

Topic 6 Randomized Complete Block Designs (RCBDs)

The Completely Randomized Design (CRD)

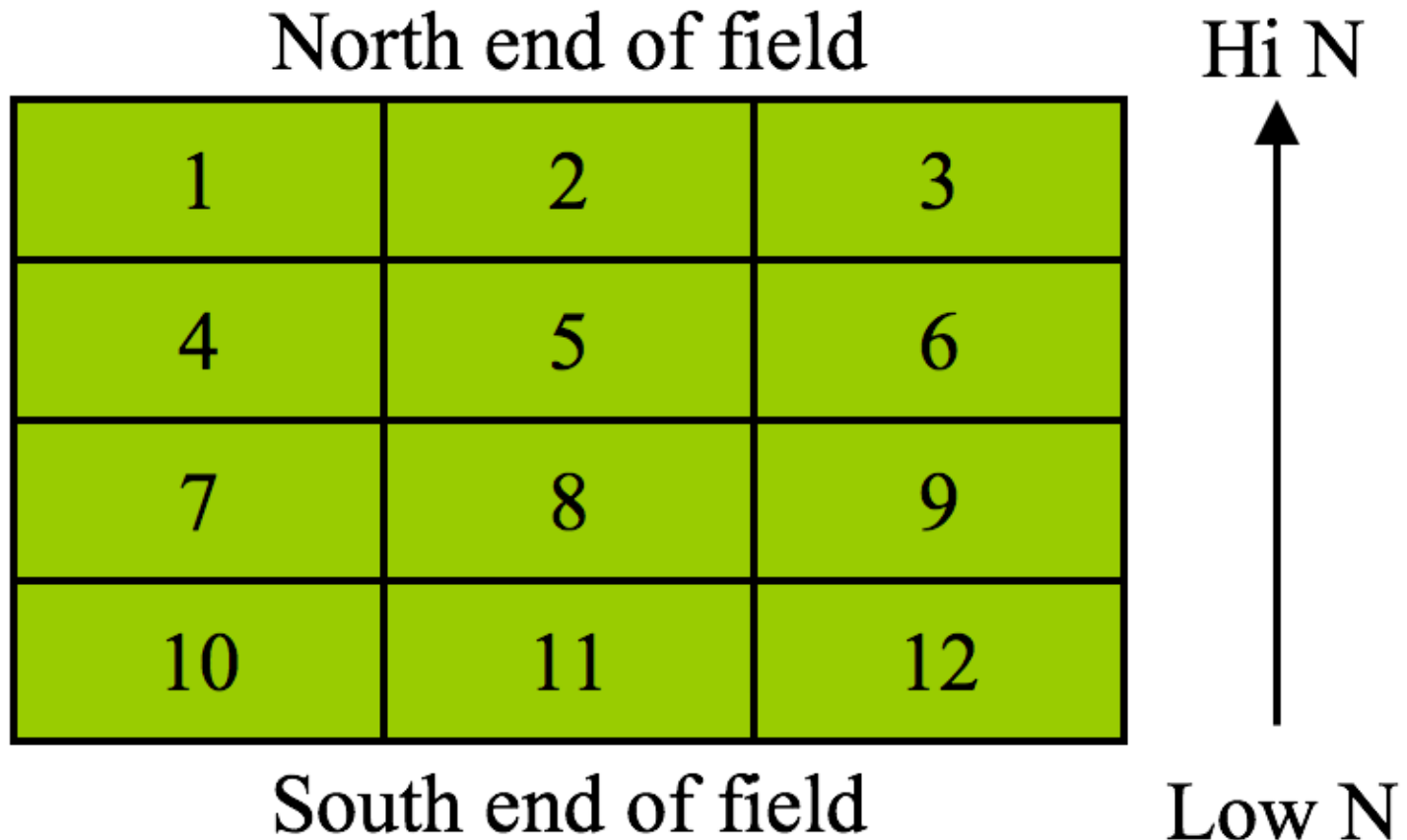
1. Experimental units (EUs) are assumed to be uniform.
2. Treatments are randomly assigned across EUs via a single randomization.
3. You should include as much of the native variability as possible *within* each EU.
4. When EUs are not uniform, experimental error (MSE) increases, F (MST/MSE) decreases, and the experiment loses sensitivity. If the experiment is replicated in a variety of situations to increase its scope, the variability increases even further.

The Randomized Complete Block Design (RCBD)

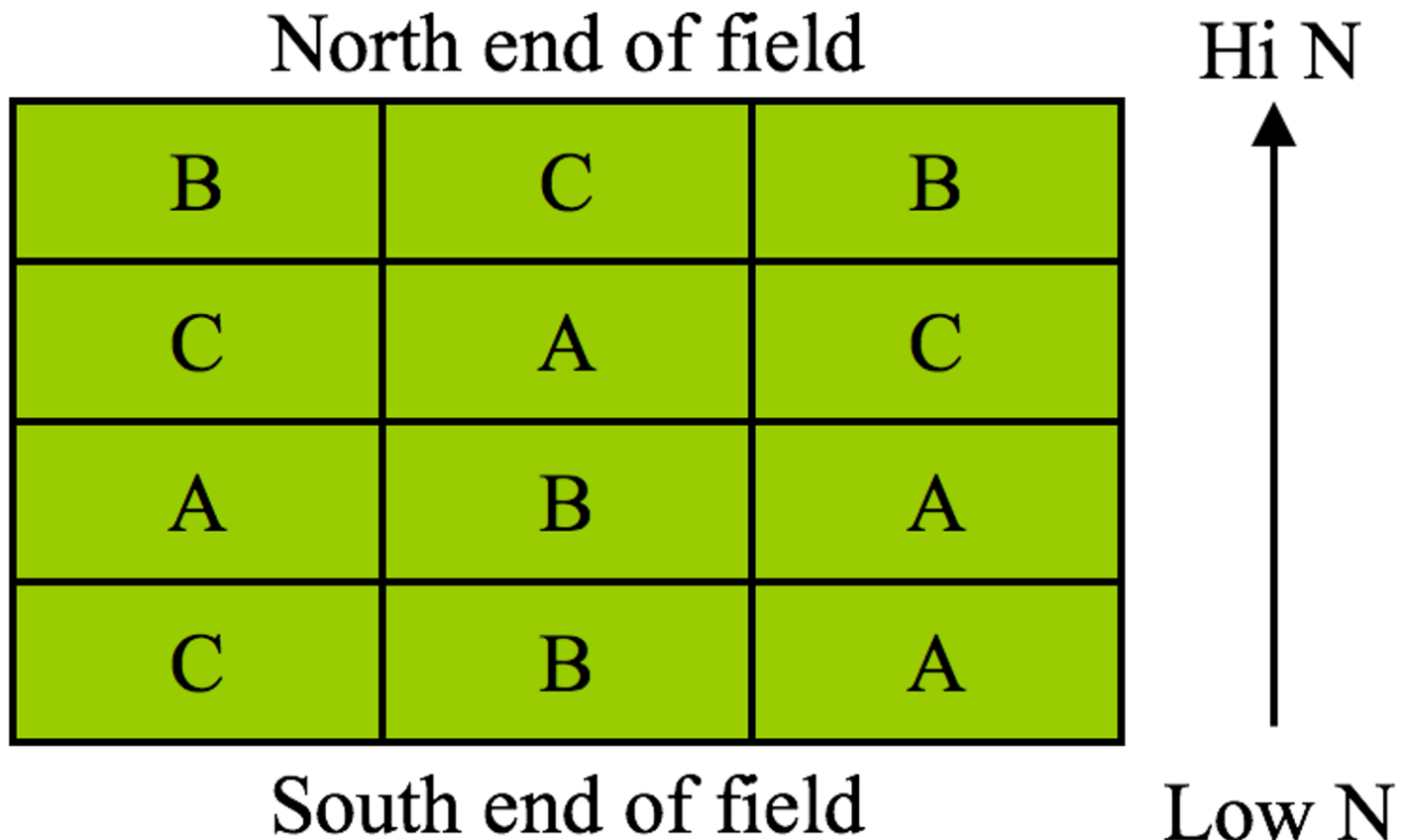
1. The population of EUs is divided into a number of relatively homogeneous subpopulations or ***blocks***, and all EUs *within a given block* are assumed to be uniform.
2. Within each block, treatments are randomly assigned to EUs such that each treatment occurs equally often (usually once) in each block (i.e. one randomization per block)
3. You should minimize the native variability as much as possible *within* blocks and maximize the native variability as much as possible *among* blocks.
4. Variation among blocks can be partitioned out of the experimental error (MSE), thereby reducing this quantity and increasing the power of the test. Additional variability introduced when increasing the scope of the experiment can also be partitioned out of the MSE.

Blocks usually represent levels of naturally-occurring differences or sources of variation that are unrelated to the treatments, and *the characterization of these differences is not of interest to the researcher.*

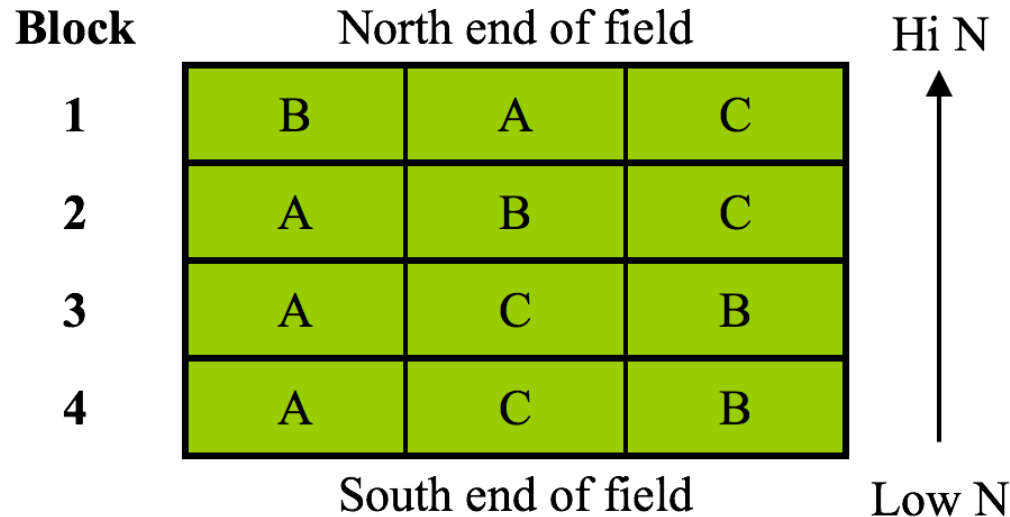
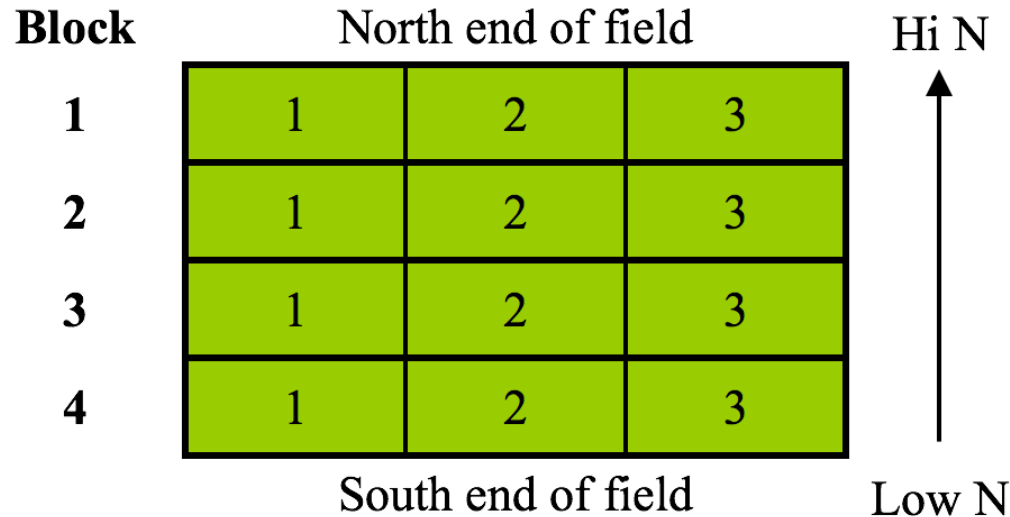
Example: A field trial comparing three clones of milkweed (A, B, and C) with four replications.



CRD: One randomization per experiment



RCBS: One randomization per block



The linear model

The model underlying each observation in the experiment:

$$Y_{ij} = \mu + \tau_i + \beta_j + \varepsilon_{ij}$$

$$Y_{ij} = \bar{Y}_{..} + (\bar{Y}_{i.} - \bar{Y}_{..}) + (\bar{Y}_{.j} - \bar{Y}_{..}) + (Y_{ij} - \bar{Y}_{i.} - \bar{Y}_{.j} + \bar{Y}_{..})$$

And the sums of squares:

$$\sum_{i=1}^t \sum_{j=1}^r (Y_{ij} - \bar{Y}_{..})^2 = r \sum_{i=1}^t (\bar{Y}_{i.} - \bar{Y}_{..})^2 + t \sum_{j=1}^r (\bar{Y}_{.j} - \bar{Y}_{..})^2 + \sum_{i=1}^t \sum_{j=1}^r (Y_{ij} - \bar{Y}_{i.} - \bar{Y}_{.j} + \bar{Y}_{..})^2$$

TSS SST SSB SSE

This *partitioning* of variance is possible because the sums of squares of treatments, blocks, and error are *orthogonal* to one another.

This orthogonality is a direct result of the *completeness* of the block design.

CRD

















Source	df	SS	MS	F
Total	$rt - 1$	TSS		
Treatments	$t - 1$	SST	$SST/(t-1)$	MST/MSE
Error	$t(r - 1)$	TSS-SST	$SSE/r(t-1)$	

RCBD (one replication per block-treatment combination)

Source	df	SS	MS	F
Total	$rt - 1$	TSS		
Treatments	$t - 1$	SST	$SST/(t-1)$	MST/MSE
Blocks	$r - 1$	SSB	$SSB/(r-1)$	
Error	$(t-1)(r-1)$	TSS-SST-SSB	$SSE/(t-1)(r-1)$	

1. RCBD has $(r - 1)$ fewer df_e than the CRD.
2. If there are no differences among blocks ($SSB = 0$), $MSE_{CRD} < MSE_{RCBD}$.
3. If there are large enough differences among blocks ($SSB \gg 0$), $MSE_{CRD} > MSE_{RCBD}$.

Example: An experiment was conducted to investigate the effect of estrogen on weight gain in sheep. The treatments are combinations of sex of sheep (M, F) and level of estrogen (Est₀, Est₁). The sheep are blocked by ranch, with one replication of each treatment level at each ranch.

Trtmt	Ranch			
	1	2	3	4
M Est ₀				
M Est ₁				
F Est ₀				
F Est ₁				

Effect of estrogen on weight gain in sheep (lbs)

	Ranch (i.e. block)				Treatment	
Treatment	I	II	III	IV	Total	Mean
M Est ₀	47	52	62	51	212	53
M Est ₁	50	54	67	57	228	57
F Est ₀	57	53	69	57	236	59
F Est ₁	54	65	74	59	252	63
Block Total	208	224	272	224	928	
Block Mean	52	56	68	56		58

CRD ANOVA (treating blocks as reps)

Source	df	SS	MS	F
Totals	15	854		
Treatment	3	208	69.33	1.29 NS
Error	12	646	53.83	

RCBD ANOVA

Source	df	SS	MS	F
Total	15	854		
Treatment	3	208	69.33	8.91**
Blocks	3	576	192.00	24.69**
Error	9	70	7.78	

Expected mean squares and F tests

EMS table for this two-way (RCBD) classification experiment, featuring t treatments, b blocks, and 1 replication per Block:Trtmt combination:

Source	df	MS	EMS
Trtmt	$t-1$	MST	$\sigma_{\varepsilon}^2 + b \sum \frac{\tau^2}{t-1}$
Block	$b-1$	MSB	$\sigma_{\varepsilon}^2 + t\sigma_{\beta}^2$
Error	$(t-1)(b-1)$	MSE	σ_{ε}^2

The appropriate test statistic (F) is a ratio of mean squares that is chosen such that the expected value of the *numerator* differs from the expected value of the *denominator* only by the specific factor being tested.

Relative efficiency: When to block?

$$F = \frac{MST}{MSE}$$

$$MSE = \frac{SSE}{df_e}$$

$$F_{crit} = F_{\alpha, df_{trt}, df_e}$$

Blocking reduces SSE, which reduces MSE.

Blocking reduces df_e , which increases MSE and increases F_{crit} .

The concept of *relative efficiency* formalizes the comparison between two experimental methods by quantifying this balance between loss of degrees of freedom and reduction in experimental error.

The information per replication in a given design is:

$$I = \frac{1}{\sigma_{\varepsilon}^2} \approx \left(\frac{df_{MSE} + 1}{df_{MSE} + 3} \right) \frac{1}{MSE}$$

$$RE_{1:2} = \frac{I_1}{I_2} \approx \frac{\left(\frac{df_{MSE1} + 1}{df_{MSE1} + 3} \right) \frac{1}{MSE_1}}{\left(\frac{df_{MSE2} + 1}{df_{MSE2} + 3} \right) \frac{1}{MSE_2}} = \frac{(df_{MSE1} + 1)(df_{MSE2} + 3)MSE_2}{(df_{MSE2} + 1)(df_{MSE1} + 3)MSE_1}$$

The main complication is how to estimate MSE for the alternative design.

If an experiment was conducted as an RCBD, MSE_{CRD} can be *estimated* by the following formula:

$$\hat{MSE}_{CRD} \cong \frac{df_B MSB_{RCBD} + (df_T + df_e) MSE_{RCBD}}{df_B + df_T + df_e}$$

Assume TSS of the two designs is the same.
Rewrite TSS in terms of its components and simplify the expression.

For the interested: Derivation of the expected MSE_{CRD}

1. Set the total sums of squares of each design equal to each other and rewrite in terms of mean squares and degrees of freedom:

$$TSS_{RCBD} = TSS_{CRD}$$

$$SST_{RCBD} + SSB_{RCBD} + SSE_{RCBD} = SST_{CRD} + SSE_{CRD}$$

$$df_{T(R)}MST_R + df_{B(R)}MSB_R + df_{e(R)}MSE_R = df_{T(C)}MST_C + df_{e(C)}MSE_C$$

$$(t-1)MST_R + (r-1)MSB_R + (t-1)(r-1)MSE_R = (t-1)MST_C + t(r-1)MSE_C$$

2. Replace each mean square with the variance components of its expected mean square:

$$(t-1)(\sigma_{e(R)}^2 + r\sigma_{T(R)}^2) + (r-1)(\sigma_{e(R)}^2 + t\sigma_{B(R)}^2) + (t-1)(r-1)\sigma_{e(R)}^2 = (t-1)(\sigma_{e(C)}^2 + r\sigma_{T(C)}^2) + t(r-1)\sigma_{e(C)}^2$$

$$[(t-1) + (r-1) + (t-1)(r-1)]\sigma_{e(R)}^2 + t(r-1)\sigma_{B(R)}^2 + r(t-1)\sigma_{T(R)}^2 = [(t-1) + t(r-1)]\sigma_{e(C)}^2 + r(t-1)\sigma_{T(C)}^2$$

$$t(r-1)\sigma_{B(R)}^2 + (tr-1)\sigma_{e(R)}^2 = (tr-1)\sigma_{e(C)}^2$$

$$\sigma_{e(C)}^2 = \sigma_{e(R)}^2 + \frac{t(r-1)\sigma_{B(R)}^2}{(tr-1)}$$

3. Finally, rewrite this expression in terms of mean squares and degrees of freedom:

$$MSE_{CRD} = MSE_{RCBD} + t(r-1) \frac{MSB - MSE_{RCBD}}{t(tr-1)}$$

$$MSE_{CRD} = MSE_{RCBD} + (r-1) \frac{MSB}{tr-1} - (r-1) \frac{MSE_{RCBD}}{tr-1}$$

$$MSE_{CRD} = [(tr-1) - (r-1)] \frac{MSE_{RCBD}}{tr-1} + (r-1) \frac{MSB}{tr-1}$$

$$MSE_{CRD} = \frac{r(t-1)MSE_{RCBD} + (r-1)MSB}{tr-1}$$

$$MSE_{CRD} = \frac{(df_{T(R)} + df_{e(R)})MSE_{RCBD} + df_B MSB}{df_{T(R)} + df_{B(R)} + df_{e(R)}}$$

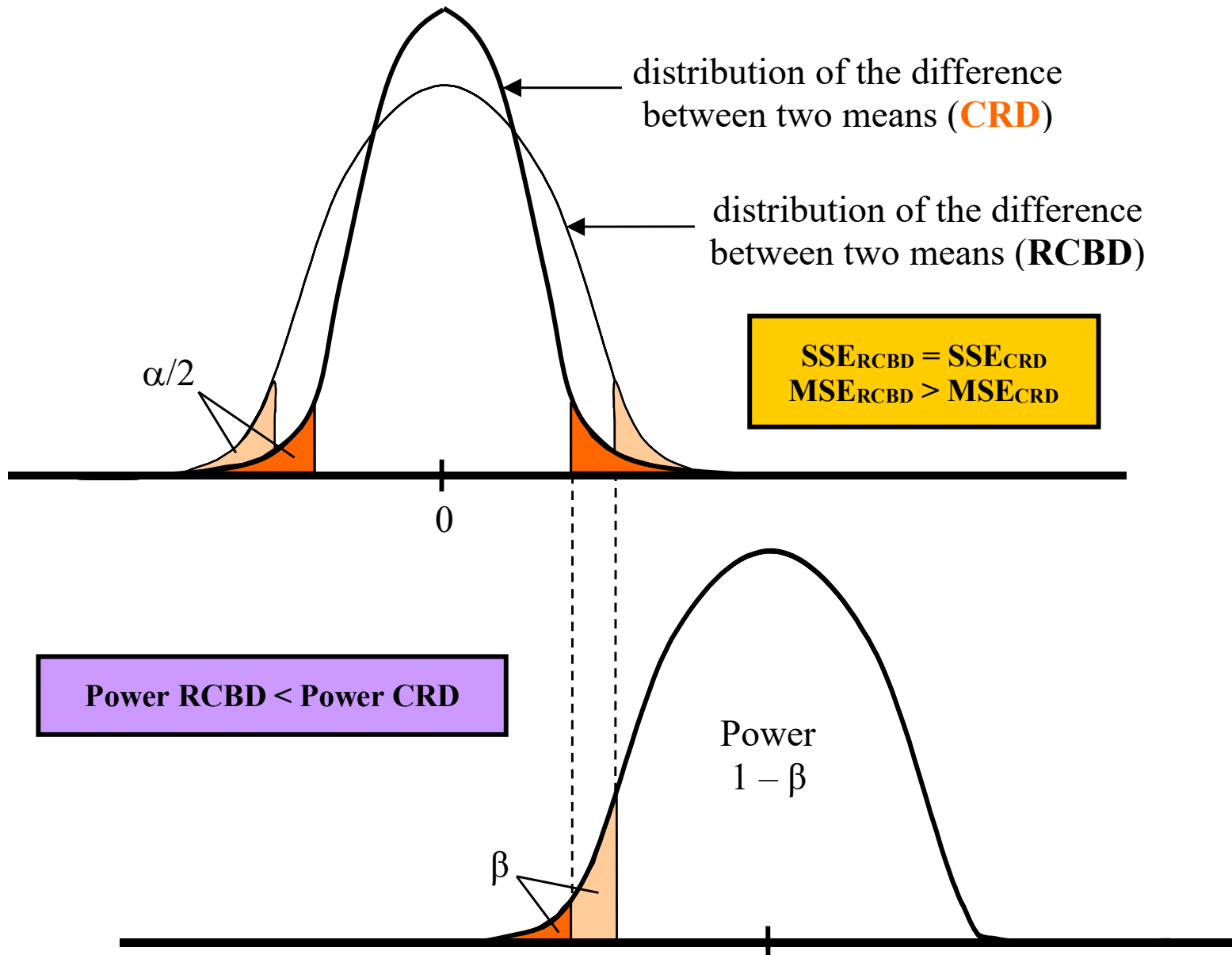
Example: From the sheep experiment, $MSE_{RCBD} = 7.78$ and $MSB_{RCBD} = 192.0$. Therefore:

$$M\hat{S}E_{CRD} \cong \frac{df_B MSB_{RCBD} + (df_T + df_e) MSE_{RCBD}}{df_B + df_T + df_e} = \frac{3(192) + (3 + 9)7.78}{3 + 3 + 9} = 44.62$$

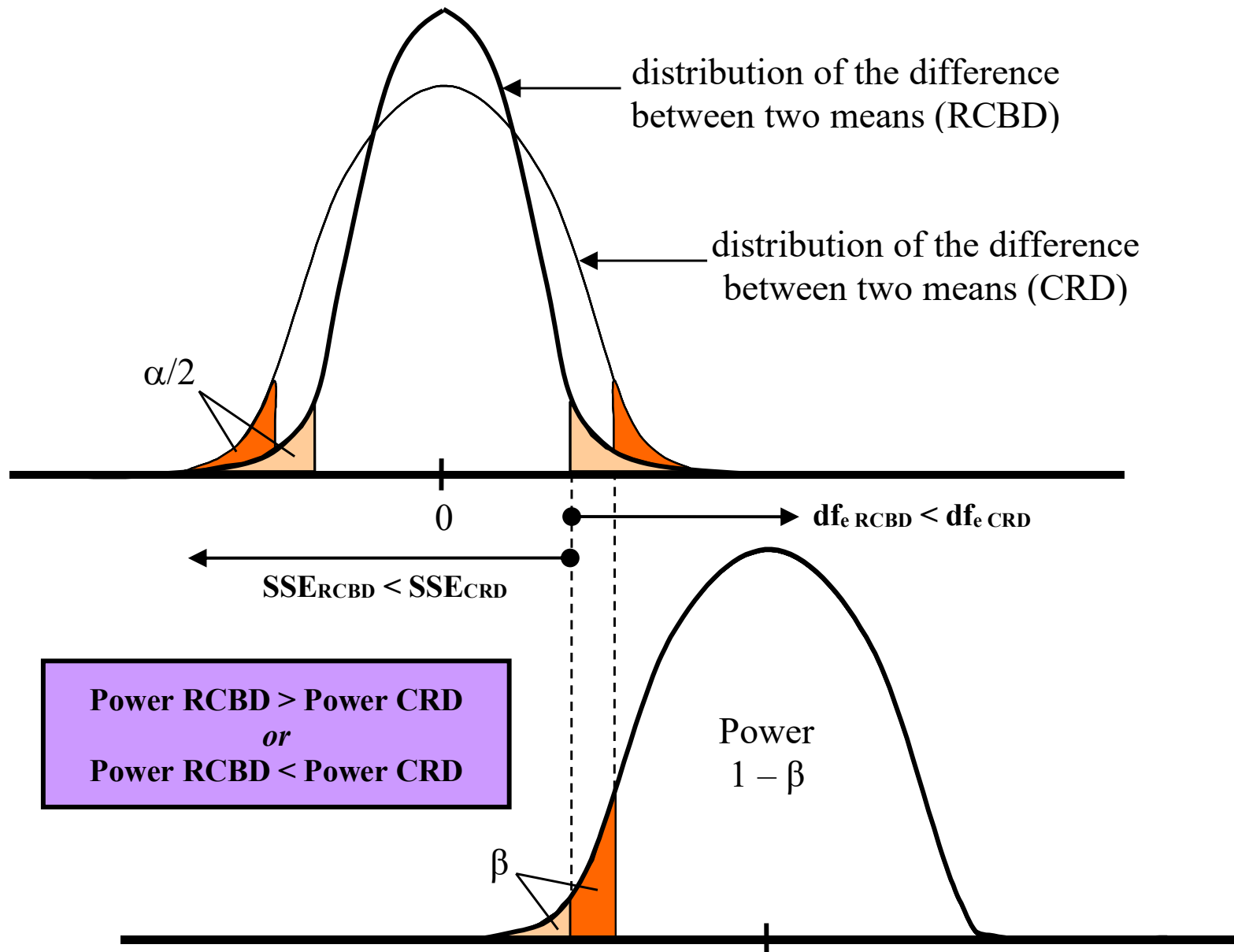
$$RE_{RCBD:CRD} \cong \frac{(df_{MSE1} + 1)(df_{MSE2} + 3)M\hat{S}E_{CRD}}{(df_{MSE2} + 1)(df_{MSE1} + 3)MSE_{RCBD}} = \frac{(9 + 1)(12 + 3)44.62}{(12 + 1)(9 + 3)7.78} = 5.51$$

Interpretation: It takes 5.51 replications in the CRD to produce the same amount of information as one replication in the RCBD. Or, the RCBD is 5.51 times more efficient than the CRD in this case.

When there are no significant differences among blocks



When there *are* significant differences among blocks



















Assumptions of the model

The model for the RCBD with a single replication per block-treatment combination:

$$Y_{ij} = \mu + \tau_i + \beta_j + \varepsilon_{ij}$$

1. The residuals (ε_{ij}) are independent, homogeneous, and normally distributed.
2. The variance within each treatment levels is homogeneous across all treatment levels.
3. The main effects are additive.

Recall that experimental error is defined as the variation among experimental units *that are treated alike*.

	Ranch			
Trtmt	1	2	3	4
M Est ₀				
M Est ₁				
F Est ₀				
F Est ₁				

There is an *expected* value for each sheep, given by:

$$\text{Expected } Y_{ij} = \mu + \tau_i + \beta_j$$

$$\text{Observed } Y_{ij} = \mu + \tau_i + \beta_j + \varepsilon_{ij}$$

With only one replication per cell (i.e. treatment-block combination), the residuals are the combined effects of experimental error **and** any non-additive treatment:block interactions:

$$\varepsilon_{ij} = \tau_i \cdot \beta_j + \text{error}_{ij}$$

So when we use ε_{ij} as estimates of the true experimental error, we are assuming that $\tau_i \cdot \beta_j \simeq 0$.

This assumption of no interaction is referred to as *the assumption of additivity of the main effects*.

If this assumption is violated, it's an indication that your blocks are not behaving as you expect (i.e. additively). In other words, something of great interest is lurking within your blocking variable that you need to better understand.

Tukey's 1-df test for nonadditivity

Under our linear model, each observation is characterized as:

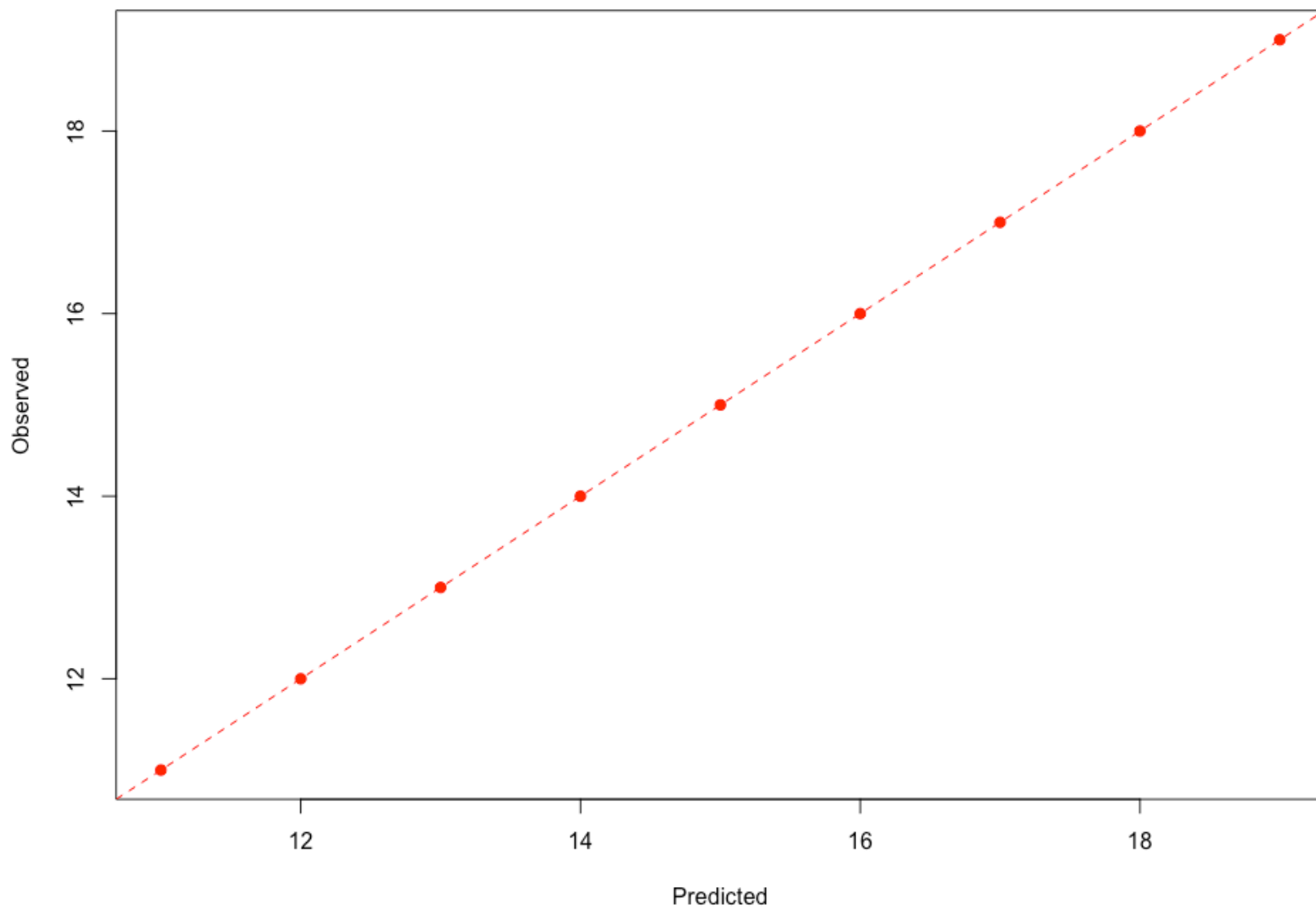
$$y_{ij} = \mu + \beta_i + \tau_j + \varepsilon_{ij}$$

The predicted value of each individual is given by:

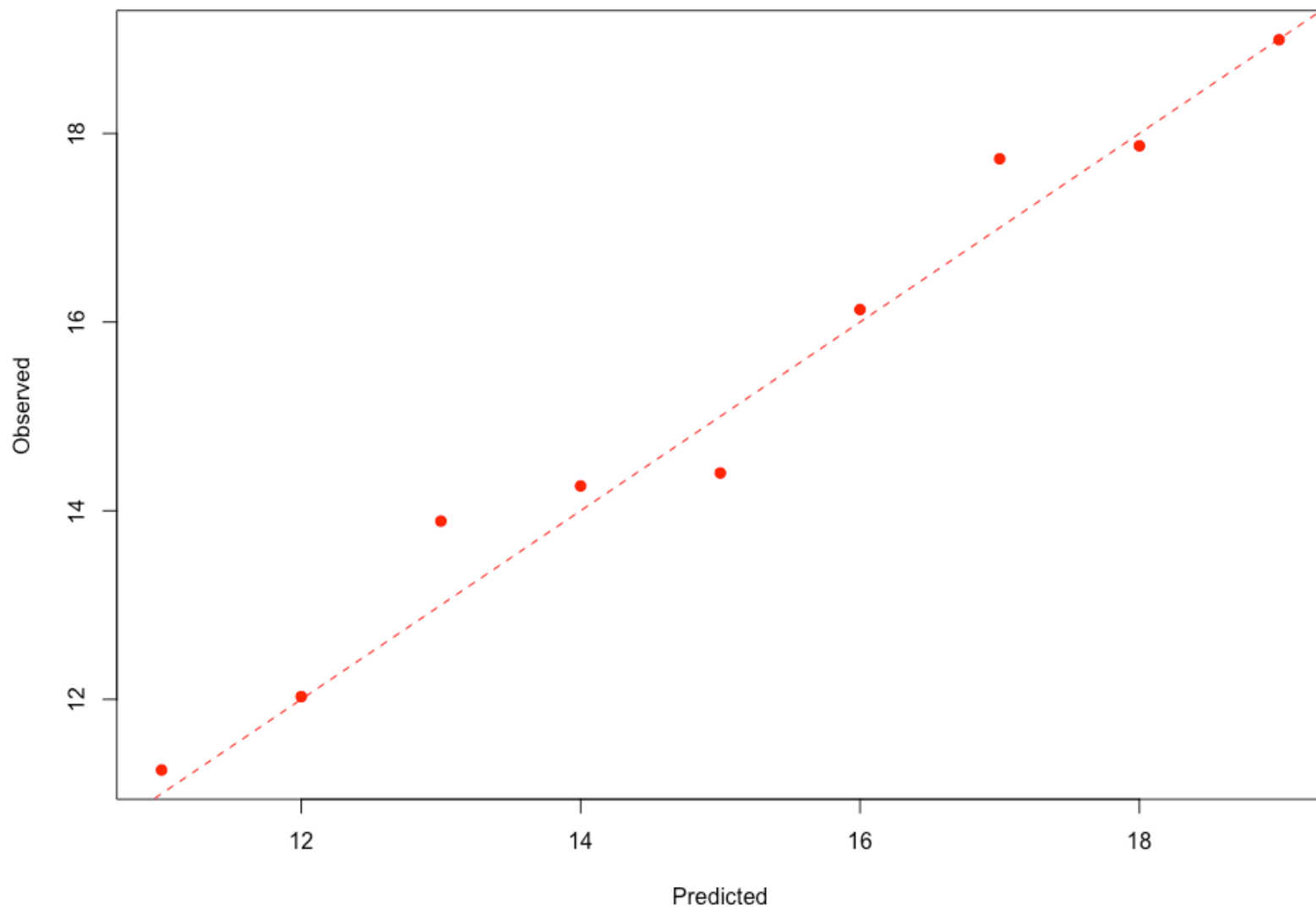
$$pred_{ij} = \mu + \beta_i + \tau_j$$

So, if we had no error in our experiment (i.e. if $\varepsilon_{ij} = 0$), the observed data would exactly match its predicted values and a correlation plot of the two would yield a perfect line with slope = 1.

Observed vs. Predicted, no error

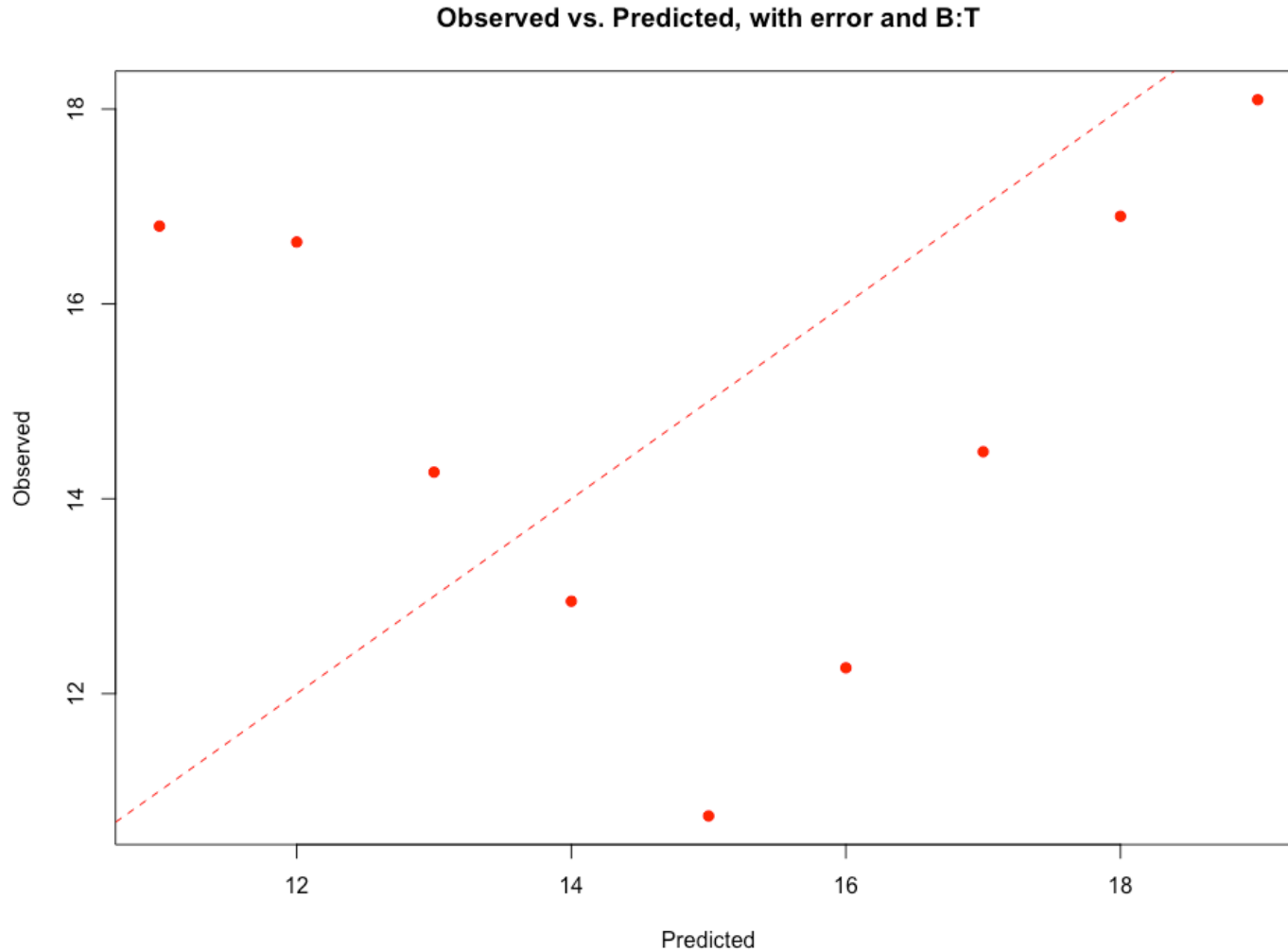


Observed vs. Predicted, with error



But what happens when you have an interaction (e.g. Block:Treatment) but lack the degrees of freedom necessary to include it in the linear model?

$$\varepsilon_{ij} = \varepsilon_{RANDOMij} + \text{B:T Interaction Effects}$$



SO, if the observed and predicted values obey a linear relationship, then the non-random Interaction Effects buried in the error term are sufficiently small to uphold our assumption of additivity of main effects.

This test is easily implemented using R

#The ANOVA [RCBD]

```
sheep_mod<-lm(Gain ~ Sex_Est + Ranch, sheep_dat)
```

```
sheep_dat$sq_preds <- predict(sheep_mod)^2
```

#The Tukey 1-df Test for Non-additivity

```
sheep_1df_mod<-lm(Gain ~ Sex_Est + Ranch + sq_preds, sheep_dat)  
anova(sheep_1df_mod)
```

Output

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Sex_Est	3	208.00	69.333	8.3307	0.0076360	**
Ranch	3	576.00	192.000	23.0696	0.0002716	***
preds2	1	3.42	3.419	0.4108	0.5394942	
Residuals	8	66.58	8.323			

This test is necessary **ONLY** when
there is **one observation** per
block-treatment combination.

If there are two or more replications
per block-treatment combination,
the block:treatment interaction can be tested
directly in the model.

Example: Yield of penicillin from four different protocols (A – D). Blocks are different stocks of an important reagent. The numbers below each observation (**O**) are the predicted values (**P** = Grand Mean + Treatment effect + Block effect) and the residuals (**R**).

Block	Treatment				Block Mean	Block Effect
	A	B	C	D		
Stock 1	O: 89	O: 88	O: 97	O: 94	92	+6
	P: 90	P: 91	P: 95	P: 92		
	R: -1	R: -3	R: 2	R: 2		
Stock 2	O: 84	O: 77	O: 92	O: 79	83	-3
	P: 81	P: 82	P: 86	P: 83		
	R: 3	R: -5	R: 6	R: -4		
Stock 3	O: 81	O: 87	O: 87	O: 85	85	-1
	P: 83	P: 84	P: 88	P: 85		
	R: -2	R: 3	R: -1	R: 0		
Stock 4	O: 87	O: 92	O: 89	O: 84	88	2
	P: 86	P: 87	P: 91	P: 88		
	R: 1	R: 5	R: -2	R: -4		
Stock 5	O: 79	O: 81	O: 80	O: 88	82	-4
	P: 80	P: 81	P: 85	P: 82		
	R: -1	R: 0	R: -5	R: 6		
Treatment mean	84	85	89	86	Mean = 86	
Treatment effect	-2	-1	3	0		

The R script for a full analysis of this dataset:

#The ANOVA

```
Penicillin_mod<-lm(Penicillin ~ Protocol + Stocks, Penicillin_dat)  
anova(Penicillin_mod)
```

#TESTING ASSUMPTIONS

#Generate residual and predicted values

```
Penicillin_dat$resids <- residuals(Penicillin_mod)  
Penicillin_dat$preds <- predict(Penicillin_mod)  
Penicillin_dat$sq_preds <- Penicillin_dat$preds^2
```

#Look at a plot of residual vs. predicted values

```
plot(resids ~ preds, data = Penicillin_dat,  
     xlab = "Predicted Values",  
     ylab = "Residuals")
```

```
#Perform a Shapiro-Wilk test for normality of residuals  
shapiro.test(Penicillin_dat$resids)
```

```
#Perform a Levene's Test for homogeneity of variances  
library(car)  
leveneTest(Penicillin ~ Variety, data = Penicillin_dat)
```

```
#Perform a Tukey 1-df Test for Non-additivity  
Penicillin _1df_mod<-lm(Penicillin ~ Protocol + Stocks + sq_preds,  
  Penicillin_dat)  
anova(Penicillin _1df_mod)
```

This dataset meets all assumptions: normality, variance homogeneity, and additivity:

Shapiro-Wilk normality test

data: Penicillin_dat\$resids

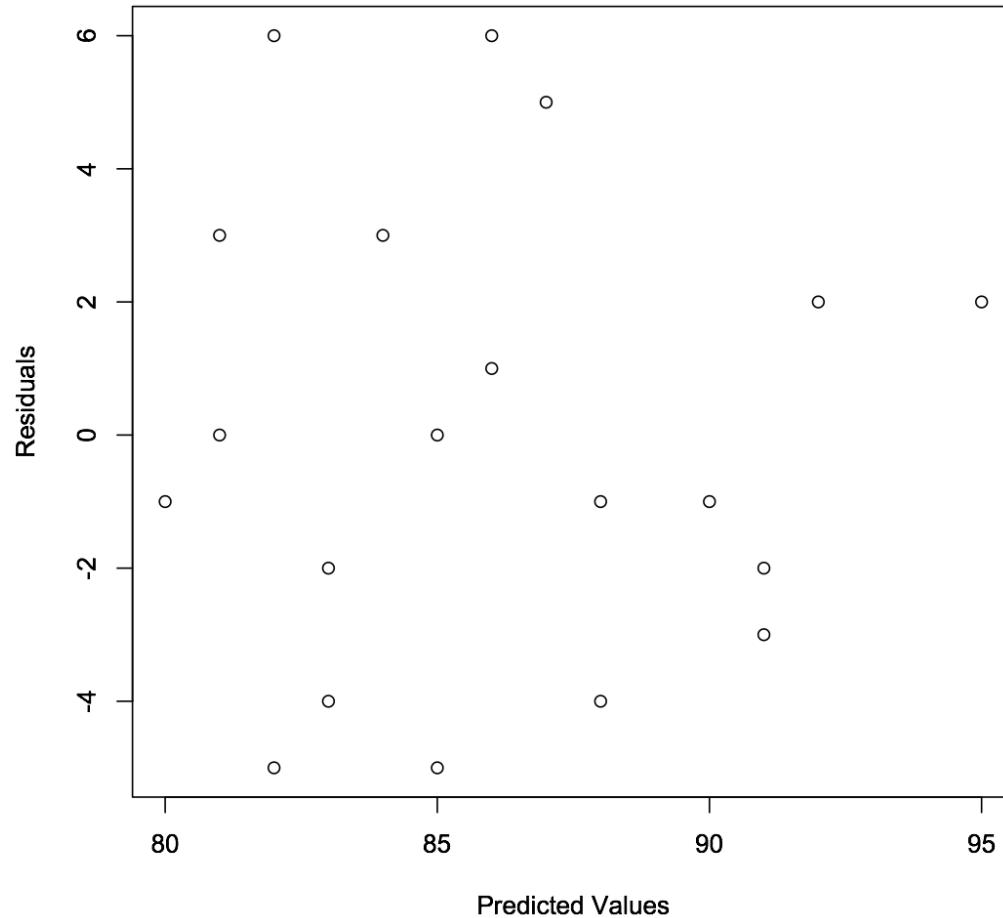
W = 0.9505, p-value = **0.3743** <- NS

Levene's Test for Homogeneity of Variance (center = median)

	Df	F value	Pr(>F)
group	3	0.1333	0.9388 <- NS













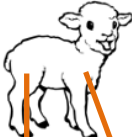



Tukey 1-df Test for Non-Additivity

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Protocol	3	70.000	23.333	1.1458	0.37360
Stocks	4	264.000	66.000	3.2411	0.05488
sq_preds	1	2.001	2.001	0.0983	0.75978 <- NS
Residuals	11	223.999	20.364		



**No particular pattern presents itself
in the plot of residuals.**

Nesting within an RCBD

Trtmt	Ranch			
	1	2	3	4
M Est ₀				
M Est ₁				
F Est ₀				
F Est ₁				

two
measurements

Nested RCBD, table of Expected Mean Squares (EMS)

Source of variation	Expected MS	F
Blocks (β_i)	$\sigma_\delta^2 + 2\sigma_\varepsilon^2 + 8\sigma_\beta^2$	MSB / MSEE
Treatments (τ_j)	$\sigma_\delta^2 + 2\sigma_\varepsilon^2 + 8\Sigma\tau^2/3$	MST / MSEE
Exp. Error ($\varepsilon_{k(ij)}$)	$\sigma_\delta^2 + 2\sigma_\varepsilon^2$	MSEE / MSSE
Samp. Error ($\delta_{l(ijk)}$)	σ_δ^2	

R script for calculating components of variance

#Calculating components of variance

```
library(lme4)
```

```
sheep1_mod<-lmer(gain ~ sex_est + (1|ranch) +  
                  (1|animal:ranch:sex_est), data = sheep_dat)
```

```
summary(sheep1_mod)
```

Random effects:

Groups	Name	Variance	Std.Dev.
animal:ranch:sex_est	(Intercept)	6.778	2.603
ranch	(Intercept)	46.056	6.786
Residual		2.000	1.414

Number of obs: 32, groups: animal:ranch:sex_est, 16; ranch, 4

Another way, using the `within()` function:

```
sheep_dat<-within(sheep_dat, animal<-(ranch:sex_est)[drop=TRUE])  
  
sheep2_mod<-lmer(gain ~ sex_est + (1|ranch) +  
                  (1|animal), data = sheep_dat)  
summary(sheep2_mod)
```

Random effects:

Groups	Name	Variance	Std.Dev.
animal	(Intercept)	6.778	2.603
ranch	(Intercept)	46.056	6.786
Residual		2.000	1.414

Number of obs: 32, groups: animal, 16; ranch, 4

Reminder

The objective of analyzing an experiment with the individual subsample values is to better understand the sources of variation, not to test their significances. Hypothesis testing is much simpler if you first average the subsamples within each experimental unit.

The optimal allocation of resources

If one animal (EU) costs \$150 US to establish and maintain, and one subsample (weighing) costs \$5 to do...

$$n_{sub} = \sqrt{\frac{C_{e.u.} * s_{sub}^2}{C_{sub} * s_{e.u.}^2}} = \sqrt{\frac{150 * 2.00}{5 * 6.778}} = 2.97$$

...the optimum allocation of resources would be to weigh each sheep three times.

#The ANOVA

#Note: ALL Trtmt F-tests must be done BY HAND, using the correct error term (this includes contrasts)

```
sheep_mod<-lm(gain ~ sex_est + ranch + animal, sheep_dat)  
anova(sheep_mod)
```

```
contrastmatrix<-cbind(c(1,1,-1,-1),c(1,-1,1,-1),c(1,-1,-1,1))  
contrasts(sheep_dat$sex_est)<-contrastmatrix
```

```
sheep_contrast_mod<-aov(gain ~ sex_est + ranch + animal, sheep_dat)  
summary(sheep_contrast_mod, split = list(sex_est = list("Sex" = 1,  
"Estrogen" = 2, "Sex*Estrogen" = 3)))
```

































Output

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
sex_est	3	416	138.7	69.333	2.19e-09	***
sex_est: Sex	1	128	128.0	64.000	5.55e-07	***
sex_est: Estrogen	1	288	288.0	144.000	2.06e-09	***
sex_est: Sex*Estrogen	1	0	0.0	0.000	1.000000	
ranch	3	1152	384.0	192.000	9.39e-13	***
animal	9	140	15.6	7.778	0.000223	***
Residuals	16	32	2.0			

The correct error term for all Trtmt and Block F-tests is the MSEE (15.6). These F- and p-values need to be calculated manually (e.g. using the **pf()** function). The corrected table:

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
sex_est	3	416	138.7	8.89	0.00469	**
sex_est: Sex	1	128	128.0	8.21	0.0186	*
sex_est: Estrogen	1	288	288.0	18.46	0.002	**
sex_est: Sex*Estrogen	1	0	0.0	0.000	1.000000	
ranch	3	1152	384.0	24.62	0.000113	***
animal	9	140	15.6	7.778	0.000223	***
Residuals	16	32	2.0			

RCBD with multiple replications per block-treatment combination

	Ranch			
Trtmt	1	2	3	4
M Est ₀	 	 	 	 
M Est ₁	 	 	 	 
F Est ₀	 	 	 	 
F Est ₁	 	 	 	 

σ_e^2

#The Exploratory ANOVA

```
sheep_mod<-lm(gain ~ sex_est*ranch, sheep_dat)
anova(sheep_mod)
```

Response: gain

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
sex_est	3	951.63	317.21	20.716	9.323e-06	***
ranch	3	176.12	58.71	3.834	0.03039	*
sex_est:ranch	9	137.12	15.24	0.995	0.48114	
Residuals	16	245.00	15.31			

```
pTrtmt<-pf(317.21/15.24,3,9,lower.tail=FALSE)
```

```
pBlock<-pf(58.71/15.24,3,9,lower.tail=FALSE)
```

#The final ANOVA

Response: gain

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
sex_est	3	951.63	317.21	20.814	0.00022	***
ranch	3	176.12	58.71	3.852	0.05031	
sex_est:ranch	9	137.12	15.24	0.995	0.48114	
Residuals	16	245.00	15.31			

#TESTING ASSUMPTIONS

#Generate residual values

```
sheep_dat$resids <- residuals(sheep_final_mod)
sheep_dat
```

#Perform a Shapiro-Wilk test for normality of residuals

```
shapiro.test(sheep_dat$resids)
```

#Perform Levene's Test for homogeneity of variances

```
library(car)
```

#Testing H0V among treatments

```
leveneTest(gain ~ sex_est, data = sheep_dat)
```

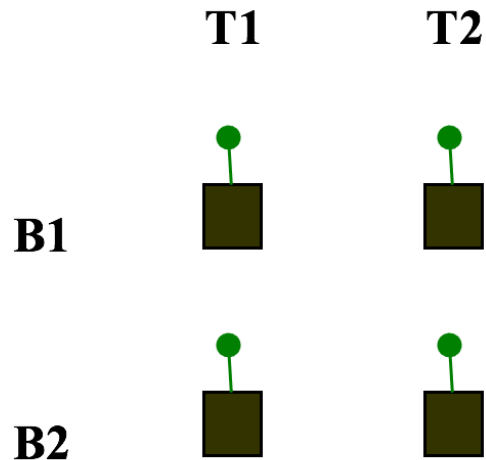
Shapiro-Wilk normality test

```
data:  sheep_dat$resids  
W = 0.9662, p-value = 0.4022
```

Levene's Test for Homogeneity of Variance (center = median)

	Df	F value	Pr(>F)
group	3	1.601	0.2114

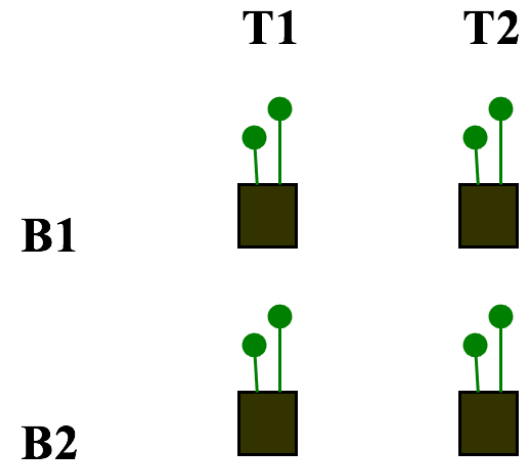
RCBD 1 rep/cell



`lm(Y ~ Block + Trtmt, X_dat)`

Tukey Test Required

RCBD 1 rep/cell with subsamples



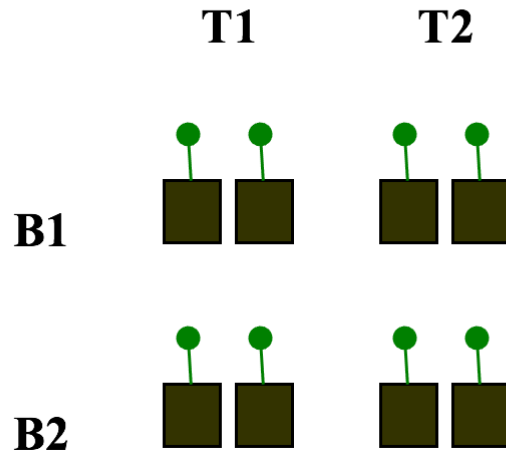
`within(X_dat, Pot <- (Block:Trtmt))`
`lm(Y ~ Block + Trtmt + Pot, X_dat)`

Custom F tests for Block and Trtmt

$MSEE = MS_{Pot}$

Tukey Test Required

RCBD >1 rep/cell



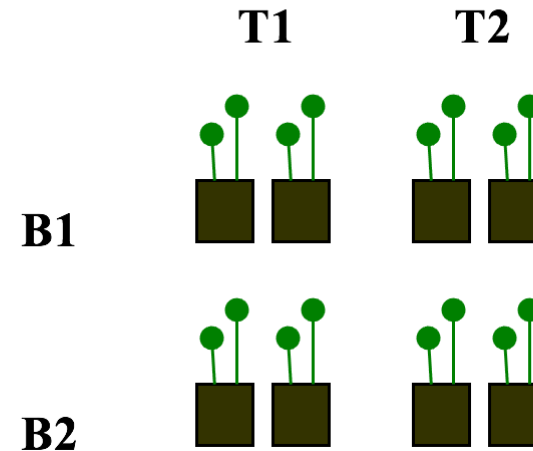
`lm(Y ~ Block*Trtmt, X_dat)`

Tukey Test not Required

Custom F tests for Block and Trtmt

error = $MS_{Block:Trtmt}$

RCBD >1 rep/cell with subsamples



`within(X_dat, Pot <- (Block:Trtmt))`
`lm(Y ~ Block*Trtmt + Pot, X_dat)`

Tukey Test not Required

Custom F test for Block:Trtmt

MSEE = MS_{Pot}

Custom F tests for Block and Trtmt

error = $MS_{Block:Trtmt}$