000 (Alsen)	11	9	12	11
2004 (Steele-ND)	9	8	6	7
2005 (Glenn)	5	2	4	2
2006 (Howard)	3	3	3	4

The effect can be seen for example with Reeder. Under the split-block model, it is ranked 1st when a fungicide is applied and 6th when none is applied; under the spatial model it is top

ranked under both fungicide and control. A comparison of means plots from the two analyses is given on the following page, with the change in rank for Reeder highlighted.



7. Practical Summary

Statistical

Initial data analysis indicated a significant variety effect and non significant fungicide effect (P = 0.085) and variety by fungicide interaction (P = 0.055). The lack of significance was surprising to both agronomist and statistician, as a simple plot of varietal means suggests there should be a detectable interaction. Residual analysis indicated failure in assumptions when using a tradition ANOVA approach for analysis. The residuals were not particularly random which suggested that an alternative model should be fitted. A row × column analysis was completed and various correlations structures explored. Deviance was used to compare the models and an AR1 × AR1 correlation structure was chosen as the best fit. The fungicide effect (P = 0.001) and variety × fungicide interaction (P = 0.009) were significant when a better statistical model were used. Standard errors were decreased and ranks of varieties changed. The linear mixed model (REML) approach provided an improved model and analysis of this field experiment.

Agronomic

The significant variety x fungicide interaction indicates that farmers should not apply fungicide treatment to every variety of wheat and expect similar yield responses. Consideration must be made as to what variety is grown and to what the potential yield response is to fungicide treatment. Certain varieties will provide greater return on investment than others, and this risk must be considered as actual market price and yield fluctuate. In this trial, Reeder (1999) and Stoa (1984) had the greatest yield responses and were the top two yielding varieties when treated with fungicide. Waldron (1969) had the third largest yield response from fungicide treatment, but only ranked 11th in grain yield when treated with fungicide. Red Fife (1841) and Marquis (1803), the oldest varieties and considered by some the true heritage type wheats in this experiment, did not respond as well to fungicide application as Reeder, Stoa or Waldron. Yield response of wheat to fungicides is variable. Evaluation of potential yield response must be based on specific variety information and not generalized based on historical time of development.

Appendix 1. REML variance components analysis assuming an AR1×Ar1 spatial model

Response variate: Yield
Fixed model: Constant + Variety + Fungicide + Variety.Fungicide
Random model: X.Y
Number of units: 96

X.Y used as residual term with covariance structure as below

Sparse algorithm with AI optimisation

Covariance structures defined for random model

Covariance structures defined within terms:

Term	Factor	Model	Order	No. rows
X.Y	X	Auto-regressive (+ scalar)	1	6
	Y	Auto-regressive	1	16

Residual variance model

Term	Factor	Model(order)	Parameter	Estimate	s.e.
X.Y			Sigma2	0.114	0.0379
	X	AR(1)	phi_1	0.6478	0.0947
	Y	AR(1)	phi_1	0.6992	0.0819

Estimated covariance models

Variance of data estimated in form:

V(y) = Sigma2.R

where: V(y) is variance matrix of data
Sigma2 is the residual variance
R is the residual covariance matrix

Residual term: X.Y

Sigma2: 0.1137

R uses direct product construction

Factor: X
Model: Auto-regressive

Covariance matrix:

1	1.000				
2	0.648	1.000			
3	0.420	0.648	1.000		
4	0.272	0.420	0.648	1.000	
5	0.176	0.272	0.420	0.648	1.000

6	0.114	0.176	0.272	0.420	0.648	1.000
	1	2	3	4	5	6

Factor: Y
Model: Auto-regressive

Covariance matrix (first 10 rows only):

1	1.000									
2	0.699	1.000								
3	0.489	0.699	1.000							
4	0.342	0.489	0.699	1.000						
5	0.239	0.342	0.489	0.699	1.000					
6	0.167	0.239	0.342	0.489	0.699	1.000				
7	0.117	0.167	0.239	0.342	0.489	0.699	1.000			
8	0.082	0.117	0.167	0.239	0.342	0.489	0.699	1.000		
9	0.057	0.082	0.117	0.167	0.239	0.342	0.489	0.699	1.000	
10	0.040	0.057	0.082	0.117	0.167	0.239	0.342	0.489	0.699	1.000
	1	2	3	4	5	6	7	8	9	10

Deviance: -2*Log-Likelihood

Deviance	d.f.
-100.29	61

Note: deviance omits constants which depend on fixed model fitted.

Tests for fixed effects

Sequentially adding terms to fixed model

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Variety	212.98	15	14.11	44.7	<0.001
Fungicide	20.26	1	20.26	10.3	0.001
Variety.Fungicide	40.64	15	2.71	31.5	0.009