Topic 6: Factorial Treatment Structures

ENVX2001 Applied Statistical Methods

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Introduction

Learning Outcomes

- Demonstrate proficiency in designing sample schemes and analysing data from them using using R;

- **Describe and identify the basic features of an experimental design; replicate, treatment structure and blocking structure;**

- **Demonstrate proficiency in the use or the statistical programming language R to apply an ANOVA** and fit regression models to experimental data;

- Demonstrate proficiency in the use or the statistical programming language R to use multivariate methods to find patterns in data

- **Interpret the output and understand conceptually how its derived** of a regression, **ANOVA** and multivariate analysis that have been calculated by R;

- Write statistical and modelling results as part of a scientific report;

- Appraise the validity of statistical analyses used in publications.
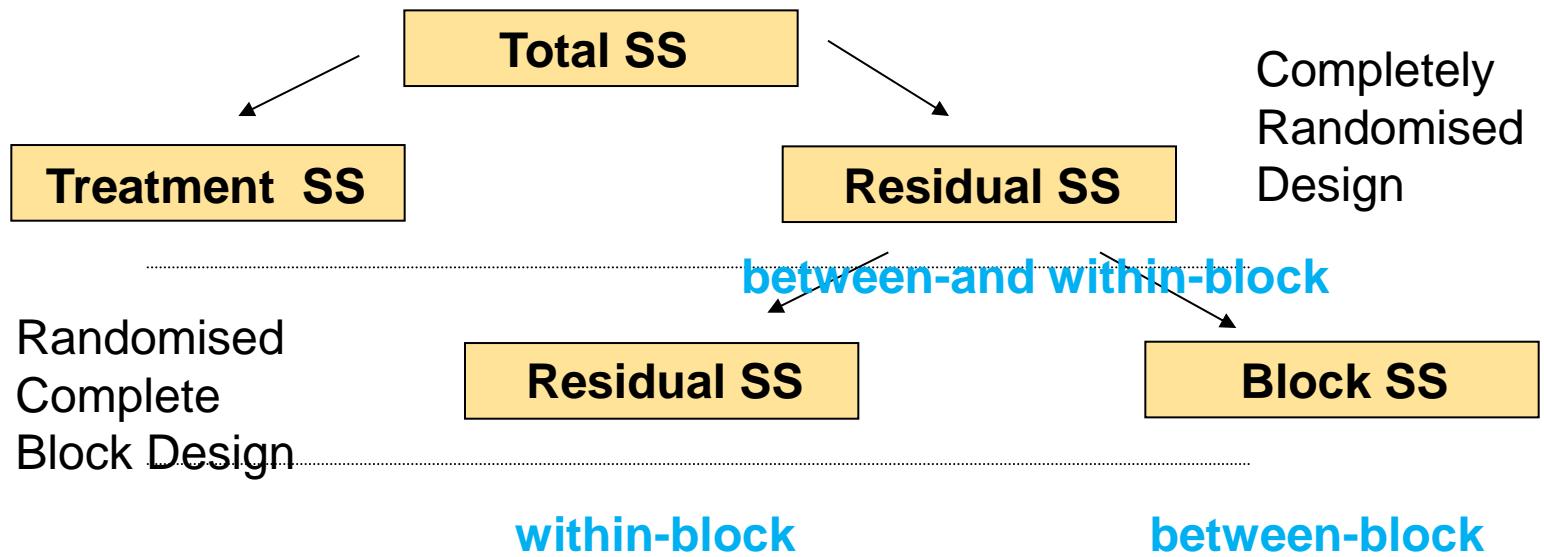
Introduction

Outline

- Treatment Designs and Experimental Designs

- Factorial Treatment Structure

- Main Effects and Interactions

- ANOVA with Factorial Treatment Designs

Introduction

Concept

	Total SS	

Treatment SS　　　　　　　　**Residual SS**　　　　Completely Randomised Design

between-and within-block

Randomised Complete Block Design　　　**Residual SS**　　　　　**Block SS**

within-block　　　　　　**between-block**

– Topic 5: About splitting up Residual SS

– Topic 6: How can we split up Treatment SS?

– Rice experiment: 3 Varieties (A, B, C) with 5 N Fertiliser Rates (0, 40, 70, 100, 130)

Introduction

Topic 6 Learning Outcomes

– At the end of this topic students should be able to:

– Demonstrate proficiency in the use of R (and interpretation of the output) for generating and analysing experiments with a factorial treatment structure

Introduction

Designs

– Treatment Design is the selection of treatments for an experiment, both the factors, as well as the levels of each factor

   – also called Treatment Structure

– Experimental Design refers to the way we allocate treatments to the experimental units (e.g. plot of land, animals)

   – also called Blocking Structure

Factorial Treatment Structure

Definition

– Previously we considered simple treatment designs:

  – one "kind" of treatment (called a factor) $\Rightarrow$ One-way ANOVA

  – varying number of levels of that factor (t).

– *"No aphorism is more frequently repeated in connection with field trials, than that we must ask Nature few questions, or, ideally, one question, at a time. The writer is convinced that this view is wholly mistaken."* R. Fisher

– Many experiments involve several factors, usually in full combinations

  – these are called **factorial treatment designs**

# Factorial Treatment Structure

Example: 2-way factorial
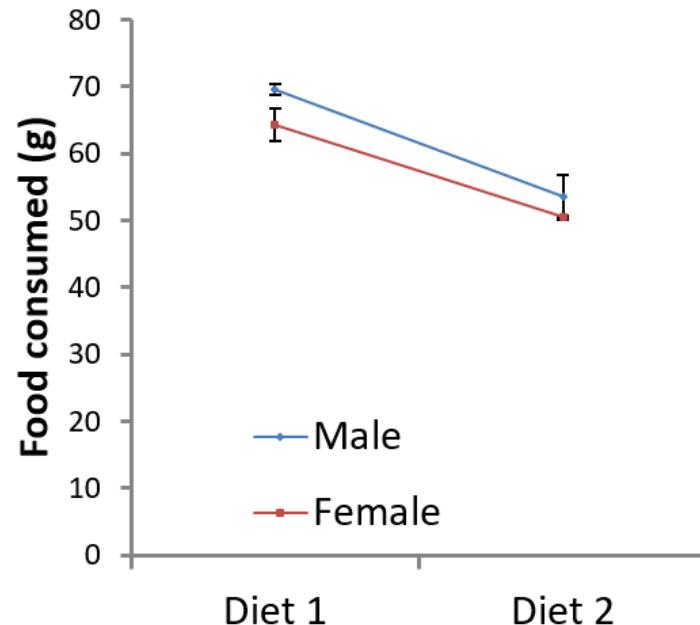
- Feeding experiment in birds.

- Two diets were prepared (Diet 1 and 2), and these were given to both male and female birds.

- Diet × Sex factorial treatment design:

    - Diet 1-Male; Diet 1-Female

    - Diet 2-Male; Diet 2-Female

Factorial Treatment Structure

Example: 2-way factorial

- Data: amount of food consumed (g)

	Diet 1	Diet 2
Male	70.9	59.2
	67.9	53.8
	69.9	47.6
Mean =	69.6	53.5
Female	65.7	50.8
	59.4	50.5
	67.7	50.5
Mean =	64.3	50.6

- Now have **3 research questions/hypotheses:**

  - What is the effect of diet?

  - What is the effect of sex?

  - Is there a different Diet effect for male and female birds (interaction)?

Factorial Treatment Structure

Example: 2-way factorial, randomised complete block design (RCBD)

– Recall from Topic 5: Pearce *et al.* (1988) outlines an experiment where 5 levels of nitrogen are applied to 3 varieties of rice (resulting in $5 \times 3 = 15$ treatment combinations).

– RCBD: 4 blocks of land were used with 15 plots per block

Image source: http://www.mars.com/global/about-mars/mars-pia/our-supply-chain/rice.aspx

Factorial Treatment Structure

Example: 2-way factorial, randomised complete block design (RCBD)

– Data are rice yield (t ha⁻¹)
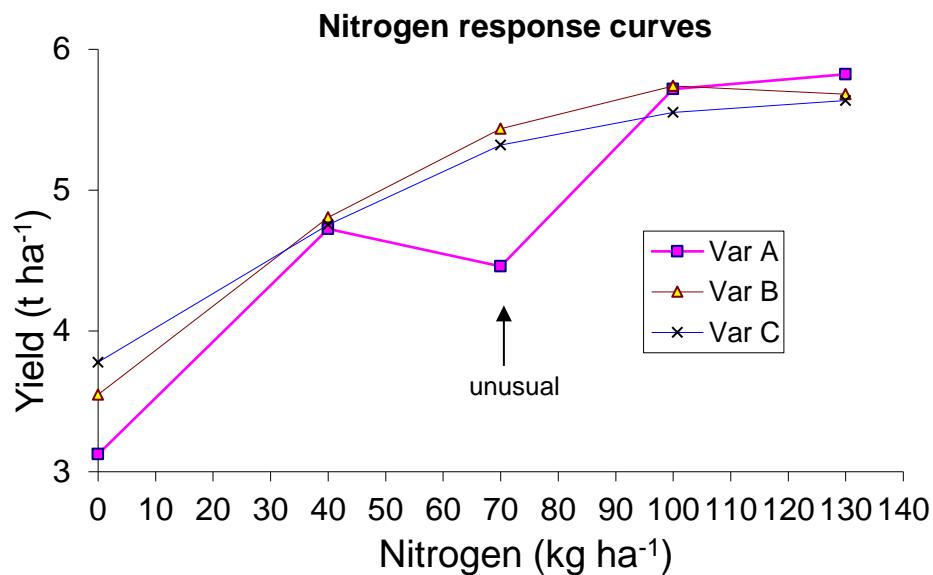
Just exploring the interaction

Treatment	Variety	Nitrogen (kg ha⁻¹)	Block 1	Block 2	Block 3	Block 4	Treatment mean
1	A	0	3.852	2.606	3.144	2.894	3.124
2	A	40	4.788	4.936	4.562	4.608	4.724
3	A	70	4.576	4.454	4.884	3.924	4.460
4	A	100	6.034	5.276	5.906	5.652	5.717
5	A	130	5.874	5.916	5.984	5.518	5.823
6	B	0	2.846	3.794	4.108	3.444	3.548
7	B	40	4.956	5.128	4.150	4.990	4.806
8	B	70	5.928	5.698	5.810	4.308	5.436
9	B	100	5.664	5.362	6.458	5.474	5.740
10	B	130	5.458	5.546	5.786	5.932	5.681
11	C	0	4.192	3.754	3.738	3.428	3.778
12	C	40	5.250	4.582	4.896	4.286	4.754
13	C	70	5.822	4.848	5.678	4.932	5.320
14	C	100	5.888	5.524	6.042	4.756	5.553
15	C	130	5.864	5.264	6.056	5.362	5.637
Block mean			5.133	4.846	5.147	4.634	4.940

Factorial Treatment Structure

Example: 2-way factorial, randomised complete block design (RCBD)

– Table of treatment means

Nitrogen response curves

Nitrogen	Variety A	Variety B	Variety C
0	3.12	3.55	3.78
40	4.72	4.81	4.75
70	4.46	5.44	5.32
100	5.72	5.74	5.55
130	5.82	5.68	5.64

Research Questions
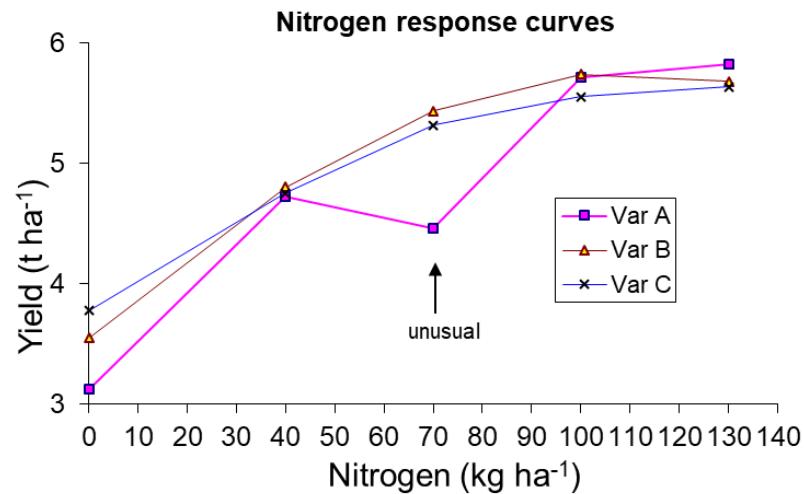
Question 1:   Is the response to nitrogen the same for each variety(Interaction)?

– If the response is not the same for each variety, the recommendation on the best level of nitrogen to use will be different from variety to variety

– The differences across varieties in the response of nitrogen is termed an **interaction**, i.e. a Nitrogen ✕ Variety interaction

– More generally, a two-factor interaction measures whether the differences among the levels of one factor are consistent across all the levels of the second factor

– An indication of the presence of an interaction between two factors is the failure of the response curves to be parallel

Research Questions

Question 2:   Is there a response to nitrogen?
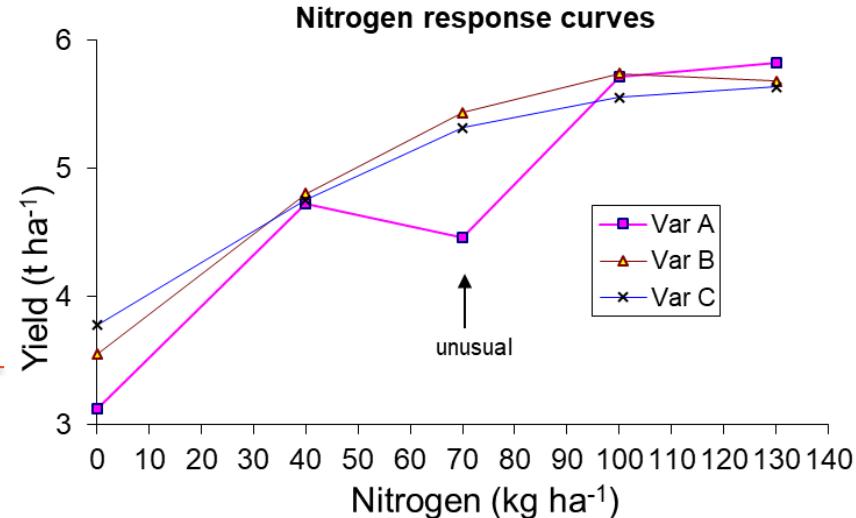
–   Or are mean yields identical no matter what level of N is used

–   Conditional on Question 1; need to answer yes: same response for each variety

–   If the response is the same for each variety, any best recommendation on the level of nitrogen to use will be the same for the three varieties

–   The *main effect* of nitrogen is a measure of the differences among the nitrogen means (averaged over varieties)

Nitrogen response curves

Research Questions

Nitrogen response curves

Question 3:   Is there a response to variety?

Do these curves separate?

- Or are mean yields identical across varieties

- Conditional on Question 1; need to answer yes: same response for each variety

- If the response is the same for each variety, the response curves are parallel across varieties, as noted. Having equal varietal means indicates that there is just one response curve (a common line), having different varietal means indicates that the response curves are simply parallel

- The *main effect* of varieties is a measure of the differences among the varietal means (averaged over nitrogen levels)

Model

Model Equation

- $y_{i,j} = \mu + \beta_i + \gamma_j + \tau_k + (\gamma\tau)_{j,k} + \epsilon_{i,j,k}$

- Observed data = Overall Mean + Block Effect + Variety Effect + Nitrogen Effect + Interaction Effect (Var × N) + Random Error

- There are two types of terms associated with this model: ones involving treatment effects and ones involving experimental layout effects, related to the experimental design.

- These are the Treatment Structure and Blocking Structure

- Treatment Structure: `Variety + Nitrogen + Variety:Nitrogen = Variety*Nitrogen`

- Blocking Structure: `Block`

- R code: `aov(Yield ~ Block + Variety*Nitrogen)`

Model
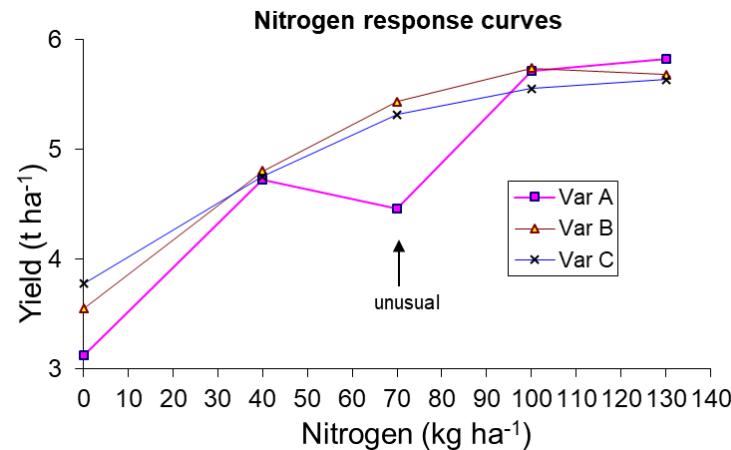
Interpretation of Model Effects

- Block effect = Block mean − Overall mean

- Variety effect = Variety mean − Overall mean = $\mu_{i\bullet} - \mu_{\bullet\bullet}$ (Main effect)

- Nitrogen effect = Nitrogen mean − Overall mean = $\mu_{\bullet k} - \mu_{\bullet\bullet}$ (Main effect)

- Variety:Nitrogen effect = Variety:Nitrogen mean − Overall mean
                                  − Variety effect − Nitrogen effect
    $$= (\mu_{ik} - \mu_{\bullet\bullet}) - (\mu_{i\bullet} - \mu_{\bullet\bullet}) - (\mu_{\bullet k} - \mu_{\bullet\bullet})$$

Model

Nitrogen response curves

Illustration

Nitrogen	Variety A	Variety B	Variety C	Overall
0	3.12	3.55	3.78	3.48
40	4.72	4.81	4.75	4.76
70	4.46	5.44	5.32	5.07
100	5.72	5.74	5.55	5.67
130	5.82	5.68	5.64	5.71
Overall	4.77	5.04	5.01	4.94

– Variety A effect = 4.77 – 4.94 = –0.17 t ha^{-1} $\Rightarrow$ below average

– Nitrogen 70 effect = 5.07 – 4.94 = +0.13 t ha^{-1} $\Rightarrow$ above average

– (Var A):(N 70) interaction effect = 4.46 – 4.94 – (–0.17 + 0.13) = –0.44 t ha^{-1}
   $\Rightarrow$ This mean yield is 0.44 t ha^{-1} below what is expected based on this Variety
   and Nitrogen level.

Model

ANOVA SS & df

– Previously, we partitioned the total variability about the grand mean as
Total SS = Block SS + Treatment SS + Residual SS

– This is now extended as
Total SS = Block SS + Variety SS + Nitrogen SS + Var.Nit SS + Residual SS
i.e. we further partition the Treatment SS in to three components,
Treatment SS = Variety SS + Nitrogen SS + Var.Nit SS

– Similarly the df are partitioned

$$\text{Total df} = \text{Block df} + \text{Variety df} + \text{Nitrogen df} + \text{Var.Nit df} + \text{Residual df}$$

$$(60-1) = (4-1) + (3-1) + (5-1) + (3-1)(5-1) + (4-1)(15-1)$$

$$59 = 3 + 2 + 4 + 8 + 42$$

4 Blocks

3×5 = 15 Treatments

Note that
$$\text{Treatment df} = \text{Variety df} + \text{Nitrogen df} + \text{Var.Nit df}$$

$$14 = 2 + 4 + 8$$

Model

Hypotheses

– As indicated by the three questions, there are three sets of H_0 / H_1:

– 1st Hypothesis: Variety $\times$ Nitrogen interaction:
H_0 :   All $(\gamma\tau)_{jk} = 0$ $(\gamma\tau)_{jk}$ = Interaction term for
H_1:   not all $(\gamma\tau)_{jk}$ equal 0   Variety j and Nitrogen k

Only if H_0 above is retained (no interaction) are main effects tested

Model

Hypotheses

- 2nd Hypothesis: Variety Main Effect:

  H_0 : $\mu_{1\bullet} = \mu_{2\bullet} = \mu_{3\bullet}$ $\mu_{i\bullet}$ = Variety i mean

  H_1:   not all $\mu_{i\bullet}$ are equal

  Equivalently:

  H_0 : $\gamma_1 = \gamma_2 = \gamma_3 = 0$ γ_i = Variety i effect

  H_1:   not all γ_i equal 0

- 3rd Hypothesis: Nitrogen Main Effect:

  H_0 : $\mu_{\bullet 1} = \mu_{\bullet 2} = \mu_{\bullet 3} = \mu_{\bullet 4} = \mu_{\bullet 5}$ $\mu_{\bullet k}$ = Nitrogen k mean

  H_1:   not all $\mu_{\bullet k}$ are equal

  Equivalently:

  H_0 : $\tau_1 = \tau_2 = \tau_3 = \tau_4 = \tau_5 = 0$ τ_k = Nitrogen k effect

  H_1:   not all τ_k equal 0

ANOVA

ANOVA Table

```
> rice.aov <- aov(Yield ~ Block + Nitrogen*Variety, data = rice)
> summary(rice.aov)
```

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Block	3	2.74	0.913	6.172	0.00143 **
Nitrogen	4	39.62	9.905	66.994	< 2e-16 ***
Variety	2	0.88	0.441	2.986	0.06132 .
Nitrogen:Variety	8	2.45	0.306	2.069	0.06099 .
Residuals	42	6.21	0.148		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

ANOVA
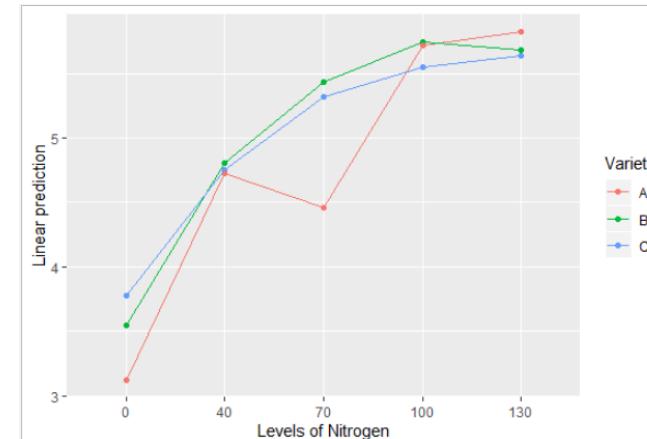
Results from F Tests

- 1. **Variety × Nitrogen interaction:** $F = 2.07$; df = 8, 42; $P = 0.061$

  - Since $P > 0.05$, conclude there is not a significant interaction

  - Note: The "unusual" results for Variety A Nitrogen = 70 (4.46 t ha^{-1}) not sufficient to make the interaction significant

  - Progress to testing main effects (assuming there is no interaction)

ANOVA

Results from F Tests

- **2. Variety: $F = 2.99$; df $= 2, 42$; $P = 0.061$**
  - Since $P > 0.05$, conclude there are no significant variety differences

- **3. Nitrogen: $F = 66.99$; df $= 4, 42$; $P < 0.001$**

  - Since $P < 0.001$, conclude there are significant nitrogen effects

 - Can do post-hoc tests
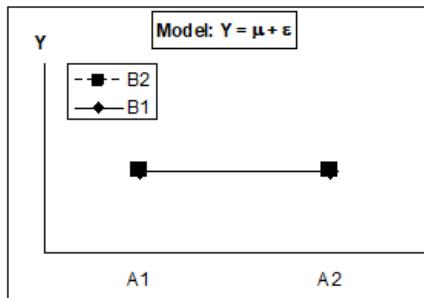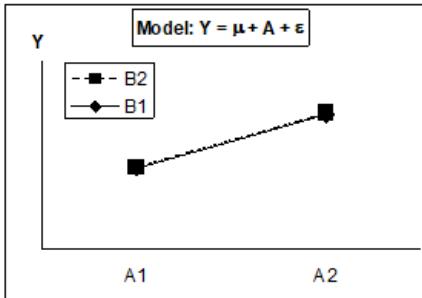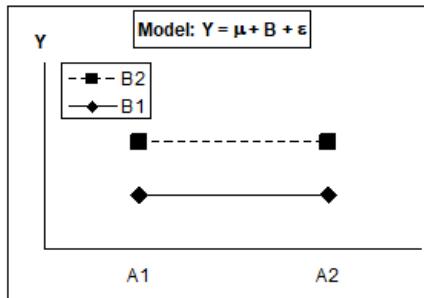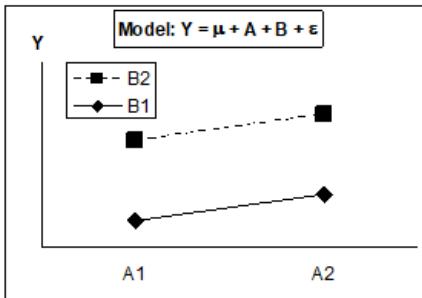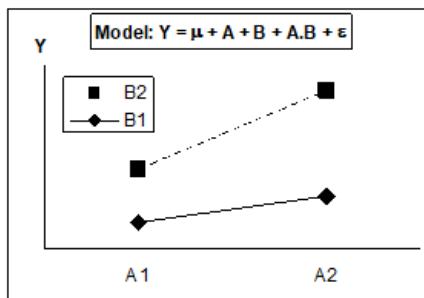
Implementation in R

Functions

– Analysis

    – `aov(Yield ~ Block + Variety*Nitrogen)`

Interaction Plots

Idealised Examples
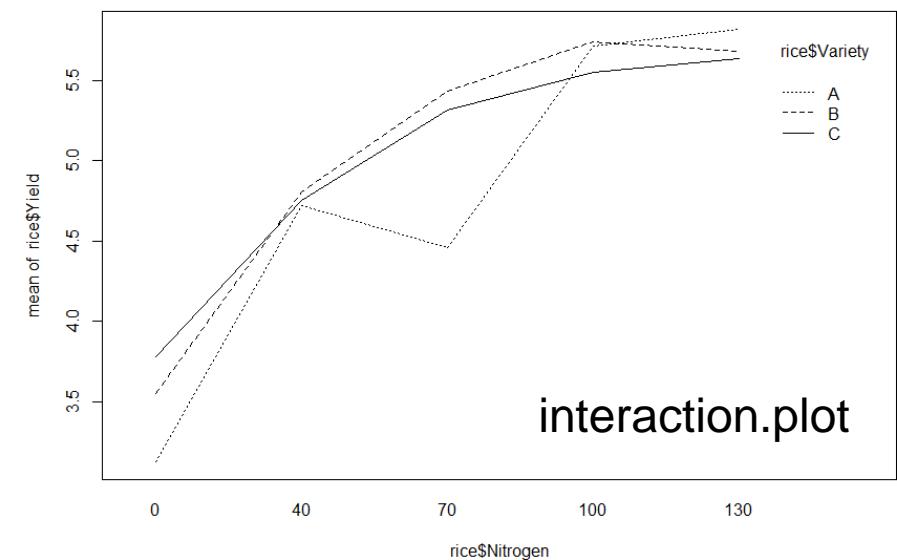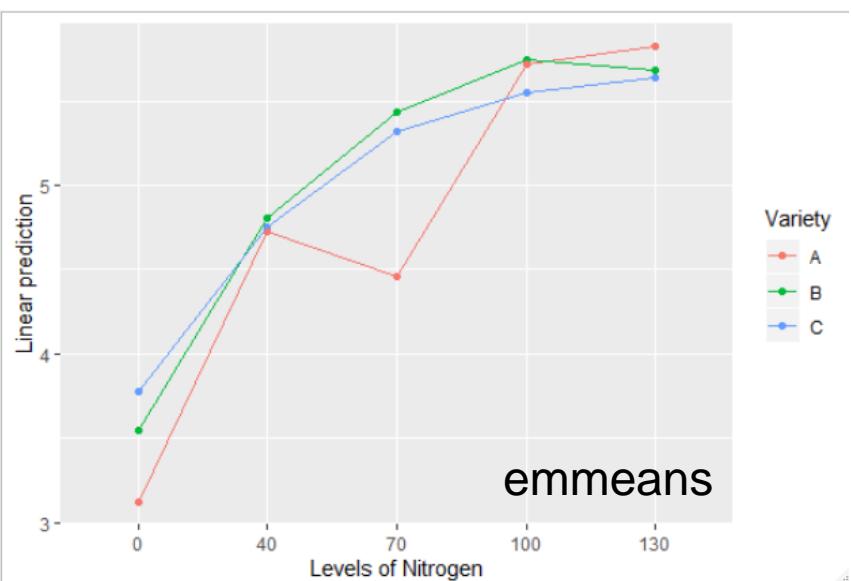
Interaction Plots

Rice Example

```
> library(emmeans)

> emmip(rice.aov, Variety ~ Nitrogen)

> interaction.plot(rice$Nitrogen,rice$Variety,rice$Yield)
```


emmeans

interaction.plot

Factorials with >2 Factors

where ^nC_r notation means C is a combination of picks of r objects from a population of n objects where order does not matter.

Structure

- The two-factor factorial treatment structure can be extended to any numbers of factors with levels in combination with each other.

- For example, suppose you have:

  - n levels of Nitrogen (N)

  - p levels of Phosphorus (P)

  - k levels of Potassium (K)

- You will have an analysis with:

  - $^3C_1 = 3$ main effects: N + P + K

  - $^3C_2 = 3$ two-factor interactions   N×P + N×K + P×K

  - $^3C_3 = 1$ three-factor interaction   N×P×K

Factorials with >2 Factors

Structure

– This constitutes a partitioning of the Treatment SS and Treatment df $(npk − 1)$ into appropriate main effects and interactions:

   – Treatment SS $(npk − 1)$

   - = Nitrogen SS $(n − 1)$

   - + Phosphorus   SS $(p − 1)$

   - + Potassium SS $(k − 1)$

   - + Nitrogen $\times$ Phosphorus SS $(n − 1)(p − 1)$

   - + Nitrogen $\times$ Potassium SS $(n − 1)(k − 1)$

   - + Phosphorus $\times$ Potassium SS $(p − 1)(k − 1)$

   - + Nitrogen $\times$ Phosphorus $\times$ Potassium $(n − 1)\ (p − 1)(k − 1)$

– Note: Higher order interactions (three-factor+) may be difficult to interpret often are ignored, and added to Residual SS component.

Factorials with >2 Factors

Example: 3-way ANOVA, RCBD

- Experiment to investigate maize yield on witchweed-affected area

- Factors included

  - 4 fertilisers: none; super only; super + manure; super + N + K

  - Infested vs Uninfested with witchweed

  - Two varieties of maize (A and B)

- So Treatment Design is a **4 × 2 × 2 Factorial** treatment structure

- Experimental Design was a RCBD

  - 2 Blocks

  - Each Block has 4 × 2 × 2 = 16 plots

Factorials with >2 Factors

Implementation in R

– Analysis

– `aov(Yield ~ Block + Variety*Witchweed*Fertiliser)`

Factorials with >2 Factors

Field plan and data from Pearce et al. (1988)

- V = *Variety* (A, B);

- W = *Witchweed* (I = infested plots, U = uninfested plots)

- F = *Fertiliser* (F1 = none, F2 = super only, F3 = super+manure, F4 = super+N+K)

- Y = *Yield*

Block	V	W	F	Y	V	W	F	Y	V	W	F	Y	V	W	F	Y
	B	I	F3	13.5	B	U	F1	12.8	A	I	F3	15.8	B	I	F4	11.6
1	A	I	F1	10.4	B	U	F4	17.1	A	I	F2	12.5	A	U	F1	14.8
	B	I	F2	11.8	B	U	F2	16.9	B	I	F1	9.5	A	I	F4	11.3
	B	U	F3	22.3	A	U	F3	24.9	A	U	F4	19.9	A	U	F2	19.7
	B	U	F2	16.0	A	I	F1	10.0	B	I	F2	9.5	A	U	F4	19.2
2	A	U	F2	18.0	B	U	F1	13.0	B	I	F1	9.6	A	U	F3	22.0
	B	I	F3	13.4	A	I	F4	11.4	B	U	F4	16.6	B	U	F3	20.0
	A	I	F2	10.1	B	I	F4	9.2	A	U	F1	14.0	A	I	F3	13.6

Factorials with >2 Factors

Model

$$Y_{ijkl} = \mu + \text{Block}_i + V_j + W_k + F_l + (V.W)_{jk} + (V.F)_{jl} + (W.F)_{kl} + (V.W.F)_{jkl} + \varepsilon_{ijkl}$$

Main effects   2-factor interactions   3-factor Interaction

– 3 factor interaction term hypothesis:
  H_0 :   All $(V.W.F)_{jkl} = 0$
  H_1:   not all $(V.W.F)_{jkl}$ equal 0

Factorials with >2 Factors

ANOVA Table

```
witch.anova<-
aov(Yield~Block+Variety*Witchweed*Fertiliser,data=witch)
anova(witch.anova)

Analysis of Variance Table

Response: Yield
                            Df  Sum Sq  Mean Sq   F value     Pr(>F)
Block                        1   11.52    11.52   19.6141  0.0004880 ***
Variety                      1   19.22    19.22   32.7242  4.052e-05 ***
Witchweed                    1  338.00   338.00  575.4824  2.230e-13 ***
Fertiliser                   3  167.74    55.91   95.2015  5.454e-10 ***
Variety:Witchweed            1    3.64     3.64    6.2060  0.0249356 *
Variety:Fertiliser           3    0.71     0.24    0.4001  0.7549378
Witchweed:Fertiliser         3   22.23     7.41   12.6135  0.0002221 ***
Variety:Witchweed:Fertiliser 3    0.33     0.11    0.1873  0.9033935
Residuals                   15    8.81     0.59
---
Signif. codes:  0 `***' 0.001 `**' 0.01 `*' 0.05 `.' 0.1 ` ' 1
```

Factorials with >2 Factors
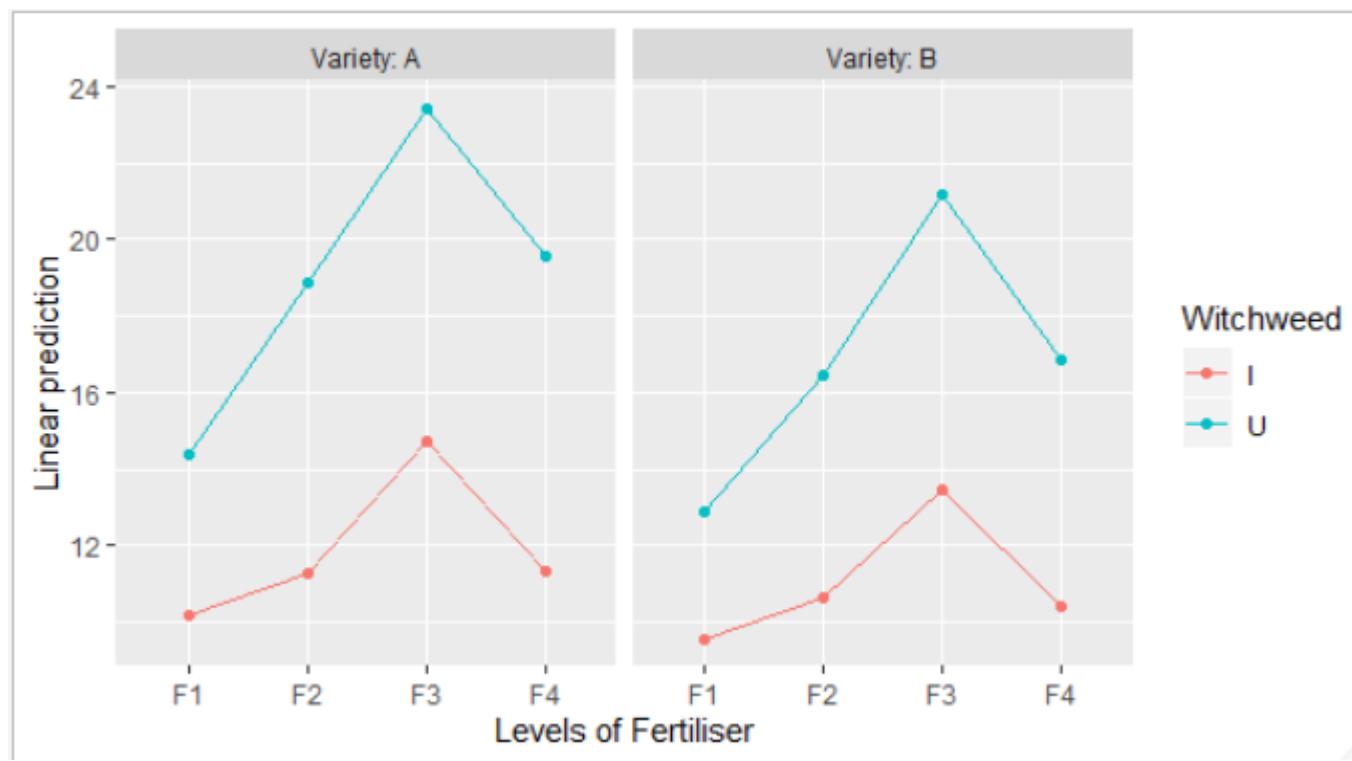
Results of F Tests

- **Looking at the highest order interaction first!**

- Variety × Witchweed × Fertiliser

- Three –way interaction is not significant ($P = 0.903$).
  ∴ the two-way interactions can be assessed

- Note: A significant 3-way interaction would indicate:

    - A separate V×W interaction for each level; of F; or equivalently,

    - A separate V×F interaction for each level; of W; or equivalently,

    - A separate W×F interaction for each level of V

Factorials with >2 Factors

Interaction Plot with 3 factors

> emmip(witch.aov, Witchweed ~ Fertiliser | Variety)

Factorials with >2 Factors
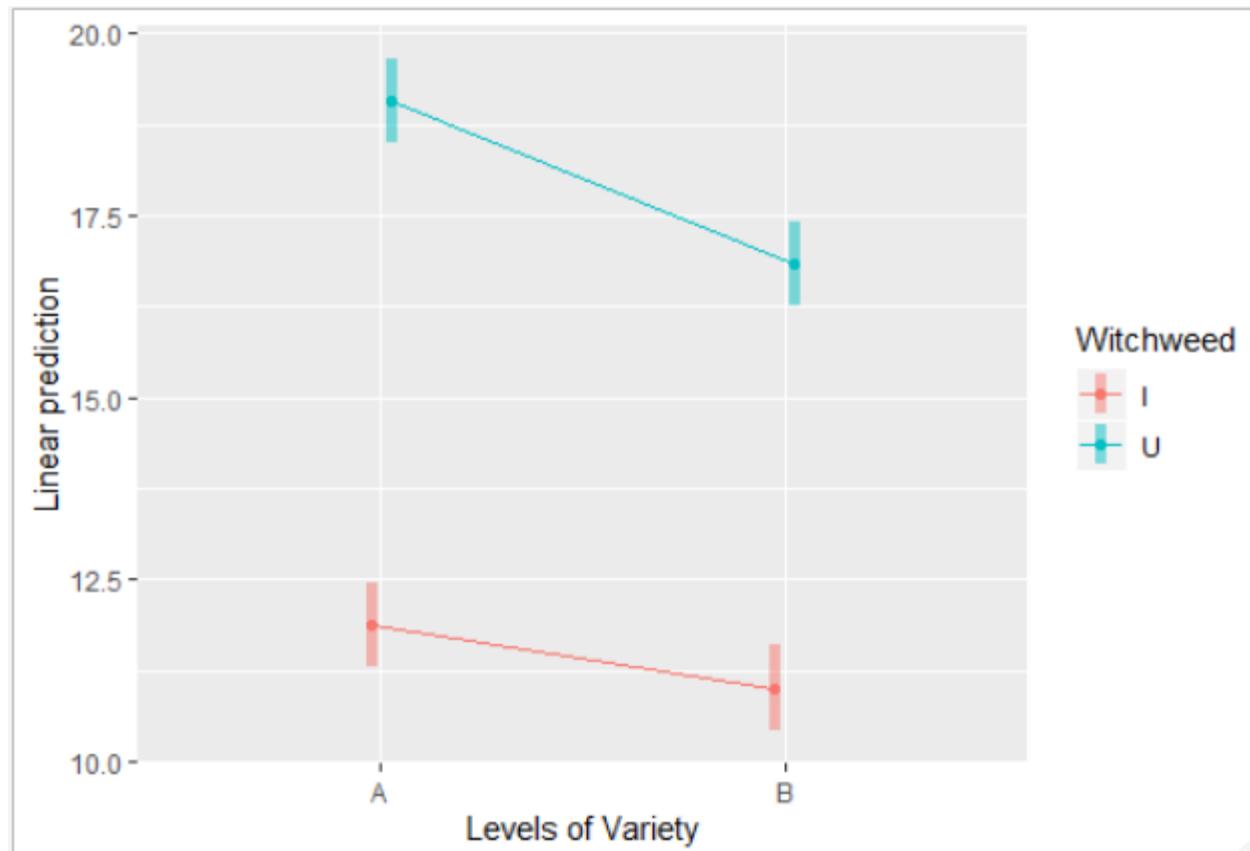
Results of F Tests

- Variety × Witchweed

  - Two–way interaction is significant ($P = 0.025$).

- Variety × Fertiliser

  - Two–way interaction is not significant ($P = 0.755$).

- Witchweed × Fertiliser

  - Two–way interaction is highly significant ($P < 0.001$).

- **Notes: Because all the main effects (V, W, F) are part of a significant interaction, they cannot be tested**

Factorials with >2 Factors

Post-hoc pairwise comparisons

– Variety x witchweed

Factorials with >2 Factors

Tukey Post-hoc test: Variety*Witchweed

```
> emmeans(witch.aov, pairwise ~ Variety*Witchweed)
NOTE: Results may be misleading due to involvement in interactions
$emmeans
 Variety Witchweed emmean    SE df lower.CL upper.CL
 A       I           11.9 0.271 15     11.3     12.5
 B       I           11.0 0.271 15     10.4     11.6
 A       U           19.1 0.271 15     18.5     19.6
 B       U           16.8 0.271 15     16.3     17.4

Results are averaged over the levels of: Block, Fertiliser
Confidence level used: 0.95


$contrasts
 contrast   estimate    SE df t.ratio p.value
 A,I - B,I     0.875 0.383 15   2.283 0.1462
 A,I - A,U    -7.175 0.383 15 -18.724 <.0001
 A,I - B,U    -4.950 0.383 15 -12.918 <.0001
 B,I - A,U    -8.050 0.383 15 -21.008 <.0001
 B,I - B,U    -5.825 0.383 15 -15.201 <.0001
 A,U - B,U     2.225 0.383 15   5.807 0.0002

Results are averaged over the levels of: Block, Fertiliser
P value adjustment: tukey method for comparing a family of 4 estimates
```
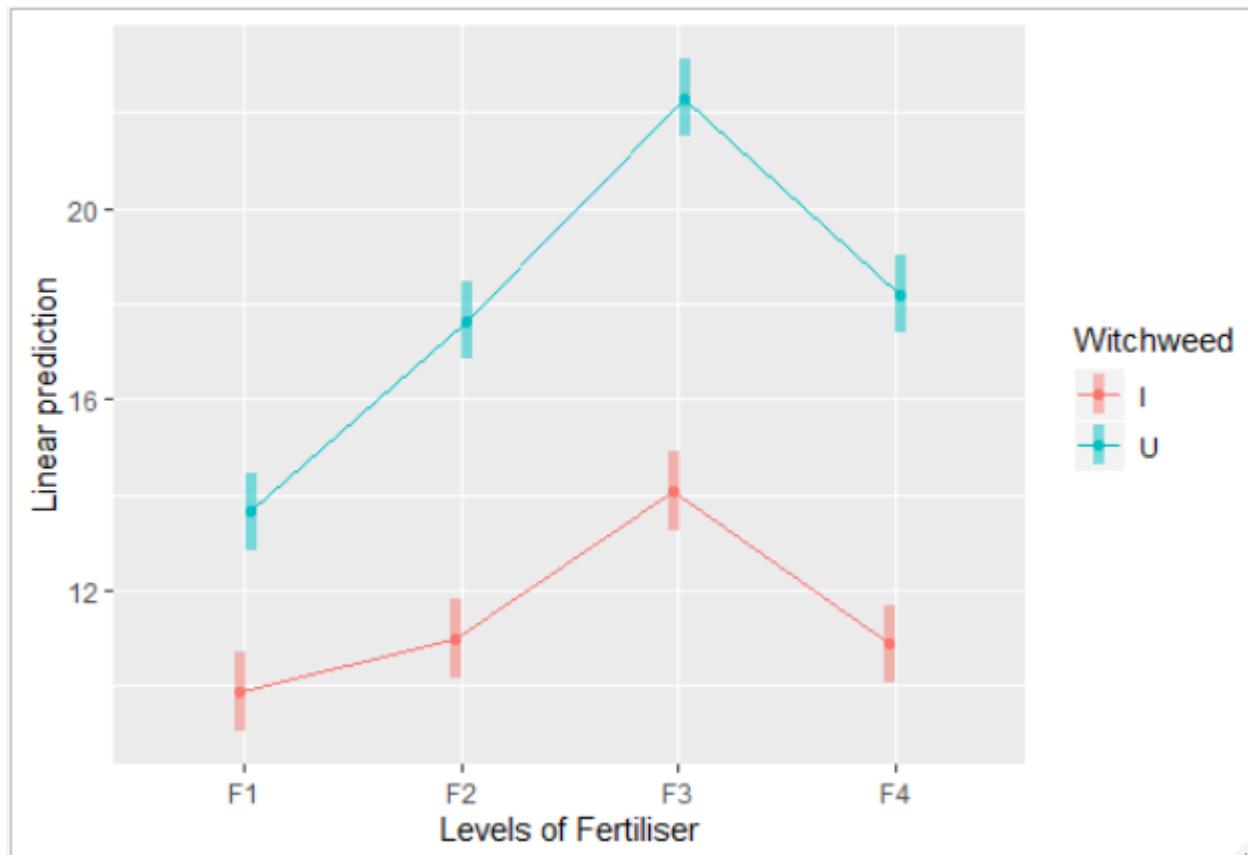
Factorials with >2 Factors

Post-hoc pairwise comparisons: Witchweed x fertiliser

Factorials with >2 Factors

Tukey Post-hoc test: Witchweed*Fertiliser

```
> emmeans(witch.aov, pairwise ~ Witchweed*Fertiliser)
NOTE: Results may be misleading due to involvement in
interactions
$emmeans
 Witchweed Fertiliser emmean    SE df lower.CL upper.CL
 I         F1           9.88 0.383 15     9.06     10.7
 U         F1          13.65 0.383 15    12.83     14.5
 I         F2          10.97 0.383 15    10.16     11.8
 U         F2          17.65 0.383 15    16.83     18.5
 I         F3          14.07 0.383 15    13.26     14.9
 U         F3          22.30 0.383 15    21.48     23.1
 I         F4          10.88 0.383 15    10.06     11.7
 U         F4          18.20 0.383 15    17.38     19.0

Results are averaged over the levels of: Block, Variety
Confidence level used: 0.95
```

```
$contrasts
 contrast      estimate     SE df t.ratio p.value
 I,F1 - U,F1    -3.775 0.542 15  -6.966 0.0001
 I,F1 - I,F2    -1.100 0.542 15  -2.030 0.4953
 I,F1 - U,F2    -7.775 0.542 15 -14.347 <.0001
 I,F1 - I,F3    -4.200 0.542 15  -7.750 <.0001
 I,F1 - U,F3   -12.425 0.542 15 -22.928 <.0001
 I,F1 - I,F4    -1.000 0.542 15  -1.845 0.6026
 I,F1 - U,F4    -8.325 0.542 15 -15.362 <.0001
 U,F1 - I,F2     2.675 0.542 15   4.936 0.0034
 U,F1 - U,F2    -4.000 0.542 15  -7.381 <.0001
 U,F1 - I,F3    -0.425 0.542 15  -0.784 0.9915
 U,F1 - U,F3    -8.650 0.542 15 -15.962 <.0001
 U,F1 - I,F4     2.775 0.542 15   5.121 0.0024
 U,F1 - U,F4    -4.550 0.542 15  -8.396 <.0001
 I,F2 - U,F2    -6.675 0.542 15 -12.318 <.0001
 I,F2 - I,F3    -3.100 0.542 15  -5.721 0.0008
 I,F2 - U,F3   -11.325 0.542 15 -20.898 <.0001
 I,F2 - I,F4     0.100 0.542 15   0.185 1.0000
 I,F2 - U,F4    -7.225 0.542 15 -13.332 <.0001
 U,F2 - I,F3     3.575 0.542 15   6.597 0.0002
 U,F2 - U,F3    -4.650 0.542 15  -8.581 <.0001
 U,F2 - I,F4     6.775 0.542 15  12.502 <.0001
 U,F2 - U,F4    -0.550 0.542 15  -1.015 0.9648
 I,F3 - U,F3    -8.225 0.542 15 -15.178 <.0001
 I,F3 - I,F4     3.200 0.542 15   5.905 0.0006
 I,F3 - U,F4    -4.125 0.542 15  -7.612 <.0001
 U,F3 - I,F4    11.425 0.542 15  21.083 <.0001
 U,F3 - U,F4     4.100 0.542 15   7.566 <.0001
 I,F4 - U,F4    -7.325 0.542 15 -13.517 <.0001

Results are averaged over the levels of: Block, Variety
P value adjustment: tukey method for comparing a family of
8 estimates
```

Summary

Factorial Designs

– Allows multiple research questions to be addressed

– "No aphorism is more frequently repeated in connection with field trials, than that we must ask Nature few questions, or, ideally, one question, at a time. The writer is convinced that this view is wholly mistaken." R. Fisher

– If interaction significant then cannot look at lower order effects (e.g. main effects)

Resources

- Quinn & Keough (2002) or (2024). Experimental Design and Data Analysis for Biologists

- Fox, G. A., S. Negrete-Yankelevich, and V. J. Sosa. (2015). *Ecological statistics: contemporary theory and application*. Oxford University Press, USA.

- Logan (2010) Biostatistical design and analysis using R a practical guide

- Ebooks in library

— **Questions/Feedback?**