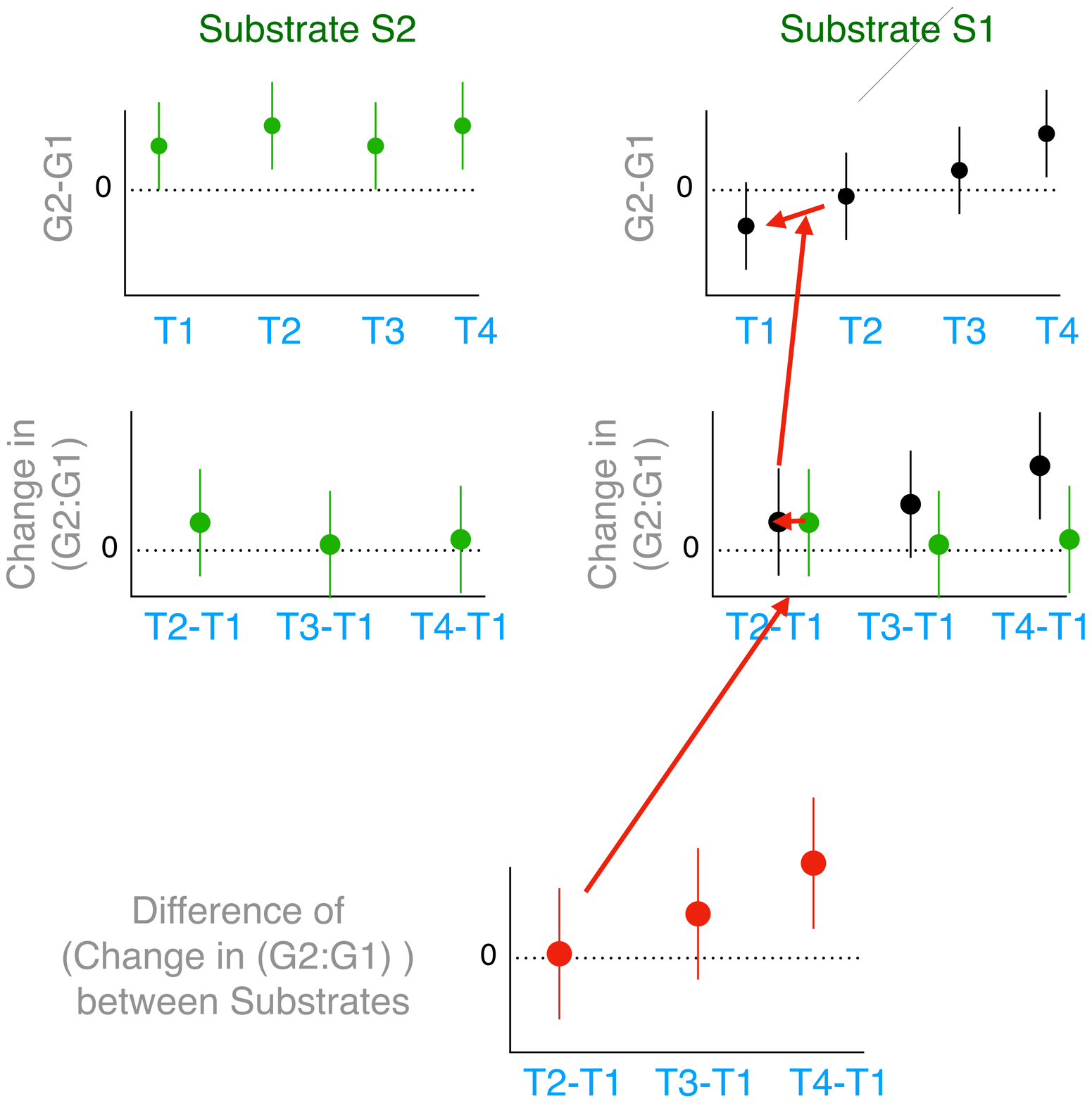
Three-way interactions are hard to interpret



Specific effects:

Differences among genotypes at each Temp

…for each substrate

2-way Interaction effects:

Change in genotype effects **among different Temps**

…for each substrate

3-way Interaction effects:

How the “change in genotype effects among different Temps” **differs depending on Substrate**

Geno:Temp:Substrate

Not: Does Genotype interact with Temp **or** Substrate

Not: Do specific combinations of Temp and Substrate alter Genotype effects

Not: Does Genotype matter in any Substrate and/or Temperature

Yes: How different are the temperature effects on genotype differences between substrates?

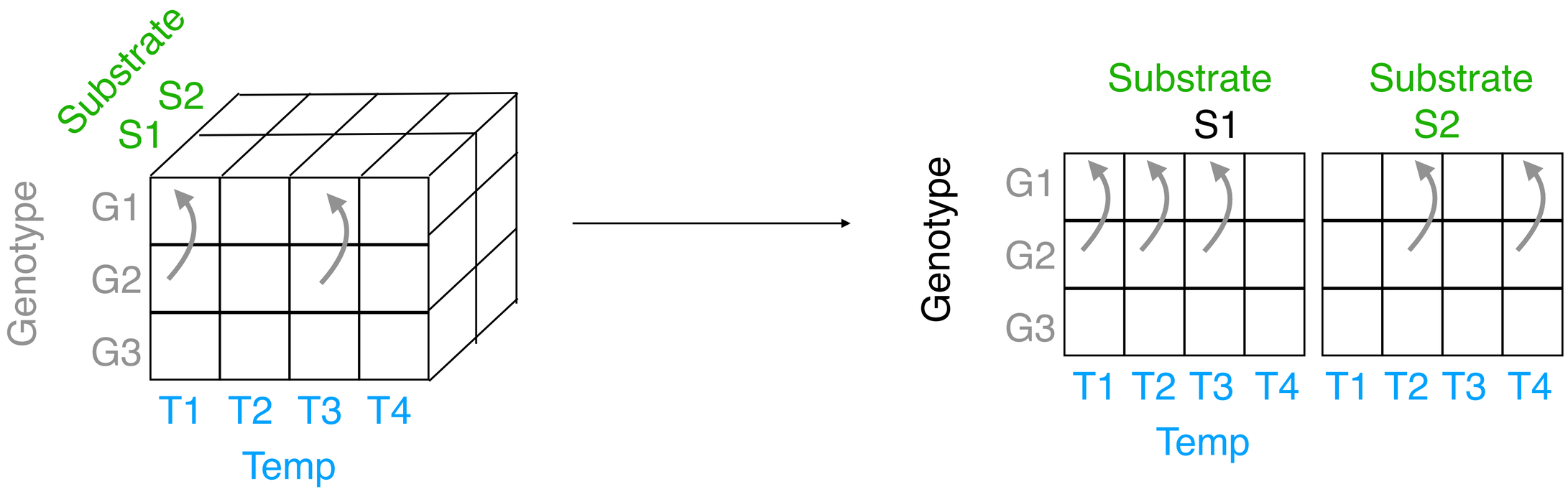
3-way (or more) factorials are very common. WHY?

Solution: Think of them as 2-way factorials

1) Think of them as a big 2-way factorial by combining moderators into 1 treatment

2) Think of the **focal effect** as the response, and **moderator 1** as the focal

Three-way factorial as a big 2-way factorial



1) Does Genotype have an effect in **any combination of Temp and Substrate?**

focal: Genotype moderator: Temp:Substrate

strategy1: create new variable Temp_Substrate

strategy2: use by = c('Temp','Substrate') in emmeans()

correct for number of levels of Temp:Substrate

2) Does any combination environment (combination of Temp and Substrate) modify Genotype effects?

focal: Genotype moderator: Temp_Substrate

Treatment

Structure	Variable	Type	#levels	Replicate	EU
Focal	Genotype	Cat	3	Temp:Substrate	Beetle
Moderator	Temp:Substrate	Cat	8	None	Beetle
Combos	Geno:Temp:Subst	Cat	24	None	Beetle
Design	Beetle	Cat	72		
Response	Activity	Num	72		

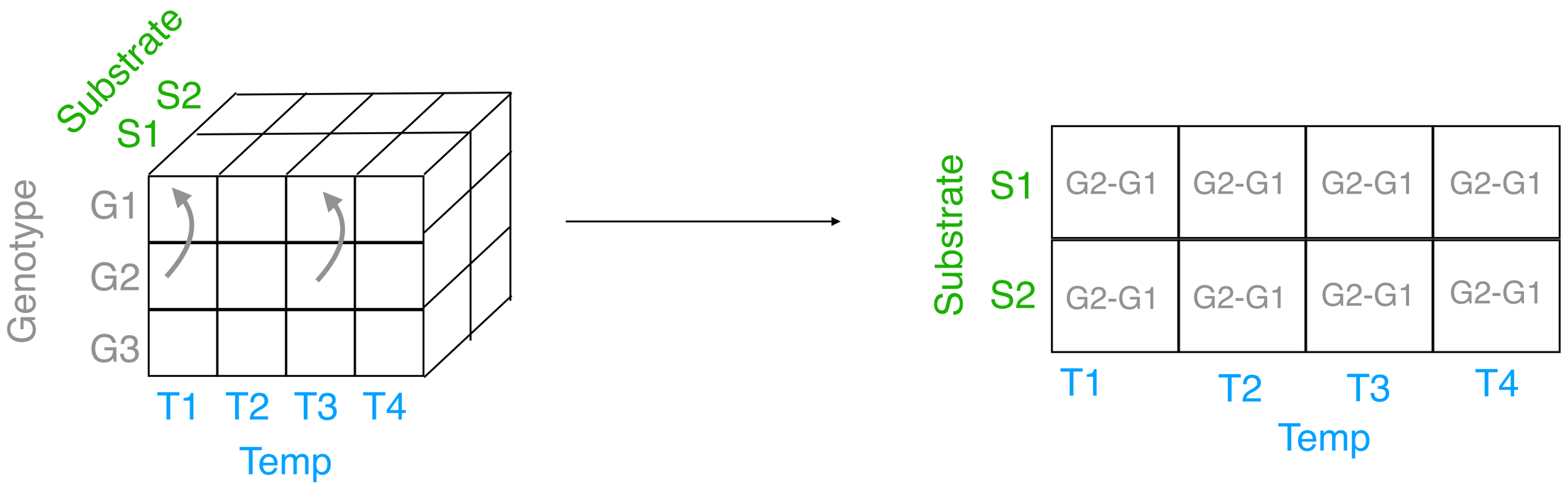
* This works for emmeans(), for ANOVA you need to create "Temp_Substrate"

Write the model for 1 (Specific Effects) and 2 (Iteractions)

1) lm(Activity ~ Temp_Substrate + Geno:Temp_Substrate)

2) lm(Activity ~ Geno + Temp_Substrate + Geno:Temp_Substrate)

Three-way factorial as 2-way with Geno_effect as the response



Think of the “Genotype effect” as a property of a beetle.

“What happens to it when you mutate a specific gene?”

Like: “What does your pulse do when you stand up”?

We could imagine measuring this on each beetle

Think of this as the response

Substrate effect (new focal treatment)

Do we see the effect of mutations in some substrates but not others?

Specific effects: Is there a substrate effect on the mutation at any temperature?

Interaction effect: Do we see substrate effects in some temperatures more than others?

Strategy

1) Estimate Genotype effects (G2-G1) in each combo of Temp and Substrate

emmeans() -> contrast()

2) Treat these effects as you would focal treatment means

1) Calculate Specific Effects of Substrate (focal) on these estimates (with by = ‘Temp’)

2) Calculate Interaction Effects of Temp:Substrate by regrouping and contrasting the specific effects

3) Report Specific effects and/or Interactions on this new trait: “Geno_effect”

ANOVA

Identify the analysis represented by each ANOVA table:

Write out a statement in words **without using the word "Interaction"**

We only look at the last row!

```
Response: Activity
                    Df Sum Sq Mean Sq F value    Pr(>F)
Temp                 3  27270  9090.1 11.4686 8.671e-06 ***
Geno                 2   2365  1182.6  1.4920 0.2351727
Substrate            1  11167 11167.3 14.0892 0.0004706 ***
Temp:Geno            6  24834  4138.9  5.2219 0.0003319 ***
Temp:Substrate       3  18170  6056.6  7.6413 0.0002831 ***
Geno:Substrate       2  10025  5012.4  6.3239 0.0036494 **
Temp:Geno:Substrate  6    157    26.2  0.0330 0.9998321
Residuals           48  38045   792.6
```

3-way interaction

How temperature modifies the effect of substrate on how much Genotype matters for a beetle

```
Response: Activity
                    Df Sum Sq Mean Sq F value    Pr(>F)
Geno                 2   2365  1182.6  1.4920  0.235173
Temp_Substrate       7  56607  8086.8 10.2027 9.467e-08 ***
Geno:Temp_Substrate 14  35015  2501.1  3.1555  0.001507 **
Residuals           48  38045   792.6
```

Big 2-way interactions

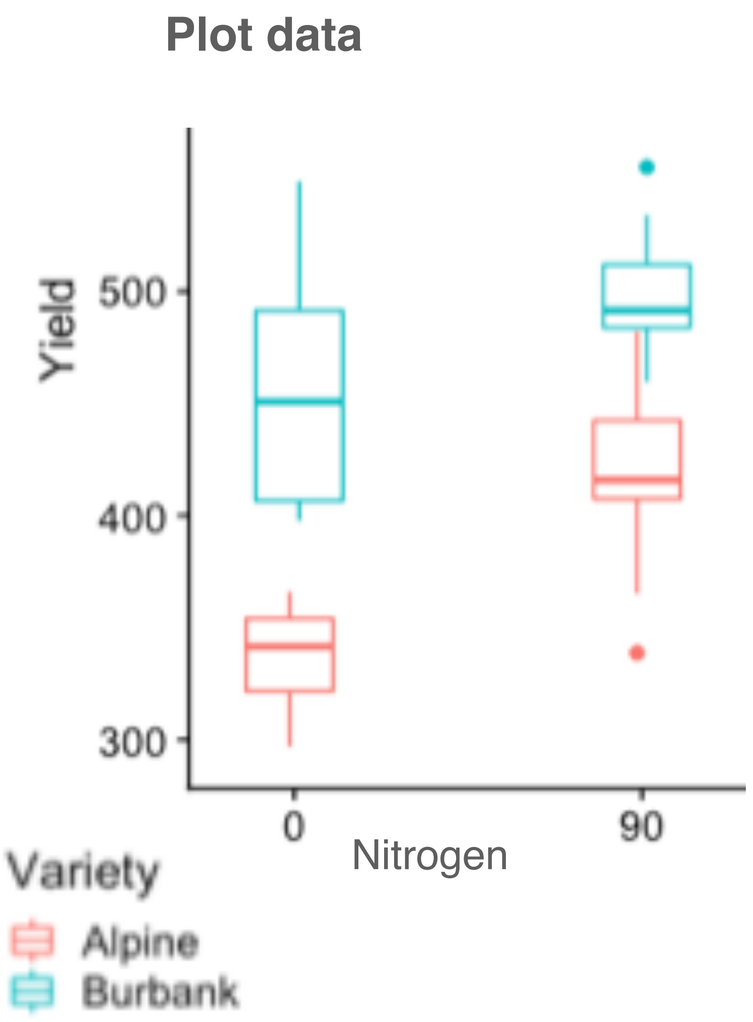
Do any combinations of Temp and Substrate alter the Genotype effects?

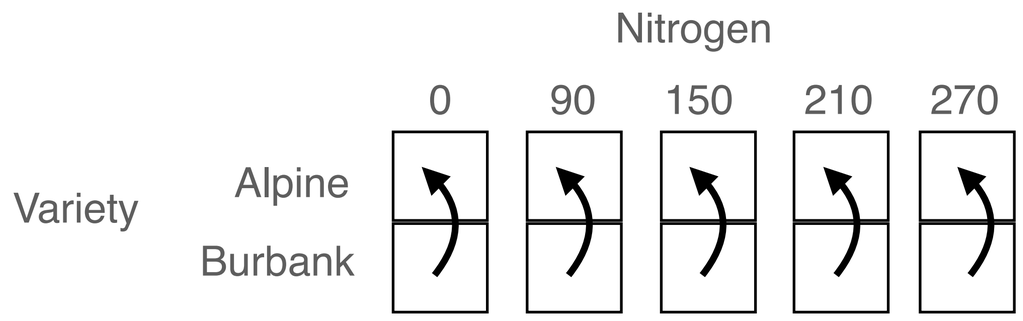
```
Response: Activity
                    Df Sum Sq Mean Sq F value    Pr(>F)
Temp_Substrate       7  56607  8086.8 10.2027 9.467e-08 ***
Temp_Substrate:Geno 16  37380  2336.3  2.9476  0.001942 **
Residuals           48  38045   792.6
```

Big 2-way specific effects

Does Genotype matter in any combination of Temp or Substrate?

Interaction effects in a factorial





Define treatments of interest

focal = Variety, moderator = Nitrogen

Calculate effects of focal **at each level** of moderator

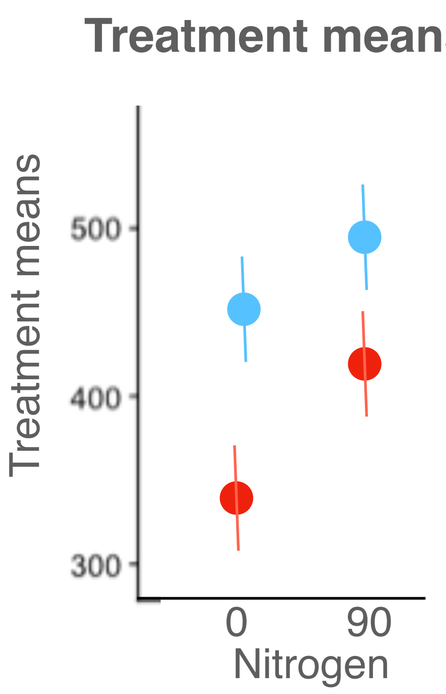
Calculate moderator effect **on** focal effects

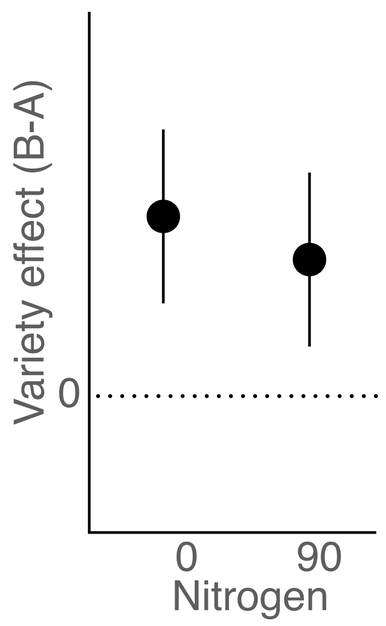
This is the Interaction

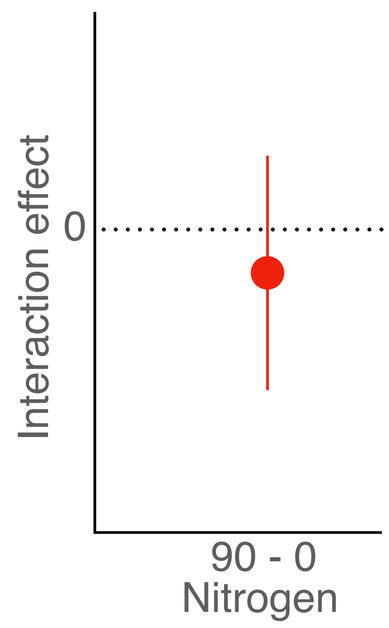
Notice what happens to the SE calculations!

points = EU estimates

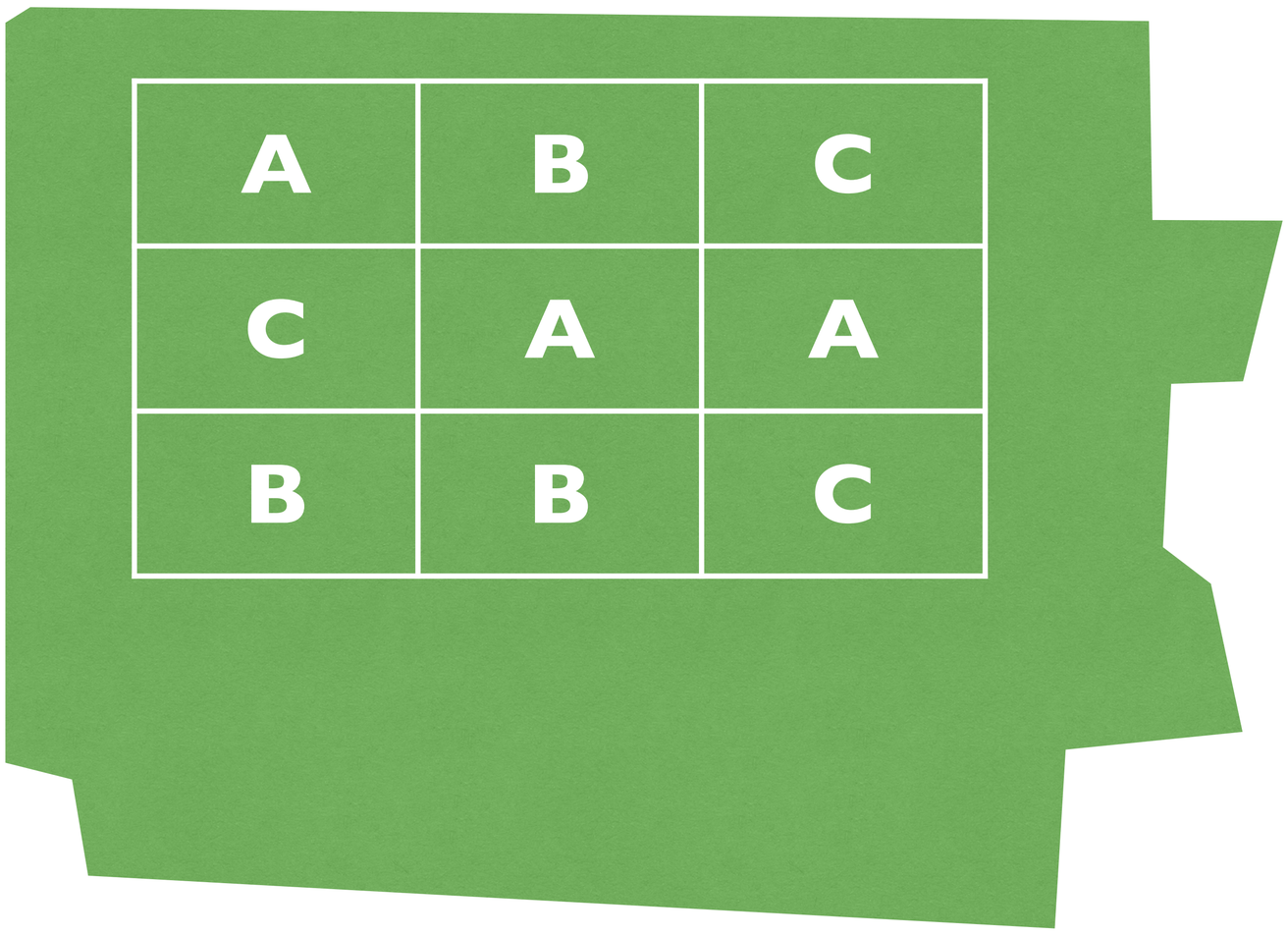
$$\sigma^2_{\hat{\mu}_i} = \sigma^2_{\mu_{ij}} + \sigma^2_m$$







	Ave value of treatment combination	**Effect** of focal trt at each level of mediator	**Effect** of mediator on focal effect
estimate	$(\hat{\mu}_i)$ mean of plots	$(\hat{\delta}_j)$ difference between trt means	$(\hat{I}_k)$ difference between variety effects
SE	$\sqrt{\sigma^2_{\hat{\mu}_i}/n_i} = \sqrt{\sigma^2_r(\hat{\mu}_i)}$	$\sqrt{\sigma^2_r(\hat{\mu}_1) + \sigma^2_r(\hat{\mu}_2)} = \sqrt{\sigma^2_r(\hat{\delta}_j)}$	$\sqrt{\sigma^2_r(\hat{\delta}_1) + \sigma^2_r(\hat{\delta}_2)}$
	averaging $n_i = 10$ plots	averaging $n_j = 1$ treatment means	



An experiment was run to compare three Insecticides applied to a field of wheat

The field was divided into 9 large plots

Each plot was randomly assigned on Insecticide

Insect counts were made 2 days later

Is this design good?

What is the EU?

Plot

How are **treatment effects** estimated? Direct or Indirect?

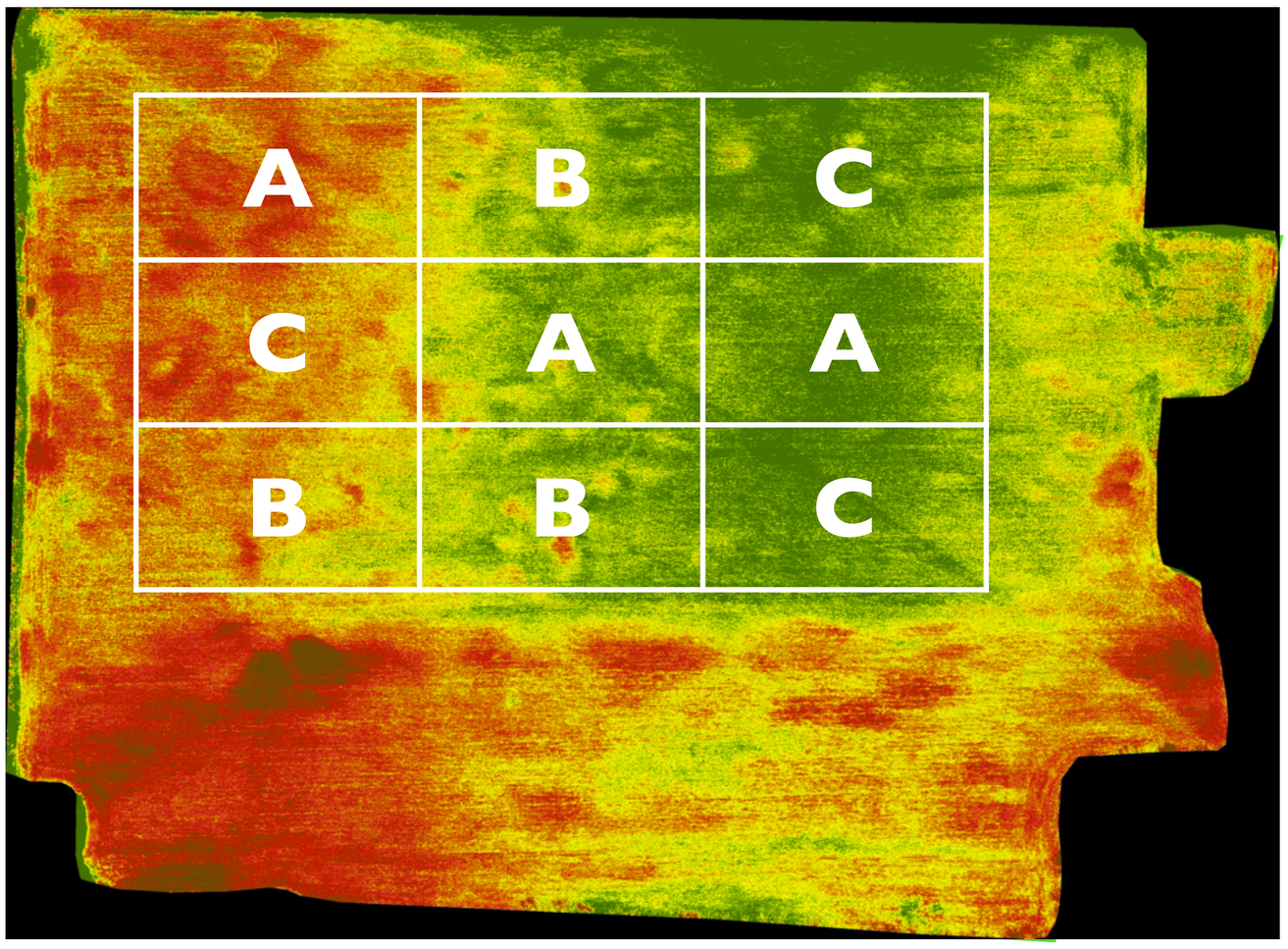
Indirect: $\hat{\mu}_B - \hat{\mu}_A$ or $\hat{\mu}_C - \hat{\mu}_A$, …

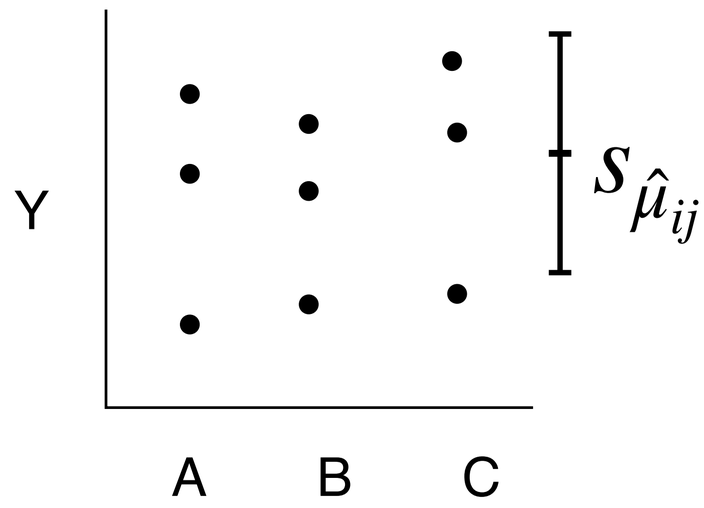
What is the formula for the standard error of the treatment effect? SED?

$$\sigma_r(\hat{\delta}) = \sqrt{\frac{\sigma^2_{plot} + \sigma^2_m}{n_B} + \frac{\sigma^2_{plot} + \sigma^2_m}{n_A}}$$

$$\text{SED} = \sqrt{\frac{s^2_{\hat{\mu}_i}}{3} + \frac{s^2_{\hat{\mu}_i}}{3}}$$

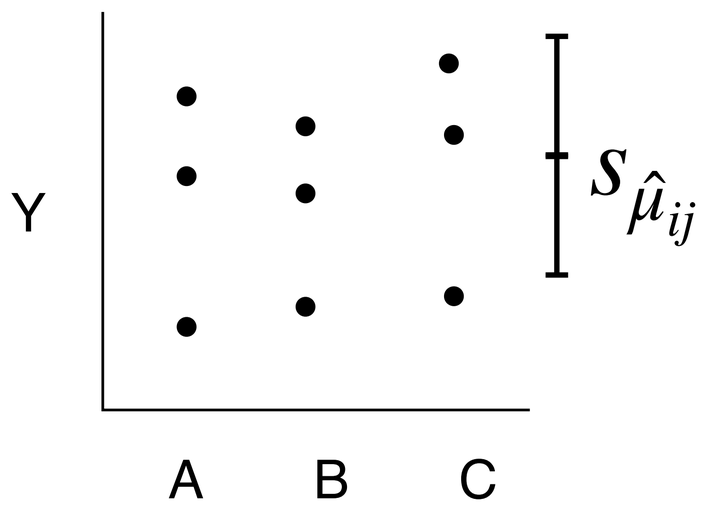
What if we assayed the wheat beforehand and saw this? Could your design be improved?



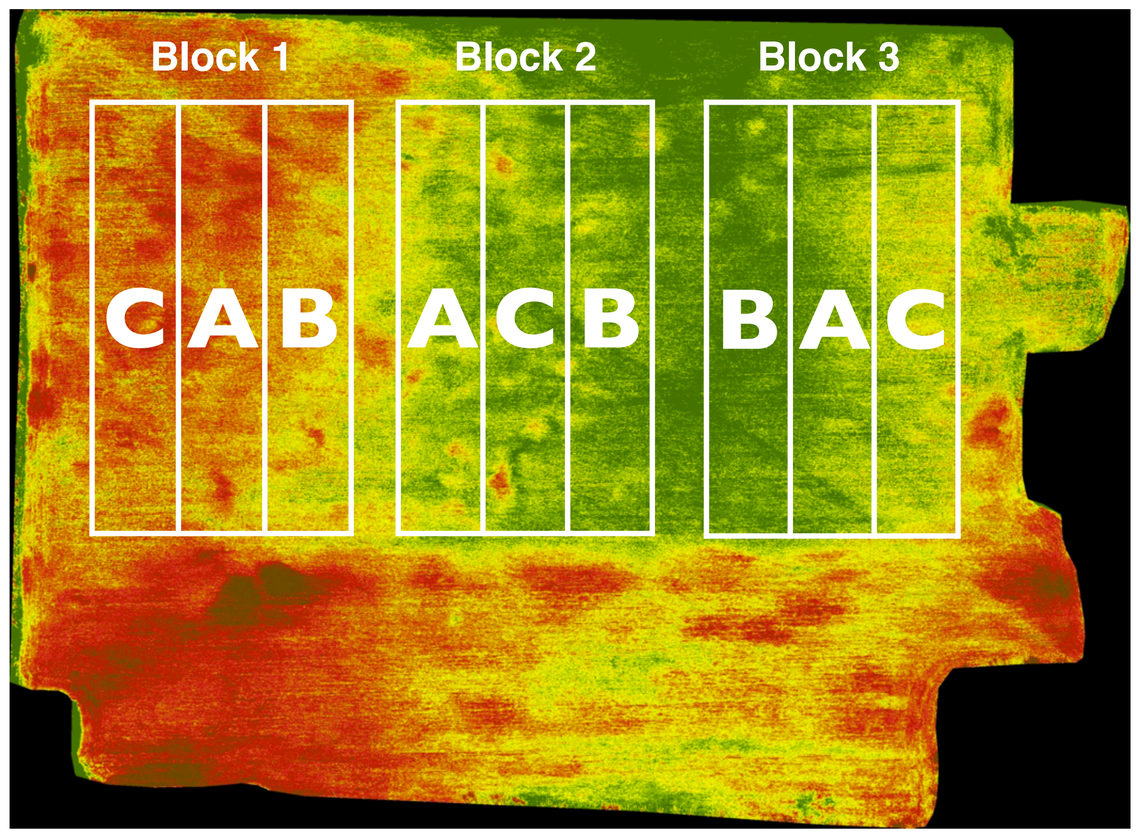


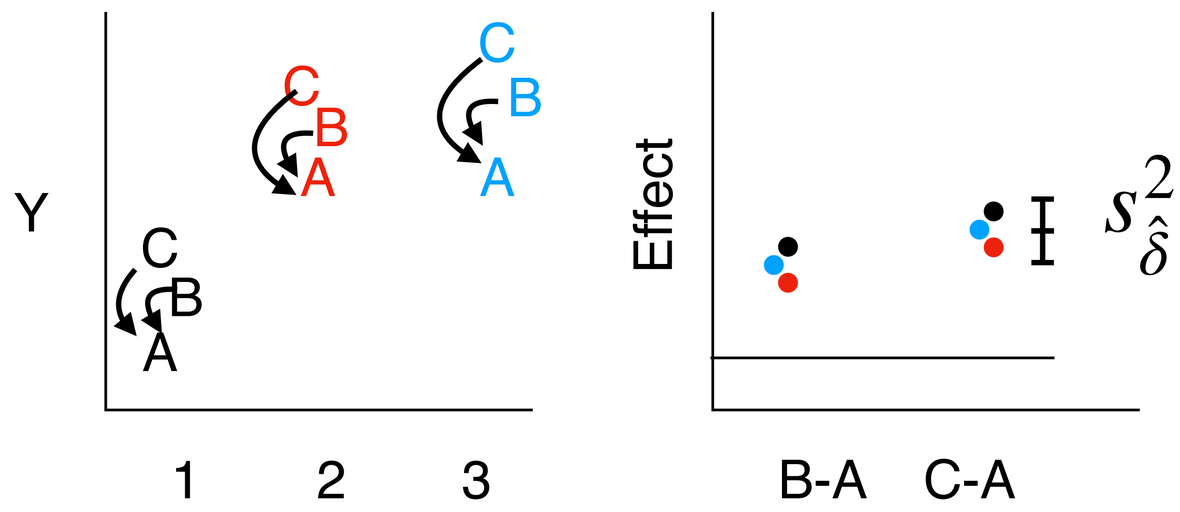
$s^2_{\hat{\mu}_i}$ will be large because of the variation among plots (within treatment levels)

Completely **R**andomized **D**esign



Randomized **C**omplete **B**lock **D**esign





Indirect design

$$\sigma_r(\hat{\delta}) = \sqrt{\frac{\sigma^2_{plot} + \sigma^2_m}{n_B} + \frac{\sigma^2_{plot} + \sigma^2_m}{n_A}}$$

$$\text{SED} = \sqrt{\frac{s^2_{\hat{\mu}_i}}{3} + \frac{s^2_{\hat{\mu}_i}}{3}}$$

Direct design

$$\sigma_r(\hat{\delta}) = \sqrt{\frac{\sigma^2_{effects} + 2\sigma^2_m}{n}}$$

$$\text{SED} = \sqrt{\frac{s^2_{\hat{\delta}}}{3}}$$

RCBD

Each Block has one plot for **each** treatment level

Blocks are **Replicates**

Direct design: Treatment effects are measured in each block

Average Treatment Effect is average across blocks

s^2 is variance of **Treatment Effect Estimates**

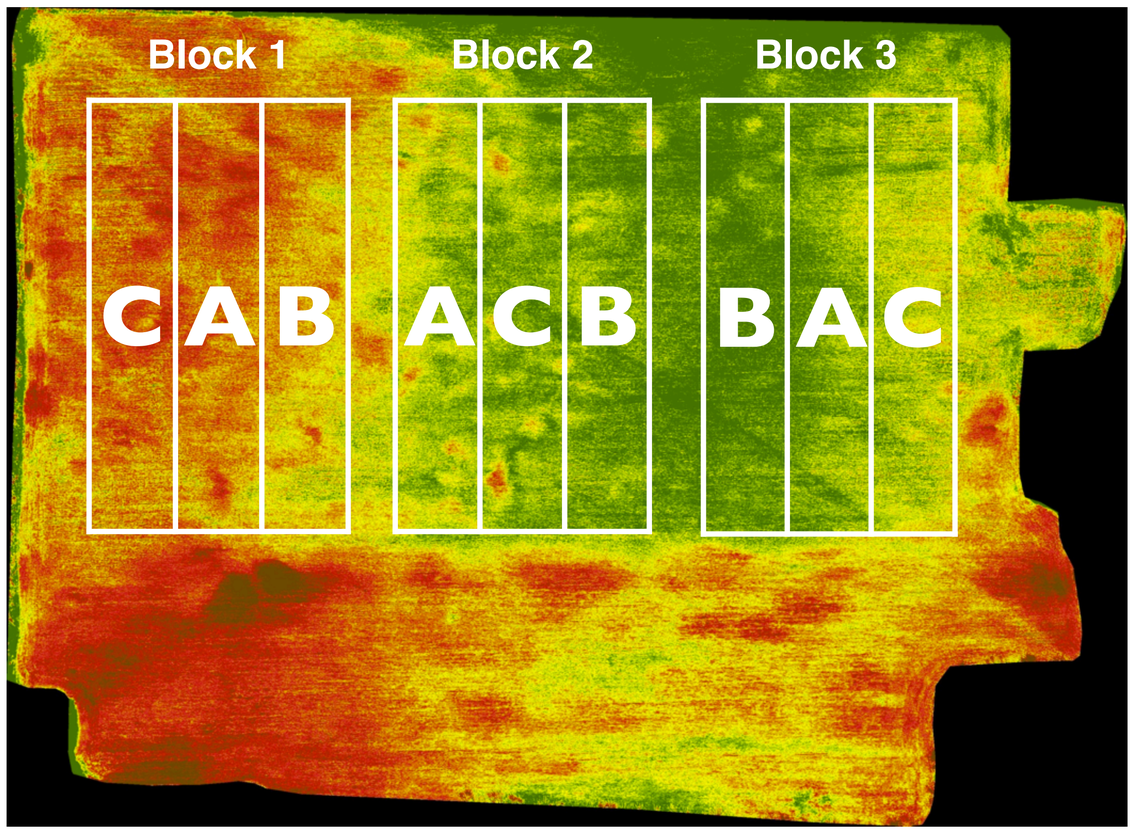
Design 2 of Pulse Experiment was a RCBD!

Completely **R**andomized **D**esign

Structure	Variable	Type	#levels	Replicate	EU
Treatment	Insecticide	Categ	3	None	Plot
Design	Plot	Categ	9		
Response	Counts	Num	9		

lm(Counts ~ Insecticide)

Randomized **C**omplete **B**lock **D**esign



Structure	Variable	Type	#levels	Replicate	EU
Treatment	Insecticide	Categ	3	Block	Plot
Design	Block	Categ	3		
	Ins:Block	Categ	9		
	Plot	Categ	9		
Response	Counts	Num	9		

lm(Counts ~ Insecticide + Block)

RCBD Design Table

EU follow the normal rules

Declare “random” if in the model

“Block” is a replicate for the Treatment

Must be a row in the Design structure

Also must include “Treatment:Block” in the Design structure

Declare random: (1|Treatment:Block) if in the model

RCBD analysis

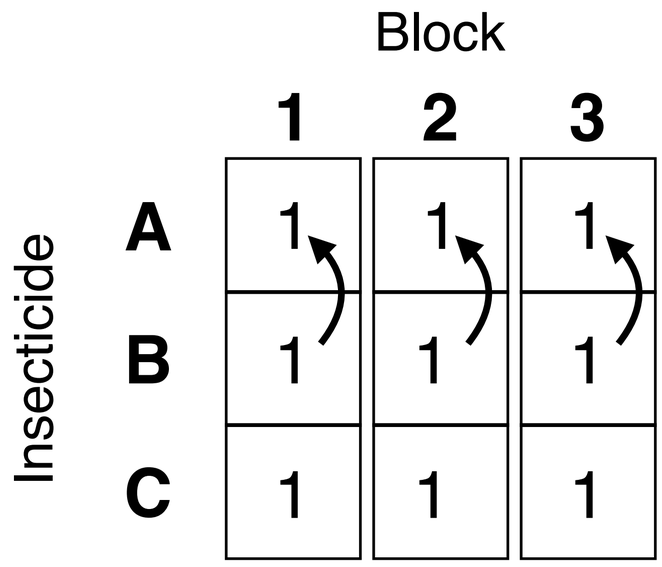
emmeans(model,specs = ‘Insecticide’)

contrast(means,’pairwise’)

```
contrast estimate    SE df t.ratio p.value
a - b      -0.901 0.515  4  -1.752  0.2949
a - c       0.474 0.515  4   0.922  0.6570
b - c       1.376 0.515  4   2.673  0.1144

Results are averaged over the levels of: B
P value adjustment: tukey method for comparing a family of 3 estimates
```

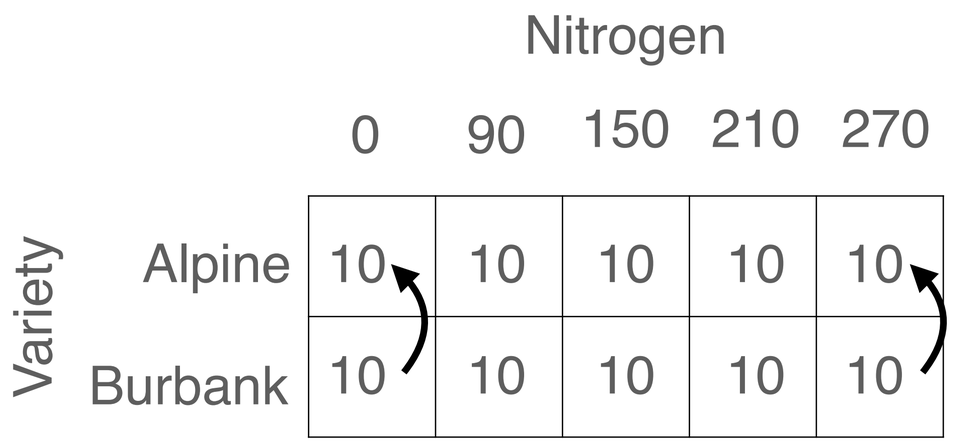
Randomized **C**omplete **B**lock **D**esign



Focal: Insecticide Moderator: Block

Structure	Variable	Type	#levels	Replicate	EU
Treatment	Insecticide	Categ	3	Block	Plot
Design	Block	Categ	3		
	Ins:Block	Categ	9		
	Plot	Categ	9		
Response	Counts	Num	9		

Factorial



Focal: Variety Moderator: Nitrogen

Structure	Variable	Type	#levels	Replicate	EU
Focal	Variety	Categ	2	Nitrogen	Plot
Moderator	Nitrogen	Categ	5	None	Plot
Combo	Var:Nitro	Categ	10	None	Plot
Design	Plot	Categ	100		
Response	Yield	Num	100		

Differences

lm(Counts ~ Insecticide + Block)

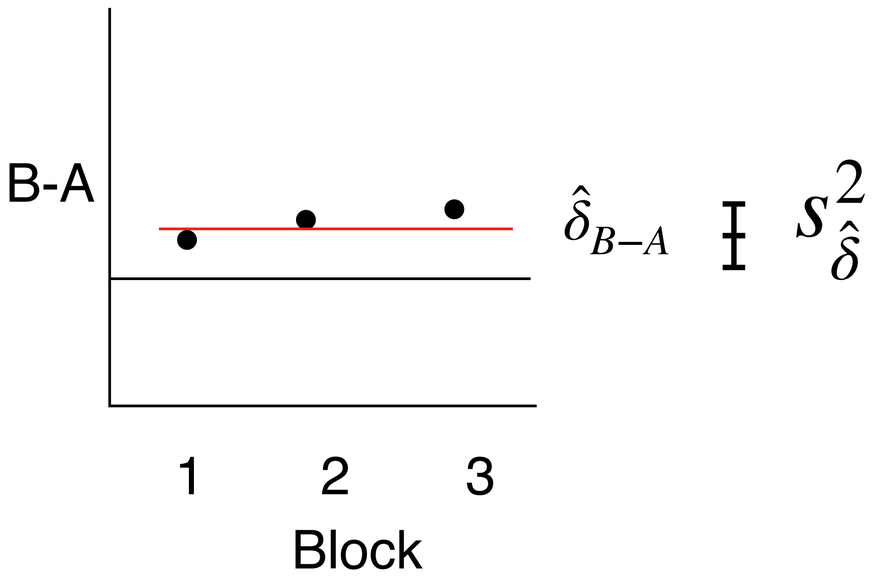
Can’t put Block:Insectide in model

Consequence: Can’t estimate **specific effects** of Insecticide using emmeans()

We can only estimate the **main effect: the average effect across blocks**

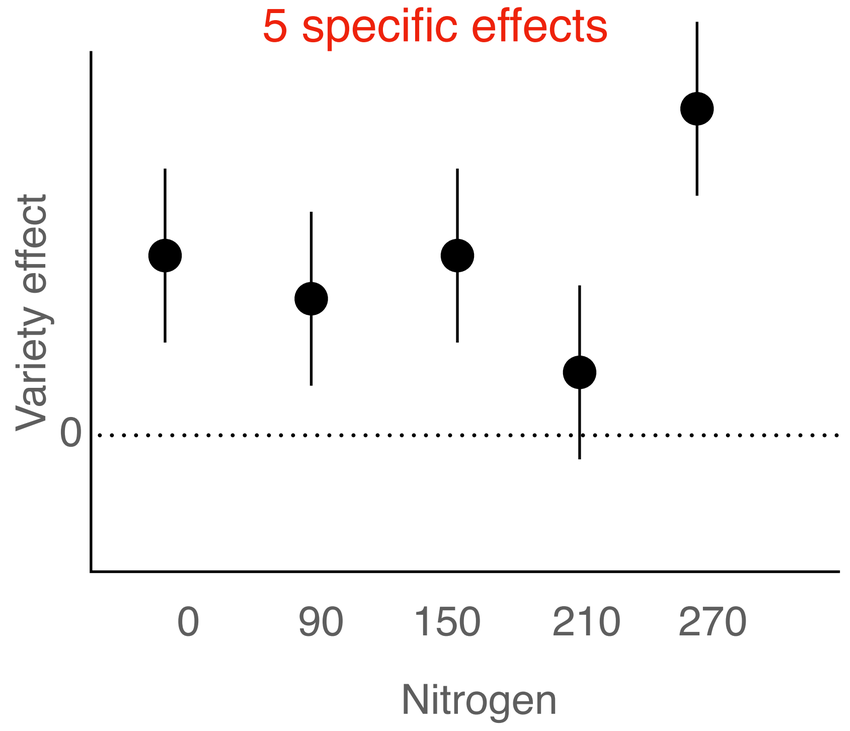
lm(Yield ~ Variety + Nitrogen + Variety:Nitrogen)

No replicates of Block:Treatment combinations



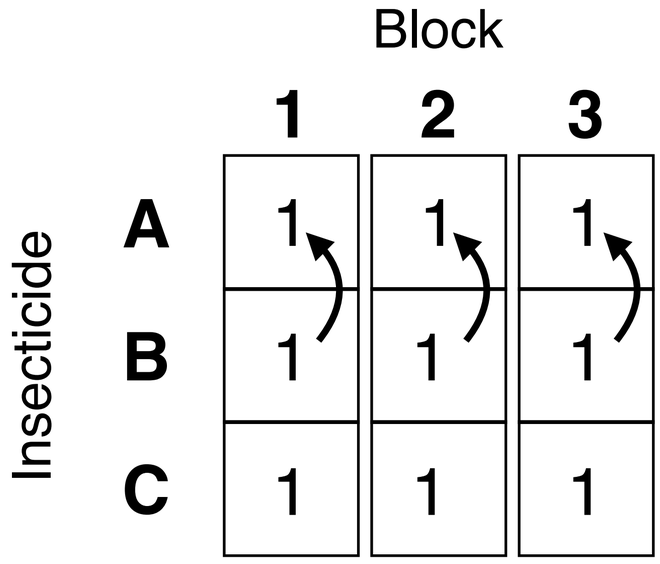
No CIs for treatment effect in each block

Variety:Nitrogen combos repeated 10 times each



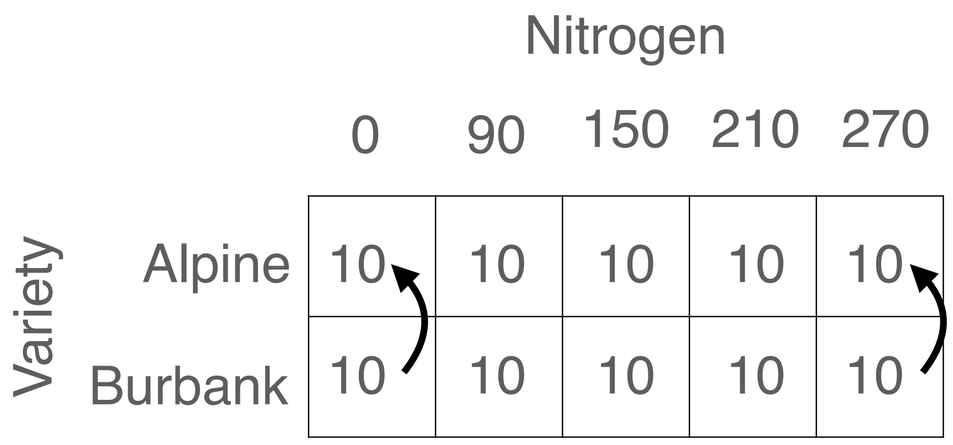
These aren’t needed for estimating the **main effect** because for this we just use $s^2_{\hat{\delta}}$

Randomized **C**omplete **B**lock **D**esign



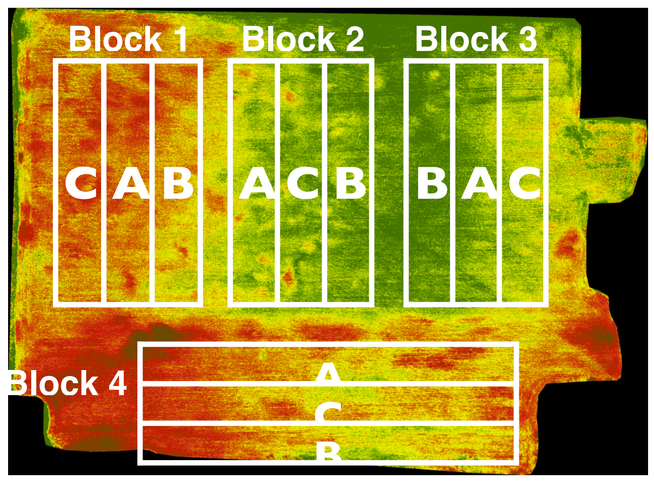
Goal: Main Effect

Factorial



Goal: Specific Effects / Interaction Effects

Key Difference



"Block" is **NOT** a treatment!

We haven't done a manipulation

We aren't trying to **explain** differences among blocks

We **don't care** about these blocks *per se*

We don't really know **which factors** that differs among the blocks are relevant

So we can't predict what will happen in a specific new block

If the treatment effect estimates are similar among blocks (low $s^2_{\hat{\delta}}$)

We will be confident predicting the response into new settings

If the treatment effect estimates are NOT similar among blocks $s^2_{\hat{\delta}}$

We will NOT be confident predicting the response into new settings

Also:

EUs of each block **are not interspersed**

So we wouldn't be confident about interpreting block differences anyway!

This is why "Block" is put in the **Design Structure,** not the **Treatment Structure**

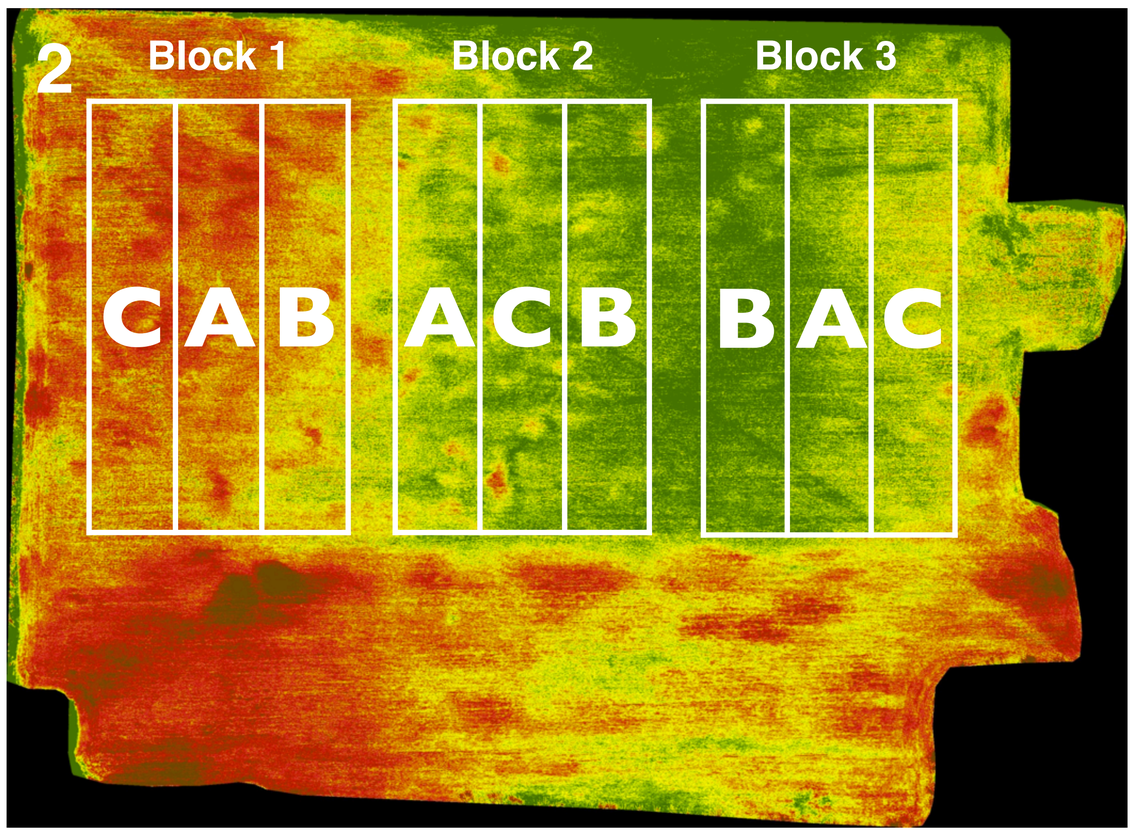
Key Questions to determine if a factor is a block or a treatment:

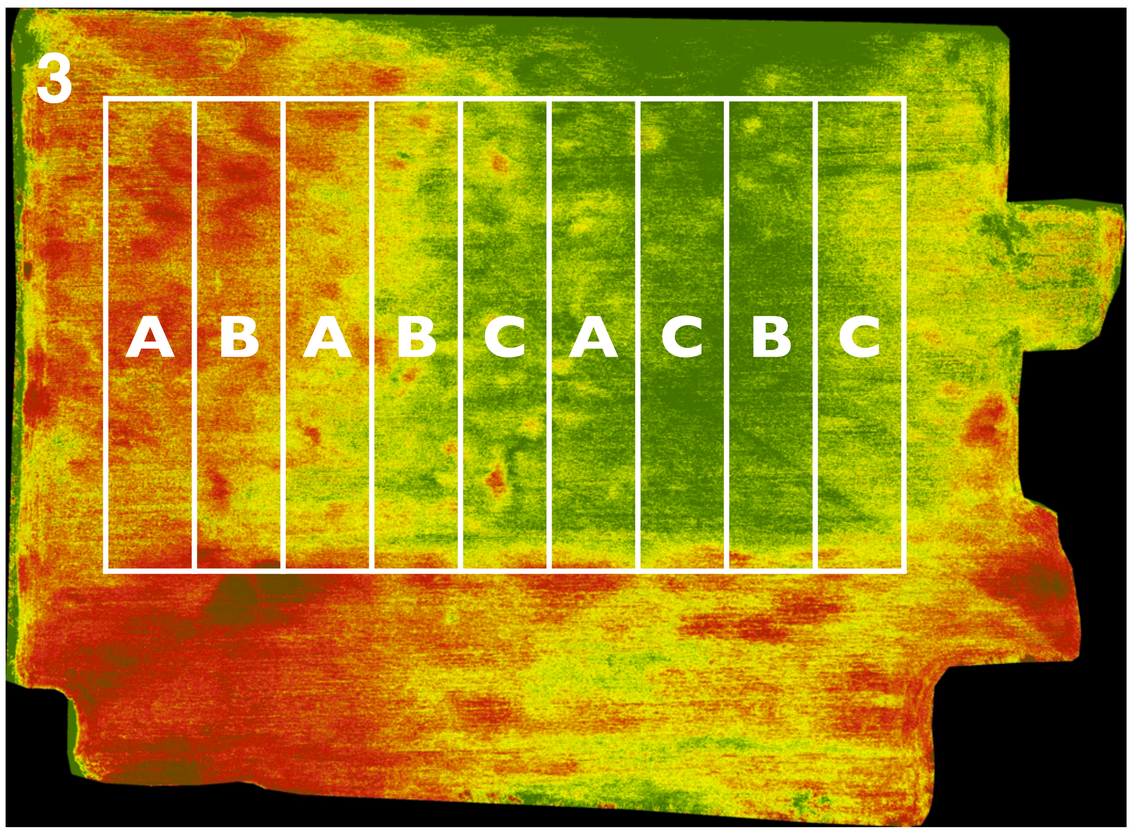
Is the factor **manipulated**?

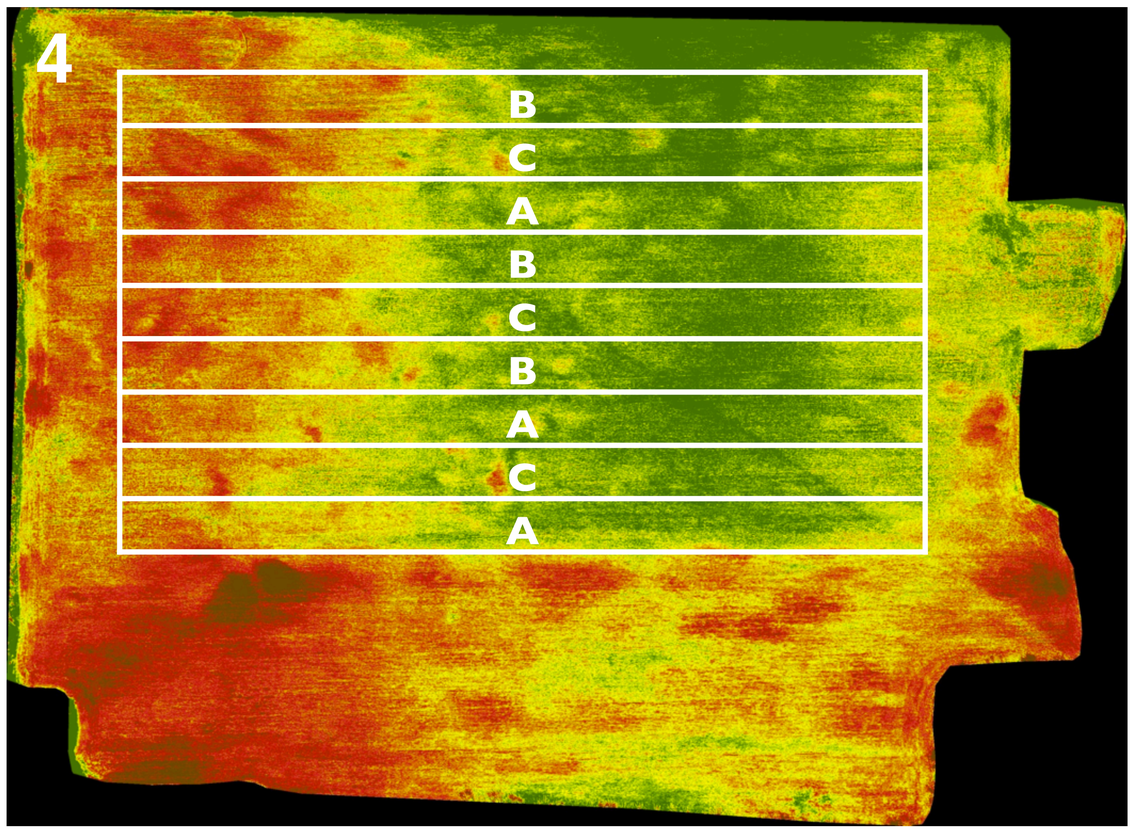
Are the EUs **interspersed**?

Are the Treatment:Factor combinations **replicated**?

Is the RCBD the best design for this experiment?







2 is a RCBD

DF = 9-3-2 = 4 $s^2_{effects}$

1, 3, 4 are all Completely Randomized Designs (CRD)

DF = 9-3 = 6 s^2_{plots}

Which Design has plots that are the **least variable**?

Design 4 - all of them span good -> bad areas of the field. s^2_{plots} would be smallest

Always good to run EU **along gradients** to average over this variation

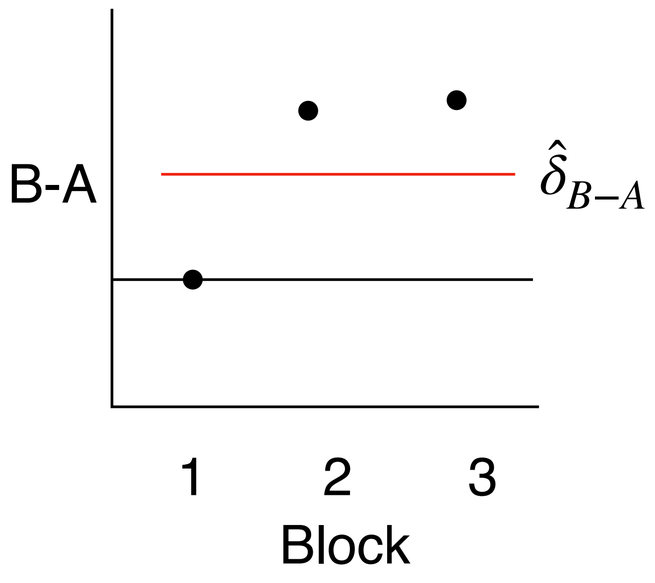
Only use RCBD if you can't do this

What about variation in **treatment effects** between good (green) and bad (red) areas?

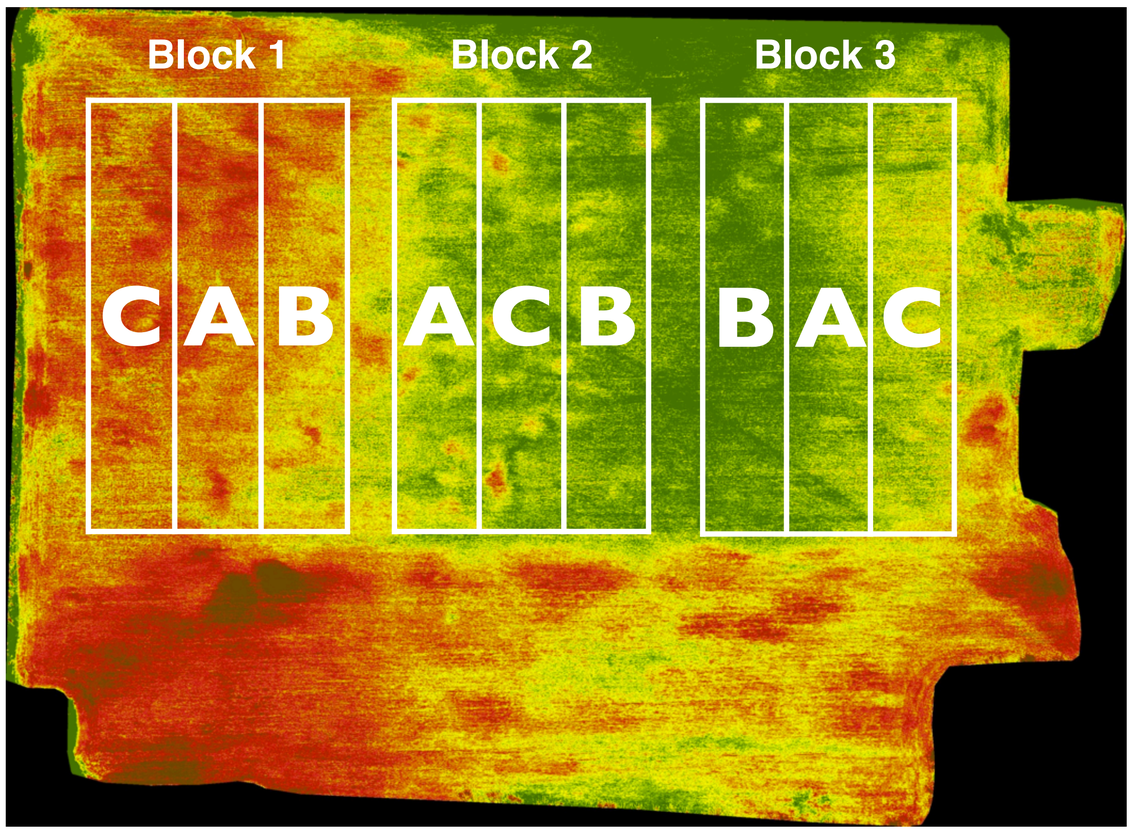
Can observe this using RCBD (but no error bars)

Increases uncertainty (s^2) in Designs 1-3

Less so in 4, because this is averaged over **within EU**



What makes good blocks?



You can block by any factor that you can observe **before the experiment**

Area of a field that you know has different water

Growth chamber

Person doing the measurements

Time of day / year

Think of Blocks as Experimental Replicates

You measure each treatment in each block, make treatment effect estimates,
then compare among blocks

So, experimental replicates **are blocks**

Blocks are most useful when the EU within blocks are similar (correlated) for their potential response

If so, $s^2_{effect} < s^2_{value}$

But, Blocks don't have to be good/useful to be **valid**

If you block by regions of the field based on a previous year's data

But this year the whole file grows well, the blocks weren't very useful

But you'll still include them in your analysis

And your analysis will still be valid