DESIGN AND MIXED-MODEL ANALYSIS OF EXPERIMENTS

XI. Split-plot experiments

(Cochran and Cox, sec. 7.3; Mead, ch. 14; Mead and Curnow, sec. 6.7)

XI.A Design of split-plot experiments

When discussing the topic of confounding, our goal was to leave main effects unconfounded, or perhaps, only partially confounded and with most of the information about main effects confounded with less variable units.

On occasion, however, it is desirable to have main effects confounded with more variable units such as blocks. This particular class of designs is called split-plot designs for reasons which will become obvious. Their defining attribute is that there is randomization to two different physical entities such that some main effects are randomized to the more variable entities.

Definition XI.1: The **standard split-plot design** is one in which two factors, say A and B with *a* and *b* levels, respectively are assigned as follows: one of the factors, A say, is randomized according to a RCBD with say *r* blocks and each of its *ra* plots, called the **main plots**, is split into *b* **subplots** and the levels of B randomized independently in each subplot. ■

The split-plot principle is very flexible and can be used to generate a large number of different types of experiments. For example, the main plots could be arranged in any of a CRD, RCBD, Latin square, BIBD, Youden Square and each plot of the particular design used then subdivided into subplots.

The subplots may utilize more complicated designs as well. For example, the main plots may be arranged in a RCBD each of which are subdivided in such a way as to allow a Latin Square to be placed in each main plot.

Also, subplots can be split into subsubplots and subsubplots into ... Nor is one restricted to applying just one factor to each type of unit. More than one factor can be randomized to main plots, more than one to subplots and so on.

The standard split-plot design is nearly the simplest possibility; only a CRD in the main plots would be simpler.

The split-plot design is useful in the following situations:

1. When the physical attributes of a factor require the use of larger units of experimental material than other factors.

   For example, land preparation treatments usually require to be performed on larger areas of land than do the sowing of different varieties (due to the different pieces of equipment). Temperature control for say storage purposes involves the use of relatively large chambers in which several samples can usually be stored. Different processing runs are often of a minimum size such that their produce can be readily subdivided for the application of further treatments. Also, some factors are relatively hard to change. For example, the temperature of a production operation is often difficult to change so that it might be better to change it less often by making it a main-plot factor.

2. When it is desired to incorporate an additional factor into an experiment.

   When an experiment, such as an agricultural experiment is run over a period of time, it sometimes occurs after the trial has been set up that it would be advantageous to incorporate an additional factor. This can be achieved by splitting the existing plots into enough subplots so that the levels of the new factor(s) can be randomized to the subplots in each subplot.

3. When it is expected that the differences amongst the levels of certain factors are larger than amongst those of other factors.

   The levels of the factors with larger differences are randomized to main plots. One effect of this may be to increase the precision of comparisons between the levels of the other factors.

4. When it is desired to ensure greater precision between some factors than others.

   Irrespective of the size of the differences between the main plot treatment factors, it is desired to increase the precision of some factors by assigning them to subplots. On the other hand, it may be that one is less interested in the main effects of some factors, in which case these factors should be assigned to main plots. A particular example of such factors are "noise" factors which are often included in production experiments to ensure the product works under a range of conditions; one is usually more interested in product differences and the interaction of products and the "noise" factors.

Note that the last two of these situations are utilising the anticipated greater variability of main plots relative to subplots. That is, we are expecting the larger units to be more variable than the smaller units. This will be expressed in the variation model for these experiments, and hence in the expected mean squares.

In describing the type of study, you need to identify the main plot and subplot design.

XI.B The standard split-plot experiment

a) Designing a standard split-plot experiment

To use Genstat to obtain a layout for the standard split-plot experiment is similar to obtaining the layout for a randomized complete block design. Use the *orthogonal hierarchical designs (randomized blocks, split-plots)* option of Genstat's *Stats > Design > Select Design* command. Specify three block factors instead of two and ask for one of the two treatment factors to be applied to the second block factor and the other to the third block factor. Other types of split-plot designs can also be obtained by varying the number of block factors and the number of treatment factors applied to the different block factors. The following responses should be used to obtain the layout for a standard split-plot experiment:

How many block factors (or strata) does your design have?	3
What would you like to call block factor 1?	name for blocks
How many treatment factors are to be applied to the units indexed by Blocks	0
How many replicates are there of Blocks?	*r*
What would you like to call block factor 2?	name for plots
How many treatment factors are to be applied to the units indexed by Plots	1
What would you like to call treatment factor 1 (in this stratum)?	name for treatments A
How many levels does treatment factor A have?	*a*
What would you like to call block factor 3?	name for subplots
How many treatment factors are to be applied to the units indexed by Subplots	1
What would you like to call treatment factor 1 (in this stratum)?	name for treatments B
How many levels does treatment factor B have?	*b*
Seed for randomization (0 for none)?	6-digit number
Do you want to print the design?	yes
Do you want to check the design by ANOVA	yes

Example XI.1 Perennial ryegrass experiment

An experiment was conducted to investigate the effect on dry matter yield of 3 varieties of perennial ryegrass (S23, NZ and Kent) which were grown in swards at each of 2 fertilizer levels. The varieties were assigned to 3 plots in 4 blocks using a randomized complete block design. The plots were split in two for the application of the two fertilizers (normal and extra fertilizer) and the two fertilizers were randomized to the two subplots within each plot. The table below gives the field layout of the experiment:

Layout for a standard split-plot experiment

Block						
I	S23		NZ		Kent	
	N	E	N	E	N	E
II	NZ		Kent		S23	
	N	E	E	N	E	N
III	NZ		S23		Kent	
	E	N	E	N	E	N
IV	S23		NZ		Kent	
	N	E	E	N	E	N

N = Normal; E = Extra

For this experiment the number of blocks is 4 and there is one factor, Varieties, of three levels randomized to the Plots and one factor, Fertilizer, of two levels randomized to the subplots. That is, $r = 4$, $a = 3$ and $b = 2$. Supplying this information and a seed of 123654 to Genstat results in the following output, which produces a different layout to that given above:

```
Genstat 5  Release 4.1  (PC/Windows NT)               18 April 2000 14:59:29
Copyright 1998, Lawes Agricultural Trust (Rothamsted Experimental Station)

                 ______________________________________________

                 Genstat 5 Fourth Edition - (for Windows)
                 Genstat 5 Procedure Library Release PL11
                 ______________________________________________


 *** Treatment combinations on each unit of the design ***


         Subplots   1     2
  Blocks    Plots
       1        1   3 2   3 1
                2   1 1   1 2
                3   2 2   2 1
       2        1   3 2   3 1
                2   2 1   2 2
                3   1 1   1 2
       3        1   1 1   1 2
                2   3 2   3 1
                3   2 2   2 1
       4        1   1 2   1 1
                2   2 1   2 2
                3   3 2   3 1

 Treatment factors are listed in the order:  Variety Fertiliz
```

```
1.........................................................................

***** Analysis of variance *****
Source of variation     d.f.

Blocks stratum             3

Blocks.Plots stratum
Variety                    2
Residual                   6

Blocks.Plots.Subplots stratum
Fertiliz                   1
Variety.Fertiliz           2
Residual                   9

Total                     23
```

b) Determining the analysis of variance table

For these experiments we will not derive the analysis in full; rather the rules for determining the analysis of variance table will be relied upon to establish the analysis. In general, the analysis for these experiments is complicated by the fact that there is no analysis with $\mathbf{V} = \sigma^2\mathbf{I}$. Consequently, generalized least squares must be employed in deriving their analysis.

Example XI.1 Perennial ryegrass experiment (continued)

What are the components of the study?

1. Observational unit – a subplot

   Variables (incl. factors) are? Ans. Blocks, Plots, Subplots, Variety, Fertilizer, Dry matter
2. Response variable – Dry matter
3. Unrandomized factors – Blocks, Plots, Subplots
4. Randomized factors – Variety, Fertilizer
5. Type of study – Standard split-plot with main plots in an RCBD and subplots completely randomized within a plot

The experimental structure is:

Structure	Formula
unrandomized	*4* Blocks/*3* Plots/*2* Subplots
randomized	*3* Variety**2* Fertilizer

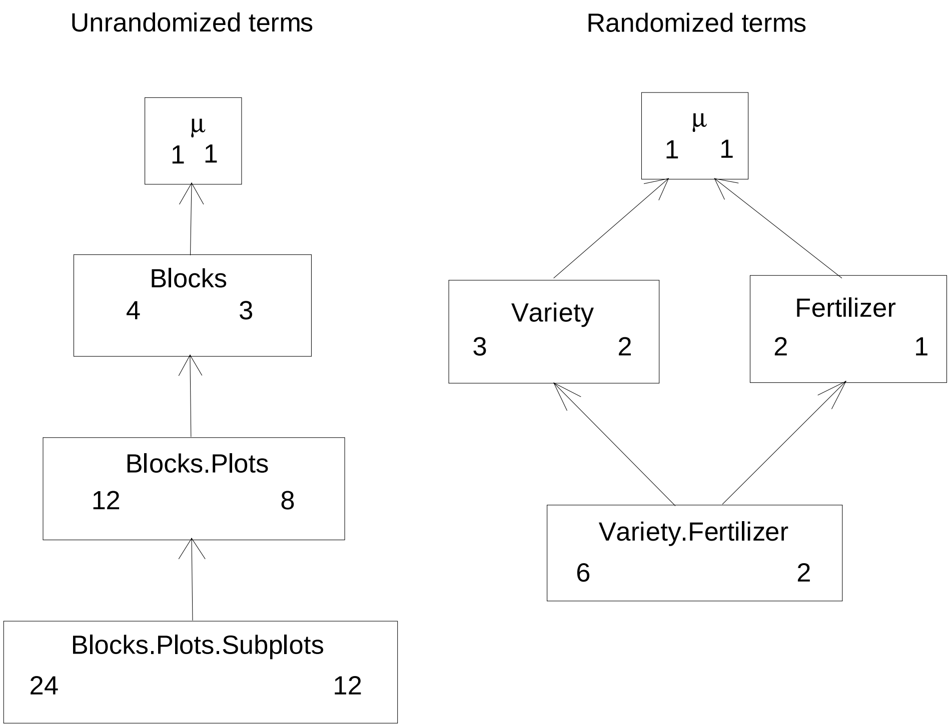
The formulae expand to give:

(Blocks + Blocks.Plots)/Subplots

= Blocks + Blocks.Plots + Blocks.Plots.Subplots

and Variety*Fertilizer = Variety + Fertilizer + Variety.Fertilizer

Hasse diagram for the standard split-plot experiment



Note, that in working out the degrees of freedom for the terms from the randomized structure, the rule for a set of crossed factors can be used. That is, for each factor in the term, calculate the number of levels minus one and multiply these together.

The models for this experiment, based on the unrandomized factors being random factors and the randomized factors being fixed factors are:

E[Y] = Variety.Fertilizer

and var[Y] = Blocks + Blocks.Plots + Blocks.Plots.Subplots.

The analysis of variance table for this experiment has the following form:

Source	df	E[MSq]
Blocks	3	$\sigma^2_{BPS} + 2\sigma^2_{BP} + 6\sigma^2_{B}$
Blocks.Plots	8	
Variety	2	$\sigma^2_{BPS} + 2\sigma^2_{BP} + f_V(\psi)$
Residual	6	$\sigma^2_{BPS} + 2\sigma^2_{BP}$
Blocks.Plots.Subplots	12	
Fertilizer	1	$\sigma^2_{BPS} + f_F(\psi)$
Variety.Fertilizer	2	$\sigma^2_{BPS} + f_{VF}(\psi)$
Residual	9	σ^2_{BPS}

The design chosen has resulted in the differences between varieties being confounded with Plots differences whereas differences between fertilizers are confounded with Subplots (the Plots do not differ in fertilizers). This has been achieved by splitting the main plots (Plots in this case) into subplots (Subplots in this case). This is evident from the above table.

Now it is likely that Plots will be more variable than Subplots so that differences between Varieties may well be estimated with less precision than Fertilizer differences.

c) Analysis of the example

Example XI.1 Perennial ryegrass experiment (continued)

The data for the experiment is as follows:

Blocks	Plots	Subplots	Variety	Fertilizer	Dry Matter
1	1	1	S23	Normal	247.0
		2	S23	Extra	299.0
	2	1	NZ	Normal	257.0
		2	NZ	Extra	315.0
	3	1	Kent	Normal	233.0
		2	Kent	Extra	382.0
2	1	1	NZ	Normal	175.0
		2	NZ	Extra	247.0
	2	1	Kent	Extra	353.0
		2	Kent	Normal	216.0
	3	1	S23	Extra	318.0
		2	S23	Normal	202.0
3	1	1	NZ	Extra	289.0
		2	NZ	Normal	188.0
	2	1	S23	Extra	284.0
		2	S23	Normal	171.0
	3	1	Kent	Extra	383.0
		2	Kent	Normal	200.0
4	1	1	S23	Normal	183.0
		2	S23	Extra	279.0
	2	1	NZ	Extra	307.0
		2	NZ	Normal	174.0
	3	1	Kent	Extra	310.0
		2	Kent	Normal	143.0

The Genstat output file for analyzing this experiment is:

```
Genstat 5  Release 4.1  (PC/Windows NT)             17 April 2000 21:45:04
Copyright 1998, Lawes Agricultural Trust (Rothamsted Experimental Station)

                ________________________________________

                Genstat 5 Fourth Edition - (for Windows)
                Genstat 5 Procedure Library Release PL11
```

```
 3  "Data taken from File: D:/ANALYSES/LM/MULTIFAC/SPLRYEAN.GSH"
 4  DELETE [redefine=yes] Blocks,Plots,SubPlots,Variety,Fertiliz,DryMatte
 5  FACTOR [modify=yes;nvalues=24;levels=4] Blocks
 6  READ Blocks; frepresentation=ordinal

  Identifier    Values   Missing    Levels
      Blocks        24         0         4

 8  FACTOR [modify=yes;nvalues=24;levels=3] Plots
 9  READ Plots; frepresentation=ordinal

  Identifier    Values   Missing    Levels
       Plots        24         0         3

11  FACTOR [modify=yes;nvalues=24;levels=2] SubPlots
12  READ SubPlots; frepresentation=ordinal

  Identifier    Values   Missing    Levels
    SubPlots        24         0         2

14  FACTOR [modify=yes;nvalues=24;levels=3;labels=!t('S23','NZ','Kent')] Variety
15  READ Variety; frepresentation=ordinal

  Identifier    Values   Missing    Levels
     Variety        24         0         3

17  FACTOR [modify=yes;nvalues=24;levels=2;labels=!t('Extra','Normal')] Fertiliz
18  READ Fertiliz; frepresentation=ordinal

  Identifier    Values   Missing    Levels
    Fertiliz        24         0         2

20  VARIATE [nvalues=24] DryMatte
21  READ DryMatte

  Identifier   Minimum      Mean   Maximum    Values   Missing
    DryMatte     143.0     256.5     383.0        24         0

24
25  PRINT Blocks,Plots,SubPlots,Variety,Fertilizer,DryMatter

    Blocks       Plots    SubPlots     Variety    Fertiliz    DryMatte
         1           1           1         S23      Normal       247.0
         1           1           2         S23       Extra       299.0
         1           2           1          NZ      Normal       257.0
         1           2           2          NZ       Extra       315.0
         1           3           1        Kent      Normal       233.0
         1           3           2        Kent       Extra       382.0
         2           1           1          NZ      Normal       175.0
         2           1           2          NZ       Extra       247.0
         2           2           1        Kent       Extra       353.0
         2           2           2        Kent      Normal       216.0
         2           3           1         S23       Extra       318.0
         2           3           2         S23      Normal       202.0
         3           1           1          NZ       Extra       289.0
         3           1           2          NZ      Normal       188.0
         3           2           1         S23       Extra       284.0
         3           2           2         S23      Normal       171.0
         3           3           1        Kent       Extra       383.0
         3           3           2        Kent      Normal       200.0
         4           1           1         S23      Normal       183.0
         4           1           2         S23       Extra       279.0
         4           2           1          NZ       Extra       307.0
         4           2           2          NZ      Normal       174.0
         4           3           1        Kent       Extra       310.0
         4           3           2        Kent      Normal       143.0

26  BLOCK Blocks/Plots/SubPlots
27  TREAT Variety*Fertilizer
28  ANOVA [FPROB=Y; PSE=LSD] DryMatter
```

```
28...............................................................................

***** Analysis of variance *****

Variate: DryMatte

Source of variation     d.f.       s.s.       m.s.    v.r.  F pr.

Blocks stratum             3     9907.5     3302.5    3.13

Blocks.Plots stratum
Variety                    2     5373.1     2686.5    2.55  0.158
Residual                   6     6331.9     1055.3    2.66

Blocks.Plots.SubPlots stratum
Fertiliz                   1    79005.4    79005.4  198.87  <.001
Variety.Fertiliz           2     5884.8     2942.4    7.41  0.013
Residual                   9     3575.4      397.3

Total                     23   110078.0


***** Tables of means *****

Variate: DryMatte

Grand mean  256.5

  Variety      S23       NZ     Kent
             247.9    244.0    277.5

 Fertiliz    Extra   Normal
             313.8    199.1

  Variety Fertiliz    Extra   Normal
      S23             295.0    200.7
       NZ             289.5    198.5
     Kent             357.0    198.0


*** Least significant differences of means (5% level) ***

Table              Variety    Fertiliz     Variety
                                          Fertiliz
rep.                     8          12           4
l.s.d.               39.74       18.41       42.25
d.f.                     6           9       10.39
Except when comparing means with the same level(s) of
 Variety                                     31.88
 d.f.                                            9



  29  CALC pB=1-FPROB(3302.5/1055.3; 3; 6)
  30  &    pBP=1-FPROB(1055.3/397.3; 6; 9)
  31  PRINT pB,pBP

          pB         pBP
      0.1089     0.09102

  32  APLOT METHOD=fit,normal
```

```
             -
             I
             I
             I
        25.0 I
r            I                         *           *
e            I                            *
s            I                     *                   *            *
i            I             *                     *
d            I                          *          *   *
u        0.0 I                 *                  *
a            I         *         *                                     *
l            I                *              *
s            I                      **                             *
             I                                              *
             I                   *                        *
       -25.0 I
             -+---------+---------+---------+---------+---------+---------+---
          100.0     150.0     200.0     250.0     300.0     350.0     400.0

                                         fitted values


                                          Normal plot

             -
             I
        25.0 I
r            I                                                 *    *
e            I                                             *
s            I                                       * * *
i            I                                    * *
d            I                                ** *
u        0.0 I                             * *
a            I                         * **
l            I                      * *
s            I                 * * *
             I               *
             I      *    *
       -25.0 I
             -+---------+---------+---------+---------+---------+---------+---
           -2.4      -1.6      -0.8       0.0       0.8       1.6       2.4

                                   expected Normal quantiles
```

```
  33  "
 -34  **** Tukey''s one-degree-of-freedom-for-non-additivity.
 -35  **** It is the term designated covariate in the following analysis
 -36  "
  37  AKEEP [FIT=Fit]
  38  CALC ResSq=Fit*Fit
  39  ANOVA [PRINT=*]  ResSq; RES=ResSq
  40  COVAR ResSq                            "A computational trick"
  41  ANOVA [PRINT=A; FPROB=Y] DryMatter
```

41..

***** Analysis of variance (adjusted for covariate) *****

Variate: DryMatte
Covariate: ResSq

Source of variation	d.f.	s.s.	m.s.	v.r.	cov.ef.	F pr.
Blocks stratum	3	9907.5	3302.5	3.13		
Blocks.Plots stratum						
Variety	2	5373.1	2686.5	2.55	1.00	0.158
Residual	6	6331.9	1055.3	2.58	1.00	

```
Blocks.Plots.SubPlots stratum
Fertiliz                  1    79005.4    79005.4  193.46    1.00  <.001
Variety.Fertiliz          2     5884.8     2942.4    7.20    1.00  0.016
Covariate                 1      308.3      308.3    0.75          0.410
Residual                  8     3267.1      408.4            0.97

Total                    23   110078.0

44  AGRAPH [METHOD=lines] XFACTOR=Fertilizer; GROUP=Variety
```

The analysis of variance table for the example is:

Source	df	MSq	E[MSq]	F	Prob
Blocks	3	3302.5	$\sigma^2_{BPS} \quad +2\sigma^2_{BP} \quad +6\sigma^2_{B}$	3.13	0.109
Blocks.Plots	8				
Variety	2	2686.5	$\sigma^2_{BPS} \quad +2\sigma^2_{BP} \quad +f_V(\psi)$	2.55	0.158
Residual	6	1055.3	$\sigma^2_{BPS} \quad +2\sigma^2_{BP}$	2.66	0.091
Blocks.Plots.Subplots	12				
Fertilizer	1	79005.4	$\sigma^2_{BPS} \quad +f_F(\psi)$	198.87	<.001
Variety.Fertilizer	2	2942.4	$\sigma^2_{BPS} \quad +f_{VF}(\psi)$	7.41	0.013
Residual	9	397.3	σ^2_{BPS}		
Nonadditivity	1	308.3		0.75	0.410
Deviations	8	408.4			

The residual-versus-fitted-values and normal probability plots appear to be satisfactory. The data is not exhibiting transformable nonadditivity as Tukey's test for nonadditivity is not significant.

There is a significant interaction as the Variety.Fertilizer term is significant ($p < 0.05$). Note also that the plots are not significantly more variable than the subplots ($p = 0.091$); however, it would be unwise to pool plots with subplots as one cannot be certain that there is no extra plot variability. Similarly, blocks are not significantly more variable than the plots ($p = 0.109$).

d) Treatment differences for the standard split-plot

As for other experiments treatment differences are investigated using multiple comparisons, polynomial submodels or contrasts. Of these, only multiple comparisons is different to the ordinary factorial experiment and we investigate the use of these in the next section.

Multiple comparisons

The calculation of multiple comparison statistics is slightly more complicated for split-plot experiments. The formulae for the standard errors of difference for a split-plot design in which one factor (A) is randomized to whole-plots and another (B) to subplots (as in the example) are:

Two overall A means (e.g. Variety means)	$\sqrt{\dfrac{2s_1^2}{rb}}$	where *rb* is the no. of replications in an A mean
Two overall B means (e.g. Fertilizer means)	$\sqrt{\dfrac{2s_2^2}{ra}}$	where *ra* is the no. of replications in a B mean
Two interaction means		
– at the same level of A	$\sqrt{\dfrac{2s_2^2}{r}}$	where *r* is the no. of replications in an A.B mean
– not at the same level of A	$\sqrt{\dfrac{2\left[(b-1)s_2^2+s_1^2\right]}{rb}}$	

Note: s_1^2 & s_2^2 are the first and second Residual MSqs, respectively, from the ANOVA table.

Example XI.1 Perennial ryegrass experiment (continued)

The two-way table of means is of interest for the example. The output containing this table (and the LSDs) is:

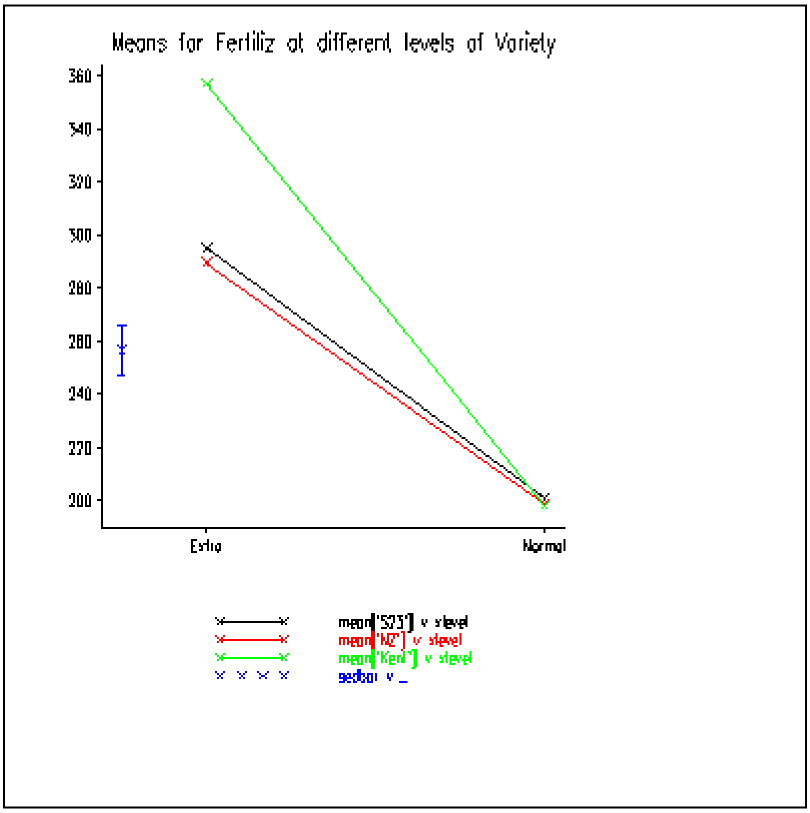
```
  Variety Fertiliz     Extra   Normal
      S23              295.0    200.7
       NZ              289.5    198.5
     Kent              357.0    198.0


*** Least significant differences of means (5% level) ***

Table              Variety    Fertiliz     Variety
                                          Fertiliz
rep.                     8          12           4
l.s.d.               39.74       18.41       42.25
d.f.                     6           9       10.39
Except when comparing means with the same level(s) of
 Variety                                     31.88
 d.f.                                            9
```

The LSD can be used in this instance as the number of treatments is just 6. There are two LSDs given for the table of Variety.Fertilizer means. The second of these is used to examine the differences between fertilizer means within each variety — it is 31.88. Using this LSD it is found that, for all varieties, there is a difference between the two fertilizers.

A plot of the table of means is given in the following diagram.



The interaction clearly arises because this difference is largest for the variety Kent.

e) Computation in Genstat

The following commands were used in analyzing the example:

```
PRINT Blocks,Plots,SubPlots,Variety,Fertilizer,DryMatter
BLOCK Blocks/Plots/SubPlots
TREAT Variety*Fertilizer
ANOVA [FPROB=Y; PSE=LSD] DryMatter
CALC pB=1-FPROB(3302.5/1055.3; 3; 6)
&    pBP=1-FPROB(1055.3/397.3; 6; 9)
PRINT pB,pBP
APLOT METHOD=fit,normal
"
**** Tukey''s one-degree-of-freedom-for-non-additivity.
**** It is the term designated covariate in the following analysis
"
AKEEP [FIT=Fit]
CALC ResSq=Fit*Fit
ANOVA [PRINT=*]  ResSq; RES=ResSq
COVAR ResSq                            "A computational trick"
ANOVA [PRINT=A; FPROB=Y] DryMatter
AGRAPH [METHOD=lines] XFACTOR=Fertilizer; GROUP=Variety
```

XI.C Systematic or Unreplicated Main Plots

(Cochran and Cox, sec. 7.3)

As was pointed out at the outset split-plot designs are often used where one of the treatments requires larger units to apply. Sometimes the difficulty in applying the larger units means that it is not possible to randomize the treatments to the main plots or even to replicate them. In this section we look at the consequences of these two variations in the layout of a standard split-plot experiment.

Example XI.2 Varieties applied systematically

Suppose an experiment is to be run to investigate the effect on the yield of five varieties of wheat and 2 levels of fertilizer. The experiment is to involve three blocks. However, it was know that the varieties would ripen in a particular order and to facilitate harvest the varieties were assigned to the main plots in the same order within each block. The following diagram shows the layout with V_1 being the earliest variety and V_5 the latest variety to ripen.

Layout for systematic main plots in a split-plot experiment

Variety

Block		V_1	V_2	V_3	V_4	V_5
	I	N	Y	Y	N	N
		Y	N	N	Y	Y

	V_1	V_2	V_3	V_4	V_5
II	N	N	Y	Y	N
	Y	Y	N	N	Y

	V_1	V_2	V_3	V_4	V_5
III	N	N	N	Y	N
	Y	Y	Y	N	Y

N = No; Y = Yes

The experimental structure is:

Structure	Formula
unrandomized	(*3* Blocks**5* Plots)/*2* Subplots
randomized	*5* Variety**2* Fertilizer

In this structure we have Plots crossed with Blocks. This is appropriate because the first plot in each block has in common that they are in the same relative position in the block and that they will be harvested first.

The analysis of variance table for this experiment has the following form:

Source	df	E[MSq]			
Blocks	2	σ^2_{BPS}	$+2\sigma^2_{BP}$		$+10\sigma^2_{B}$
Plots	4				
Variety	4	σ^2_{BPS}	$+2\sigma^2_{BP}$	$+6\sigma^2_{P}$	$+f_V(\psi)$
Blocks.Plots	8	σ^2_{BPS}	$+2\sigma^2_{BP}$		
Blocks.Plots.Subplots	15				
Fertilizer	1	σ^2_{BPS}			$+f_F(\psi)$
Variety.Fertilizer	4	σ^2_{BPS}			$+f_{VF}(\psi)$
Residual	10	σ^2_{BPS}			

The implications of this analysis are that it is not possible to separate plot variability from Variety differences. While you could test Variety differences against the Blocks.Plots line it is not possible to determine whether these differences involve Variety as well as Plot differences. However, it is possible to examine fertilizer differences both within varieties and averaged over all varieties.

Example XI.3 Unreplicated irrigation treatments

Irrigation treatments usually require large areas and it is often difficult to change between irrigated and nonirrigated areas. For example, in an experiment to investigate the effects of irrigation on the yield of different varieties of tomatoes, one area of tomatoes is irrigated and a second is not. Within each area eight varieties are randomized to 16 plots so that each variety occurs twice. In each plot is grown five tomato bushes and the yield of each is obtained.

The experimental structure is:

Structure	Formula
unrandomized	*2* Areas/*16* Plots/*5* Bushes
randomized	*2* Irrigations**8* Varieties

The analysis of variance table for this experiment has the following form:

Source	df	E[MSq]			
Areas	1				
Irrigations	1	σ^2_{APB}	$+5\sigma^2_{AP}$	$+80\sigma^2_{A}$	$+f_I(\psi)$
Areas.Plots	30				
Variety	7	σ^2_{APB}	$+5\sigma^2_{AP}$		$+f_V(\psi)$
Variety.Irrigations	7	σ^2_{APB}	$+5\sigma^2_{AP}$		$+f_{VI}(\psi)$
Residual	16	σ^2_{APB}	$+5\sigma^2_{AP}$		
Areas.Plots.Bushes	128	σ^2_{APB}			

Like the split-plot experiment with main-plot treatments applied systematically, in unreplicated experiments it is not possible to separate plot variability from Irrigation differences. While you could test Irrigation differences against the residual for Areas.Plots it is not possible to determine whether these differences involve Irrigation differences as well as Plot differences. However, it is possible to examine variety differences both within irrigations and averaged over both irrigation treatments.

XI.D A Complex Split-Plot Experiment

As was mentioned in the introduction the split-plot principle is very flexible. It can involve several levels of splitting. At each level a range of designs can be employed to assign the treatments to that level and as many factors as desired can be applied. Because of this the number of possible designs is limitless and quite complex designs are possible. To demonstrate the flexibility of such designs and the utility of the approach we use in determining the analysis of variance table, we next consider a more complicated split-plot design.

Example XI.4 Grazing experiment

An experiment is conducted to investigate the effects on pasture composition of different patterns of grazing. The different patterns were specified by three treatment factors. The three treatment factors were:

- Period: the length of the period for which plots were grazed; 3, 9 or 18 days;
- Spring grazing: the number of cycles of grazing in Spring; either 2 periods of grazing, with long gaps, or 4 periods of grazing, with short gaps;
- Summer grazing: the number of cycles in Summer; either 2 or 4 periods of grazing, with long or short gaps, respectively.

The experimental design involved the assignment of Periods to plots using a 3×3 Latin square. Each plot was split into 2 SubRows $\times$ 2 SubColumns; the 2 numbers of grazing cycles for Spring and Summer randomized to the SubRows and SubColumns, respectively, within each plot. The measured response was the percentage covered by the principal grass. The field layout was as given in the table below.

Layout for the grazing experiment

Period	Summer			Summer			Summer		
	18	2	4	**9**	4	2	**3**	4	2
Spring	4			2			2		
	2			4			4		
	9	4	2	**3**	2	4	**18**	2	4
Spring	2			2			2		
	4			4			4		
	3	2	4	**18**	2	4	**9**	4	2
Spring	2			2			2		
	4			4			4		

What are the components of the study?

1. Observational unit – a subrow-subcolumn combination
2. Response variable – %Covered by Principal Grass
3. Unrandomized factors – Rows, Columns, Subrows, Subcolumns
4. Randomized factors – Periods, Spring, Summer
5. Type of study – Split-plot with main plots in an LS and subplots arranged in rows and columns with factors randomized to them

The experimental structure is:

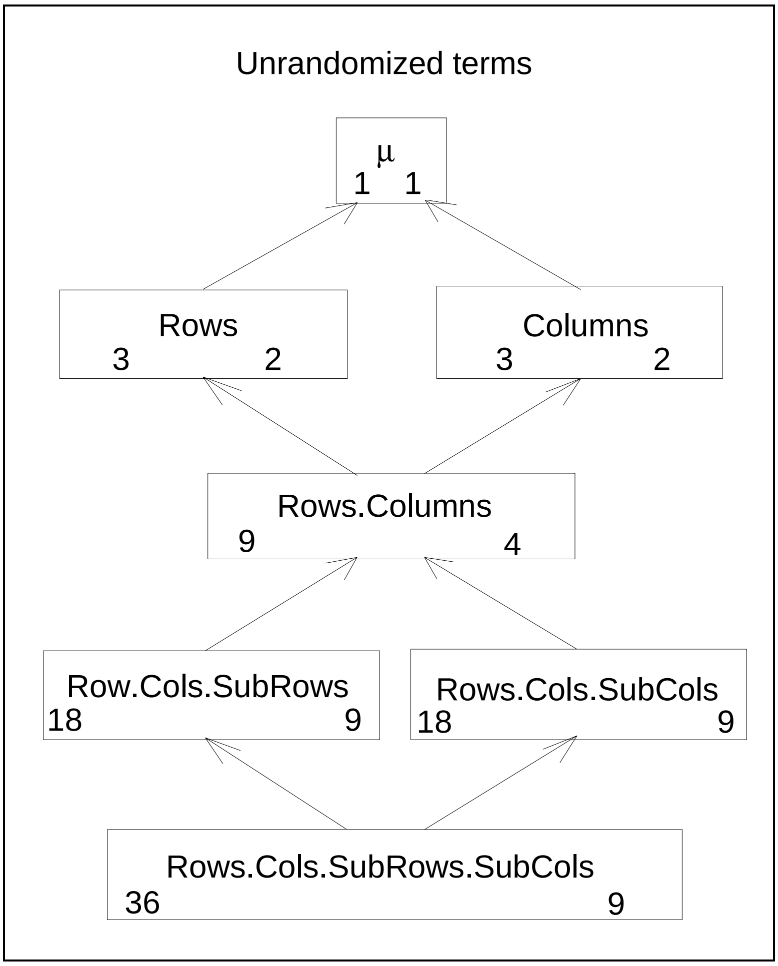
Structure	Formula
unrandomized	(*3* Rows**3* Columns)/(*2* Subrows**2* Subcolumns)
randomized	*3* Periods**2* Spring**2* Summer

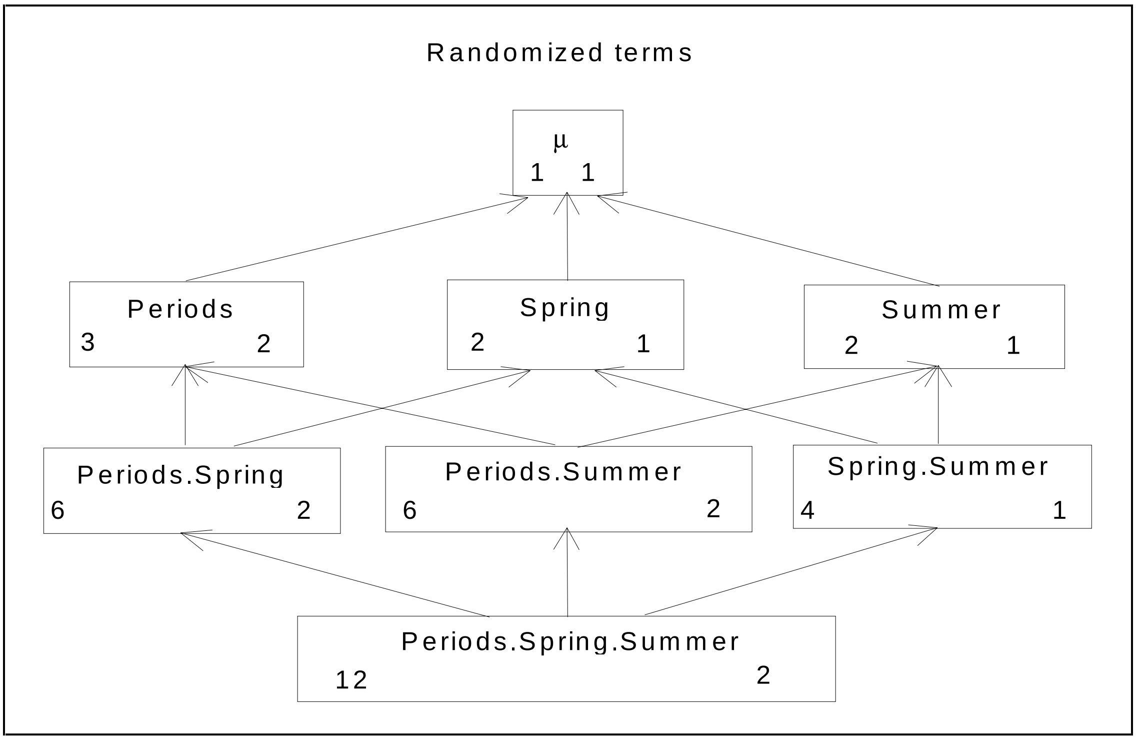
The formulae expand to give:

(Rows*Columns)/(Subrows*Subcolumns)
= (Rows + Columns + Rows.Columns)
/(Subrows + Subcolumns + Subrows.Subcolumns)
= Rows + Columns + Rows.Columns
+ Rows.Columns.Subrows + Rows.Columns.Subcolumns
+ Rows.Columns.Subrows.Subcolumns

and Periods*Spring*Summer
= (Periods + Spring + Periods.Spring)*Summer
= Periods + Spring + Periods.Spring
+ Periods.Summer + Spring.Summer + Periods.Spring.Summer

Hasse diagrams of term marginalities for the grazing experiment





Note, that in working out the degrees of freedom for the terms from the randomized structure, the rule for a set of crossed factors can be used. That is, for each factor in the term, calculate the number of levels minus one and multiply these together.

The models for this experiment, based on the unrandomized factors being random factors and the randomized factors being fixed factors are:

$$\text{E}[Y] = \text{Periods.Spring.Summer}$$

and $\text{var}[Y] = \text{Rows} + \text{Columns} + \text{Rows.Columns} + \text{Rows.Columns.Subrows} + \text{Rows.Columns.Subcolumns} + \text{Rows.Columns.Subrows.Subcolumns}$

The variance components and their multipliers are:

$$\sigma^2_{\text{RCrc}},\ 2\sigma^2_{\text{RCr}},\ 2\sigma^2_{\text{RCc}}, 4\sigma^2_{\text{RC}}, 12\sigma^2_{\text{R}},\ 12\sigma^2_{\text{C}}$$

The analysis of variance table for this experiment has the following form:

Source	df	E[MSq]
Rows	2	$\sigma^2_{\text{RCrc}} + 2\sigma^2_{\text{RCr}} + 2\sigma^2_{\text{RCc}} + 4\sigma^2_{\text{RC}} + 12\sigma^2_{\text{R}}$
Columns	2	$\sigma^2_{\text{RCrc}} + 2\sigma^2_{\text{RCr}} + 2\sigma^2_{\text{RCc}} + 4\sigma^2_{\text{RC}} + 12\sigma^2_{\text{C}}$
Rows.Columns	4	
Periods	2	$\sigma^2_{\text{RCrc}} + 2\sigma^2_{\text{RCr}} + 2\sigma^2_{\text{RCc}} + 4\sigma^2_{\text{RC}} + f_{\text{P}}(\psi)$
Residual	2	$\sigma^2_{\text{RCrc}} + 2\sigma^2_{\text{RCr}} + 2\sigma^2_{\text{RCc}} + 4\sigma^2_{\text{RC}}$
Rows.Columns.Subrows	9	
Spring	1	$\sigma^2_{\text{RCrc}} + 2\sigma^2_{\text{RCr}} + f_{\text{S}}(\psi)$
Spring.Periods	2	$\sigma^2_{\text{RCrc}} + 2\sigma^2_{\text{RCr}} + f_{\text{SU}}(\psi)$
Residual	6	$\sigma^2_{\text{RCrc}} + 2\sigma^2_{\text{RCr}}$
Rows.Columns.Subcols	9	
Summer	1	$\sigma^2_{\text{RCrc}} + 2\sigma^2_{\text{RCc}} + f_{\text{U}}(\psi)$
Summer.Periods	2	$\sigma^2_{\text{RCrc}} + 2\sigma^2_{\text{RCc}} + f_{\text{UP}}(\psi)$
Residual	6	$\sigma^2_{\text{RCrc}} + 2\sigma^2_{\text{RCc}}$
Rows.Columns.Subrows.Subcols	9	
Spring.Summer	1	$\sigma^2_{\text{RCrc}} + f_{\text{SU}}(\psi)$
Spring.Summer.Periods	2	$\sigma^2_{\text{RCrc}} + f_{\text{SUP}}(\psi)$
Residual	6	σ^2_{RCrc}

This design has problems in that the Residuals have low degrees of freedom. An alternative design would be to assign the four Spring-Summer combinations completely at random to the four subplots in each plot; this would have resulted in more degrees of freedom for the subplot residual. However, this would halve the size of the unit to which the Spring and Summer treatments were applied. It may be that practical restrictions on the size of plots prevented the use of the smaller units and forced the adoption of the design presented here.

The data, in field order, is given in the table below.

Results for the grazing experiment

			SubRows			
			1		2	
Row	Column	SubColumn	1	2	1	2
	1		12.5	26.2	33.4	44.2
1	2		59.2	47.6	49.9	15.8
	3		55.0	35.9	27.3	18.3
	1		56.2	52.3	27.5	25.1
2	2		67.7	62.2	24.1	27.5
	3		28.0	29.4	19.5	29.9
	1		57.2	69.5	16.9	19.5
3	2		30.3	26.6	11.0	17.6
	3		61.9	46.5	26.2	15.4

This data has been analyzed in Genstat with linear trends being fitted to the Period means. The output is given below.

```
Genstat 5  Release 4.1  (PC/Windows NT)              23 April 2000 12:58:58
Copyright 1998, Lawes Agricultural Trust (Rothamsted Experimental Station)

                 ______________________________________________

                 Genstat 5 Fourth Edition - (for Windows)
                 Genstat 5 Procedure Library Release PL11
                 ______________________________________________

   3  "Data taken from File: D:/ANALYSES/LM/MULTIFAC/SPLGRASS.GSH"
   4  DELETE [redefine=yes] Rows,Columns,SubRows,SubColum,Period,Spring,Summer\
   5  ,%MainGra
   6  FACTOR [modify=yes;nvalues=36;levels=3] Rows
   7  READ Rows; frepresentation=ordinal

    Identifier    Values   Missing    Levels
          Rows        36         0         3

   9  FACTOR [modify=yes;nvalues=36;levels=3] Columns
  10  READ Columns; frepresentation=ordinal

    Identifier    Values   Missing    Levels
       Columns        36         0         3

  12  FACTOR [modify=yes;nvalues=36;levels=2] SubRows
  13  READ SubRows; frepresentation=ordinal

    Identifier    Values   Missing    Levels
       SubRows        36         0         2

  15  FACTOR [modify=yes;nvalues=36;levels=2] SubColum
  16  READ SubColum; frepresentation=ordinal

    Identifier    Values   Missing    Levels
      SubColum        36         0         2
```

```
18  FACTOR [modify=yes;nvalues=36;levels=!(3,9,18)] Period
19  READ Period; frepresentation=ordinal

  Identifier    Values   Missing    Levels
      Period        36         0         3

21  FACTOR [modify=yes;nvalues=36;levels=!(2,4)] Spring
22  READ Spring; frepresentation=ordinal

  Identifier    Values   Missing    Levels
      Spring        36         0         2

24  FACTOR [modify=yes;nvalues=36;levels=!(2,4)] Summer
25  READ Summer; frepresentation=ordinal

  Identifier    Values   Missing    Levels
      Summer        36         0         2

27  VARIATE [nvalues=36] %MainGra
28  READ %MainGra

  Identifier   Minimum      Mean   Maximum    Values   Missing
    %MainGra     11.00     35.37     69.50        36         0

32
33  PRINT Rows,Columns,SubRows,SubColumns,Period,Spring,Summer,%MainGrass; \
34              FIELD=9; DEC=7(0),1

     Rows  Columns  SubRows SubColum   Period   Spring   Summer %MainGra
        1        1        1        1       18        4        2     12.5
        1        1        1        2       18        4        4     26.2
        1        1        2        1       18        2        2     33.4
        1        1        2        2       18        2        4     44.2
        1        2        1        1        9        2        4     59.2
        1        2        1        2        9        2        2     47.6
        1        2        2        1        9        4        4     49.9
        1        2        2        2        9        4        2     15.8
        1        3        1        1        3        2        4     55.0
        1        3        1        2        3        2        2     35.9
        1        3        2        1        3        4        4     27.3
        1        3        2        2        3        4        2     18.3
        2        1        1        1        9        2        4     56.2
        2        1        1        2        9        2        2     52.3
        2        1        2        1        9        4        4     27.5
        2        1        2        2        9        4        2     25.1
        2        2        1        1        3        2        2     67.7
        2        2        1        2        3        2        4     62.2
        2        2        2        1        3        4        2     24.1
        2        2        2        2        3        4        4     27.5
        2        3        1        1       18        2        2     28.0
        2        3        1        2       18        2        4     29.4
        2        3        2        1       18        4        2     19.5
        2        3        2        2       18        4        4     29.9
        3        1        1        1        3        2        2     57.2
        3        1        1        2        3        2        4     69.5
        3        1        2        1        3        4        2     16.9
        3        1        2        2        3        4        4     19.5
        3        2        1        1       18        2        2     30.3
        3        2        1        2       18        2        4     26.6
        3        2        2        1       18        4        2     11.0
        3        2        2        2       18        4        4     17.6
        3        3        1        1        9        2        4     61.9
        3        3        1        2        9        2        2     46.5
        3        3        2        1        9        4        4     26.2
        3        3        2        2        9        4        2     15.4

35  BLOCK (Rows*Columns)/(SubRows*SubColumns)
36  TREAT POL(Period;1)*Spring*Summer
37  ANOVA [FPROB=Y; PSE=LSD] %MainGrass
```

```
37..........................................................................

***** Analysis of variance *****

Variate: %MainGra

Source of variation      d.f.        s.s.        m.s.     v.r.  F pr.

Rows stratum                2      107.62       53.81     0.50

Columns stratum             2      121.20       60.60     0.56

Rows.Columns stratum
Period                      2     1677.43      838.72     7.81  0.114
  Lin                       1     1397.15     1397.15    13.01  0.069
  Deviations                1      280.28      280.28     2.61  0.248
Residual                    2      214.77      107.39

Rows.Columns.SubRows stratum
Spring                      1     5697.73     5697.73    71.52  <.001
Period.Spring               2      822.16      411.08     5.16  0.050
  Lin.Spring                1      820.57      820.57    10.30  0.018
  Deviations                1        1.59        1.59     0.02  0.892
Residual                    6      477.97       79.66     2.70

Rows.Columns.SubColum stratum
Summer                      1      696.08      696.08    11.36  0.015
Period.Summer               2       80.98       40.49     0.66  0.550
  Lin.Summer                1        1.89        1.89     0.03  0.866
  Deviations                1       79.08       79.08     1.29  0.299
Residual                    6      367.58       61.26     2.08

Rows.Columns.SubRows.SubColum stratum
Spring.Summer               1       21.31       21.31     0.72  0.428
Period.Spring.Summer        2       52.07       26.04     0.88  0.461
  Lin.Spring.Summer         1       41.23       41.23     1.40  0.282
  Deviations                1       10.84       10.84     0.37  0.566
Residual                    6      176.73       29.46

Total                      35    10513.64


***** Tables of means *****

Variate: %MainGra

Grand mean  35.37

   Period      3.00     9.00    18.00
              40.09    40.30    25.72

   Spring      2.00     4.00
              47.95    22.79

   Summer      2.00     4.00
              30.97    39.77

   Period   Spring      2.00     4.00
     3.00              57.92    22.27
     9.00              53.95    26.65
    18.00              31.98    19.45

   Period   Summer      2.00     4.00
     3.00              36.68    43.50
     9.00              33.78    46.82
    18.00              22.45    28.98

   Spring   Summer      2.00     4.00
     2.00              44.32    51.58
     4.00              17.62    27.96
```

```
            Spring     2.00              4.00
   Period   Summer     2.00     4.00     2.00     4.00
     3.00             53.60    62.23    19.77    24.77
     9.00             48.80    59.10    18.77    34.53
    18.00             30.57    33.40    14.33    24.57


*** Least significant differences of means (5% level) ***

Table                Period      Spring      Summer      Period
                                                         Spring
rep.                     12          18          18           6
l.s.d.               18.203       7.280       6.384      14.246
d.f.                      2           6           6        5.13
Except when comparing means with the same level(s) of
 Period                                                  12.609
 d.f.                                                         6

Table                Period      Spring      Period
                     Summer      Summer      Spring
                                             Summer
rep.                      6           9           3
l.s.d.               14.151       8.638      15.128
d.f.                   4.45       11.80       10.16
Except when comparing means with the same level(s) of
 Period              11.057                  14.961
 d.f.                     6                   11.80
 Spring                           7.013
 d.f.                             10.69
 Summer                           7.769
 d.f.                              9.90
 Period.Spring                               12.147
 d.f.                                         10.69
 Period.Summer                               13.456
 d.f.                                          9.90


  38  APLOT METHOD=fit,normal


             -
             I
             I
             I
         5.0 I
r            I                                 *  *
e            I              *                                        *
s            I   *                                             *
i            I     * *     *                             **             *
d            I *     *            *            *
u        0.0 I          *       * 2
a            I  *             2       *
l            I           *       2        *               *    *
s            I                   *                   *
             I                 *                                     *
             I          *                                          *
        -5.0 I
             -+---------+---------+---------+---------+---------+---------+---
           10.0      20.0      30.0      40.0      50.0      60.0      70.0

                                         fitted values
```

```
                                          Normal plot
             -
             I
         5.0 I
r            I                                                    *    *
e            I                                               * *
s            I                                            **
i            I                                     ******
d            I                                 ****
u        0.0 I                              *2*
a            I                          ****
l            I                    ******
s            I                 **
             I             * *
             I    *    *
        -5.0 I
             -+---------+---------+---------+---------+---------+---------+---
           -2.4      -1.6      -0.8       0.0       0.8       1.6       2.4

                                   expected Normal quantiles
```

```
  39  "
 -40  **** Tukey''s one-degree-of-freedom-for-non-additivity.
 -41  **** It is the term designated covariate in the following analysis
 -42  "
  43  AKEEP [FIT=Fit]
  44  CALC ResSq=Fit*Fit
  45  ANOVA [PRINT=*]  ResSq; RES=ResSq
  46  COVAR ResSq                            "A computational trick"
  47  ANOVA [PRINT=A; FPROB=Y] %MainGrass

47................................................................................
```

***** Analysis of variance (adjusted for covariate) *****

Variate: %MainGra
Covariate: ResSq

Source of variation	d.f.	s.s.	m.s.	v.r.	cov.ef.	F pr.
Rows stratum	2	107.62	53.81	0.50		
Columns stratum	2	121.20	60.60	0.56		
Rows.Columns stratum						
Period	2	1677.43	838.72	7.81	1.00	0.114
Lin	1	1397.15	1397.15	13.01	1.00	0.069
Deviations	1	280.28	280.28	2.61	1.00	0.248
Residual	2	214.77	107.39		1.00	
Rows.Columns.SubRows stratum						
Spring	1	5697.73	5697.73	71.52	1.00	<.001
Period.Spring	2	822.16	411.08	5.16	1.00	0.050
Lin.Spring	1	820.57	820.57	10.30	1.00	0.018
Deviations	1	1.59	1.59	0.02	1.00	0.892
Residual	6	477.97	79.66	2.36	1.00	
Rows.Columns.SubColum stratum						
Summer	1	696.08	696.08	11.36	1.00	0.015
Period.Summer	2	80.98	40.49	0.66	1.00	0.550
Lin.Summer	1	1.89	1.89	0.03	1.00	0.866
Deviations	1	79.08	79.08	1.29	1.00	0.299
Residual	6	367.58	61.26	1.82	1.00	
Rows.Columns.SubRows.SubColum stratum						
Spring.Summer	1	21.31	21.31	0.63	1.00	0.463
Period.Spring.Summer	2	52.07	26.04	0.77	1.00	0.510
Lin.Spring.Summer	1	41.23	41.23	1.22	1.00	0.319
Deviations	1	10.84	10.84	0.32	1.00	0.595
Covariate	1	8.06	8.06	0.24		0.646
Residual	5	168.67	33.73		0.87	
Total	35	10513.64				

```
COVAR
AGRAPH [METHOD=lines] XFACTOR=Period; GROUP=Spring
VARI Periods
CALC Periods=Period
MODEL %MainGrass
TERMS Spring/Periods
FIT Spring/Periods


54.................................................................................


***** Regression Analysis *****

 Response variate: %MainGra
     Fitted terms: Constant + Spring + Periods.Spring


*** Summary of analysis ***

              d.f.         s.s.         m.s.      v.r.
Regression       3        7915.      2638.49     32.50
Residual        32        2598.        81.19
Total           35       10514.       300.39

Percentage variance accounted for 73.0
Standard error of observations is estimated to be 9.01
* MESSAGE: The following units have large standardized residuals:
         Unit     Response    Residual
            7        49.90        3.07
           10        35.90       -2.92


*** Estimates of parameters ***

                             estimate         s.e.     t(32)
Constant                        65.80         4.05     16.26
Spring 4                       -40.65         5.72     -7.10
Periods.Spring 2               -1.785        0.345     -5.18
Periods.Spring 4               -0.236        0.345     -0.69
```

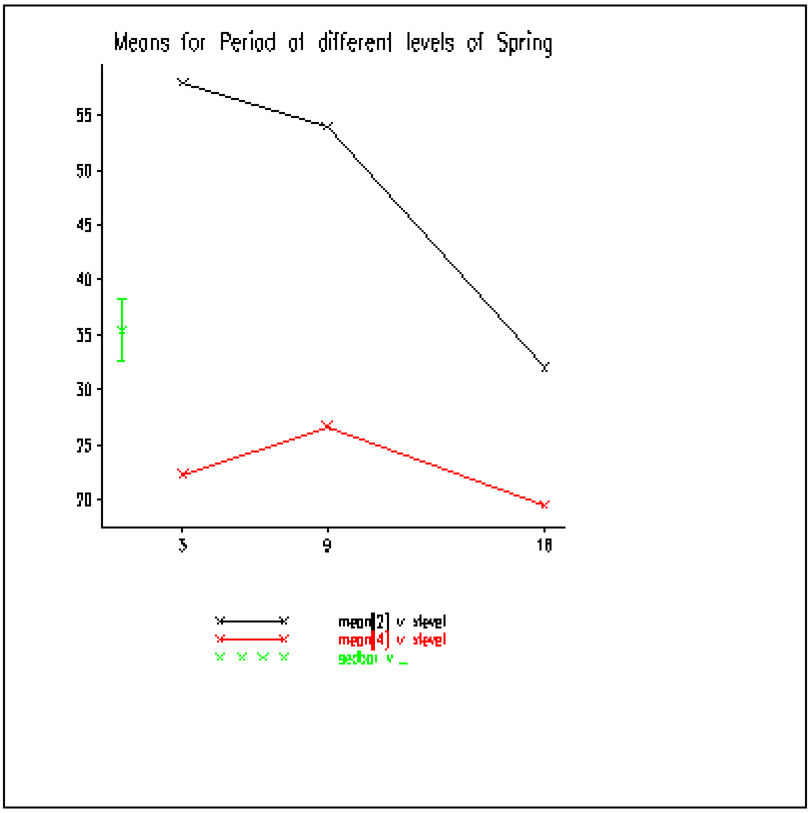
The diagnostic checking indicates that the assumptions have been met because a) the residual-versus-fitted-values plot has fairly even distribution of points as one goes from left to right, b) the normal probability plot displays a roughly straight line pattern and c) Tukey's test for nonadditivity is not significant.

From the first analysis of variance table in the output we determine the fitted model that best describes the data. Firstly, Deviations for Spring.Summer.Periods is non-significant as is the Lin source for this three-factor interaction so that there is no three-factor interaction. Of the two-factor interactions only the Lin term for Spring.Periods is significant. Given that this term involving both Spring and Periods is significant, only the main effect for Summer has to be examined. The F value for Summer is significant. Hence, the fitted expectation model is:

$$E[Y] = \text{Summer} + \text{Spring.Periods}_{\text{Lin}}$$

The interpretation of this model is that there is a linear trend in the % of the principal grass over the Periods that differs between the two numbers of spring grazing cycles and that there is an overall difference between the two numbers of summer grazing cycles that is independent of the other two treatment factors, Periods and Spring.

The trends in the period means for the two numbers of spring cycles are illustrated in the following diagram:



The equations for the fitted straight lines describing the trend in the period means for the different numbers of spring grazing cycles can only be obtained using regression. This has been done at the end of the output and the fitted equations are,

For 2 spring cycles, $\%MainGrass = 65.80 - 1.785\,Period$

For 4 spring cycles, $\%MainGrass = 25.15 - 0.236\,Period$

That is, the percentage of the main grass decreases as the number of periods of grazing increases, but the decrease is greater for two spring cycles than for 4 spring cycles.

The Summer means are given in the table below.

Summer table of means

Summer	
2	4
30.97	39.77

Clearly, 4 cycles in summer, irrespective of the number of Spring cycles and the length of grazing, result in a larger percentage of the main grass surviving.