Topic 12. The Split-plot design and its relatives [ST&D Ch 16]

12. 1. Definition

The split-plot design results from a specialized randomization scheme for a factorial experiment.

The basic split-plot design involves assigning the levels of one factor to **main plots** arranged in a CRD, RCBD, or a Latin-Square and then assigning the levels of a second factor to **subplots** within each main plot.

Note that randomization is a **two-stage** process. First, levels of factor A are randomized over the main plots and then levels of factor B are randomized over the subplots within each main plot. Each main plot may be considered as a block as far as factor B is concerned but only as an **incomplete block** as far as the full set of treatments is concerned because not every subplot has the same chance of receiving every treatment combination.

This restriction in randomization results in the presence of **two distinct error terms**, one appropriate for the main plots (i.e. for testing the effect of factor A) and one appropriate for the subplots (i.e. for testing the effect of factor B). Ordinarily, the error term for the main plots is larger than it would be in a complete design since the main plots are larger, further apart, and encompass greater heterogeneity, while the subplot error is smaller than it would be in a complete design. Since the interactions are compared using the smaller subplot error, the precision in estimating interactions is usually increased in a split-plot design relative to a simple factorial.

A typical example of a split-plot design is an irrigation experiment where irrigation levels are applied to large areas, and factors like varieties and fertilizers are assigned to smaller areas within particular irrigation treatments. The proper analysis of a split-plot design recognizes that treatments applied to main plots are subject to larger experimental errors than those applied to subplots; hence, different mean squares are used as denominators for the corresponding *F* ratios. This concept is explored here in terms of expected mean squares.

In summary, in a split-plot design, the factor assigned to the subplots is the factor that requires smaller amounts of experimental material, is of primary importance, is expected to exhibit smaller differences, or for which greater precision is desired.

12. 2. Uses of Split-plot designs

1) Split-plot designs (and a variation, the split-block) are frequently used for factorial experiments in which the nature of the experimental material or the operations involved make it difficult to handle all factor combinations in the same manner. It may be used when the treatment levels associated with one of the factors require larger amounts of experimental material than do treatment levels for other factors.
2) These designs are also used when the investigator wishes to increase precision in estimating certain effects and is willing to sacrifice precision in estimating other effects. The design usually sacrifices precision in estimating the average effects of

the treatments assigned to main plots. It often improves the precision for comparing the average effects of treatments assigned to subplots and, when interactions exist, for detecting those interactions. This arises from the fact that the experimental error for main plots is usually larger than the experimental error used to compare subplot treatments. Usually, the error term for subplot treatments is smaller than would be obtained if treatments were randomly assigned to experimental units as *combinations* of factor levels (a one-stage randomization process).

3) The design may also be useful when an additional factor is to be incorporated into an experiment to increase its scope. For example, suppose that the primary purpose of an experiment is to compare the effects of several seed protectants. To increase the scope of the experiment across a range of varieties, several varieties could be used as main plots and the seed protectants as subplots.

12. 3. The split-plot design

Suppose factor A is the main plot factor, with 3 levels, while factor B is the subplot factor, with 2 levels. There are 4 reps per main plot. We will see how such an experiment could be arranged according to 3 different designs:

1. Factorial (no split), arranged as a CRD;
2. Split-plot, with main plots arranged as a CRD; and
3. Split-plot, with main plots arranged as an RCBD.

12. 3. 1. Factorial (no split), arranged as a CRD.

This is a simple 3x2 factorial arranged as a CRD, like you've seen before. With 6 possible treatment combinations and 4 replications, 24 experimental units (e.g. plots in a field) are required. The six treatment combinations are randomly assigned to the plots in a single randomization process. The resulting field could look like this:

a1**b1**	a2b2	a2**b1**	a1b2	a3b2	a1**b1**	a2b2	a2**b1**	a1b2	a3b2	a1**b1**	a3b2
a2b2	a3**b1**	a1b2	a3**b1**	a1b2	a3b2	a2**b1**	a1**b1**	a2b2	a3**b1**	a2**b1**	a3**b1**

12. 3. 2. Split-plot, with main plots arranged as a CRD

In this scenario, the **randomization** process is divided into **2 stages**.

Stage 1: Randomize the levels of factor A over the main plots.

a2	a3	a2	a1	a2	a3	a2	a3	a1	a3	a1	a1
a2	a3	a2	a1	a2	a3	a2	a3	a1	a3	a1	a1

Stage 2: Randomize the levels of factor B over the subplots.

a2b2	a3b2	a2**b1**	a1**b1**	a2**b1**	a3b2	a2**b1**	a3b2	a1**b1**	a3**b1**	a1**b1**	a1b2
a2**b1**	a3**b1**	a2b2	a1b2	a2b2	a3**b1**	a2b2	a3**b1**	a1b2	a3b2	a1b2	a1**b1**

12. 3. 3. Split-plot, with main plots arranged as an RCBD.

In this scenario, the **randomization** process is divided into **2 stages *per block***.

Stage 1: Randomize the levels of factor A over the main blocks.

a2	a1	a3	a1	a2	a3	a1	a3	a2	a3	a2	a1
a2	a1	a3	a1	a2	a3	a1	a3	a2	a3	a2	a1

Stage 2: Randomize the levels of B over the subplots, 2 per subplot.

a2**b1**	a1**b1**	a3b2	a1b2	a2**b1**	a3b2	a1b2	a3**b1**	a2b2	a3**b1**	a2**b1**	a1**b1**
a2b2	a1b2	a3**b1**	a1**b1**	a2b2	a3**b1**	a1**b1**	a3b2	a2**b1**	a3b2	a2b2	a1b2

In split-plot designs, the effect of Factor B (i.e. the difference between b1 and b2 values) is generally more consistent across the experiment due to their proximity to one another within each main plot. Another way of saying this is that there is usually a positive correlation between b1 and b2 values within each main plot. This results in a smaller variance among levels of Factor B than in a normal factorial experiment, thereby increasing the precision with which differences among levels of Factor B are detected.

12. 4. Linear model for the split-plot

The linear model for the split-plot, with main plots arranged as a CRD is:

$$Y_{ijk} = \mu + \alpha_i + (\alpha\gamma)_{ik} + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}$$

where

i = 1, ..., a indexes the main plot levels, and
j = 1, ... , b indexes the subplot levels.
k = 1, ..., r indexes the reps,

The variance associated with $(\alpha\gamma)_{ik}$ (i.e. $\sigma^2_{\alpha\gamma}$) is the correct error term for testing the main plot effects. The variance associated with ε_{ijk} (i.e. σ^2_{ε}) is the correct error term for testing the subplot effects. Of the two, $\sigma^2_{\alpha\gamma}$ is usually larger.

The linear model for the split-plot, with main lots arranged as an RCBD is:

$$Y_{ijk} = \mu + \alpha_i + \gamma_k + (\alpha\gamma)_{ik} + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}$$

In this case,

$k = 1,...,r$ indexes the blocks

and the extra term γ_k represents the effect of the k^{th} block.

12. 5. Split-plot ANOVA

The total degrees of freedom in a sploit-plote experiment are one less than the total number of subplots. In other words, $df_{total} = rab - 1$, where

r= number of replications (is a CRD) or number of blocks (in an RCBD),

a number of main plots and,.

b= number of subplots per main plot.

The main plot (factor A) SS has $df_{MP} = a - 1$ and the subplot (factor B) SS has $df_{SP} = b - 1$.

The main plot error

The appropriate mean square error to test effects of the main plot factor is often called "error A" or MS(MPE) (i.e. mean square of the main plot error). This error is computationally equivalent to the *Main plot x Replication* interaction term in a CRD and to the *Main plot x Block* interaction in a RCBD. This error term is the appropriate error term for testing differences among levels of the main plot factor.

CRD Main plot error = *Main plot x Replication*
RCBD Main plot error = *Main plot x Block*

Why is this the correct error term? From the perspective of the main plot (i.e. Factor A), the subplots are simply subsamples; so it is reasonable to average them when testing the main plot effects.

Consider first the case of a RCBD with one observation per block-treatment combination. If the values of the subplots within each main plot are averaged, the resulting design is a simple RCBD. Remember that in this case, the appropriate error term is the *Block * Treatment* interaction. Therefore, it makes sense to use this error term in the split-plot to compare the main plot effects.

Now consider the case of the CRD. If the values of the subplots within each main plot are averaged, the resulting design is a simple CRD. We've never pointed it out, but what exactly IS the error term of a simple CRD? It's the Treatment x Replication interaction. So, like all CRD's, the appropriate error term is the Treatment x Replication interaction; but in order to use it, we must explicitly extract it from the error term and put it in the model.

The subplot error

The appropriate mean square error to test effects of the subplot factor is often called "error B" or MS(SPE) (i.e. mean square of the subplot error). This error is computationally equivalent to the [

Subplot x Replication + Main plot x Subplot x Replication] in a CRD and to

[*Subplot x Block + Main plot x Subplot x Block*] in a RCBD.

In either model, this is the *residual error*; in other words, it is the variation that is left after all other factors have been accounted for. This error term is the appropriate error term for testing significance of the subplot effect and the subplot x main plot interaction effect.

CRD Subplot error = *Subplot x Replication + Main plot x Subplot x Replication*

RCBD Subplot error = *Subplot x Block + Main plot x Subplot x Block*

The general ANOVA table for the split-plot CRD:

Source	df	SS	MS	F
Main plots total	ra - 1	SS(MP)		
Factor A	a - 1	SSA	MSA	MSA/MS(MPE)
Main plot error	a(r - 1)	SS(MPE)	MS(MPE)	
Factor B	b - 1	SSB	MSB	MSB/MS(SPE)
A x B	(a - 1)(b - 1)	SS(AxB)	MS(AxB)	MS(AxB)/MS(SPE)
Subplot error	a(r - 1)(b - 1)	SS(SPE)	MS(SPE)	
Total (subplots)	rab - 1	TSS		

The general ANOVA tables for the split-plot RCBD and the split-plot LS are similar to the CRD case and are given in Table 16.1 of ST&D (page 402). These different designs have no effect on the last four rows of the previous table. But the upper lines, corresponding to the main plot effects, do change:

CRD		RCBD		Latin Square	
A Error A	a-1 a(r-1)	Blocks A Error A	r-1 a-1 (r-1)(a-1)	Rows Columns A Error A	a-1 a-1 a-1 (a-1)(a-2)
Total	ra-1	Total	ra-1	Total	ra-1
Factor B **A x B** **Error B** **Total**	b-1 (a-1)(b-1) a(r-1)(b-1) rab-1	**Factor B** **A x B** **Error B** **Total**	b-1 (a-1)(b-1) a(r-1)(b-1) rab-1	**Factor B** **A x B** **Error B** **Total**	b-1 (a-1)(b-1) a(r-1)(b-1) rab-1

Example of RCBD Error B df calculation:

Error B (B*Block+A*B*Block)

df = (b-1)*(r-1) + (b-1)*(r-1)*(a-1)= (b-1)*(r-1)*[1+(a-1)]= a*(b-1)*(r-1)

And the corresponding SAS codes for these models:

CRD

```
Proc GLM;
   Class Rep A B;
   Model Y = A Rep*A B A*B;
   Test h = A e = Rep*A;
```

RCBD

```
Proc GLM;
   Class Block A B;
   Model Y = Block A Block*A B A*B;
   Test h = A e = Block*A;
```

LS

```
Proc GLM;
   Class Row Col A B;
   Model Y = Row Col A Row*Col*A B A*B;
   Test h = A e = Row*Col*A;
```

Replicated LS (shared rows and columns)

```
Proc GLM;
   Class Square Row Col A B;
   Model Y = Square Row Col A Square*Row*Col*A B A*B;
   Test h = A e = Square*Row*Col*A;
```

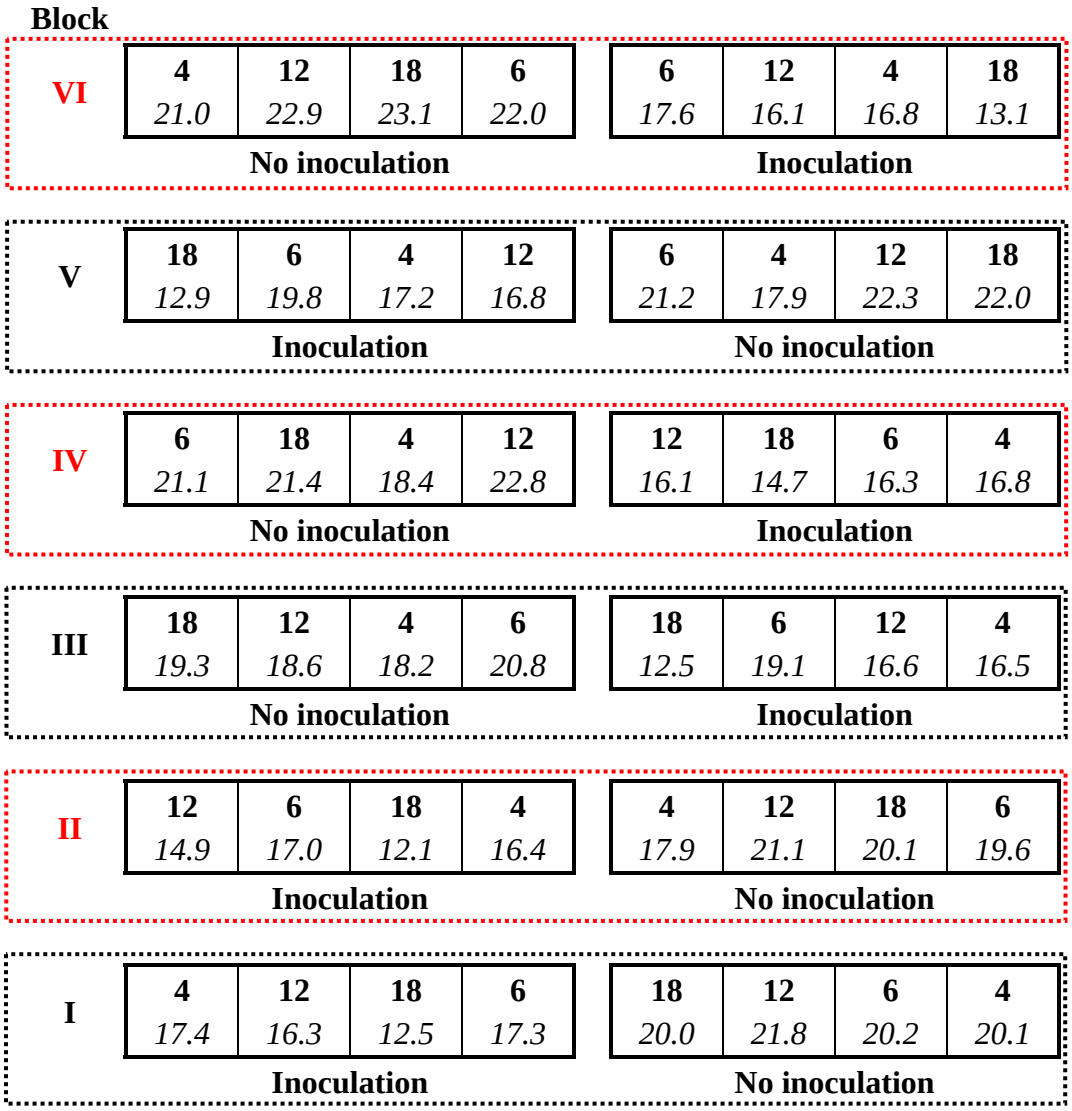
12. 6. Example of a split-plot with main plots arranged as an RCBD

To illustrate this design, we will consider an experiment from Thomson *et al.* (Phytopathology **71**: 605-608) carried out to determine the effect of bacterial vascular necrosis on the root yield of sugar beets planted at different in-row spacings. The two factors in the experiment were inoculation (inoculated versus not inoculated with *Erwinia carotovora*) and in-row spacing between plants (4, 6, 12, and 18 inches). The layout of this field experiment is shown on the next page.

Note that in this experiment, the bacterial inoculation levels were applied to large plots (main plot or whole plot) and the spacing levels were assigned to small plots (subplots) within the main plots. There were two reasons for assigning inoculation levels to main plots: 1) To confine the inoculum as well as possible to its assigned plots (i.e. to avoid contaminating non-inoculated plants); and 2) To allocate precision in the experiment to where it is needed most (i.e. while large differences in yield are expected between healthy and diseased plants, relatively smaller differences in yield are expected due to in-row spacing effects).

The two inoculation levels were randomly assigned to the main plots within each of the six blocks. As far as the main plot treatments are concerned, then, this is a simple RCBD. The subplot treatment levels (spacings) were then randomly assigned within each main plot. A separate randomization of subplot levels occurred within *each* main plot.

Figure: Split-plot field layout of the sugar beet root rot study. Each block contains 2 main plots, to which the inoculation treatment levels were assigned (Inoculation, No Inoculation). Each main plot is split into 4 subplots, to which the in-row spacing levels were assigned (4, 6, 12, and 18 inches). The yields of the subplots are shown in italics.



12. 6. 1. The SAS code for this experiment

```
Data SplitBeets;
   Input A_Inoc Block B_Space Yield @@;
Cards;
1 1 4 17.4 1 1 6 17.3 1 1 12 16.3 1 1 18 12.5 0 1 4 20.1 0 1 6 20.2 0 1 12 21.8 0 1 18 20.0
1 2 4 16.4 1 2 6 17.0 1 2 12 14.9 1 2 18 12.1 0 2 4 17.9 0 2 6 19.6 0 2 12 21.1 0 2 18 20.1
1 3 4 16.5 1 3 6 19.1 1 3 12 16.6 1 3 18 12.5 0 3 4 18.2 0 3 6 20.8 0 3 12 18.6 0 3 18 19.3
1 4 4 16.8 1 4 6 16.3 1 4 12 16.1 1 4 18 14.7 0 4 4 18.4 0 4 6 21.1 0 4 12 22.8 0 4 18 21.4
1 5 4 17.2 1 5 6 19.8 1 5 12 16.8 1 5 18 12.9 0 5 4 17.9 0 5 6 21.2 0 5 12 22.3 0 5 18 22.0
1 6 4 16.8 1 6 6 17.6 1 6 12 16.1 1 6 18 13.1 0 6 4 21.0 0 6 6 22.0 0 6 12 22.9 0 6 18 23.1
;
Proc GLM;
```

```
   Class Block A_Inoc B_Space;
   Model Yield = Block A_Inoc Block*A_Inoc B_Space A_Inoc*B_Space;
      Test h = A_Inoc e = Block*A_Inoc;
      Test h = Block  e = Block*A_Inoc;
   Means A_Inoc;
   Means B_Space;
   Means A_Inoc*B_Space;
Run;
Quit;
```

The first **test** statement specifies that the hypothesis concerning the main plots (h = A_Inoc) must be tested using the **Block*A_Inoc** interaction as the error term (e = Block*A_Inoc). In other words, this statement constructs a custom F test whose numerator is the A_Inoc mean square and whose denominator is the Block*A_Inoc mean square. The test statement is necessary in a split-plot analysis because all the default F tests use the residual mean square (MSE) in the denominator, which is not statistically valid when testing main plot effects. It is not necessary to construct a custom F test for the subplot effects because the correct subplot error is the residual MSE.

12. 6. 2. SAS Output

```
Class    Levels    Values
BLOCK         6    1 2 3 4 5 6
A_INOC        2    0 1
B_SPACE       4    4 6 12 18
Number of observations in data set = 48
```

Dependent Variable: YIELD

Source	DF	SS	Mean Square	F Value	Pr > F
Model	17	388.5475	22.8557	29.17	0.0001
Error	30	23.5050	0.7835		
Corrected Total	47	412.0525			

Source	DF	Type III SS	Mean Square	F Value	Pr > F
BLOCK	5	16.2500	3.2500	4.15	~~0.0055~~
A_INOC	1	256.6875	256.6875	327.62	~~0.0001~~
BLOCK*A_INOC	5	11.5350	2.3070	2.94	0.0280
B_SPACE	3	39.6375	13.2125	16.86	0.0001
A_INOC*B_SPACE	3	64.4375	21.4791	27.41	0.0001

Tests of Hypotheses using Type III MS for BLOCK*A_INOC as an error term

Source	**DF**	**Type III SS**	**Mean Square**	**F Value**	**Pr > F**
A_INOC	1	256.6875	256.6875	111.26	0.0001
BLOCK	5	16.2500	3.2500	1.41	0.3580

These F values indicate significant yield differences due to *Inoculations*, as well as *Spacings*. There also is a significant *Inoculation * Spacing* interaction. Note that when the appropriate error term is used, the differences among blocks are not significant. This is quite different from the conclusion one reaches using the incorrect F value of 4.15 from the ANOVA Table (significant differences among *Blocks*).

The final ANOVA table for the sugar beet root-rot study:

Source	df	SS	MS	F
Total (subplots)	47	412.06		
Block	5	16.26	3.25	1.41 NS
Inoculation (A)	1	256.69	256.69	111.26 ***
Error A (Block * A)	5	11.54	2.31	
Spacing (B)	3	39.64	13.21	16.86 ***
Interaction (A x B)	3	64.44	21.48	27.41 ***
Error B	30	23.50	0.78	

Interpretation: Note that the mean square for **error A** (2.31) is greater than the mean square for **error B** (0.78). The coefficient of variation (CV) for the main plots is 8.3% [(√2.31/18.26) x 100] and for the subplots it is 4.8% [(√0.78/18.26) x 100]. This is usual for split plot experiments. In a factorial experiment, the interaction of the treatment factors is usually of primary importance, and this reduced **error-B** increases the chance of detecting such interactions. In this particular case, the interaction between inoculation and spacing is highly significant. This indicates that the magnitude of the difference between inoculation treatments depends on in-row spacing, and vice-versa. For example, the difference for the 4- inch spacing between inoculated and non-inoculated plots was 2.1 tons/acre, while 8-tons/acre difference was observed at the 18-inch spacing. The significant interaction effect implies that the difference between levels of one factor depends on the level of the other factor. For this fixed-effects study, such a result dictates that subsequent analysis be performed on the *simple effects* of each factor.

12. 6. 3. Appropriate error terms

To confirm that the tests above are in fact the appropriate F tests for the split-plot design, we can add the Random statement to the above code and inspect the resultant table of expected mean squares:

```
Proc GLM;
   Class Block A_Inoc B_Space;
   Model Yield = Block A_Inoc Block*A_Inoc B_Space A_Inoc*B_Space;
   Random Block Block*A_Inoc;
```

The output:

Source	Type III Expected Mean Square
BLOCK	**Var(Error) + 4 Var(BLOCK*A_INOC)** + 8 Var(BLOCK)
A_INOC	**Var(Error) + 4 Var(BLOCK*A_INOC)** + Q(A_Inoc)
BLOCK*A_INOC	**Var(Error) + 4 Var(BLOCK*A_INOC)**
B_SPACE	Var(Error) + Q(B_SPACE)
A_INOC*B_SPACE	**Var(Error) + Q(A_INOC*B_SPACE)**

These expected means squares indicate that the appropriate error term for Block and for the main plot factor A is indeed the BLOCK*A_INOC interaction. The appropriate error term for B_SPACE and A_INOC*B_SPACE is the residual VAR(ERROR) term.

12. 6. 4. Mean comparisons

Comparing group means in a split-plot design is considerably more complicated than in those designs which involve only a single error term for all factors. When performing a means separation analysis, there are **four** distinct minimum significant differences that are possible, depending on the nature of the desired comparisons. The possible comparisons:

If the **interaction** between main plot * subplot is **not significant**

12. 6. 4. 1. Comparison among main plot levels.
12. 6 .4. 2. Comparison among subplot levels.

If **interaction** between main plot * subplot is **significant**

12. 6 .4. 3. Comparison among subplot levels within a main plot level.
12. 6 .4. 4. Comparison among main plot levels within a subplot level.
12. 6 .4. 5. Comparison among subplot levels for different main plot levels.

12. 6. 4. 1. Main plot comparisons in the absence of a significant interaction

If no significant interaction is detected between main plot and the subplot effects, it is valid to compare each factor across all levels of the other factor (main effects). For didactic purposes, we will use the previous example even though a significant interaction was found.

A valid comparison among the means of the main plot levels requires the appropriate error variance. If we chose to use the LSD method of mean separation, this would be done by using the following MEANS statement after the TEST statement:

```
Means A_inoc / lsd e=block*A_inoc;
or
Contrast 'Example' A_inoc 1 -1 /e=block*A_inoc;
```

SAS Output:

```
T tests (LSD) for variable: YIELD
Alpha= 0.05  df= 5  MSE= 2.31
Critical Value of T= 2.57
Least Significant Difference= 1.1271

                T Grouping          Mean       N  A_INOC

                      A           20.575      24  0
                      B           15.950      24  1
```

Note that the MSE used (2.31) is the **block*A_inoc** mean square. In this particular case, this test is uninformative because there are only two main plots. It is included here only as an example.

12. 6. 4. 2. Subplot comparisons in the absence of a significant interaction

To compare subplots, it is not necessary to specify ERROR B because it is the residual error (the default MSE for all F tests). The SAS statement in this case is simple:

```
      Means B_Space / LSD;
```

The output:

```
 T tests (LSD) for variable: YIELD  Alpha= 0.05  df= 30  MSE= 0.784
 Critical Value of T= 2.04   Least Significant Difference= 0.738

               T Grouping              Mean      N  B_SPACE
                        A           19.3333     12   6
                        A           18.8583     12  12
                        B           17.8833     12   4
                        C           16.9750     12  18
```

Note the different MSE used.

If the main plot * subplot interaction **is** significant, we are not justified in carrying out the above analyses of main effects. Instead, we are interested in the simple effects of each factor as described below.

12. 6 .4. 3. Comparisons among subplot levels *within* a common main plot

When the A*B interaction is significant, the most usual subsequent analysis is that of subplot effects within the different levels of the main plot factor. In the previous example, there is a significant interaction between main plot and subplot effects; so it is appropriate to analyze the simple effects. To analyze the differences among the four spacing treatments *within each inoculation level*, the following SAS code can be used:

```
Proc Sort Data = SplitBeets;
   By A_Inoc;
Proc GLM;
   Class Block B_space;
   Model Yield = Block B_Space;
   Means B_Space / LSD;
      By A_Inoc;
```

The output:

The ANOVAS for Inoculation = 0 and Inoculation = 1 both showed a significant effect of in-row spacing on yield. The LSD means separations obtained in each case are:

```
------------------------------ A_INOC=0 ------------------------------
T tests (LSD) for variable: YIELD
Alpha= 0.05  df= 15  MSE= 0.846556 (MS error for ANOVA Inoc=0)
Critical Value of T= 2.13
Least Significant Difference= 1.1322

                  T Grouping           Mean       N  B_SPACE
                        A          21.5833       6  12
                        A          20.9833       6  18
                        A          20.8167       6   6
                        B          18.9167       6   4

------------------------------ A_INOC=1 ------------------------------
T tests (LSD) for variable: YIELD
Alpha= 0.05  df= 15  MSE= 0.720444 (MS error for ANOVA Inoc=1)
Critical Value of T= 2.13
Least Significant Difference= 1.0445

                  T Grouping             Mean       N  B_SPACE
                          A          17.8500       6   6
                  B       A          16.8500       6   4
                  B                  16.1333       6  12
                          C          12.9667       6  18
```

Notice that the subplot error used in the original analysis (MSE = 0.7835) is just the average of the MSE's of these two simple effects ANOVAs.

12. 6 .4. 4. Comparisons among main plot levels *within* common subplot levels

Another possible set of comparisons to make is among main plots levels within a common subplot level. The following SAS statements can be added to the previous program to test the differences between the inoculation levels within each spacing level:

```
Proc Sort Data = SplitBeets;
   By B_Space;
Proc GLM;
   Class Block A_Inoc;
   Model Yield = Block A_Inoc;
   Means A_Inoc / LSD;
      By B_Space;
```

Note that the residual error here is automatically the mean square of the Block*A interaction, which is the correct error for main plot comparisons.

12.6.4.5 Mixed Comparisons: Comparisons between subplot levels across different main plot levels

The comparison of subplot means across different main plot levels is more difficult because the comparisons are across two separate levels of the experiment (across subplots and across main plots), each of which has its own appropriate error term. In the case of such mixed comparisons, the accepted protocol is to create an error term

(MSE_{Mix}) that is a weighted average of MSE_A and MSE_B, with emphasis on MSE_B. Such comparisons require hand computations.

The appropriate weighted error is:

$$MSE_{Mix} = \frac{(b-1)*MSE_B + MSE_A}{b} = \frac{(4-1)*0.7835 + 2.307}{4} = 1.164375$$

Each of the two error terms in the original analysis (MSEA and MSEB) also each have their critical t values, based on their different degrees of freedom. The accepted protocol for such mixed comparisons is to generate an intermediate *t* value between the *t* value for the main plot ($t_{A,\ 5\ df} = 2.571$) and that for the subplot ($t_{B,\ 30\ df} = 2.042$). The formula to calculate this intermediate *t* value is (ST&D page 404):

$$t_{Mix} = \frac{(b-1)*t_B*MSE_B + t_A*MSE_A}{(b-1)MSE_B + MSE_A} = \frac{3*2.042*0.78 + 2.571*2.31}{3*0.78 + 2.31} = 2.305$$

Note that this t_{Mix} value is between t_A and t_B.

With this weighted error term and its associated weighted critical t value, the LSD minimum significant difference can be calculated:

$$LSD_{\alpha=0.05} = t_{Mix}\sqrt{\frac{2MSE_{Mix}}{r}} = 2.305*\sqrt{\frac{2(1.164375)}{6}} = 1.436$$

If the absolute value of the difference between the means being compared is larger than this critical value, H_0 is rejected (i.e. one concludes that there are significant differences between the subplot means in the different main plot levels).

For example, if we want to compare the mean of inoculated / spacing 4 = 16.85 with the mean of not inoculated / spacing 6 = 20.82:

$$|20.82 - 16.85| = 4.32$$

Since 4.32 > **1.436** $\Rightarrow$ This difference is significant

12.7. Split-split plot design

The concept of the split-plot design is easily extended to three factors. Here, more options present themselves, based on the manner in which these three factors are assigned to the hierarchy of plots:

1. **Split-plot with factorial main plot:** Combinations of levels of Factors A and B are assigned to main plots, levels of Factor C to subplots within each mainplot.

2. **Split-plot with factorial subplot:** Levels of Factor A are assigned to main plots, combinations of levels of Factors B and C are assigned to subplots.
3. **Split-split plot:** Levels of Factor A are assigned to main plots, levels of Factor B to subplots within each main-plot, and levels of Factor C to sub-subplots within each subplot.

The designs 1 and 2 can be solved in the same way as the split-plots discussed before, except now either the levels of Factor A (#1 above, the mainplot) or the levels of Factor B (#2 above, the subplot) are combinations of two factors. This additional factorial structure can be solved by contrasts among the appropriate levels within a 2-way split-plot design.

The addition of a third factor by **splitting subplots** of a split-plot design using a third level of randomization, results in a **split-split plot design** (#3 above). This technique is often quite useful for a three-factor experiment to facilitate field operations or when it is desirable to keep certain treatment combinations together. However, the additional restriction on randomization makes it necessary to compute a **third unique error term** that is used to test for main effects of the factor applied to the second split and for all interactions involving this factor. So, while the design may have certain advantages in terms of physical operations with the experimental units, the necessity of a third error term can make means separations quite complicated.

The randomization procedure follows the procedure for the split-plot design. Then, the subplots are split into sub-subplots, equal in number to the levels of the third factor, to which the levels of the third factor are randomly assigned. This operation requires an independent randomization within each subplot.

The following figure from Little & Hills illustrates the layout of a split-split plot to evaluate the effects of dates of planting (A), aphid control (B), and date of harvest (C) on the control of an aphid-borne sugar beet virus. The diagram is presented such that each block shows the results of each stage of the randomization process.

Block I			A_1			**II** **A_3**			A_2		
	III						**IV**				
A_3	A_1	A_2	A_2	A_2	A_2	A_1	A_1	A_1	**A_3**	**A_3**	**A_3**
B_1	B_1	B_2	B_1	B_1	B_1	B_2	B_2	B_2	B_2	B_2	B_2
			C_1	C_3	C_2	C_3	C_1	C_2	C_1	C_3	C_2
A_3	A_1	A_2	A_2	A_2	A_2	A_1	A_1	A_1	**A_3**	**A_3**	**A_3**
B_2	B_2	B_1	B_2	B_2	B_2	B_1	B_1	B_1	B_1	B_1	B_1
			C_3	C_2	C_1	C_1	C_3	C_2	C_3	C_1	C_2

The analysis of variance for the split-split-plot design is basically a straightforward extension of the split-plot case. The different error terms are constructed by pooling together different sources of variation.

Level one

Block

A

Block*A= **Error A**

Level two

B

A*B

Block*B + Block*A*B= **Error B**

Level three

C

A*C

B*C

A*B*C

Block*C + Block*A*C + Block*B*C + Block*A*B*C= residual = **Error C**

The following SAS code produces an ANOVA and correct tests for the split-split-plot design.

```
proc glm;
    class Block a b c;
    model response= Block a Block*a  b a*b Block*b*a c a*c b*c a*b*c;
    test h=a   e=Block*a;
    test h=b   e=Block*b*a;
    test h=a*b e=Block*b*a;
```

In the above code, the Sum of Squares for the **`Block*b*a`** `interaction` is computationally equivalent to the Sum of Squares **`Block*b + Block*a*b`**.

The reason for this is that, in SAS, a feature of **Proc GLM** is that interaction terms in the MODEL statement **contain the SS of all the associated main effects and lower-order interactions as well, unless such effects are listed separately in the model**. Examples:

A feature of PROC GLM is that MODEL statements containing interaction terms without one or more of the corresponding lower-effect terms listed in the model, produce Sum of Squares that contain the nonspecified main-effect sums of squares. For example the SAS statements:

model y= a*b → produces a SS labeled a*b that is actually SS(A)+SS(B)+SS(A*B)
model y= a a*b → produces a SS labeled a*b that is actually SS(B)+SS(A*B)

model y= Block*a*b → produces a SS labeled Block*a*b that is actually SS(Block*b) + SS(Block*a*b), because all the factors in the Block*b interaction are within Block*a*b and the Block*b interaction is not listed in the model statement.

In the split-split plot code above, the term **Block*a*b** produces a SS labeled Block*a*b that is actually [SS(Block*b) + SS(Block*a*b)] because all the factors in the Block*b interaction are within Block*a*b and the Block*b interaction is not listed separately in the model statement.

Since Block, a, b, Block*a, and a*b *are* listed separately in the model, these SS are not integrated into the SS labeled Block*A*B. A complete analysis of this split-split plot example is discussed in Little and Hills (1978).

12. 8. Split-block (or strip-plot) design

In the strip-plot or split-block design the subunit treatments are applied in strips across a complete set (replication) of main plot levels.

Here is a comparison of the layout for a 5x4 split-plot design and a 5x4 split-block design (only one replication or block is shown). Although the terms main plot and subplot are still used, from a theoretical perspective there is no longer a difference between the two (i.e. they are symmetric; there is no logical hierarchy to them).

A3	A2	A1	A5	A4
B2	B1	B2	B3	**B4**
B1	B3	B1	B2	B3
B3	B2	**B4**	**B4**	B1
B4	**B4**	B3	B1	B2
Split-plot				

A3	A2	A1	A5	A4
B2	B2	B2	B2	B2
B4	**B4**	**B4**	**B4**	**B4**
B1	B1	B1	B1	B1
B3	B3	B3	B3	B3
Split-block or Strip-plot				

Note that the subunit treatments are contiguous across the entire block or main plot, and thus each subunit treatment "splits" the block. This design is also called **strip-plot,** as both A and B treatments are in strips. The A and B treatments are independently randomized within each replication.

12. 8. 1. Reasons for arranging an experiment as a split-block

1) **Physical operations** (e.g. tractor manipulation, irrigation, harvesting) may be easier.
2) The design tends to sacrifice precision in testing the main effects but **improves precision in detecting interaction effects,** which may be the most important objective of the experiment.

12. 8. 2 Linear model for the split-block design

The linear model for the split-block with main plots arranged according to an RCBD (this is the most common design) is:

$$Y_{ijk} = \mu + \rho_k + \alpha_i + \beta_j + \gamma_{ik} + \theta_{jk} + (\alpha\beta)_{ij} + \varepsilon_{ijk},$$

where

i = 1, ..., a indexes the main plot levels,

j = 1, ..., b indexes the subplot levels, and.

k = 1, ..., r indexes the blocks,

The extra term θ_{jk} represents the interaction of blocks with subplot levels.

In the previous split-plot model this θ_{jk} term was not specified; so the variation ascribed to this term was included in the Subplot error: MS (SPE)= *Subplot x Replication* + *Main plot x Subplot x Replication* (see topic 12.5).

12. 8. 3. ANOVA for the split-block design

In the strip-plot design, the subplot error for testing the main effect of Factor B is MS(StPE) = *Subplot x Block*. This test is symmetric to the test for Factor A, where SS(MPE) = *Main plot x Block* is the denominator of the *F* test. This is a reasonable result, considering that in the split-block design the randomization procedures for both factors are symmetric. Another way to think about this error term is to consider the average of all main plots within each subplot. Averaging in this way results in an RCBD for Factor B with one replication per cell. As we have seen before, the appropriate error term in this case is the MS of *Factor B x Block*.

The general ANOVA table for the RCBD split-block design is

Source	df	SS	MS	F
Block	r - 1	SS(Block)		
Factor A	a - 1	SSA	MSA	MSA / MS(MPE)
Error A= A*Block	(a - 1)(r - 1)	SS(MPE)	MS(MPE)	
Factor B	b - 1	SSB	MSB	MSB / MS(StPE)
Error B= B*Block	(b - 1)(r - 1)	SS(StPE)	MS(StPE)	
A x B	(a - 1)(b - 1)	SS(AxB)	MS(AxB)	MS(AxB) / MS(SPE)
Error C= MS(StPE) =A*B*Block	(a-1)(r-1)(b-1)	SS(SPE)	MS(SPE)	
Total (subplots)	rab - 1	TSS		

This new error term, the mean square of the strip-plot error MS(StPE), is subtracted from the subplot error (MSE_{AB}), taking (r-1)(b-1) degrees of freedom from that error. The result of this subtraction is a smaller MSE_{AB}, which is the error term used to test the interaction AxB. This results in an **improved precision in the tests for interaction effects.**

12. 8. 4. Example of a split-block (modified from Little and Hills, Chapter 10)

The following figure gives the layout of an experiment designed to examine the effect of nitrogen fertilizer rate on sugar beet root yield at various harvest times. The main plots are four nitrogen fertilizer rates, arranged as an RCBD with two blocks. The subplot treatment levels are five dates of harvest. The subplots to be harvested at each date span continuous strips across a full set of main plot levels. The harvest date strips, orthogonal to the N fertilizer strips, are also randomized within each of the two blocks. The strip-plot design is helpful here because harvest operations are easier to conduct when the plots to be harvested on a certain date lie along one continuous pass.

The root yield in tons per acre for each subplot are given in the diagram below. Internal dashed lines emphasizing the main plot randomization (Nitrogen levels N0, 80, 160, and 320) are shown in Block I; internal dashed lines emphasizing the split-plot randomization (Harvest levels 1 – 4) are shown in Block II.

	Block I				
	H4	**H5**	**H1**	**H3**	**H2**
N80	26.4	29.3	10.1	23.1	18.2
N320	31.2	34.2	10.3	25.9	19.2
N160	28.0	31.2	10.2	22.3	16.9
N0	10.1	11.4	2.3	9.8	8.8

	Block II				
	H4	**H2**	**H3**	**H5**	**H1**
N160	34.2	18.5	22.4	30.3	10.8
N0	21.3	12.5	16.7	19.1	5.2
N80	29.5	16.9	20.4	26.6	9.5
N320	31.9	17.8	22.8	29.2	7.4

12. 8. 4. 1. SAS code for a split-block (=strip-plot) design

```
Data StripPlot;
   Input Block Harvest_B Nitrogen_A Yield @@;
Cards;
1 1   0  2.3 1 2   0  8.8 1 3   0  9.8 1 4   0 10.1 1 5   0 11.4
1 1  80 10.1 1 2  80 18.2 1 3  80 23.1 1 4  80 26.4 1 5  80 29.3
1 1 160 10.2 1 2 160 16.9 1 3 160 22.3 1 4 160 28.0 1 5 160 31.2
1 1 320 10.3 1 2 320 19.2 1 3 320 25.9 1 4 320 31.2 1 5 320 34.2

2 1   0  5.2 2 2   0 12.5 2 3   0 16.7 2 4   0 21.3 2 5   0 19.1
2 1  80  9.5 2 2  80 16.9 2 3  80 20.4 2 4  80 29.5 2 5  80 26.6
2 1 160 10.8 2 2 160 18.5 2 3 160 22.4 2 4 160 34.2 2 5 160 30.3
2 1 320  7.4 2 2 320 17.8 2 3 320 22.8 2 4 320 31.9 2 5 320 29.2
;
Proc GLM;
   Class Block Nitrogen_A Harvest_B;
   Model yield = Block
                 Nitrogen_A Nitrogen_A*Block
                 Harvest_B Harvest_B*Block Harvest_B*Nitrogen_A;
   Test h = Nitrogen_A e = Nitrogen_A*Block;
   Test h = Harvest_B e = Harvest_B*Block;
Run; Quit;
```

The output

```
BLOCK          2    1 2
N_A            4    0 80 160 320
H_B            5    1 2 3 4 5
Number of observations in data set = 40
```

Dependent Variable: YIELD

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	27	3027.2658	112.1210	88.67	0.0001
Error	12	15.1740	1.2645		
Corrected Total	39	3042.4398			

Source	DF	Type I SS	Mean Square	F Value	Pr > F
BLOCK	1	14.5203	14.5203	11.48	~~0.0054~~
N_A	3	838.2988	279.4329	220.98	~~0.0001~~
BLOCK*N_A	3	111.6847	37.2282	29.44	0.0001
H_B	4	1898.9460	474.7365	375.43	~~0.0001~~
BLOCK*H_B	4	42.7860	10.6965	8.46	0.0017
N_A*H_B	12	121.0300	10.0858	7.98	0.0005

Tests of Hypotheses using the Type III MS for BLOCK*N_A as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
N_A	3	838.29875	279.43292	7.51	**0.0660**

Tests of Hypotheses using the Type III MS for BLOCK*H_B as an error term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
H_B	4	1898.9460	474.7365	44.38	**0.0014**

Our conclusions? The interaction Nitrogen * Harvest date is highly significant. Therefore, even though no significant differences were detected among Nitrogen levels, we do not accept this result; it is necessary to examine the simple effects. Similarly, even though significant differences were found among harvest dates, we do not accept this result; it is necessary to examine the simple effects. In both cases, such examination is straightforward, requiring the use of the "by" statement, as illustrated in Topic 9.7.6. The code is simple:

```
Proc Sort;
   By Harvest_B;
Proc GLM;
   Class Nitrogen_A Block;
   Model Yield = Nitrogen_A Block;
   Means Nitrogen_A;
      By Harvest_B;

Proc Sort;
   By Nitrogen_A;
Proc GLM;
   Class Harvest_B Block;
   Model Yield = Harvest_B Block;
   Means Harvest_B;
      By Nitrogen_A;
```

For the sake of covering one more concept here, let's assume the interaction was found to be non-significant, thereby justifying an analysis of the main effects. In this scenario, notice that the *F* test for the nitrogen levels is *almost* significant. Since four levels of nitrogen were tested, the resulting SS potentially includes a linear, a quadratic, and a cubic component. It is not easy to write a contrast for these effects because the selected

levels of nitrogen are not equally spaced. However, as we showed in the lab handout for Topics 4&5, this problem can be overcome using a multiple regression approach. In this example, the following simplified program could be used to partition the Nitrogen sum of squares into its three components:

```
Proc GLM;
   Class Block;
   Model Yield = Block Nitrogen_A Nitrogen_A*Nitrogen_A
                                          Nitrogen_A*Nitrogen_A*Nitro
gen_A;
```

The output

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Block	1	14.5202500	14.5202500	0.23	0.6330
~~Nitrogen_A~~	~~1~~	~~508.2087500~~	~~508.2087500~~	~~8.12~~	~~0.0073~~
~~Nitrogen_*Nitrogen_A~~	~~1~~	~~290.1897727~~	~~290.1897727~~	~~4.64~~	~~0.0382~~
~~Nitrog*Nitrog*Nitrog~~	~~1~~	~~39.9002273~~	~~39.9002273~~	~~0.64~~	~~0.4299~~

Things look good. The addition of the linear component (508.21), the quadratic component (290.19), and the cubic component (39.90) equals the total sum of squares for Nitrogen (838.30) from the previous model. So why is everything crossed out? Because, like before, the appropriate error term for these tests is the Block*A interaction. But now we are in a quandary. The **test** option uses Type III SS, which are not appropriate for a regression analysis; so we cannot use this option to declare custom F tests. Also, Nitrogen_A is not a class variable (it is a regression variable), so we have no way of telling SAS to use the Block*A interaction as an error term. The result? It must be done by hand.

The manually-adjusted ANOVA table, featuring the appropriate F tests, shows a significant ($p < 0.05$) linear effect:

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Nitrogen_A	1	508.2087500	508.2087500	13.65	**0.0073 ***
Nitrogen_*Nitrogen_A	1	290.1897727	290.1897727	7.79	**0.0382 NS**
Nitrog*Nitrog*Nitrog	1	39.9002273	39.9002273	1.07	**0.4299 NS**
Block*Nitrogen_A	3	111.684750	37.228250		

As mentioned above, the study of the simple effects of Nitrogen at each Harvest date and the simple effects of Harvest date at each Nitrogen level would be the appropriate continuation of this study. This same partitioning of the Nitrogen SS could be conducted within that simple effects analysis.

Finally, the output from the **Random** statement confirms that the errors we have used for the main effects are correct. It also illustrates that testing for the Block effect in a strip-plot design requires a synthetic error term. The code:

```
Proc GLM;
   Class Block Nitrogen_A Harvest_B;
   Model yield = Block
                 Nitrogen_A Nitrogen_A*Block
                 Harvest_B Harvest_B*Block Harvest_B*Nitrogen_A;
   Random Block Nitrogen_A*Block Harvest_B*Block;
```

Source	Type III Expected Mean Square
Block	Var(Err) + **4 Var(Block*B)** + **5 Var(Block*A)** + 20 Var(Block)
Nitrogen_A	**Var(Err) + 5 Var(Block*A)** + Q(A,A*B)
Block*Nitrogen_A	**Var(Err) + 5 Var(Block*A)**
Harvest_B	**Var(Err) + 4 Var(Block*B)** + Q(B,A*B)
Block*Harvest_B	**Var(Err) + 4 Var(Block*B)**
Nitrogen_A*Harvest_B	Var(Err) + Q(A*B)

Block*A is the correct error for A
Block*B is the correct error for B
A synthetic error term [Block*A + Block*B – Error] is the correct error for Block

Review: Pooling of interaction components to construct Split-plot, Split-split-plots and Split Block error terms

Two way factorial

```
Class Block A B;
Model Y= Block A B A*B;
```

A
B
A*B
Block

Split Plot **Split Block**

Error Factorial

- Block*A } **Error A Main plot** } **Error A**
- Block*B } **Error B Subplot** } **Error B**
- Block*A*B } **Error Interaction**

Three way factorial

```
Class Block A B;
Model Y= Block A B A*B
```

A
B
C
A*B
A*C
B*C
A*B*C
Block

Split-split Plot

Error Factorial

- Block*A } **Error A**
- Block*B, Block*A*B } **Error B**
- Block*C, Block*C*A, Block*C*B, Block*C*A*B } **Error C**