



Design and Analysis of Agricultural Experiments

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Talk Outline

- Statistics & Variation
- Principles of Good Design
- Ideas for Student Experiments
- “GenStat for Teaching and Learning”

Coin Exercise

- those who have a coin, please toss it 10x and count the number of heads.
- lets record the number of heads
- graph results (histogram)
- calculate
 - mean [Excel=AVERAGE(...)]
 - standard deviation [=STDEV(...)]
 - standard error of mean [=STDEV(...)/SQRT(n)]

Average, SD, SE

- Average – estimate of the true unknown average
- Standard Deviation (SD)
 - measure of variation/spread in the data

Average ± 1 SD

Includes $\sim 70\%$ of the data

Average $\pm 2 \times \text{SD}$

Includes $\sim 95\%$ of the data

- Standard Error (SE) of Average
 - measure of error/uncertainty in the average

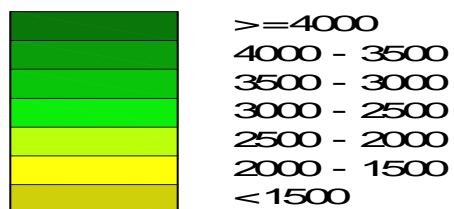
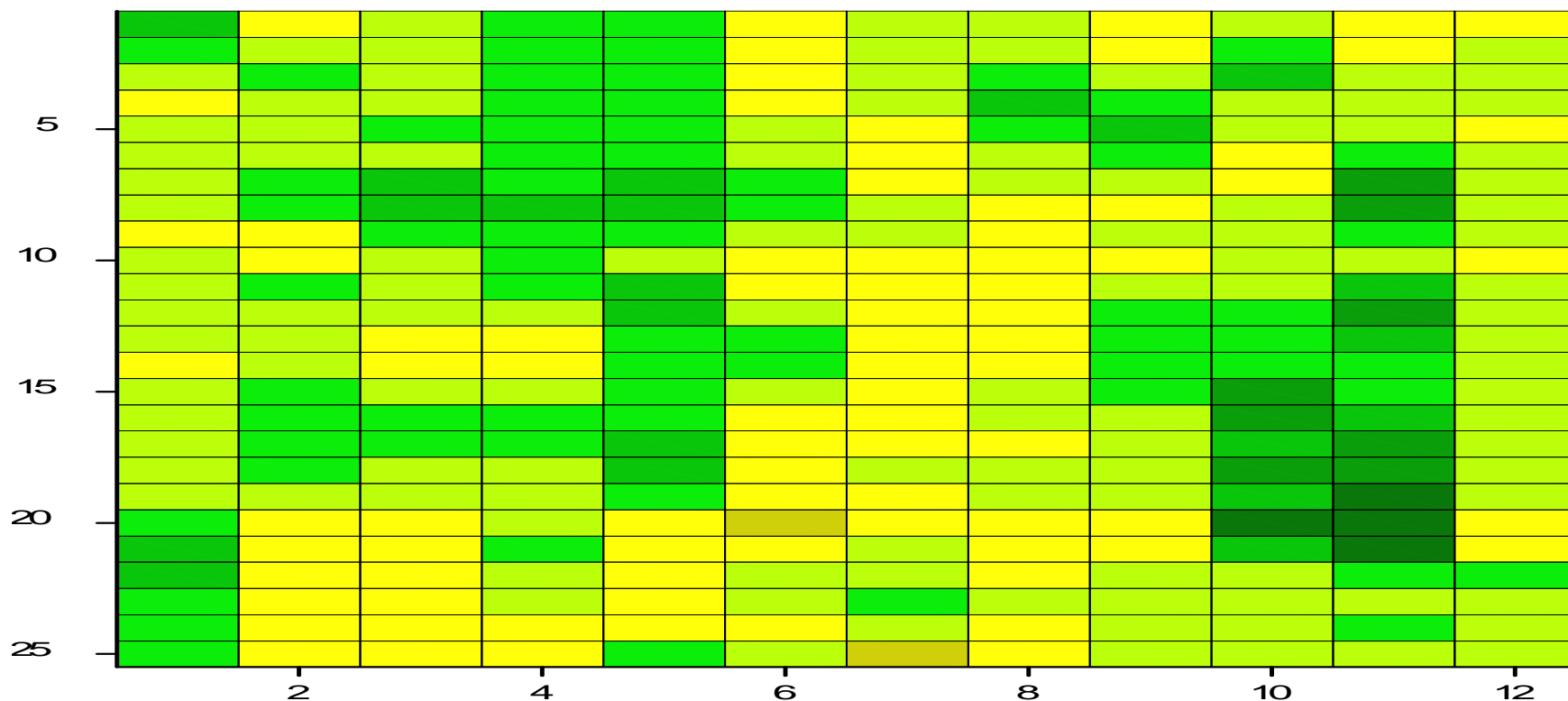
Average $\pm 2 \times \text{SE}$

$\sim 95\%$ confident includes true average (“Confidence Interval”)

Measurement Error

- A skilled Condition Score operator will often give a different (but close) score a 2nd time
 - Variation due to operator error
- Weigh scales also give different 2nd readings
 - Variation due to machine error
- Possible student exercise?
 - Weigh &/or CS sheep
 - Repeat in different order.

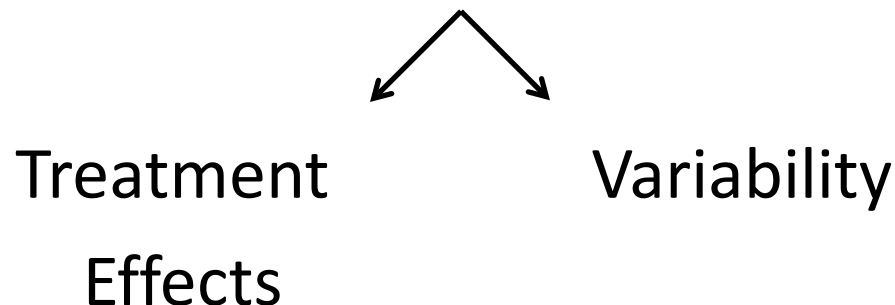
Field Variation



kg/ha wheat yield (5m x 1.25m plots)

Agricultural Experiments

- Purpose:
 - to compare the effect of different treatments
- Problem/Challenge:
 - many sources of variability so results include both:



What is required?

- A design where variation is minimised and measureable
- An analysis method to objectively test if treatments are different
- One approach:
 - Randomised Block Design
 - Analysis of Variance (ANOVA)

Randomised Block Design

E	A	D	B
B	C	A	D
C	D	B	E
D	E	C	A
A	B	E	C

Block1

Block2

Block3

Block4

← BLOCKING

- Treatments:

- A = 0 Nitrogen

← CONTROL

- B/C/D/E = 10/20/30/40 kg/ha Nitrogen

○ REPLICATION

- Blocks: each treatment once in random order

RANDOMISATION →

Why Replication?

- Most important ingredient for a good design
- Assume the following result (no replication)

Control 2.1	Treated 2.4
----------------	----------------

- Difference could be the result of different soil or disease variation or human/machine error.
- Replication – gives confidence the result is real an not a once-off.

Results from more Reps

	Situation 1		Situation 2				
Rep 1:	<table><tr><td>Control 2.1</td><td>Treated 2.4</td></tr></table>	Control 2.1	Treated 2.4		<table><tr><td>Control 2.1</td><td>Treated 2.4</td></tr></table>	Control 2.1	Treated 2.4
Control 2.1	Treated 2.4						
Control 2.1	Treated 2.4						
Rep 2:	<table><tr><td>Control 2.2</td><td>Treated 2.6</td></tr></table>	Control 2.2	Treated 2.6		<table><tr><td>Control 2.5</td><td>Treated 2.3</td></tr></table>	Control 2.5	Treated 2.3
Control 2.2	Treated 2.6						
Control 2.5	Treated 2.3						
Rep 3:	<table><tr><td>Control 1.9</td><td>Treated 2.1</td></tr></table>	Control 1.9	Treated 2.1		<table><tr><td>Control 2.2</td><td>Treated 2.0</td></tr></table>	Control 2.2	Treated 2.0
Control 1.9	Treated 2.1						
Control 2.2	Treated 2.0						
Conclusion:	treatment has positive effect		no conclusive treatment effect				

Poor Designs

- can give wrong conclusions:

Real Treatment Effect	Conclusion from Experiment	Practical Consequence
Yes	No	Lost opportunity
No	Yes	Waste \$ applying treatment

– better one experiment done well, than several poorly

“Consulting a statistician after the experiment has been conducted, is like performing an autopsy. You may be able to say what the experiment died of.” (R.A. Fisher)

Repeat how many times?

- More reps required when there is:
 - Higher variability
 - Smaller treatment differences
- 2 or 3 reps is often ok when there is a large number of treatments

Randomisation

- With no randomisation:

A	A	A	A
B	B	B	B
C	C	C	C
D	D	D	D
E	E	E	E

- Treatment A is always on the top edge and next to B which may give it an (dis)advantage.
- Randomisation is also insurance against unknown sources of variation

Blocking

- Grouping of plots that are more similar
- Improves comparisons (done within blocks)

Exercise: Which of these is blocked better?

– Assuming differences in environment left to right

E	A	D	B	C
B	C	A	D	E

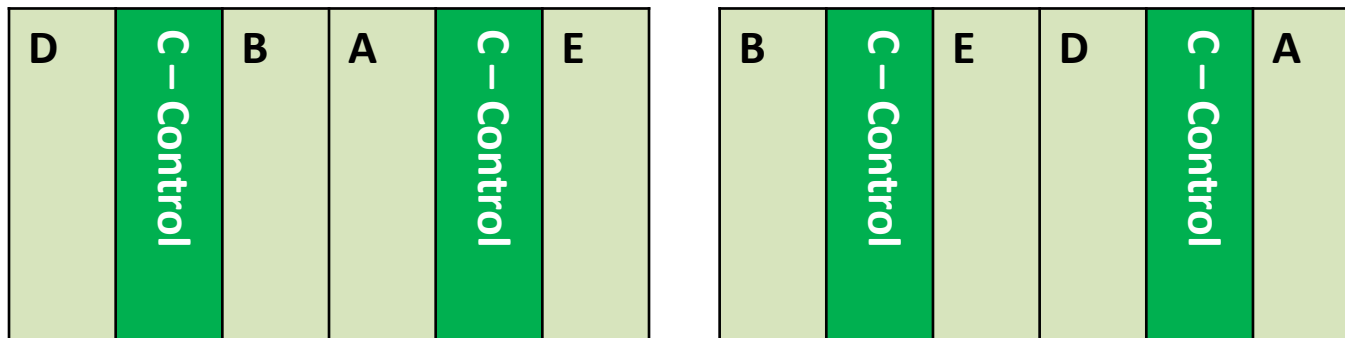
Option 1

E	B
A	C
D	A
B	D
C	E

Option 2

Controls

- Base treatment for comparisons
 - eg. standard practice; 0 rate
- Can be used to deal with spatial trends:



- Each treated plot can be compared to a neighbouring control

Stratification

- Preferred method for allocating animals to treatments
- Eg. Stratification by weight to 5 treatments
 - weight all animals
 - sort animal weights
 - randomly allocate 5 heaviest, one to each of the treatments
 - randomly allocate next 5, etc...

Factorial Treatments

- Assess 2+ treatments
- Include all combinations

Example “2x3 Factorial”

- 2 levels of Phosphorus (0, +P)
- 3 levels of Nitrogen (0, +N, +2N)
- 6 treatments (all combinations):

Nil, +P, +N, +P+N, +2N, +P+2N

Common Designs

1. Randomised Block Design (RBD):

Rep 1		Rep 2		Rep 3	
Nil	+P+N	+P+N	+N	+P+N	+P
+N	+2N	+P	Nil	+N	+2N
+P+2N	+P	+P+2N	+2N	Nil	+P+2N

Common, simple design – generally works well

Common Designs

2. Split Block Design:

Rep 1		Rep 2		Rep 3	
Nil	+P+N	+P+N	+N	+P+2N	Nil
+N	+P+2N	+P	Nil	+P+N	+N
+2N	+P	+P+2N	+2N	+P	+2N
+P		+P		+P	

Practical benefits in applying one treatment (eg. P) together but comes at some statistical cost

Common Designs

3. “Latinised” RBD:

Column Rep 1		Column Rep 2		Column Rep 3		
Nil	+P+N	+P	+N	+2N	+P+2N	Row Rep 1
+N	+2N	Nil	+P+2N	+P+N	+P	Row Rep 2
+P+2N	+P	+P+N	+2N	Nil	+N	Row Rep 3

Blocking in 2 directions improves design by better control of spatial variation.

Exercise

- How can this RBD be improved?

B	D	C	A	Rep 1
C	B	D	A	Rep 2
B	C	A	D	Rep 3
B	D	C	A	Rep 4

B	D	C	A
C	B	A	D
A	C	D	B
D	A	B	C

Latin Square

Student Exercise 1

- Plant Response to Nutrient Application
 - Treatments: 6 levels of Thrive: (or say Nitrogen)

Nil (Control)	$\frac{1}{4}$ x Label Rate	$\frac{1}{2}$ x Label Rate
Label Rate	$1\frac{1}{2}$ x Label Rate	2 x Label Rate

- Wheat planted in pots with saucers
 - In case of over-watering, can use water in saucers at the next watering to not lose nutrients.
- Randomised Block Design, 3 reps, 18 pots
- Layout/block according to environment
 - eg. Location in shade house can have an impact

Timing

- Week 1 – Design, prepare and seed pots
- Week 3 – Apply treatments
- Week 5 – Measurements (eg. dry matter cuts)
- Week 6 – Analyse results

Measurements

- Key Measure:
 - Dry matter cuts
- Other options:
 - No. Tillers
 - Height
 - Stem thickness
 - Visual observations (eg. yellowing)
 - indicate different nutrient deficiencies
 - Tissue tests (after sub-sampling)

Alternatives

- An outdoor plot experiment
 - Representative dry matter sampling
- Factorial treatments (Nitrogen & Phosphorus)
 - As well as the response to N and P, can assess if the response of N is different when P is applied
- Different plant eg. vegetable or pasture

Student Exercise 2

- Plant response to Grazing Level
 - Treatments: use lawnmower to simulate animal grazing down to different heights
 - Randomised Block Design, 3 reps
 - Layout/block according to environment
- Measurements
 - Dry matter cuts at beginning, middle and end

Timing

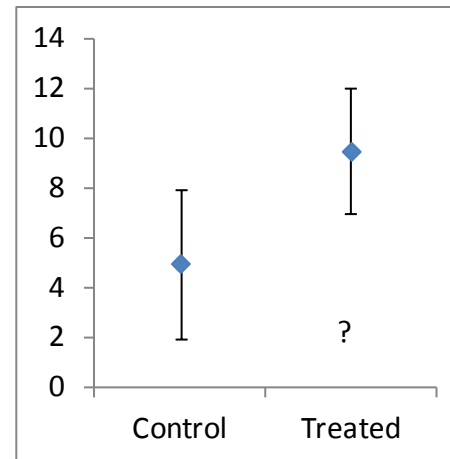
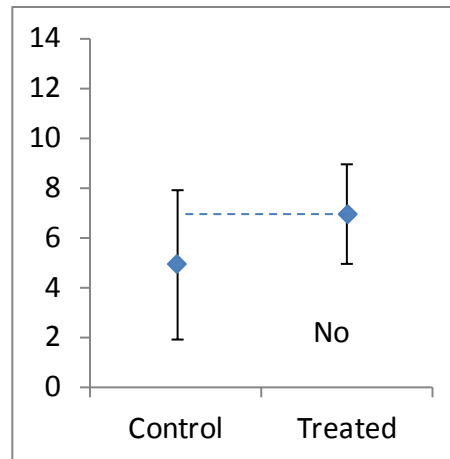
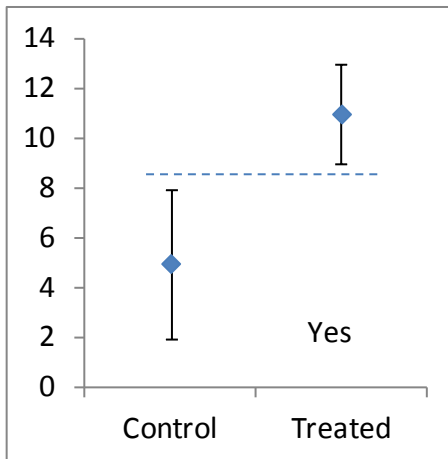
- Week 1 – layout trial and mow plots
- Week 1 – dry matter cuts
- Week 3 – dry matter cuts
- Week 5 – dry matter cuts
- Week 6 – analyse results

Exercises with Animals

- A couple of ideas:
 - “Faecal Egg Count Reduction Test” measuring reduction in worms two weeks after drenching.
 - Farmnote: http://www.agric.wa.gov.au/objtwr/imported_assets/content/pw/ah/par/fn2006_drenchresist_devans.pdf
 - Weight gain of egg-laying verses meat chickens fed the same for the first 6 weeks of their lives.
 - <http://www.chicken.org.au/page.php?id=233>

Analysis

- Key Question: Does the observed difference between treatments indicate a real difference, rather than just variability?
- Get some idea from mean and $2 \times \text{SE}$ of mean:



Analysis (cont)

- 2 treatments – Excel: TTEST(...)
- >2 treatment – Analysis of Variance (ANOVA)
 - ANOVA is an extension of T-test
 - Excel – has very limited/clumsy ANOVA capability
 - GenStat – great ANOVA capability (software used by the Department)

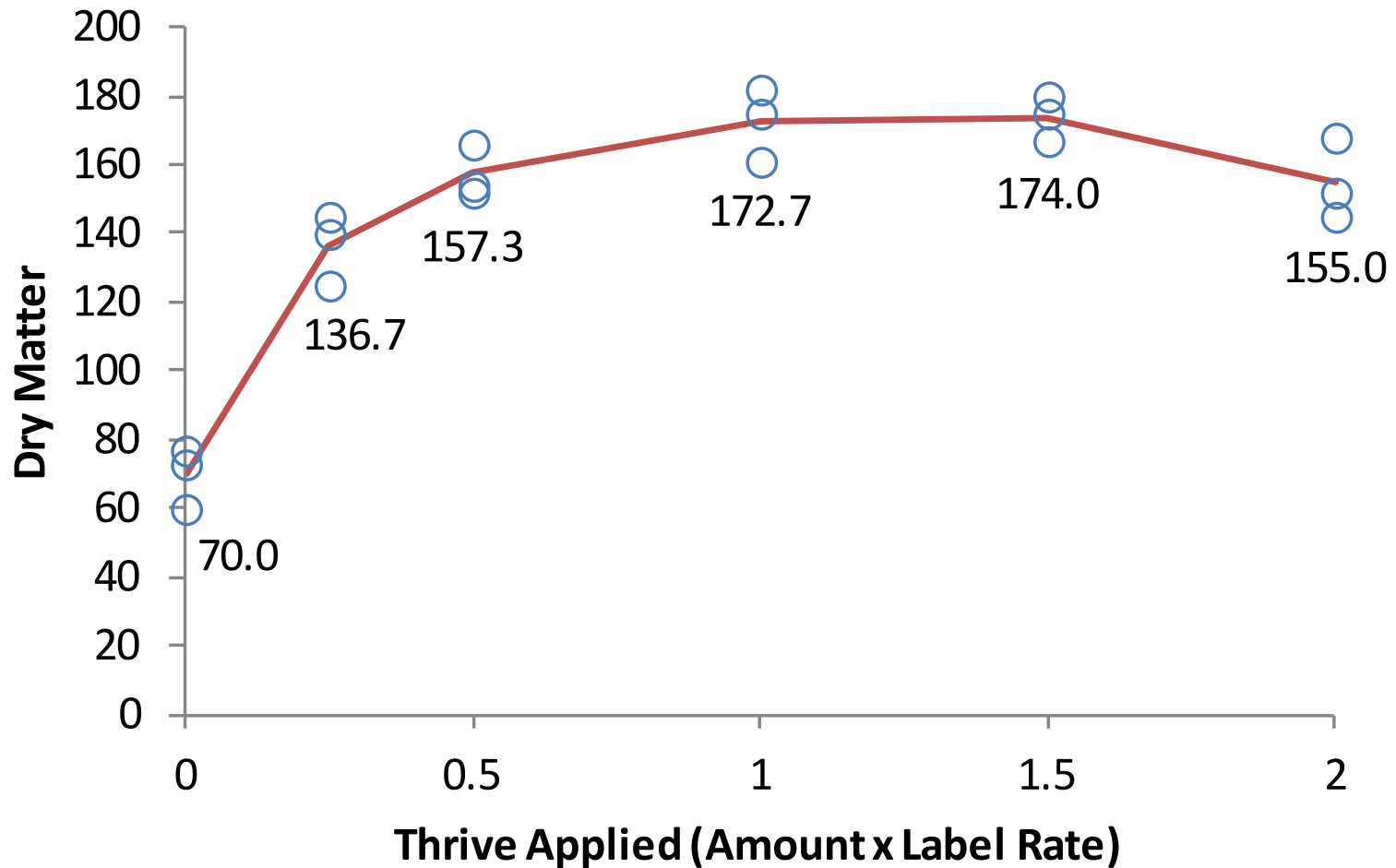
GenStat

- “GenStat for Teaching and Learning”
 - free for schools & universities
 - simple to receive and install – see:
<http://www.vsni.co.uk/software/teaching/genstat-teaching>
- Run in “undergraduate”, not “school” mode
 - Change via Options in Tools Menu

Design in GenStat

- Randomised Block Design:
 - In “Stats” Menu select “Design” and then “Generate a Standard Design”
 - Enter the number of blocks and treatments (the default number showing for each is 4).
 - Press Run
- Alternative methods:
 - Excel (using the =RAND() function)
 - “picking numbers out of a hat”

Example Results



Analysis in GenStat

- Analysis of Randomised Block Design
 - Prepare data in Excel in 3 columns:
 - Block! (Note: you need the !)
 - Treatment! (Note: you need the !)
 - Response (eg. dry matter) (Note: no ! here)
 - Load Data into GenStat
 - In Data menu, select “Load” and then “Data file”
 - Change “Files of type” to “Other Spreadsheet Files”
 - In “Stats” Menu select “Analysis of Variance” and then “General”...

Analysis in GenStat (cont)

- Enter “Y-variate” (eg. Dry matter), “Treatment” and “Block”
- In Options select “LSD’s”
- Press Run
- In Window Menu, select “Output”

Tables of means

Treatment	0.00	0.25	0.50	1.00	1.50	2.00
	70.0	136.7	157.3	172.7	174.0	155.0

Least significant differences of means (5% level)

l.s.d. 12.02

Analysis of Variance

- 2 key values:

	Tests:	Significant if:
1. p-value	<i>overall</i> treatment differences	$p < 0.05$
2. LSD	if <i>two treatments</i> different	difference $>$ LSD



Thank you

- Questions?