

FACTORIALS

Advantages: efficiency & analysis of interactions

PROBLEM

SOLUTION

Random or mixed trt. New sources of variance Complex EMS and F tests	→	Predicted EMS Special F tests Satterthwaite linear combination of MS SAS: RANDOM
Missing data Contamination of means and differences between means	→	Partial Sum of Squares Least Squares adjusted means SAS: Type III SS LSMEANS
Limitations in randomization Complex EMS and F tests	→	Predicted EMS Special F tests Split plots SAS: TEST

Topic 12. The Split-Plot and its Relatives [S&T Ch 16]

12. 1. Definition

The split-plot design involves assigning the treatments of one factor to **main plots** and then assigning the second factor to **subplots** within each main plot.

- **Specialized** randomization scheme for a **factorial** experiment.
- **Two-stage randomization**: factor A is randomized over the main plots and then factor B is randomized over the subplots.
- 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.
- **Two error-terms**, one for main plots and one for subplots. Error term for the main plots > subplot error.
- Precision in estimating interactions is usually increased.
- Example: Main plots to irrigation levels and varieties to subplots
- The factors that require **smaller amount of experimental material**, that are of **major importance**, that are expected to exhibit **smaller differences**, or for which **greater precision** is desired are assigned to the **subplots**.

12. 2. Uses of Split-plot designs

- 1) 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. For example one factor requires larger amounts of experimental material in an e. u.
- 2) To **increase precision** in estimating certain effects at the expense of a decrease in the precision in estimating the others. The design usually sacrifices precision in the main plots. It often improves the precision for comparing the average effects of treatments assigned to subplots and interactions.
- 3) The design may be used when an additional factor is to be incorporated in an experiment to **increase its scope**. For example, suppose that the major purpose of an experiment is to compare the effects of several seed protectants. To increase the scope of the experiment several **varieties are used as main plots** and the **seed protectants are used as subplots**.

12. 3. Split-Plot design

A: main plot factor, 3 levels. **B: subplot** factor, 2 levels. 4 reps.

12. 3. 1. Factorial (no split) with CRD treatment combinations.

6 combinations times 4 reps = 24 plots. The six treatment combinations are applied randomly across the plots.

a1b1	a2b2	a2b1	a1b2	a3b2	a1b1	a2b2	a2b1	a1b2	a3b2	a1b1	a3b2
a2b2	a3b1	a1b2	a3b1	a1b2	a3b2	a2b1	a1b1	a2b2	a3b1	a2b1	a3b1

12. 3. 2. Split-plot with completely randomized main plots.

The randomization process is divided into 2 stages;

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

a 2	a 3	a 2	a 1	a 2	a 3	a 2	a 3	a 1	a 3	a 1	a 1
a 2	a 3	a 2	a 1	a 2	a 3	a 2	a 3	a 1	a 3	a 1	a 1

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

a2b2	a3b2	a2b1	a1b1	a2b1	a3b2	a2b1	a3b2	a1b1	a3b1	a1b1	a1b2
a2b1	a3b1	a2b2	a1b2	a2b2	a3b1	a2b2	a3b1	a1b2	a3b2	a1b2	a1b1

12. 3. 3. Split-plot with randomized complete block main plots.

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

a 2	a 1	a 3	a 1	a 2	a 3	a 1	a 3	a 2	a 3	a 2	a 1
a 2	a 1	a 3	a 1	a 2	a 3	a 1	a 3	a 2	a 3	a 2	a 1

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

a2b1	a1b1	a3b2	a1b2	a2b1	a3b2	a1b2	a3b1	a2b2	a3b1	a2b1	a1b1
a2b2	a1b2	a3b1	a1b1	a2b2	a3b1	a1b1	a3b2	a2b1	a3b2	a2b2	a1b2

12. 4. The linear model for the split-plot

The linear model for the split-plot with CRD main plots is

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

Where: $k = 1, \dots, r$ indexes the reps,

$i = 1, \dots, a$ indexes the main plot levels, and

$j = 1, \dots, b$ indexes the subplot levels.

γ_{ik} : error associated with the main plots

ϵ_{ijk} error associated with the subplots.

The variance σ^2_γ is usually larger than σ^2_ϵ .

The sum of squares equations for the CRD are

SS = **SS(MP)** + **SS(SP)**, where

SS(MP) = SSA + SS(MPE)

SS(SP) = SSB + SS(AxB) + SS(SPE)

12. 5. Split-plot ANOVA

Main plot error, MS (MPE), or error A.

In a **CRD**, Error A = *Main plots x Replication* interaction

In a **RCBD**, Error A = *Main plots x Block* interaction.

Appropriate error term in testing for differences among main plots.

If the responses of the **subplots** within a main plot are **averaged**, the resulting design is a RCBD with one observation per block-treatment combination. In this case the appropriate error term is the *Block * Treatment*.

Subplot error, MS (SPE), or error B.

In a **CRD**, Error B = *Subplot x Replication + Main plot x Subplot x Replication*

In a **RCBD**, Error B = *Subplot x Block + Main plot x Subplot x Block*

Appropriate error term to estimate the significance of the subplot effect and the subplot x main plot interaction effect.

This is the residual error. The variation left after the variation of all variables and interactions included in the model is extracted from the total variation.

The ANOVA table for the CRD split-plot is

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	rab - 1	SS		

The formulas for the split plot with main plots organized in RCBD or LS are similar. These different designs will not affect the last four rows of the previous table. The three upper lines are:

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

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

$$df = (B-1)*(r-1) + (B-1)*(A-1)*(r-1)$$

$$df = (B-1)*(r-1) * [1 + (A-1)]$$

$$df = (B-1)*(r-1) * A$$

Why is B not NESTED in A?

B is a real factor. B1 has a similar treatment in A1 A2 or A3.

12. 6. Example of a split-plot with randomized complete block main plots

Experiment (Phytopathology 71:605-608) to determine the effect of bacterial vascular necrosis on root yield of sugar beet at different in-row spacing.

Main plot: inoculated versus not inoculated. The reasons for assigning inoculation to main plots were to avoid the contamination of healthy plants in non-inoculated plots during the process of inoculation, and because large differences in yield are expected between healthy and diseased plants.

If we only consider the main plot treatments, this is a RCBD.

Subplots: in-row spacing between plants (4, 6, 12, and 18 inches).

The subplot treatments (**spacing**) are randomly assigned independently within each main plot. Each main plot was split into 4 subplots.

Block	No inoculation				Inoculation			
VI	4	12	18	6	6	12	4	18
	21.0	22.9	23.1	22.0	17.6	16.1	16.8	13.1
V	18	6	4	12	6	4	12	18
	12.9	19.8	17.2	16.8	21.2	17.9	22.3	22.0
IV	6	18	4	12	12	18	6	4
	21.1	21.4	18.4	22.8	16.1	14.7	16.3	16.8
III	18	12	4	6	18	6	12	4
	19.3	18.6	18.2	20.8	12.5	19.1	16.6	16.5
II	12	6	18	4	4	12	18	6
	14.9	17.0	12.1	16.4	17.9	21.1	20.1	19.6
I	4	12	18	6	18	12	6	4
	17.4	16.3	12.5	17.3	20.0	21.8	20.2	20.1

12. 6. 1. SAS Program

```
data split;
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
1 2 4 16.4 1 2 6 17.0 1 2 12 14.9 1 2 18 12.1
1 3 4 16.5 1 3 6 19.1 1 3 12 16.6 1 3 18 12.5
1 4 4 16.8 1 4 6 16.3 1 4 12 16.1 1 4 18 14.7
1 5 4 17.2 1 5 6 19.8 1 5 12 16.8 1 5 18 12.9
1 6 4 16.8 1 6 6 17.6 1 6 12 16.1 1 6 18 13.1
0 1 4 20.1 0 1 6 20.2 0 1 12 21.8 0 1 18 20.0
0 2 4 17.9 0 2 6 19.6 0 2 12 21.1 0 2 18 20.1
0 3 4 18.2 0 3 6 20.8 0 3 12 18.6 0 3 18 19.3
0 4 4 18.4 0 4 6 21.1 0 4 12 22.8 0 4 18 21.4
0 5 4 17.9 0 5 6 21.2 0 5 12 22.3 0 5 18 22.0
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;
run; quit;
```

- The **TEST** statement specifies that the hypothesis concerning the main plots (h= A_inoc) is tested using the **BLOCK*A_INOC** interaction as the error term (e=blocks*A_inoc).
- This statement request an *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 in the denominator, and that is not statistically valid when testing main-plot effects.
- It is not necessary to specify **ERROR B** because it is the residual.

12. 6. 2. SAS Output

Dependent Variable: YIELD

Source	DF	SS	MS	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 I SS	MS	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 MS for **BLOCK*A_INOC** as an error term

Source	DF	Type III SS	MS	F Value	Pr > F
A_INOC	1	256.6875	256.6875	111.26	0.0001

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

Source	DF	Type III SS	MS	F Value	Pr > F
BLOCK	5	16.2500	3.2500	1.41	0.3580

ANOVA table of sugar beet root rot of Figure 1.

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	

- F values indicate significant differences between *Inoculations*, between *Spacing* and a significant *Inoculation * Spacing* interaction. The magnitude of the difference between inoculation treatments depends on spacing.
- Note that when the appropriate error term is used the differences among blocks are not significant. Do not use the inappropriate F values!
- **MS Error A > MS Error B** (CV main plot= 8.3% CV subplot= 4.8%)
- The smaller **Error-B** increases the chance of detecting true interactions.

12. 6. 3. Appropriate error terms

To verify the appropriate tests the following **RANDOM** statement can be added to the previous program:

```
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 of this statement is:

Source	Type III Expected Mean Square
BLOCK	Var(E.) +4 Var(BLOCK*A_INOC) +8 Var(BLOCK)
A_INOC	Var(E.) +4 Var(BLOCK*A_INOC) +Q(A_INOC, A_INOC*B_SPACE)
BLOCK*A_INOC	Var(E.) +4 Var(BLOCK*A_INOC)
B_SPACE	Var(E.) +Q(B_SPACE, A_INOC*B_SPACE)
A_INOC*B_SPACE	Var(E.) +Q(A_INOC*B_SPACE)

The expected means squares indicate that the appropriate error term for block and the **main plot** is the **BLOCK * A_INOC interaction**.

The appropriate error term for **B_SPACE** and **A_INOC * B_SPACE** is the residual **VAR(E.)**

Significant interactions

When A*B **interactions are significant** we will test the **simple effects**

- 1) Between subplot treatments within each main plot treatment
- 2) Between main plots within each subplot treatment
- 3) Between subplot treatments for different main plot treatments.

12. 6. 4. 3. Mean comparisons among subplots within main plots

To test the simple effects of the 4 spacing within each inoculation.

```
proc sort data=split;
  by A_inoc;
proc glm;
  class block B_space;
  model yield=block B_space;
  means B_space/ lsd;
  by A_inoc;
```

----- 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

12. 6. 4. 4. Mean comparisons among main plots within each subplot

To test the main effect of inoculation within each spacing.

```
proc sort data=split;
  by B_space;
proc glm;
  class block A_inoc;
  model yield=block A_inoc;
  means A_inoc/ lsd;
  by B_space;
```

Note that the error here will automatically be **block*A** which is the correct error for main plot comparisons.

12. 6. 4. 5. Mixed comparisons

The comparison of subplot means between different main plots uses a weighted average of MSE_A and MSE_B with emphasis on MSE_B .

These comparisons require hand computations.

The appropriate square root of the MS_{A-B} is:

$$MS_{AB} = \sqrt{\frac{(b-1) * MSE_B + MSE_A}{b * r}} = \sqrt{\frac{(4-1) * 0.78 + 2.31}{4 * 6}} = 0.44$$

For the mean comparisons we need 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 average t_{AB} value is presented below (ST&D page 404).

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

Note that this t value is between t_A and t_B

Now the LSD can be calculated as

$$LSD_{\alpha=0.05} = t_{ab} * \sqrt{2} * MS_{AB} = 2.305 * 1.4142 * 0.44 = 1.434$$

If the absolute value of the difference between two means is larger than the critical value $\Rightarrow H_0$ is rejected.

Example:

If we want to compare the mean of inoculated / spacing 4= 16.85 with the mean of not inoculated/ spacing 6= 20.82

$|20.82-16.85|= 4.32$ and since $4.32 > 1.434$ (LSD) \Rightarrow This difference is significant

Mean comparisons when **no significant interaction** is detected

12. 6. 4. 1. Main plot comparisons: If no interactions are detected between main plot effect and subplot effect, it is valid to compare each factor across all levels of the other factor

A valid comparison among means of the main plots requires the appropriate error variance.

```
means A_inoc / lsd e=block*A_inoc;
```

Contrast statements can also specify error terms

```
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 is the **block*A_inoc** mean square error.

12. 6. 4. 2. Subplot comparisons: To compare subplots it is not necessary to specify ERROR B because it is the residual.

```
means B_space / lsd;
```

SAS Output:

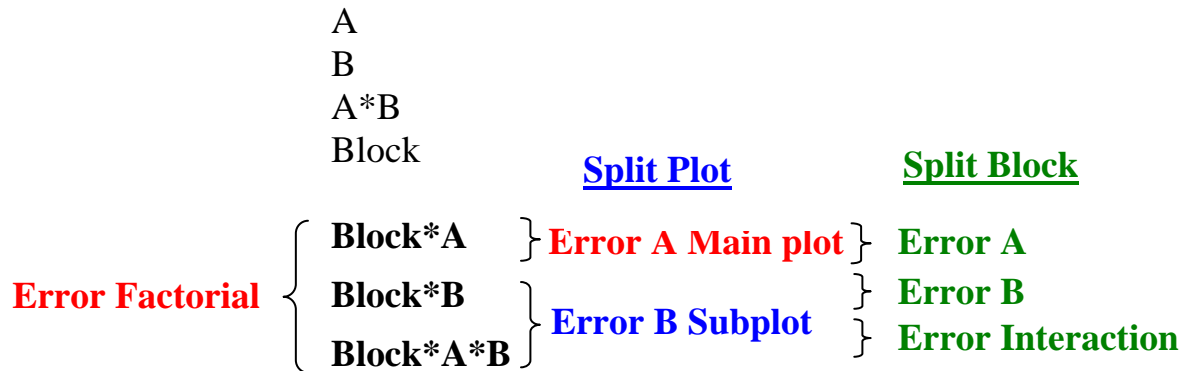
```
T tests (LSD) for variable: YIELD
Alpha= 0.05  df= 30  MSE= 0.784
Critical Value of T= 2.04
Least Significant Difference= 0.738
      T Grouping      Mean      N  B_SPACE
      -----
          A          19.3333      12    6
          A          18.8583      12   12
          B          17.8833      12    4
          C          16.9750      12   18
```

Note the different **MSE** used.

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

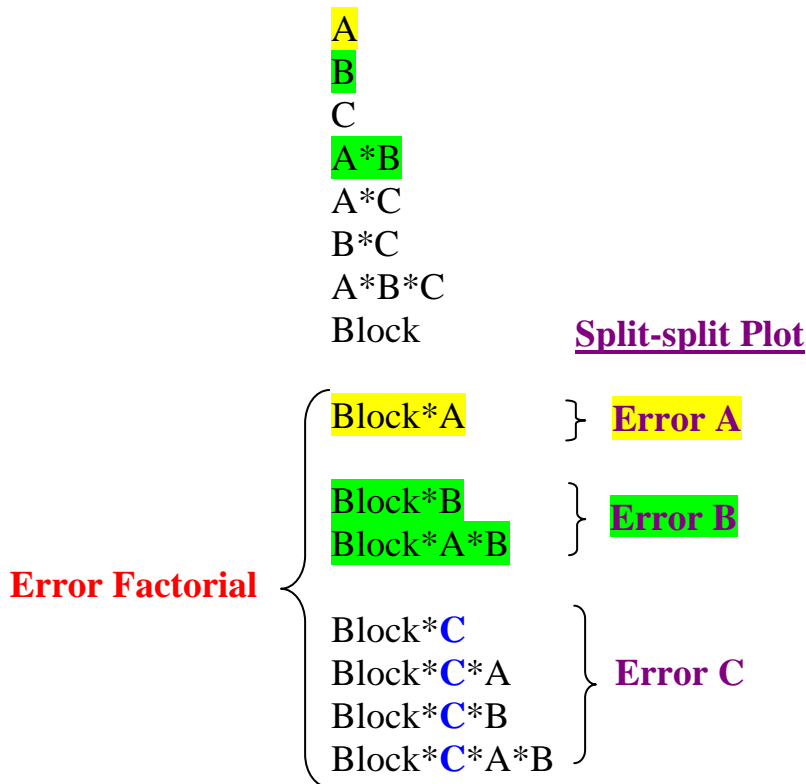
Two way factorial

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



Three way factorial

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



12.7. Split-split plot design

- In testing the interaction of **3 factors** one can arrange them in a hierarchy of subplots.
- The addition of a third factor by splitting subplots of a split-plot design results in a **split-split plot design**.
- This technique is often quite useful for a **three-factor experiment** to facilitate field operations or when it is desirable to keep treatment combinations together.
- The additional restriction on randomization makes it necessary to compute a **third 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.
- The necessity for the third error term can make mean separation quite complicated.
- The **randomization procedure** is the same as for the split-plot design, with the subplots being split into sub-subplots, equal in number to the levels of factor three, to which the third factor is randomly assigned- a new randomization for each set of sub-subplots.

Example: The following figure illustrates the partial 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 aphid-borne sugar beet viruses.

Block I			A ₁			II A ₃			A ₂		
A ₃ B ₁	III A ₁ B ₁	A ₂ B ₂	A ₂ B ₁ C ₁	A ₂ B ₁ C ₃	A ₂ B ₁ C ₂	A ₁ B ₂ C ₃	IV A ₁ B ₂ C ₁	A ₁ B ₂ C ₂	A ₃ B ₂ C ₁	A ₃ B ₂ C ₃	A ₃ B ₂ C ₂
A ₃ B ₂	A ₁ B ₂	A ₂ B ₁	A ₂ B ₂ C ₃	A ₂ B ₂ C ₂	A ₂ B ₂ C ₁	A ₁ B ₁ C ₁	A ₁ B ₁ C ₃	A ₁ B ₁ C ₂	A ₃ B ₁ C ₃	A ₃ B ₁ C ₁	A ₃ B ₁ C ₂

ANOVA of a split-split plot: extension of the split-plot. The different error terms can be constructed by pooling together different sources of variation.

Block

A

Block*A = Error A

B

A*B

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

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 statements produce an analysis of variance and correct tests.

```
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;
```

SS **Block*b*a** is computationally equivalent to SS **Block*b** + **Block*a*b**.

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 **non-specified** main-effect sums of squares.

For example the SAS statements:

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

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

In the example above:

Block*b*a → produces a SS that is **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.

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

In the **strip-plot** or **split-block** design the subunit treatments are applied in strips across an entire replication of main plot treatments.

Comparison of a 5 x 4 **split-plot** and a 5 x 4 **strip-plot** (only one replication is shown). In the **strip-plot** the terms main plot and subplot but there is no difference between the two (i.e., they are **symmetric**).

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 continuous across the entire block or main plot, and thus each subunit treatment splits the block. Another term applicable to this layout is **strip-plot**, as both A and B treatments are in strips.
- The A and B treatments are independently randomized in each replication.

12. 8. 1. Reasons for doing a split-block design

- 1) **Physical operations** (e.g. tractor manipulation, irrigation, harvesting)
- 2) The design tends to sacrifice precision in the main effects and **improve precision in the interaction effects**.

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

The linear model for the split-block with RCB main plots is

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

where $k = 1, \dots, r$ indexes the blocks, $i = 1, \dots, a$ indexes the main plot levels, and $j = 1, \dots, b$ indexes the subplot levels.

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

This θ_{jk} term was not specified in the **split-plot** model and consequently its variation was included in the Subplot error, **MS(SPE)**, estimated from the interaction [*Subplot x Replication + Main plot x Subplot x Replication*].

12. 8. 3. ANOVA for the strip-plot design

- **CRD**: the **strip-plot** error ($MS (STPE) = Subplot \times Replication$) is the denominator of the F statistic for the main effects of factor B.
- **RCBD**: Consider the **average of all main plots in each subplot**. This will result in a RCBD for factor B with one replication per cell, and the appropriate error term is the MS of *Factor B x Block*.

A2	A3	A5	A4	A1	Avg.	A3	A2	A1	A5	A4	Avg.
B4	B4	B4	B4	B4	B4	B2	B2	B2	B2	B2	B2
B2	B2	B2	B2	B2	B2	B4	B4	B4	B4	B4	B4
B3	B3	B3	B3	B3	B3	B1	B1	B1	B1	B1	B1
B1	B1	B1	B1	B1	B1	B3	B3	B3	B3	B3	B3
Block I						Block II					

- This test is **symmetric** to the test for factor A, where $SS(MPE) = Main \text{ plot} \times Replication$ is the denominator of the F test. This is a reasonable result considering that both factors have similar randomization.
- The error term, $MS (STPE)$, is subtracted from the subplot error (Error C) and it takes $(r-1)(b-1)$ degrees of freedom from this error. The result is a smaller error C, used to test the interaction $A \times B$. This results in an **improved precision in the tests for interaction effects.**

The ANOVA table for the RCBD split block design is

Source	df	SS	MS	F
Blocks	$r - 1$	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=A*B*block	$(a-1)(r-1)(b-1)$	SS(SPE)	MS(SPE)	
Total (subplots)	$rab - 1$	SS		

12. 8. 4. Example of split-block

- The following figure gives the layout of an experiment designed to examine the effect of **nitrogen fertilizer rate** on sugar beet root yield for different **harvest times**.
- The **main plots** are four **nitrogen fertilizer rates** arranged in a **RCBD** with two blocks.
- **Subunit treatments** are five **dates of harvest**.
- The subplots to be harvested at each date are in **strips** through an entire column of main plots.
- The harvest date strips are randomized for each column of main plots.
- Harvest operations are easier to conduct when the plots to be harvested on a certain date form a continuous column.
- The root yield in tons per acre for each subplot, and the means for each N main plot are given in the next figure:

Block I					Block II				
H4	H5	H1	H3	H2	H4	H2	H3	H5	H1
N 80 (plot total = 107.1)					N 160 (plot total = 116.2)				
26.4	29.3	10.1	23.1	18.2	34.2	18.5	22.4	30.3	10.8
N 320 (plot total = 120.8)					N 0 (plot total = 74.8)				
31.2	34.2	10.3	25.9	19.2	21.3	12.5	16.7	19.1	5.2
N 160 (plot total = 109.6)					N 80 (plot total = 102.9)				
28.0	31.2	10.2	22.3	16.9	29.5	16.9	20.4	26.6	9.5
N 0 (plot total = 42.4)					N 320 (plot total = 109.1)				
10.1	11.4	2.3	9.8	8.8	31.9	17.8	22.8	29.2	7.4

12. 8. 4. 1. SAS Program for a split block

```

data splitblk;
input block H_B N_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 N_A H_B;
model yield = block
            N_A N_A*block
            H_B H_B*block
            N_A*H_B;
test h=N_A e=N_A*block;
test h=H_B e=H_B*block;
run; quit;

```

Output

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 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 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

Conclusions

There are **no significant differences among Nitrogen levels** but **differences among harvest dates are highly significant.**

The interaction **Nitrogen * Harvest** date is also **highly significant.**

The study of simple effects of Nitrogen at each Harvest date or the simple effects of Harvest date at each Nitrogen level would be an appropriate continuation of this study.

Trend analysis of the Nitrogen levels

- The *F* test for the nitrogen levels is almost significant.
- Since four level of nitrogen were tested, the resulting SS includes a linear, a quadratic and a cubic response.
- It is not easy to write a contrast for these effects because the levels of nitrogen selected are not equally spaced.

This problem can be overcome using a multiple regression approach. The following program can be used to partition the Nitrogen sum of squares:

```
proc GLM;
  class block;
  model yield = block N_A N_A*N_A N_A*N_A*N_A;
```

Source	DF	Type I SS	Mean Square	F Value	Pr > F
BLOCK	1	14.52025	14.52025	0.23	0.6330
N_A	1	508.20875	508.20875	8.12	0.0073
N_A*N_A	1	290.18977	290.18977	4.64	0.0382
N_A*N_A*N_A	1	39.90023	39.90023	0.64	0.4299

The addition of the linear effect (508.21), the quadratic effect (290.19), and the cubic effect (39.90) equals the total sum of squares for Nitrogen (838.30) from the previous model.

The appropriate *F* tests show a significant ($p < 0.05$) lineal effect.

Source	DF	Type I SS	Mean Square	F Value
N_A	1	508.20875	508.20875	13.65 *
N_A*N_A	1	290.18977	290.18977	7.79 NS
N_A*N_A*N_A	1	39.90023	39.90023	1.07 NS
BLOCK*N_A	3	111.6847	37.2282	

12. 9. Repeated measures analysis

Sometimes researchers take observations repeatedly on **the same experimental units**:

- Measuring plant height **over time**
- Yields of perennial crops over seasons
- Animal growth
- Population dynamics over years etc.

These observations **are not replications** and are called **repeated measurements**.

Furthermore, because these observations are taken from the same experimental units, they are **not independent**.

Time series or multivariate methods can be used to analyze these designs. However, time series methods are more appropriate to analyze long series of data (>20 repeated measures, e.g. stock data or weather data).

The **split-plot** principle can be applied to experiments where successive observations are made on the same experimental unit over a period of time.

Examples:

- A fertilizer trial or variety trial with a perennial crop like alfalfa harvested several times.
- Repeated picking of fruit from the same trees
- Repeated sampling of soil plots over time for nutrient content.

The plots to which the treatments are assigned be called **main plots**, and the several harvests (**repeated measurements**) can be called **subplots**.

A subplot in this case, however, differs from the usual subplot in that it consists of data taken from the entire main plot rather than from a designated portion of the main plot, as is the case with the usual split-plot.

The dependency among the observations is used to adjust the degree of freedoms in the analysis of variance to give an approximate test for the repeated measurements.

12. 9. 1. ANOVA of the repeated measures analysis

Approximate ANOVA of repeated measurement analysis

Source	df	SS	MS	Conservative df
Between e.u.				
Treatment (A)	a-1	SSA	SSA/(a-1)	
Replic.* Trt (Error A)	a (n-1)	SS(MPE)	SS(MPE)/a(n-1)	
Within e. u.				
Response in time (B)	b-1	SSB	SSB/(b-1)	1
Response by Trt. (A*B)	(b-1)(a-1)	SSAB	SSAB(a-1)(b-1)	a-1
Error B	a(b-1)(n-1)	SS(SPE)	SS(SPE)/a(n-1)(b-1)	a(n-1)

- **Similar to a split-plot** analysis, except **conservative degrees of freedoms** are used in the F -tests for the repeated responses and the interactions.
- No unusual problems in analyzing data on a main plot (A) basis for a single replication or for the totals over several dates of observation.
- However, F values arising from testing the effects of successive observations (B) and the interaction of main plots treatments with successive observations (A*B) may not be distributed as F , and too many significant effects may result.

What distinguishes **repeated-measures** data from any other **multivariate** data is not so much the existence of the repeated measurements but the desire to examine changes in the measurements taken on each subject.

- **Split-plot model**: observations within the same main unit are equally correlated.
- **Repeated-measures**: observations on the same unit are not necessarily equally correlated. Measures close in time can be more highly correlated than measurements far apart in time.

Since the unequal correlation between the repeated measurements is ignored in a split-plot analysis, the tests derived in this manner may not be valid.

Conservative approach: use $> F$ values for significance of B and A*B effects.

- **Divide df** B, A*B, and Error B **by df of response in time B**.
- Select tabular F values on the basis of the resulting **df**
- The uncorrected df are appropriate for independent replications within main plots.
- The corrected df are appropriate for totally dependent replications.

Total dependence would be equivalent to have all responses represented by 1 response, and explains why the corrected df is one. The dependence in a real experiment will probably be somewhere between these two extremes.

12. 9. 2. Example of a repeated measurements experiment

Yields of 4 alfalfa cultivars tested in a CRD with 5 replications, with repeated measurements (4 cuts). Split-plot design for repeated measurements, in a CRD.

```
data rem_mes;
input rep A_var B_cut yield @@;
cards;
1 1 1 2.80191 1 1 2 3.73092 1 1 3 3.09856 1 1 4 2.50965
1 2 1 2.76212 1 2 2 5.40530 1 2 3 3.82431 1 2 4 2.72992
1 3 1 2.29151 1 3 2 3.81140 1 3 3 2.92575 1 3 4 2.39863
1 4 1 2.56631 1 4 2 4.96070 1 4 3 2.81734 1 4 4 2.05752
2 1 1 2.96602 2 1 2 4.43545 2 1 3 3.10607 2 1 4 2.57299
2 2 1 3.09636 2 2 2 3.90683 2 2 3 3.26229 2 2 4 2.58614
2 3 1 2.54027 2 3 2 3.82716 2 3 3 2.86727 2 3 4 2.16287
2 4 1 2.31630 2 4 2 3.96629 2 4 3 2.91461 2 4 4 2.15764
3 1 1 2.43232 3 1 2 4.32311 3 1 3 2.81030 3 1 4 2.07966
3 2 1 3.09917 3 2 2 4.08859 3 2 3 3.13148 3 2 4 2.60316
3 3 1 2.41199 3 3 2 4.08317 3 3 3 3.03906 3 3 4 2.07076
3 4 1 2.65834 3 4 2 3.71856 3 4 3 2.92922 3 4 4 2.15684
4 1 1 2.93509 4 1 2 3.99711 4 1 3 2.77971 4 1 4 2.44033
4 2 1 2.65256 4 2 2 5.42879 4 2 3 2.70891 4 2 4 2.30163
4 3 1 2.30420 4 3 2 3.27852 4 3 3 2.72711 4 3 4 2.04933
4 4 1 2.47877 4 4 2 3.92048 4 4 3 3.06191 4 4 4 2.35822
5 1 1 2.42277 5 1 2 3.85657 5 1 3 3.24914 5 1 4 2.34131
5 2 1 2.63666 5 2 2 3.77458 5 2 3 3.09734 5 2 4 2.30082
5 3 1 2.36941 5 3 2 3.44835 5 3 3 2.50562 5 3 4 2.08980
5 4 1 2.23595 5 4 2 4.02985 5 4 3 2.85279 5 4 4 1.85736
;
proc glm;
  class rep A_var B_cut;
  model yield= A_var rep*A_var B_cut A_var*B_cut;
  test h=A_var e=rep*A_var;
  means A_var / lsd e= rep*A_var;
  means B_cut/ lsd;
run; quit
```

Source	DF	SS	MS	F Value	Pr > F
Model	31	42.88	1.383	14.46	0.0001
Error	48	4.59	0.096		
Corrected Total	79	47.47			

Source	DF	SS	MS	F Value	Pr > F
A_VAR	3	2.84	0.95	9.90	0.0001
REP*A_VAR	16	2.05	0.13	1.34	0.2141
B_CUT	3	37.45	12.48	130.46	0.0001 F_{3,48}
A_VAR*B_CUT	9	0.55	0.06	0.64	0.7606 F_{9,48}

Tests of Hypotheses using the MS for REP*A_VAR as an error term					
Source	DF	SS	MS	F Value	Pr > F
A_VAR	3	2.84	0.95	7.40	0.0025

The **adjusted degrees of freedom are**

Source	DF	SS	MS	F Value	P
B_CUT	1	37.45	37.45	130.46	F_{1,16} **
A_VAR*B_CUT	3	0.55	0.18	0.64	F_{3,16} NS
Error	16	4.59	0.29		

- The *F* test for cuttings is significant using the most conservative df.
- NS interactions ⇒ compare means of the main effects.

T tests (LSD) for variable: YIELD

Alpha= 0.05 df= 16 **MSE= 0.13**

Critical Value of T= 2.12

Least Significant Difference= 0.2399

T Grouping	Mean	N	A_VAR
A	3.26985	20	2
B A	3.04445	20	1
C B	2.90075	20	4
C	2.76011	20	3

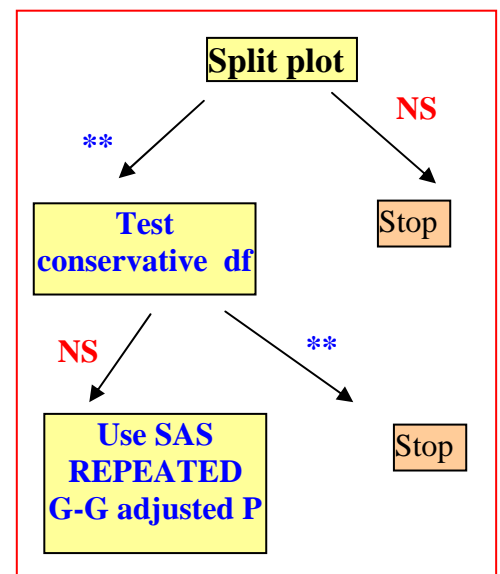
T tests (LSD) for variable: YIELD

Alpha= 0.05 df= 48 **MSE= 0.096**

Critical Value of T= 2.01

Least Significant Difference= 0.1967

T Grouping	Mean	N	B_CUT
A	4.09959	20	2
B	2.98544	20	3
C	2.59890	20	1
D	2.29123	20	4



Univariate Repeated-measures analysis using the REPEATED statement

If adjusted and unadjusted df produce different conclusions, a more precise calculation can be obtained using **SAS REPEATED** statement.

Repeated-measures is a **special case of multivariate analysis of variance (MANOVA)**. The 4 cuttings are treated as 4 response variables and the blocks and varieties are the classification variables.

The previous **CRD** data set reorganized for the **REPEATED** statement is:

```
data repeated;
input rep var cut1 cut2 cut3 cut4 @@;
cards;
1 1 2.80191 3.73092 3.09856 2.50965
1 2 2.76212 5.40530 3.82431 2.72992
1 3 2.29151 3.81140 2.92575 2.39863
1 4 2.56631 4.96070 2.81734 2.05752
2 1 2.96602 4.43545 3.10607 2.57299
2 2 3.09636 3.90683 3.26229 2.58614
2 3 2.54027 3.82716 2.86727 2.16287
2 4 2.31630 3.96629 2.91461 2.15764
3 1 2.43232 4.32311 2.81030 2.07966
3 2 3.09917 4.08859 3.13148 2.60316
3 3 2.41199 4.08317 3.03906 2.07076
3 4 2.65834 3.71856 2.92922 2.15684
4 1 2.93509 3.99711 2.77971 2.44033
4 2 2.65256 5.42879 2.70891 2.30163
4 3 2.30420 3.27852 2.72711 2.04933
4 4 2.47877 3.92048 3.06191 2.35822
5 1 2.42277 3.85657 3.24914 2.34131
5 2 2.63666 3.77458 3.09734 2.30082
5 3 2.36941 3.44835 2.50562 2.08980
5 4 2.23595 4.02985 2.85279 1.85736
;
proc anova;
  class var;
  model cut1 cut2 cut3 cut4= block var/ nouni;
  repeated time /nom;
run; quit;
```

The 4 cuttings are now to the left of the model statement as 4 dependent variables.

/nouni prevents SAS to perform one univariate analysis for each cutting.

After the statement REPEATED, *time* is the name of the within-subjects factor. This name must not have been previously defined in the data set.

The option “**/nom**” is to perform a univariate ANOVA, **not** a **MANOVA**.

SAS OUTPUT for REPEATED statement

Repeated Measures Analysis of Variance				
Dependent Variable	CUT1	CUT2	CUT3	CUT4
Level of TIME	1	2	3	4

Tests of Hypotheses for Between Subjects Effects					
Source	DF	SS	MS	F Value	Pr > F
VAR	3	2.84	0.95	7.40	0.0025
Error	16	2.05	0.13		

Note that this analysis produces the appropriate F values for Varieties and Blocks and **does not need a TEST statement**

Univariate Tests of Hypotheses for **Within** Subject Effects

- **Source: TIME** (same as previous B_cut)

Adjusted Pr>F						
DF	SS	MS	F Value	Pr > F	G - G	H - F
3	37.448	12.48	130.46	0.0001	0.0001	0.0001

- **Source: TIME*VAR** (same as previous B_cut*A_var)

Adjusted Pr>F						
DF	SS	MS	F Value	Pr > F	G - G	H - F
9	0.548	0.061	0.64	0.7606	0.6585	0.6948
Epsilon					0.50	0.64
REPEATED statement df =					9*0.50= 4.5	9*0.64= 5.8
conservative df =					9/3= 3.0	
split plot df =					9.0	

Greenhouse-Geisser df are more conservative than the Huynh-Feldt

- **Source: Error(TIME)**

DF	SS	MS
48	4.593	0.096

Note that this analysis produces the same F values the previous split plot analysis but **“correct”** probability values for TIME (previous B_cut) and TIME*VAR (previous B_cut*A_var)

G-G (Greenhouse-Geisser) and H-F (Huynh-Feldt)

- These tests present two adjusted probabilities that attempt to **correct for the unequal correlations** among pairs of repeated measures.
- The **G-G adjustment is the more conservative** one.
- They estimate “**Epsilon**” and then multiply the numerator and denominator df by Epsilon
- **This is a less drastic reduction of the df than the adjusted df that divides the d.f. of B, B*A and Error by the degrees of freedom of B.** However, is more conservative than the split plot with unadjusted d.f.grees of freedom.

Assumptions of the test

- The *F* tests will be correct if the assumptions are correct.
- Assumptions can be tested by the **PRINTE** option after “/ nom”
- The test labeled “**Applied to Orthogonal Components**” is the one that is important in determining whether the univariate *F* tests for the within-subjects effects are valid.
- It tests the assumptions that the variances and correlations are the same among the various dependent variables.

12. 9. 3. 1. Mean comparison (Cody & Smith 1991, Chapter 8))

- The MEANS statement cannot be used with the repeated factor name.
- The only way to compute pair-wise comparisons in this case is to use the keyword **CONTRAST** (n) with the REPEATED statement.

REPEATED factor_name **CONTRAST**(n)

Where (n) is a number from 1 to k, with k= n of levels of the repeated factor. **CONTRAST**(1) compares 1st level of the factor with each of the other levels.

Which to use?

- If there is a **clear trend** to the data: the split plot method with unadjusted df is not appropriate. If responses are significant even when the conservative adjusted df are used, those conclusions are valid.
- For the cases where **different conclusions are obtained with the adjusted and unadjusted**, the REPEATED statement would solve the problem of correlation, but the interpretation of the results requires a number of assumptions.
- **If many observations are missing**, the split-plot approach might be the only way of analyzing a repeated-measures design, because the REPEATED statement ignores cases where **any** of the repeated measures is missing.