# Stat 412/512 ANOTHER MULTIFACTOR STUDY

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**Charlotte Wickham** 

stat512.cwick.co.nz

# Without replication

The estimate of  $\sigma$  depends on the model chosen.

We can't estimate a saturated model.

$$df = n - p = n - n = 0$$

Buy back some degrees of freedom by:

- assuming interactions don't exist fri
- some numerical categorical variables can be modelled as continuous

## case1402: Soybeans and ozone

Total of 30 combinations of treatment: Ozone (5 levels), sulfur dioxide (three levels) and water stress (two levels).

One chamber per treatment. I.e. one no replicate!

Two soybean cultivars in each chamber (we'll analyse them separately)

Response: yield in kg/hectare

### Seed yields for soybean cultivars *Forrest* and *Williams* from chambers kept under varying conditions of ozone, sulphur dioxide and water stress

			Yields (kg/ha)		
Water Stress	$SO_2(\mu L/L)$	0, (µL/L)		Forrest	Williams
Well-Watered	0.0045	0.017		4376 40	5561
(WW) (-0.05 MPa)		0.049		4544	5947
		0.067		2806	4273
		0.084		3339	3470
		0.099		3320	3080
	0.0170	0.017		3747	5092
		0.049	'	4570	4752
		0.067		4635	4232
		0.084		3013	2867
		0.099		3259	3106
	0.0590	0.017		4179	4736
		0.049		5077	3672
		0.067		3401	3386
		0.084		3371	2854
		0.099		2158	2557
<ul> <li>Soil Moisture Stress</li> </ul>	0.0045	0.017		4977	4520
(SMS) (-0.40 MPa)		0.049		3780	3047
		0.067		3804	3526
		0.084		3941	3337
		0.099		2863	2663
	0.0170	0.017		5573	4869
		0.049		3555	3774
		0.067		3340	2955
		0.084		3243	3513
		0.099	$\square$	2802	2838
	0.0590	0.017		4589	4056
		0.049		3250	2758
		0.067		3045	3094
		0.084		2827	2398
	L	0.099	4	2979	2101

halt star

### Questions

Do the three factors interact?

can't assume them away because they are what we are interested in

Does water stress affect yield?

Are there differences between the cultivars?



# Your turn

What would the saturated model be:  $\mu$  {log Yield | SO2, O3, Water } = Bo+B, (full model form) Int 502+ 03+WATER + 562×03 + 2 + 4 + 1 + 562×WATER + 03×WATER + 2×1 + ×1 8 (in shorthand) 03×502 × WATER 2×4×1 562-3 levels WATTER-Zlevely 03 - 5 levels paramete

### Tentative model

Looks like we can save some parameters by modeling ozone as continuous

(1 slope parameter, instead of 4 parameters on indicators)

 $\mu$  {log Yield | SO2, O3, Water } =

SO2 + WATER + ozone + SO2xozone + WATER×SO2 + WATERxozone + WATER×SO2xozone

## Soz - 3hrun, Yourturn Water - Zhevels

 $\mu$  {log Yield | SO2, O3, Water } =

How many parameters are in this model?



### Residuals from tentative model



### Checking linearity of Ozone





Ozone, SO2 interaction would mean a different slope for ozone at each level of SO2.

Ozone, water stress interaction would mean a different slope for ozone at each level of stress.

A ozone, SO2, water interaction, allows the effect of sulphur on the relationship between yield and ozone to depend on water stress.

#### Forrest Cultivar

Source	df	Sum of Squares	Mean Square	F-stat	p-value
ozone	1	.7208	.7208	30.050	<.0001
SULPHUR	2	.0635	.0317	1.356	.2827
water	1	.0080	.0080	.343	.5651
ozone×SULPHUR	2	.0173	.0087	.370	.6956
ozone×water	1	.0136	.0136	.583	.4552
SULPHUR×water	2	.0285	.0143	.610	.5543
ozone×SULPHUR×water	2	.0683	.0342	1.461	.2583
residuals	18	.4211	.0234		

#### Williams Cultivar

Source	df	Sum of Squares	Mean Square	F-stat	p-value
ozone	1	1.150	1.150	86.7999	<.00001
SULPHUR	2	.2780	.1390	10.495	.001
water	1	.2376	.2376	17.943	.0005
ozone×SULPHUR	2	.0037	.0019	.140	.8702
ozone×water	1	.0128	.0128	.964	.3392
SULPHUR×water	2	.0263	.0131	.994	.3896
ozone×SULPHUR×water	2	.0093	.0047	.352	.7079
residuals	18	.2384	.0132		

Full model is: µ {log Yield | SO2, O3, Water } =

SO2 + WATER + ozone + SO2xozone + WATERxSO2 + WATERxozone + WATERxSO2xozone

# I think it's easier to think about simplifying the model

#### three way interaction?

Compare:

SO2 + WATER + ozone + SO2×ozone + WATER×SO2 + WATER×ozone +

WATER×SO2×ozone

#### to

SO2 + WATER + ozone + SO2xozone + WATERxSO2 + WATERxozone

#### two way interactions?

Compare:

SO2 + WATER + ozone + SO2×ozone + WATER×SO2 + WATER×ozone

to

SO2 + WATER + ozone

big p-value

sig p-value

model SO2 as linear too (after checking with a test).

#### Coefficient estimates and standard errors for the linear soybean models, with Y = log(soybean seed yield)

	Forrest		a eta. a	Williams		a eta. a
Variable	Coefficient	St. Error	2-Sided p-Value	Coefficient	St. Error	2-Sided p-Value
CONSTANT	8.608	0.080		8.825	0.058	
ozone	-5.397	0.929	<.0001	-6.806	0.679	<.0001
sulphur	-1.566	0.989	.1252	-3.512	0.723	<.0001
water	0.094	0.153	.5453	0.507	0.112	.0001

1page summary in Sleuth.

Includes estimates of possible size of interactions (even though we have no evidence for them).

I.e. The ozone effect when SO2 is 0.0590 is estimated to be only 14.7% of the ozone effect when SO2 is 0.0045 (95% CI, 0.16% and 1365%)

2ndEd

I.e. The ozone effect when SO2 is 0.0590 is estimated to be only 14.7% of the ozone effect when SO2 is 0.0045 (95% CI, 0.16% and 1365%)

Fit:  $\mu$  {log Yield | SO2, O3, Water } = so2+ WATER + ozone + so2×ozone =  $\beta_0$  +  $\beta_1$ ozone +  $\beta_2$ so2 +  $\beta_3$ water +  $\beta_4$ ozone × so2

	Estimate St	d. Error t	value H	?r(> t )	
(Intercept)	8.54408	0.10229	83.528	< 2e-16	***
S02	0.34845	2.77868	0.125	0.90121	
StressStressed	-0.03274	0.05364	-0.610	0.54718	
03	-4.50785	1.42371	-3.166	0.00404	**
SO2:03	-34.81736	40.07680	-0.869	0.39324	

Slope on ozone is :  $\beta_1 + \beta_4$ so2 Slope when SO2 is 0.0590:  $\beta_1 + \beta_4 0.0590$ Slope when SO2 is 0.0045:  $\beta_1 + \beta_4 0.0045$ Difference in slope:  $\beta_4$  (0.0590 - 0.0045)

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2ndEd
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 $\exp(-34.81736^{*}(0.0590 - 0.0045)) = 0.1499361$ 

### Difference in slope: $\beta_4$ (0.0590 - 0.0045)

<pre>&gt; confint(fit_f</pre>	for_int)	
	2.5 %	97.5 %
(Intercept)	8.3334107	8.75475270
S02	-5.3743554	6.07125724
StressStressed	-0.1432094	0.07773829
03	-7.4400394	-1.57565236
SO2:03	-117.3570749	47.72235133

exp(c(-117.3570749, 47.7223513) \* (0.0590 - 0.0045)) [1] 0.001668283 13.475431598

I.e. The ozone effect when SO2 is 0.0590 is estimated to be only 14.7% of the ozone effect when SO2 is 0.0045 (95% CI, 0.16% and 1365%) 2ndEd

### 3rd ed

I.e. The detrimental effect of increasing ozoneby 0.01 when SO2 is 0.0590 is estimated to be1.9% greater than when SO2 is 0.0045 (95% CI,2.6% smaller and 6.7% larger)

A much more sensible comparison! exp(-34.81736\*(0.0590 - 0.0045)\*0.01) = 0.981

# To replicate or not?

If interactions are of interest, then replicate!

When experimental units are expensive, you can sometimes gain more by reducing variability, than increasing your replicates.

> SE of cell average =  $\sigma / \sqrt{\text{number in cell}}$ reduce this or increase this

### Hypothetical example

- New method for reducing high blood pressure.
- Blood pressure tends to depend on age. With no treatment the researcher expects something like ->



#### Option 1:

Ignore age, randomly assign treatment to six people aged 50 to 75. Six replicates, can make causal inferences. Expected variability = 6.1

#### **Option 2:**

Pick 6 people of the same age, randomly assign to treatment. Six replicates, can still make causal inferences (to a much reduced population). Expected variability = 3.8

#### **Option 3:**

Pick 6 people from 50-75 but pair them by similar ages, within each pair randomly assign to treatment (i.e. block by age). No replicates, can still make causal inferences. Expected variability ~ closer to 3.8

### Lesson: Include important sources of variation in the design.

## Identifying false replicates

a.k.a pseudo replication

- The replication needs to be at the level of experimental unit (the items that are randomly assigned to treatment).
- The replicates need to be **independent** applications of the same treatment.

# Examples

**Pygmalion study**: platoon was randomized to treatment. It would be inappropriate to treat individual soldiers scores on the test as replicates.

**Soybean study:** chambers were randomized to treatment. It would be inappropriate to treat individual soybean plants as replicates.

in both cases we used the average within the experimental unit our estimate of  $\sigma$  tells us about the variability expected between experimental units

# Experimental Design

### ST513

### read chapters 23 & 24

### A good read:

Hurlbert, Stuart H. (1984). "Pseudoreplication and the design of ecological

field experiments". Ecological Monographs (Ecological Society of America) 54

(2): 187–211. <u>doi:10.2307/1942661</u>