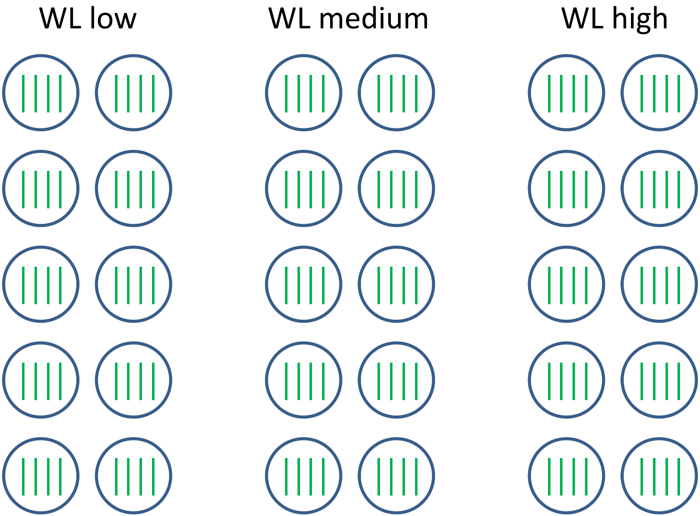
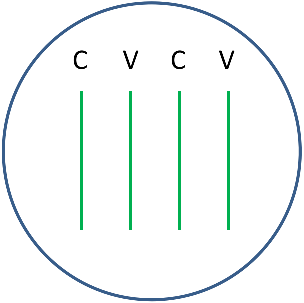
18. More Example Split-Plot Experiments

Example 1

Researchers were interested in studying how soil moisture level affects the ability of plants to respond to a virus infection. A total of 30 pots were assigned to three watering levels (1 = low, 2 = medium, 3 = high) using a balanced and completely randomized design. Each of the 30 pots contained four seedlings. Two randomly selected seedlings within each pot were injected with a virus. The remaining two seedlings in each pot were “mock infected” by injection with a harmless substance. Two weeks after treatment, each seedling was individually weighed, and these weights served as the response variable for subsequent analysis.


WL low
WL medium
WL high

WL medium



A Model

$i = 1, 2, 3$ watering levels

$j = 1, \ldots, 10$ pots within each watering level

$k = 1, 2$ infections (control, virus)

$l = 1, 2$ seedlings within watering level, pot, and infection.

$$y_{ijkl} = \mu_{ik} + p_{ij} + e_{ijkl},$$

where ...

A Model (continued)

$$p_{ij} \sim N(0, \sigma_p^2),$$

$$e_{ijkl} \sim N(0, \sigma_e^2),$$

and all random effects are independent.

ANOVA Table

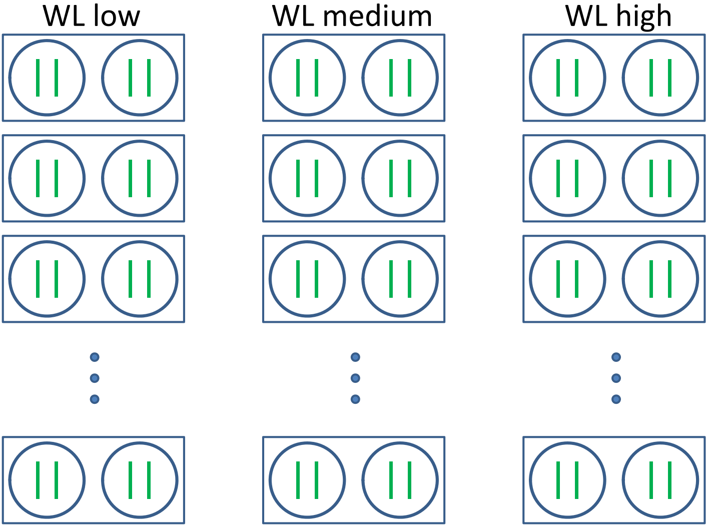
Source	DF
wl	2
pot(wl)	27
inf	1
wl$\times$inf	2
error	87
c.total	119

SAS Code

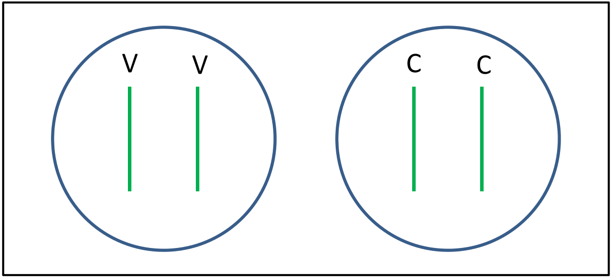
```
proc mixed;
  class wl pot inf;
  model y=wl inf wl*inf / ddfm=satterth;
  random pot(wl);
run;
```

Example 2

Researchers were interested in studying how soil moisture level affects the ability of plants to respond to a virus infection. A total of 30 trays were assigned to three watering levels (1 = low, 2 = medium, 3 = high) using a balanced and completely randomized design. Each of the 30 trays contained two pots. Each of the 60 pots contained two seedlings. The two seedlings in one randomly selected pot in each tray were injected with a virus. The two seedlings in the other pot on a given tray were "mock infected" by injection with a harmless substance. Two weeks after treatment, each seedling was individually weighed, and these weights served as the response variable for subsequent analysis.


WL low
WL medium
WL high

WL medium



A Model

$i = 1, 2, 3$ watering levels

$j = 1, \ldots, 10$ trays within each watering level

$k = 1, 2$ infections (control, virus)

$l = 1, 2$ seedlings within watering level, pot, and infection

$$y_{ijkl} = \mu_{ik} + t_{ij} + p_{ijk} + e_{ijkl},$$

where ...

A Model (continued)

$$t_{ij} \sim N(0, \sigma_t^2),$$

$$p_{ijk} \sim N(0, \sigma_p^2),$$

$$e_{ijkl} \sim N(0, \sigma_e^2),$$

and all random effects are independent.

ANOVA Table

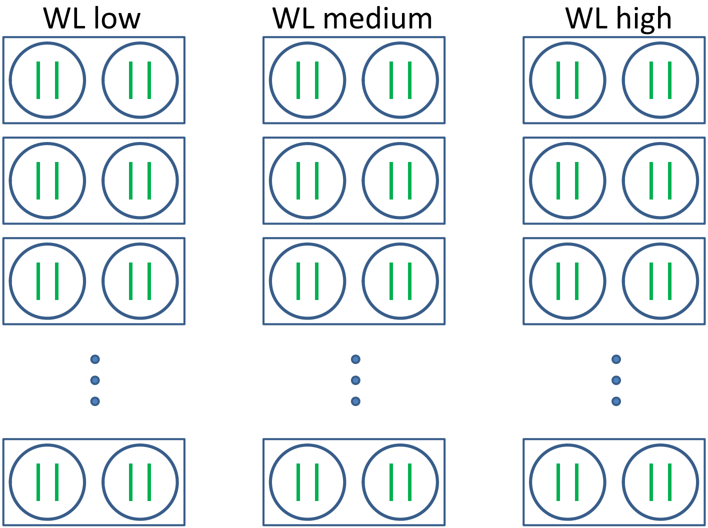
Source	DF
wl	2
tray(wl)	27
inf	1
wl$\times$inf	2
inf$\times$tray(wl)	27
error	60
c.total	119

SAS Code

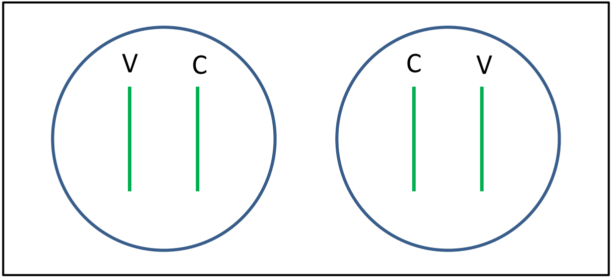
```
proc mixed;
  class wl tray inf;
  model y=wl inf wl*inf / ddfm=satterth;
  random tray(wl) inf*tray(wl);
run;
```

Example 3

Researchers were interested in studying how soil moisture level affects the ability of plants to respond to a virus infection. A total of 30 trays were assigned to three watering levels (1 = low, 2 = medium, 3 = high) using a balanced and completely randomized design. Each of the 30 trays contained two pots. Each of the 60 pots contained two seedlings. In each pot, one of the two seedlings was randomly selected and injected with a virus; the other seedling in the pot was “mock infected” by injection with a harmless substance. Two weeks after treatment, each seedling was individually weighed, and these weights served as the response variable for subsequent analysis.


WL low
WL medium
WL high

WL medium



A Model

$i = 1, 2, 3$ watering levels

$j = 1, \ldots, 10$ trays within each watering level

$k = 1, 2$ pots within watering levels and trays

$l = 1, 2$ infections (control, virus)

$$y_{ijkl} = \mu_{il} + t_{ij} + p_{ijk} + e_{ijkl},$$

where …

A Model (continued)

$$t_{ij} \sim N(0, \sigma_t^2),$$

$$p_{ijk} \sim N(0, \sigma_p^2),$$

$$e_{ijkl} \sim N(0, \sigma_e^2),$$

and all random effects are independent.

ANOVA Table

Source	DF
wl	2
tray(wl)	27
pot(wl tray)	30
inf	1
wl$\times$inf	2
error	57
c.total	119

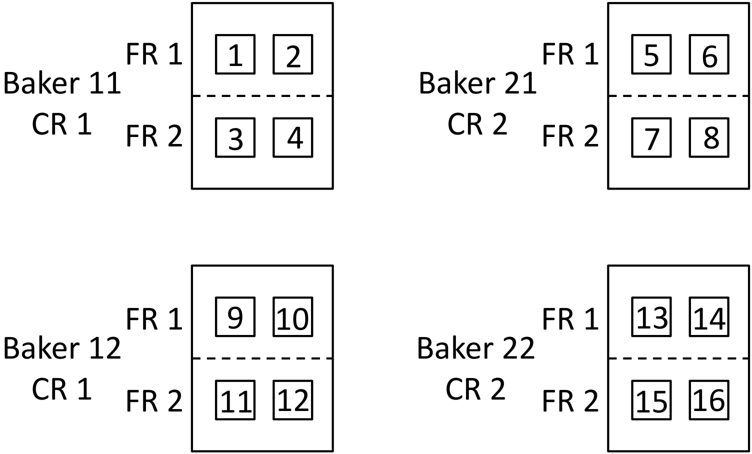
SAS Code

```
proc mixed;
  class wl tray pot inf;
  model y=wl inf wl*inf / ddfm=satterth;
  random tray(wl) pot(wl tray);
run;
```

Example 4: Researchers were interested in determining which combination of cake recipe and frosting recipe would yield the best tasting frosted cake. Two cake recipes (labeled CR1 and CR2) and two frosting recipes (labeled FR1 and FR2) were considered. The two cake recipes were randomly assigned to four bakers with two bakers for each cake recipe. Each baker prepared and baked a cake according to the recipe he or she was assigned. While cakes were baking and cooling, each baker prepared one batch of frosting using each of the frosting recipes. Half of each cake was randomly selected and covered with frosting prepared using FR1. The other half of each cake was covered with frosting prepared using FR2.

Example 4 (continued)

Two pieces of frosted cake from each half of each cake were scored for taste, with higher scores indicating better tasting frosted cake. The following diagram shows the four cakes baked by the four bakers as large rectangles. The dashed line on each rectangle shows the dividing point that separates FR1 frosting from FR2 frosting. The numbered squares within each rectangle show the pieces of frosted cake that were scored for taste.


Baker 11
CR 1
FR 1
1
2
FR 2
3
4
Baker 21
CR 2
FR 1
5
6
FR 2
7
8
Baker 12
CR 1
FR 1
9
10
FR 2
11
12
Baker 22
CR 2
FR 1
13
14
FR 2
15
16

A Model for the Frosted Cake Data

$i = 1, 2$ cake recipes

$j = 1, 2$ bakers within each cake recipe

$k = 1, 2$ frosting recipes

$l = 1, 2$ pieces of cake within each half cake

$$y_{ijkl} = \mu_{ik} + b_{ij} + h_{ijk} + e_{ijkl},$$

where ...

A Model for the Frosted Cake Data (continued)

$$b_{11}, b_{12}, b_{21}, b_{22} \sim N(0, \sigma_b^2),$$

$$h_{111}, h_{112}, h_{121}, h_{122}, h_{211}, h_{212}, h_{221}, h_{222} \sim N(0, \sigma_h^2),$$

$$e_{ijkl} \sim N(0, \sigma_e^2),$$

and all random effects are independent.

SAS Code for Frosted Cake Example

```
proc mixed;
  class cr baker fr;
  model y=cr fr cr*fr / ddfm=satterth;
  random baker(cr) fr*baker(cr);
run;
```

ANOVA Table for Frosted Cake Example

Source	DF
cr	1
baker(cr)	2
fr	1
cr×fr	1
fr×baker(cr)	2
error	8
c.total	15

ANOVA Table for Frosted Cake Example

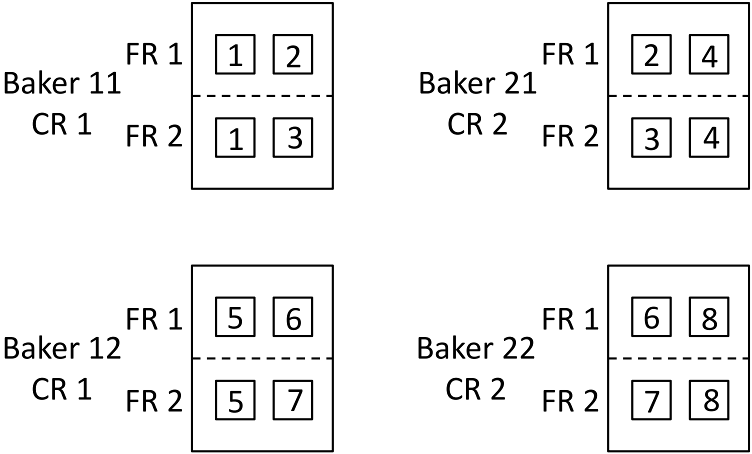
Source	DF
cr	1
baker(cr)	2
fr	1
cr$\times$fr	1
fr$\times$baker(cr)	2
piece(cr, baker, fr)	8
c.total	15

ANOVA Table for Frosted Cake Example

Source	DF
cr	1
whole-plot error	2
fr	1
cr×fr	1
split-plot error	2
piece(cr, baker, fr)	8
c.total	15

Example 5: In the context of the frosted cake example, a statistician is asked to analyze the data. The statistician asks, "How was the score for each piece of cake determined?"

Answer: Two pieces of frosted cake from each half of each cake were cut and delivered to eight trained judges for evaluation. Each judge tasted and separately assigned a score to two pieces of frosted cake. In the following diagram, the numbers 1 through 8 indicate the pieces of cake scored by judges 1 through 8, respectively.


Baker 11
CR 1
FR 1
FR 2
1
2
1
3
Baker 21
CR 2
FR 1
FR 2
2
4
3
4
Baker 12
CR 1
FR 1
FR 2
5
6
5
7
Baker 22
CR 2
FR 1
FR 2
6
8
7
8

A Model

$i = 1, 2$ cake recipes

$j = 1, 2$ bakers within each cake recipe

$k = 1, 2$ frosting recipes

$l = 1, \ldots, 8$ judges

$$y_{ijkl} = \mu_{ik} + b_{ij} + h_{ijk} + t_l + e_{ijkl},$$

where $\ldots$

A Model (continued)

$$b_{11}, b_{12}, b_{21}, b_{22} \sim N(0, \sigma_b^2),$$

$$h_{111}, h_{112}, h_{121}, h_{122}, h_{211}, h_{212}, h_{221}, h_{222} \sim N(0, \sigma_h^2),$$

$$t_1, \ldots, t_8 \sim N(0, \sigma_t^2),$$

$$e_{ijkl} \sim N(0, \sigma_e^2),$$

and all random effects are independent.

SAS Code

```
proc mixed;
  class cr baker fr judge;
  model y=cr fr cr*fr / ddfm=satterth;
  random baker(cr) fr*baker(cr) judge;
  lsmeans cr fr cr*fr;
  estimate ...
run;
```

R Code

```
library(lme4)
library(lmerTest)
o = lmer(y ~ cr + fr + cr:fr +
             (1 | baker) + (1 | baker:fr) + (1 | judge))
anova(o)
ls_means(o)
contest(...)
```

This code assumes all variables (except y) are factors with cr $\in \{1,2\}$, fr $\in \{1,2\}$, baker $\in \{1,2,3,4\}$, and judge $\in \{1,\ldots,8\}$.

One ANOVA Table

Source	DF
cr	1
baker(cr)	2
fr	1
cr×fr	1
fr×baker(cr)	2
judge	6
error	2
c.total	15

How do we determine the df for judge and df for error?

Our df “rules” do not allow us to easily compute the df for judges.

If we sequentially form matrices with successively larger and larger column spaces corresponding to the ordered terms in the ANOVA table, it can be shown that adding indicator columns for judges will increase the rank of the resulting matrix from 8 to 14.

Thus, the factor judge gets $14 - 8 = 6$ df, and the leftover error gets $16 - 14 = 2$ df.

ANOVA is not particularly useful in Example 5

We have examined just one of several possible sequential ANOVA tables in this case.

Other ANOVA tables would be obtained by introducing the judge factor earlier in the sequence of progressively larger column spaces that determines the sequential ANOVA table.

This experimental design is complex, in part, because the judge factor is neither nested nor crossed with other factors in this experiment.

Reliability of SAS and R Code

Although ANOVA-based analysis is not particularly useful in this example, the SAS and R code provided should work reasonably well when the number of bakers (number of whole-plot experimental units) and the number of judges are sufficiently large.

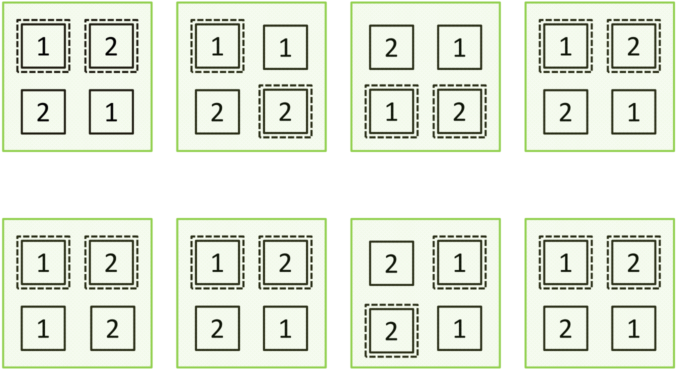
A simulation study could be used to assess the performance of the SAS and R code for various sample sizes and experimental designs.

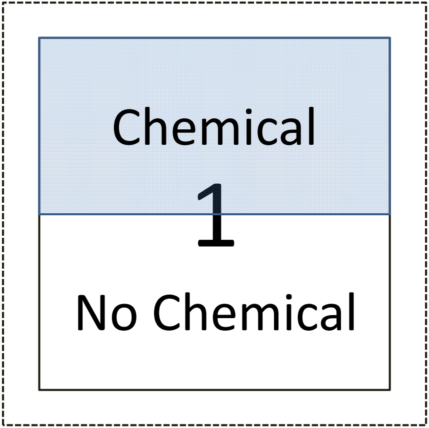
Example 6

Researchers are interested in determining ways to deter deer from eating ornamental plants. An experiment was conducted in eight fields. Within each field, four 15 meter x 15 meter squares of land were studied. Within each field, researchers used the the following procedure:

- Two of the four squares were randomly selected to be planted with plant type 1. The other two squares were planted with plant type 2.
- One of the two squares planted with plant type 1 was randomly selected, and a fence was placed around the selected square. The other plant type 1 square was not surrounded by a fence.
- One of the two squares planted with plant type 2 was randomly selected, and a fence was placed around the selected square. The other plant type 2 square was not surrounded by a fence.

- Each of the four squares was divided into two rectangles of equal size. One rectangle within each square was randomly selected, and the plants growing in that rectangle were treated with a chemical. The other rectangle in each square was not treated with a chemical.
- At the conclusion of the study, the amount of living plant biomass was measured for each rectangle.


1
2
2
1
1
1
2
2
2
1
1
2
1
2
2
1
1
2
1
2
1
2
2
1
2
1
2
1
1
2
2
1


Chemical
1
No Chemical

A Model

$i = 1, 2$ plant types

$j = 1, 2$ fences (yes, no)

$k = 1, 2$ chemicals (yes, no)

$l = 1, \ldots, 8$ fields

$$y_{ijkl} = \mu_{ijk} + f_l + s_{ijl} + e_{ijkl},$$

where ...

A Model (continued)

$$f_l \sim N(0, \sigma_f^2),$$

$$s_{ijl} \sim N(0, \sigma_s^2),$$

$$e_{ijkl} \sim N(0, \sigma_e^2),$$

and all random effects are independent.

Source	DF
field	7
ptype	1
fence	1
ptype $\times$ fence	1
field$\times$ptype+field$\times$fence+field$\times$ptype$\times$fence	21
chem	1
ptype$\times$chem	1
fence$\times$chem	1
ptype$\times$fence$\times$chem	1
error	28
c.total	63

SAS Code

```
proc mixed;
  class field ptype fence chem;
  model y=ptype|fence|chem/ddfm=satterth;
  random field field*ptype*fence;
run;
```

DF and EMS for All Terms Involving Field

Source	DF	EMS
field	7	$8\sigma_f^2 + 2\sigma_s^2 + \sigma_e^2$
field×ptype	7	$2\sigma_s^2 + \sigma_e^2$
field×fence	7	$2\sigma_s^2 + \sigma_e^2$
field×ptype×fence	7	$2\sigma_s^2 + \sigma_e^2$
field×chem	7	σ_e^2
field×ptype×chem	7	σ_e^2
field×fence×chem	7	σ_e^2
field×ptype×fence×chem	7	σ_e^2

Combine Lines with Common EMS

Source	DF	EMS
field	7	$8\sigma_f^2 + 2\sigma_s^2 + \sigma_e^2$
whole-plot error	21	$2\sigma_s^2 + \sigma_e^2$
split-plot error	28	σ_e^2

Source	DF
field	7
ptype	1
fence	1
ptype $\times$ fence	1
whole-plot error	21
chem	1
ptype$\times$chem	1
fence$\times$chem	1
ptype$\times$fence$\times$chem	1
split-plot error	28
c.total	63

Additional Comments

- Use fixed effects to specify the mean of the response vector, i.e., $E(\boldsymbol{y})$.
- Use random effects to specify the variance of the response vector, i.e., $\text{Var}(\boldsymbol{y})$.

Additional Comments

- When there is more than one observation for a given type of experimental unit, include random effects in the model for experimental units of the given type.

- When there is only one observation for each experimental unit of a given type, the error terms in the model are the random effects for those experimental units.

Additional Comments

- For balanced experiments with positive variance component estimates, the fixed-effects inferences obtained from the linear mixed-effects model analyses of observational units match the inferences obtained from simpler analyses of experimental unit averages.

Additional Comments

- If our model implies that multiple sums of squares have the same expected mean square, we pool those mean squares together by taking a weighted average of the means squares with degrees of freedom values as weights.

- This weighted averaging is equivalent to adding the sums of squares and dividing by the sum of their degrees of freedom values.

Additional Comments

- When coding a model in SAS or writing terms in an ANOVA table, use interactions between previously listed terms when possible to specify effects that you wish to include in your model.
- When levels of a factor B are different for each level of a factor A, we say that *B is nested within A*. In SAS, this is indicated by B(A). If C is nested within B, and B is nested within A, this is indicated by C(A B) in SAS.

Additional Comments

- If a term like pot(tray) is specified, it should be the case that pots within each tray are replicates that are not treated differently within a tray.
- For example, pot(tray) is correct for slide 18 (Example 3) but not for slide 11 (Example 2).
- See nesting.sas for an example that shows why I like to specify nesting.

Additional Comments

- Our model in the cake example involved 8 parameters.
- We had only 16 observations.
- We should encourage researchers to obtain far more than two observations per parameter.

Additional Comments

- You might think the examples in these slides are crazy scenarios that I made up.
- You would be right, but all examples are based on real experiments that I have seen.

Additional Comments

- In most cases, the real experiments that inspired these examples are quite a bit more complicated than my examples.
- For instance, imagine two more splits in the last example with repeated measures over time on the split-split-split-plot experimental units.
- Federer and King (2007) present an ANOVA table with 259 lines and 62 error terms for the analysis of a real experiment.

Additional Comments

- I have presented ideas that I find useful for specifying linear mixed-effect models for designed experiments.
- Following my advice is not guaranteed to lead to the “true” data-generating model for any given dataset.
- The advice is intended to provide you with a good starting point for modeling data and making inferences from designed experiments.