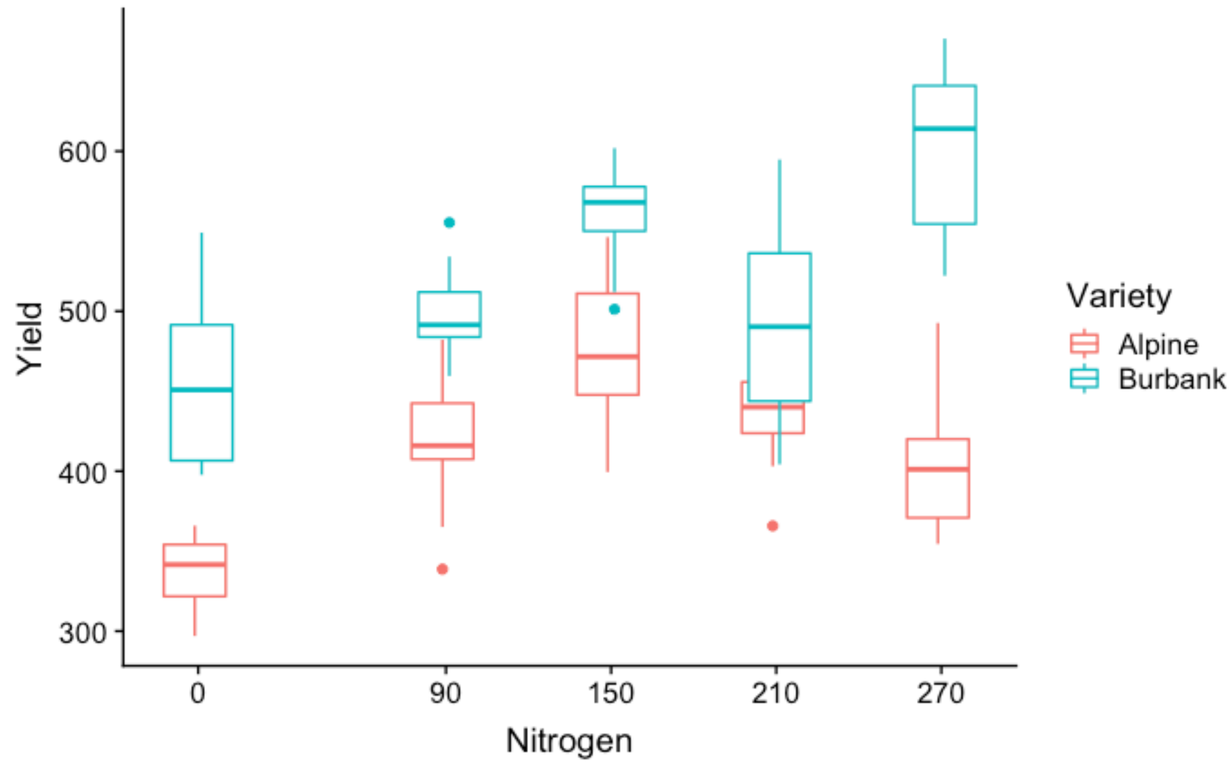
Modified potato experiment:

- 100 plots. Each receive either Alpine or Burbank variety and one of the 5 levels of Nitrogen
- Response = Yield



What was the experimental unit?

List Research Questions:

Does the effect of +Nitrogen depend on which Variety you grow?

Does +Nitrogen modify the difference between Varieties?

Is there an effect of Variety at any level of Nitrogen?

Factorial experiment

2+ class of treatments

focal treatment (Variety), **moderator** treatment (Nitrogen)

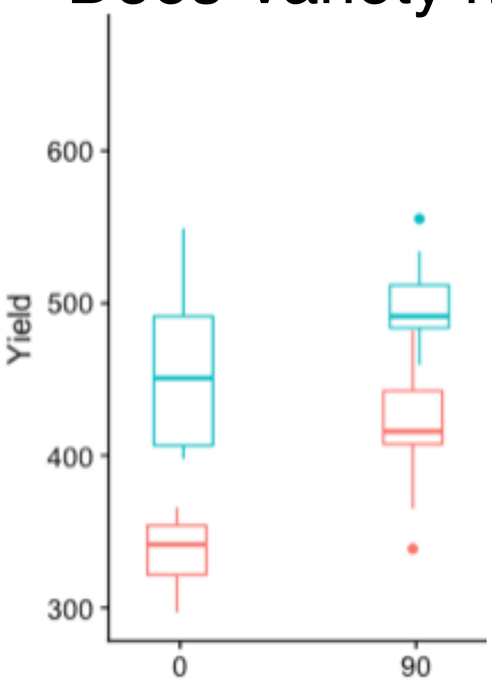
Each level of **focal treatment** tested in combination with every level of **moderator treatment**

		Nitrogen				
		0	90	150	210	270
Variety	Alpine					
	Burbank					

“2 x 5 Factorial” 10 total treatments: **A:0, A:90, B:0, B:210,…**

5 experiments of Variety under different conditions (Nitrogen)

Does Variety have an effect at **any level** of Nitrogen?



	Nitrogen	
$\hat{\mu}_i$	0	90
Alpine	344	416
Burbank	454	598
$\hat{\delta}_{Var}$	110	82
SED	18.5	18.5
CI_L	67.5	39.5

$n_i = 10$

$s^2 = 1707$

Think of this like 2 parallel experiments:

1. Estimate $\hat{\delta}_{Var}$ for **each level** of Nitrogen
2. Calculate the two SEDs

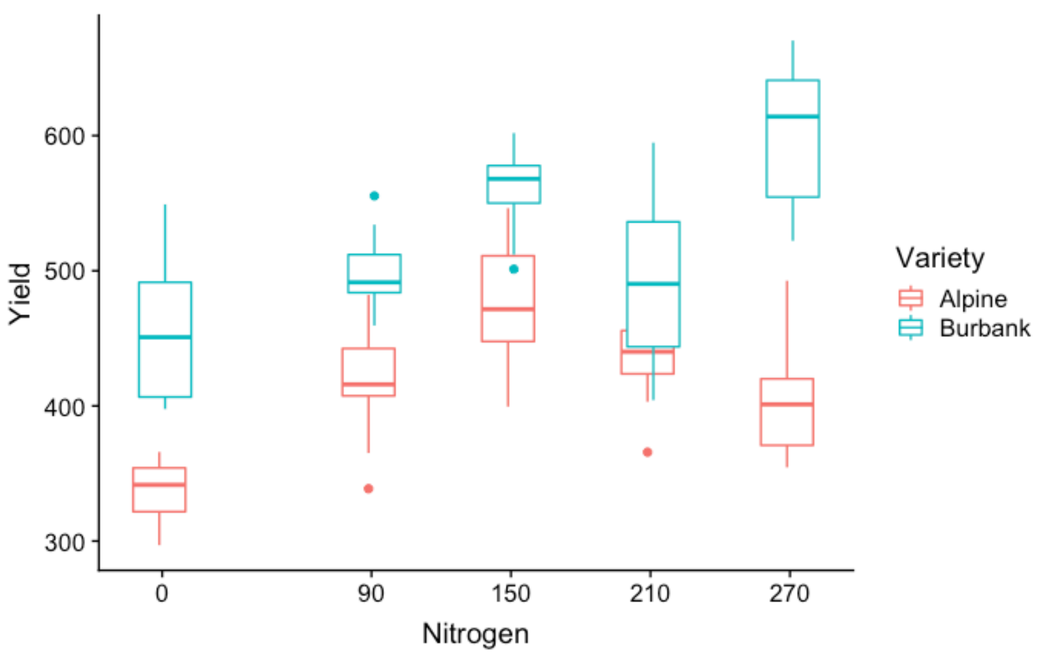
   Indirect design
3. See if any confidence interval doesn't cross zero

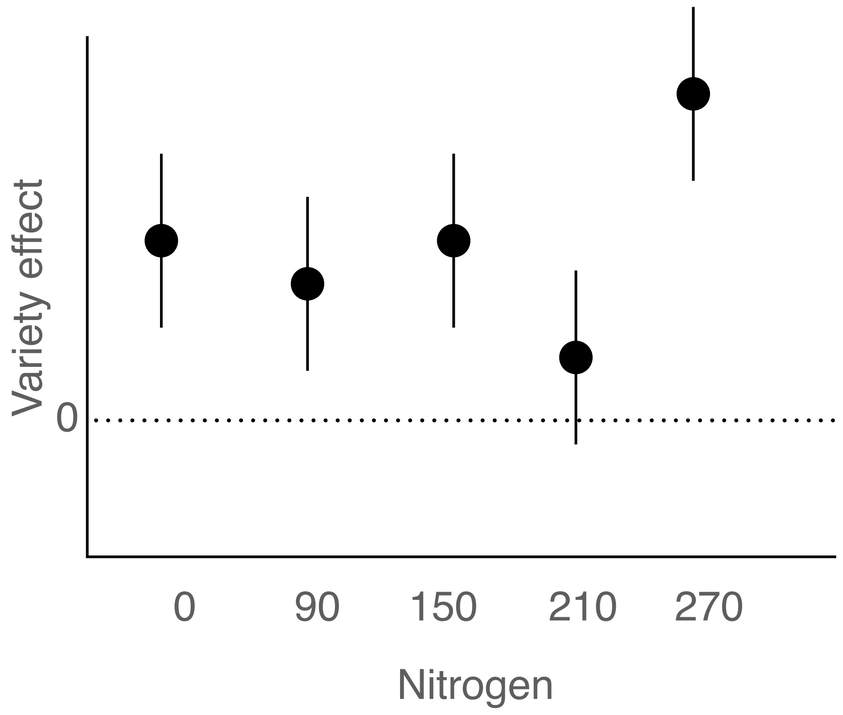
   * accounting for multiple testing by the Bonferroni method

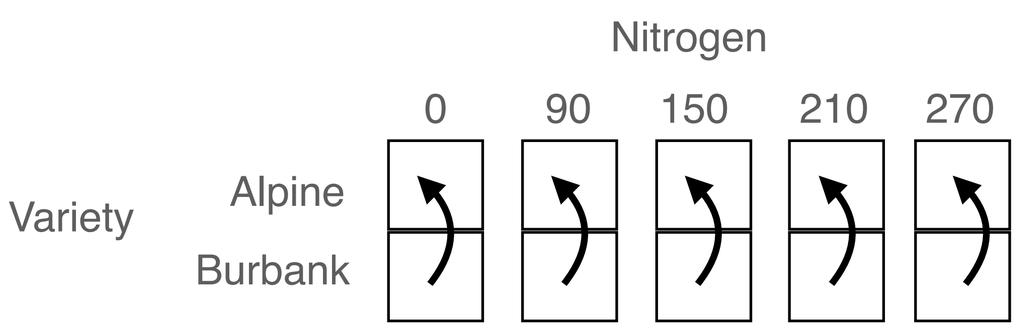
   replace α with α/k

$$t_{\frac{(0.05/2)}{2},90} = 2.3$$

Think of this like 5 parallel experiments:







With 5 levels of Nitrogen, we can estimate the **Variety effect** (Burbank - Alpine) 5 times

Specific effects — effect of Variety at **each specific** level of Nitrogen

ANOVA: Is there ever evidence of a Variety effect?

Does Variety have an effect at **any level** of Nitrogen?

Goal: Measure **specific effects** of Variety (Variety effects at each level of Nitrogen)

Treatment

Structure	Variable	Type	#levels	Replicate	EU
Focal	Variety	Cat	2	Nitrogen	Plot
Moderator	Nitrogen	Cat	5	None	Plot
Combos	Variety:Nitrogen	Cat	10	None	Plot
Design	Plot	Cat	100		
Response	Yield	Num	100		

levels = **actual** # levels in your dataset

In some experiments all possible combinations are not created

Treatment

List Focal, Moderator treatments

Moderator is a **replicate** for the focal treatment

Form Combos with replicates: Focal:Moderator

Combos of 2 treatment variables are **also** treatment variables

EU: go through the EU rules for each, ignoring others

Replicate: Don’t use focal variable as a replicate

Only Design or Moderator/Combo treatments

Design table:

Variables in the **EU** section **must be** Design variables and declared Random

Variables in the **Replicate** section could be Moderators or Design

Need Treatment:Replicate as a Variable

Model

Drop all rows with #levels < # responses

Only EU need to be random. Treatment:Replicate **can** be random if not a treatment

For specific effect ANOVA: **drop focal treatment variable**

Doesn’t matter for emmeans() analysis

lm(Yield ~ ~~Variety~~ + Nitrogen + Variety:Nitrogen)

Does Variety have an effect at **any level** of Nitrogen?

Goal: Measure **specific effects** of Variety

Treatment

Structure	Variable	Type	#levels	Replicate	EU
Focal	Variety	Cat	2	Nitrogen	Plot
Moderator	Nitrogen	Cat	5	None	Plot
Combos	Variety:Nitrogen	Cat	10	None	Plot
Design	Plot	Cat	100		
Response	Yield	Num	100		

Analysis

1) Fit model: lm() or lmer()

2) Model diagnostics: pls205_diagnostics(), specify EUs if they are a term in the model

3) (optional) ANOVA

```
Response: Yield
                 Df Sum Sq Mean Sq F value    Pr(>F)
Nitrogen          4 167984   41996  21.062  2.79e-12 ***
Variety:Nitrogen  5 340605   68121  34.164 < 2.2e-16 ***
Residuals        90 179456    1994
```

NumDF =
moderator levels *
Focal levels - 1

= # focal effects

4) Estimate the Variety effects at each level of Nitrogen using emmeans() and contrast()

a) Calculate means for Variety at each level of Nitrogen

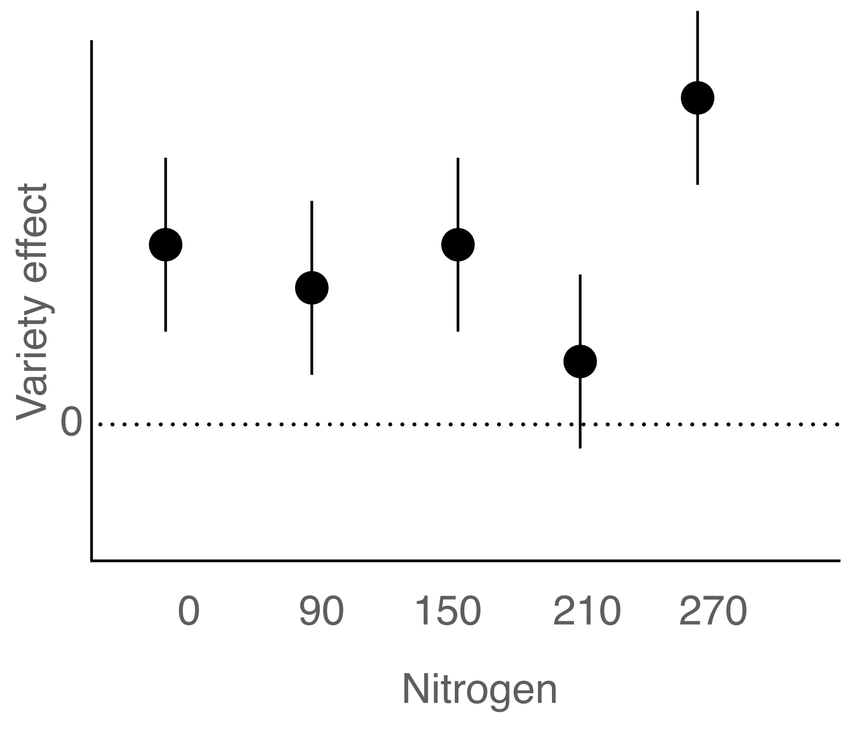
emmeans(model,specs = ‘Variety’, by = ‘Nitrogen’)

```
Nitrogen = 0:
 Variety emmean   SE df lower.CL upper.CL
 Alpine     345 14.1 90      316      373
 Burbank    455 14.1 90      427      483

Nitrogen = 90:
 Variety emmean   SE df lower.CL upper.CL
 Alpine     416 14.1 90      388      444
 Burbank    499 14.1 90      471      527
```

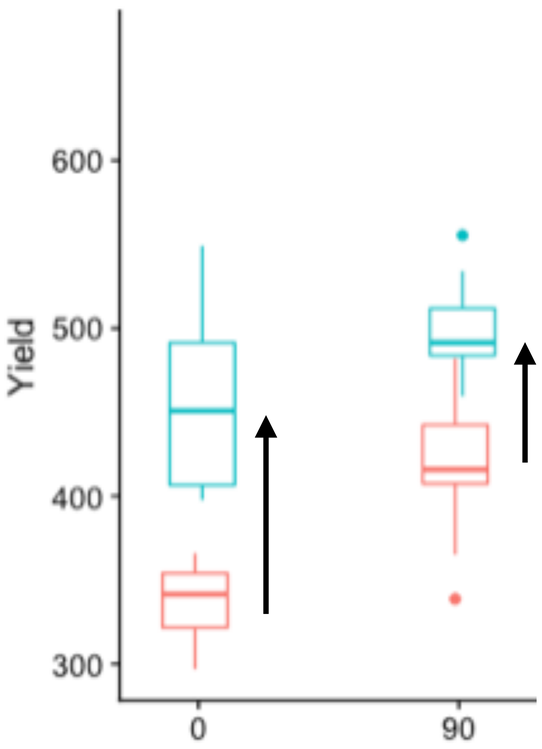
b) Contrast means within each Nitrogen level

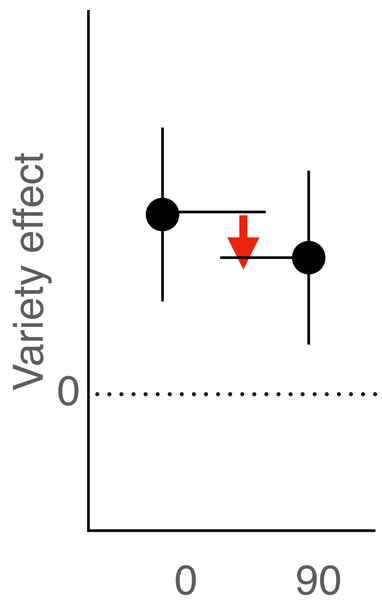
contrast(means,’pairwise’)

```
Nitrogen = 0:
 contrast         estimate SE df t.ratio p.value
 Burbank - Alpine    110.3 20 90   5.522  <.0001

Nitrogen = 90:
 contrast         estimate SE df t.ratio p.value
 Burbank - Alpine     82.3 20 90   4.120  0.0001
```



Does +Nitrogen **modify** the Variety effect?





Think of this like an indirect experiment

but comparing **effects** instead of **means**

Interaction — **change in** effect of Variety **between different** levels of Nitrogen

Difference of differences

"How much more is the Variety effect at 90lbs vs 0lbs Nitrogen?"

Estimated variety effects (Burbank - Alpine)

	Nitrogen 0	Nitrogen 90
$\hat{\delta}_{Var}$	110	82
SEs	18.5	18.5

$\hat{\delta}_{Var,90} - \hat{\delta}_{Var,0}$ -28

"Interaction"

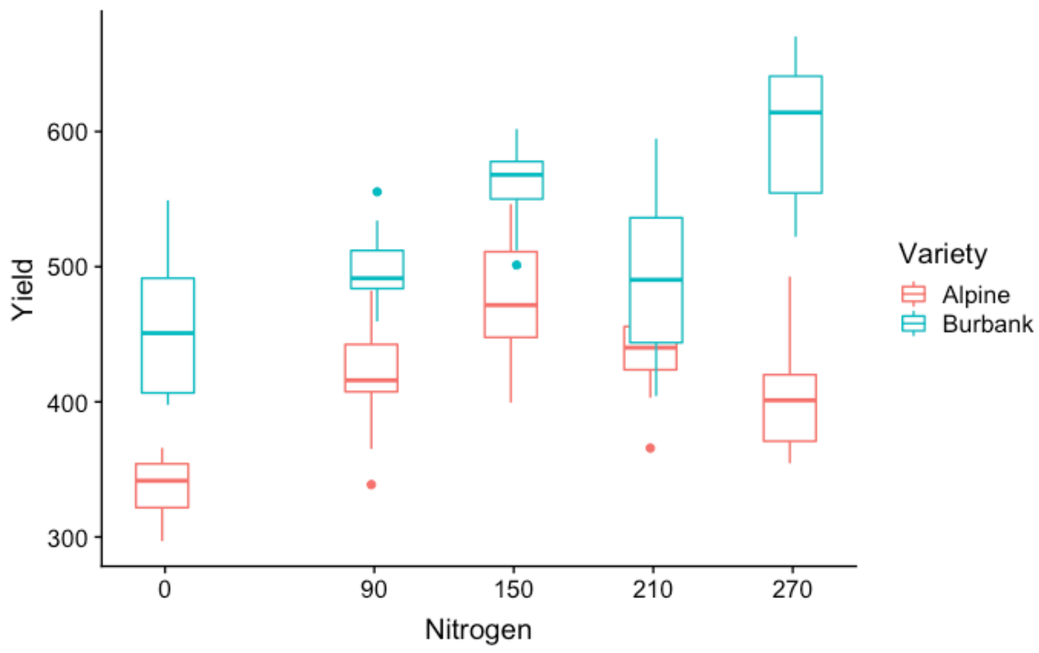
SEI 26.1

estimate = subtract: (treatment effect 2) - (treatment effect 1)

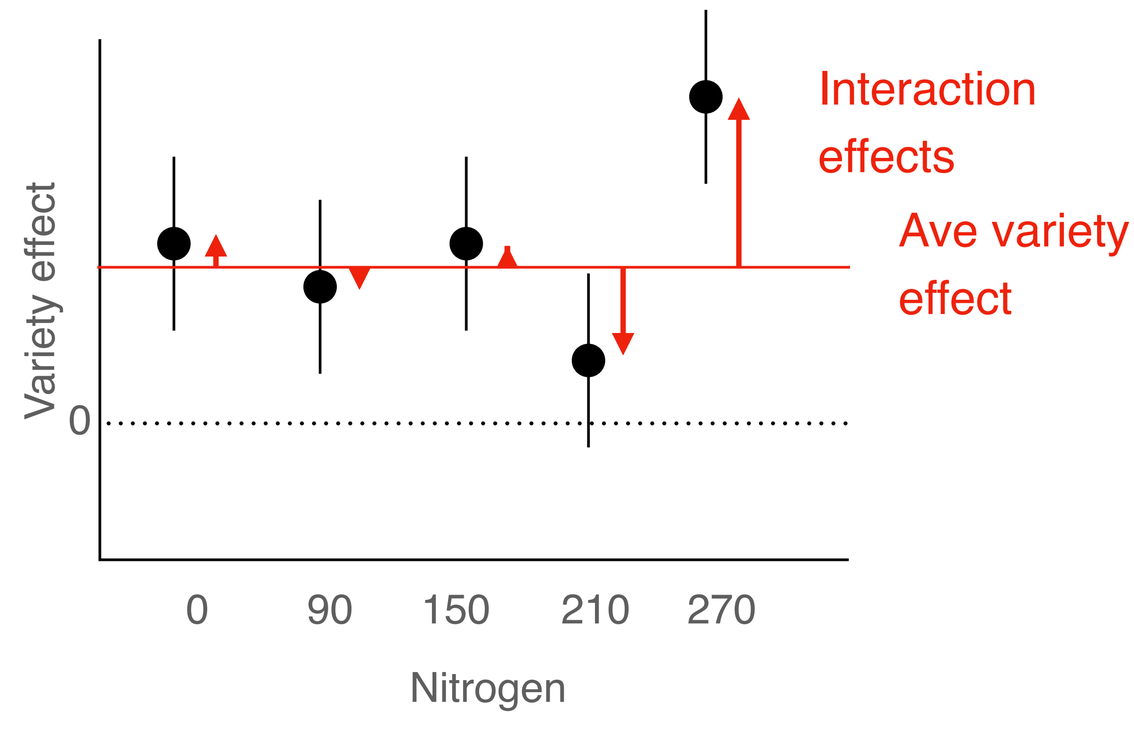
$$\sigma_r(\hat{I}) = \sqrt{\sigma_r^2(\hat{\delta}_2) + \sigma_r^2(\hat{\delta}_1)}$$

$$\sigma_r(\hat{\delta}) = \sqrt{\sigma_r^2(\hat{\mu}_2) + \sigma_r^2(\hat{\mu}_1)}$$

Which SE is larger? by how much?



$(\bar{\hat{\delta}})$



With 5 levels of Nitrogen, we can estimate the **Variety effect** (Burbank - Alpine) 5 times

Interaction effects — Deviations of the **specific effects** from the average

Tukey: Are any **pairwise contrasts** significant?

ANOVA: Are all variety effects the same?

Does +Nitrogen **modify** the Variety effect?

Goal: Measure **Interaction Effects** (changes in Variety effects between levels of Nitrogen)

Treatment

Structure	Variable	Type	#levels	Replicate	EU
Focal	Variety	Cat	2	Nitrogen	Plot
Moderator	Nitrogen	Cat	5	None	Plot
Combos	Variety:Nitrogen	Cat	10	None	Plot
Design	Plot	Cat	100		
Response	Yield	Num	100		

Same table

Model

Drop all rows with #levels < # responses

Only EU need to be random. Treatment:Replicate **can** be random if not a treatment

For interaction effect ANOVA: **keep focal treatment variable!**

Doesn't matter for emmeans() analysis

lm(Yield ~ Variety + Nitrogen + Variety:Nitrogen)

Analysis

1) Fit model: lm() or lmer()

2) Model diagnostics: pls205_diagnostics(), specify EUs if they are a term in the model

3) (optional) ANOVA

```
Response: Yield
                 Df Sum Sq Mean Sq  F value    Pr(>F)
Nitrogen          4 167984   41996  21.0617 2.790e-12 ***
Variety           1 280604  280604 140.7279 < 2.2e-16 ***
Variety:Nitrogen  4  60001   15000   7.5229 2.831e-05 ***
Residuals        90 179456    1994
```

NumDF =
(# moderator levels - 1) *
(# Focal levels - 1)

Does +Nitrogen **modify** the Variety effect?

Goal: Measure **Interaction Effects** (changes in Variety effects between levels of Nitrogen)

Treatment

Structure	Variable	Type	#levels	Replicate	EU
Focal	Variety	Cat	2	Nitrogen	Plot
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Same table

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a) Calculate means for Variety at each level of Nitrogen

emmeans(model,specs = ‘Variety’, by = ‘Nitrogen’)

```
Nitrogen = 0:
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b) Contrast means within each Nitrogen level

effects = contrast(means,’pairwise’)

```
Nitrogen = 0:
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 Burbank - Alpine      82.3 20 90   4.120  0.0001
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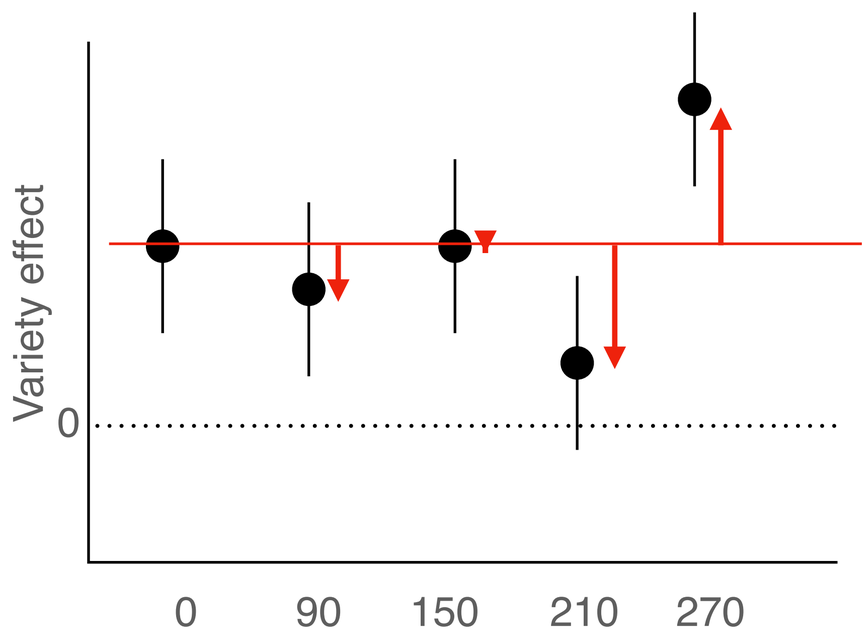


5) Contrast treatment effects (Burbank - Alpine) among levels of Nitrogen

contrast(effects,’trt.vs.ctrl’)

```
contrast = Burbank - Alpine:
 contrast1                estimate   SE df t.ratio p.value
 Nitrogen90 - Nitrogen0      -28.0 28.2 90  -0.991  0.6906
 Nitrogen150 - Nitrogen0     -24.2 28.2 90  -0.856  0.7706
 Nitrogen210 - Nitrogen0     -56.3 28.2 90  -1.995  0.1565
 Nitrogen270 - Nitrogen0      86.9 28.2 90   3.077  0.0103

P value adjustment: dunnettx method for 4 tests
```

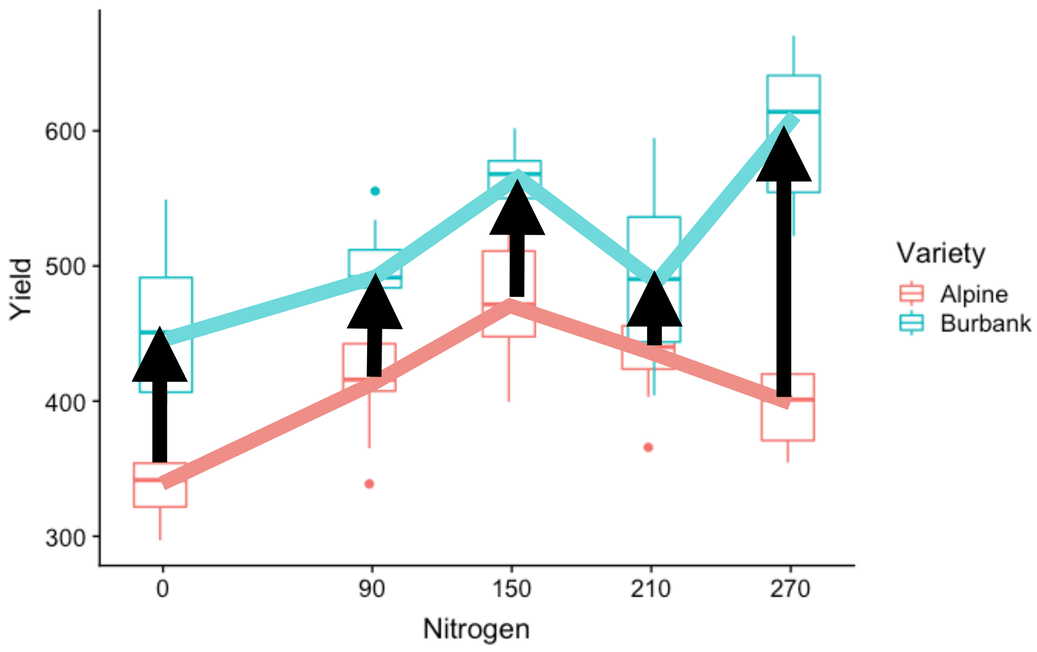
Visualizing Factorials

Treatment matrix:

Variety	Nitrogen 0	90	150	210	270
Alpine	10	10	10	10	10
Burbank	10	10	10	10	10

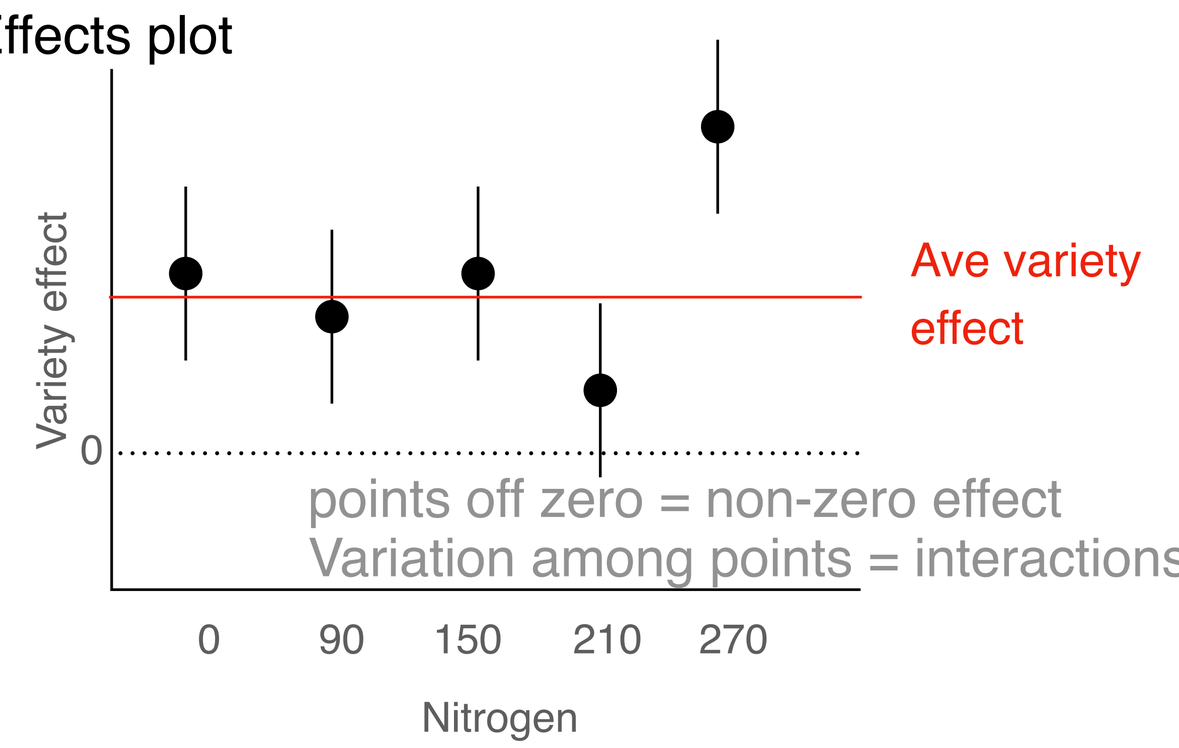
plots / treatment combo

Interaction plot

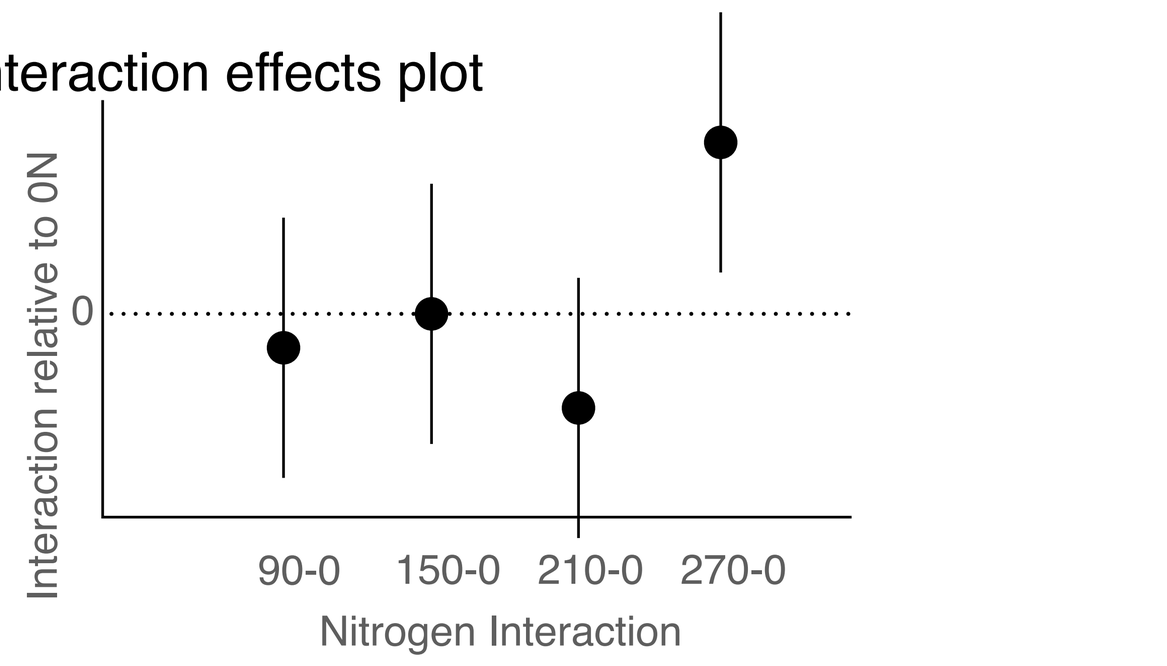


parallel lines = no interaction

Effects plot



Interaction effects plot



Challenge:

Write the model table, model, and effects plot for specific effects of Nitrogen with Variety as the moderator

How many specific effects using ‘trt.vs.ctrl’ contrasts? ‘pairwise’?

How many Interactions using ‘trt.vs.ctrl’ contrasts? ‘pairwise’?