STAT 401A - Statistical Methods for Research Workers

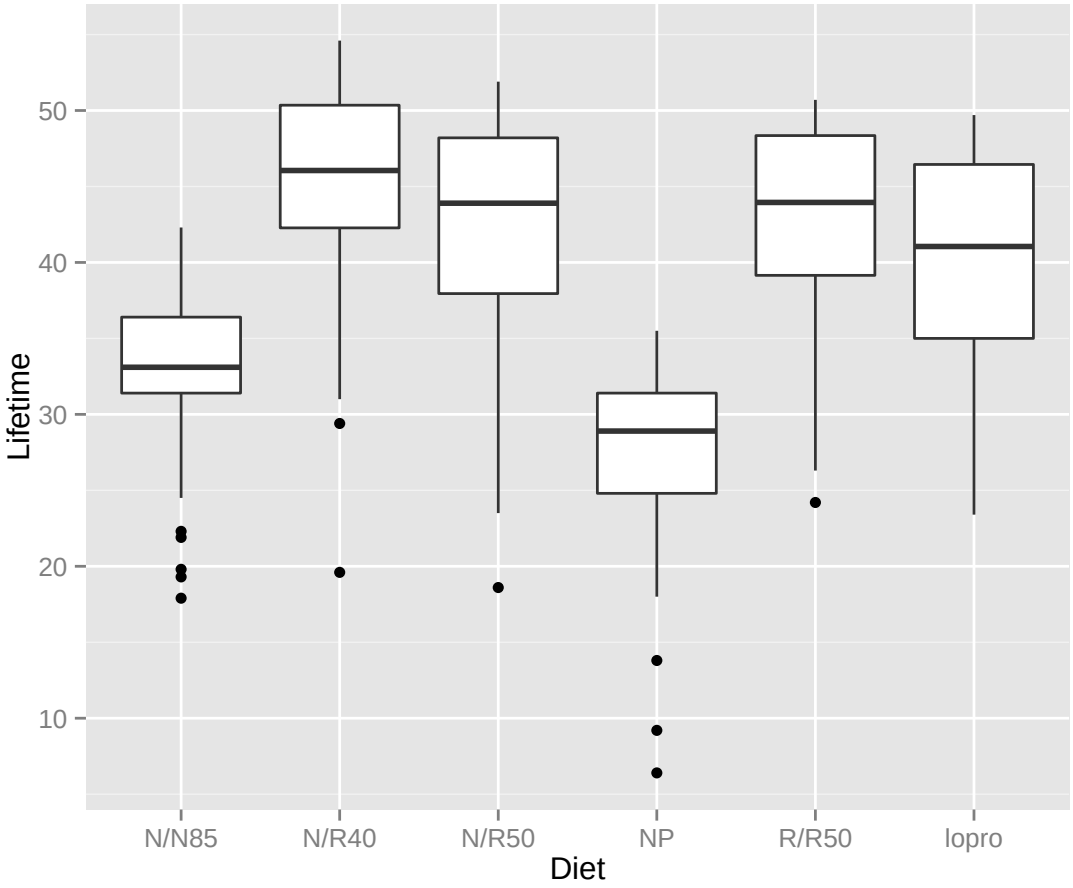
One-way ANOVA (contrasts and multiple comparisons)

Jarad Niemi (Dr. J)

Iowa State University

last updated: October 10, 2014

Mice lifetimes



Simple hypothesis

Consider the one-way ANOVA model: $Y_{ij} \sim N(\mu_j, \sigma^2)$ where $j = 1, \ldots, J$.

Here are a few simple alternative hypotheses:

1. Mean lifetimes for N/R50 and R/R50 diet are different.
2. Mean lifetimes for N/R40 is different than for N/R50 and R/R50 combined.
3. Mean lifetimes for high calorie (NP and N/N85) diets is different than for low calorie diets combined.

$H_0 : \gamma = 0 \qquad H_1 : \gamma \neq 0 :$

$$\begin{aligned}
\gamma_1 &= \mu_{R/R50} - \mu_{N/R50} \\
\gamma_2 &= \mu_{N/R40} - \frac{1}{2}(\mu_{N/R50} + \mu_{R/R50}) \\
\gamma_3 &= \frac{1}{4}(\mu_{N/R50} + \mu_{R/R50} + \mu_{N/R40} + \mu_{lopro}) - \frac{1}{2}(\mu_{NP} + \mu_{N/N85})
\end{aligned}$$

Contrasts

Definition

A linear combination of group means has the form

$$\gamma = C_1\mu_1 + C_2\mu_2 + \ldots + C_J\mu_J$$

where C_j are known coefficients and μ_j are the unknown population means.

Definition

A linear combination with $C_1 + C_2 + \cdots + C_J = 0$ is a contrast.

Remark Contrast interpretation is usually best if $|C_1| + |C_2| + \cdots + |C_J| = 2$, i.e. the positive sum to 1 and the negative coefficients sum to -1.

Inference on contrasts

$$\gamma = C_1\mu_1 + C_2\mu_2 + \cdots + C_J\mu_J$$

Estimated by

$$g = C_1\overline{Y}_1 + C_2\overline{Y}_2 + \cdots + C_J\overline{Y}_J$$

with standard error

$$SE(g) = s_p\sqrt{\frac{C_1^2}{n_1} + \frac{C_2^2}{n_2} + \cdots + \frac{C_J^2}{n_J}}$$

t-statistic (compare to t_{n-J}) and CI:

$$t = \frac{g}{SE(g)} \qquad g \pm t_{n-J}(1-\alpha/2)SE(g)$$

Contrasts for mice lifetime dataset

For these contrasts:

1. Mean lifetimes for N/R50 and R/R50 diet are different.
2. Mean lifetimes for N/R40 is different than for N/R50 and R/R50 combined.
3. Mean lifetimes for high calorie (NP and N/N85) diets is different than for low calorie diets combined.

$H_0 : \gamma = 0 \qquad H_1 : \gamma \neq 0 :$

$$\begin{aligned}
\gamma_1 &= \mu_{R/R50} - \mu_{N/R50} \\
\gamma_2 &= \mu_{N/R40} - \frac{1}{2}(\mu_{N/R50} + \mu_{R/R50}) \\
\gamma_3 &= \frac{1}{4}(\mu_{N/R50} + \mu_{R/R50} + \mu_{N/R40} + \mu_{lopro}) - \frac{1}{2}(\mu_{NP} + \mu_{N/N85})
\end{aligned}$$

	N/N85	N/R40	N/R50	NP	R/R50	lopro
early rest - none @ 50kcal	0.00	0.00	-1.00	0.00	1.00	0.00
40kcal/week - 50kcal/week	0.00	1.00	-0.50	0.00	-0.50	0.00
lo cal - hi cal	-0.50	0.25	0.25	-0.50	0.25	0.25

Mice liftime examples

	Diet	n	mean	sd
1	N/N85	57	32.69	5.13
2	N/R40	60	45.12	6.70
3	N/R50	71	42.30	7.77
4	NP	49	27.40	6.13
5	R/R50	56	42.89	6.68
6	lopro	56	39.69	6.99

Contrasts:

	g	SE(g)	t	p	L	U
early rest - none @ 50kcal	0.59	1.19	0.49	0.62	-1.76	2.94
40kcal/week - 50kcal/week	2.53	1.05	2.41	0.02	0.46	4.59
lo cal - hi cal	12.45	0.78	15.96	0.00	10.92	13.98

```
DATA case0501;
  INFILE 'case0501.csv' DSD FIRSTOBS=2;
  INPUT lifetime diet $ ;

PROC MEANS DATA=case0501;
  CLASS diet;
  VAR lifetime;
  RUN;
```

The MEANS Procedure
Analysis Variable : lifetime

diet	N Obs	N	Mean	Std Dev	Minimum	Maximum
N/N85	57	57	32.6912281	5.1252972	17.9000000	42.3000000
N/R40	60	60	45.1166667	6.7034058	19.6000000	54.6000000
N/R50	71	71	42.2971831	7.7681947	18.6000000	51.9000000
NP	49	49	27.4020408	6.1337010	6.4000000	35.5000000
R/R50	56	56	42.8857143	6.6831519	24.2000000	50.7000000
lopro	56	56	39.6857143	6.9916945	23.4000000	49.7000000

```
PROC GLM;
  CLASS diet;
  MODEL lifetime = diet / CLPARM;
  ESTIMATE 'early rest - none @ 50kcal' diet  0  1 -1  0  0  0 ;
  ESTIMATE '40kcal/week - 50kcal/week'  diet  0  2 -1  0 -1  0 / DIVISOR = 2;
  ESTIMATE 'lo cal - hi cal'            diet -2  1  1 -2  1  1 / DIVISOR = 4 ;
  RUN;
  QUIT;
```

The GLM Procedure

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	12733.94181	2546.78836	57.10	<.0001
Error	343	15297.41532	44.59888		
Corrected Total	348	28031.35713			

Parameter	Estimate	Standard Error	t Value	Pr > |t|
early rest - none @ 50kcal	0.5885312	1.19355007	0.49	0.6223
40kcal/week - 50kcal/week	2.5252180	1.04854904	2.41	0.0166
lo cal - hi cal	12.4496851	0.78001425	15.96	<.0001

Parameter	95% Confidence Limits	
early rest - none @ 50kcal	-1.7590676	2.9361299
40kcal/week - 50kcal/week	0.4628224	4.5876136
lo cal - hi cal	10.9154718	13.9838985

```
library(multcomp)
m = lm(Lifetime~Diet-1, case0501) # The -1 indicates no intercept (see Ch 7)
summary(m)
```

```
Call:
lm(formula = Lifetime ~ Diet - 1, data = case0501)

Residuals:
    Min      1Q  Median      3Q     Max
-25.517  -3.386   0.814   5.183  10.014

Coefficients:
          Estimate Std. Error t value Pr(>|t|)
DietN/N85   32.691      0.885    37.0   <2e-16 ***
DietN/R40   45.117      0.862    52.3   <2e-16 ***
DietN/R50   42.297      0.793    53.4   <2e-16 ***
DietNP      27.402      0.954    28.7   <2e-16 ***
DietR/R50   42.886      0.892    48.1   <2e-16 ***
Dietlopro   39.686      0.892    44.5   <2e-16 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 6.68 on 343 degrees of freedom
Multiple R-squared:  0.972, Adjusted R-squared:  0.972
F-statistic: 2.01e+03 on 6 and 343 DF,  p-value: <2e-16
```

```
K
```

```
                          N/N85 N/R40 N/R50   NP R/R50 lopro
early rest - none @ 50kcal   0.0  0.00 -1.00  0.0  1.00  0.00
40kcal/week - 50kcal/week    0.0  1.00 -0.50  0.0 -0.50  0.00
lo cal - hi cal             -0.5  0.25  0.25 -0.5  0.25  0.25
```

```
t = glht(m, linfct=K)
summary(t)
```

```
 Simultaneous Tests for General Linear Hypotheses

Fit: lm(formula = Lifetime ~ Diet - 1, data = case0501)

Linear Hypotheses:
                               Estimate Std. Error t value Pr(>|t|)
early rest - none @ 50kcal == 0    0.589      1.194    0.49    0.946
40kcal/week - 50kcal/week == 0     2.525      1.049    2.41    0.049 *
lo cal - hi cal == 0              12.450      0.780   15.96   <1e-05 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
(Adjusted p values reported -- single-step method)
```

```
confint(t, calpha=univariate_calpha())
```

```
 Simultaneous Confidence Intervals

Fit: lm(formula = Lifetime ~ Diet - 1, data = case0501)

Quantile = 1.967
95% confidence level

Linear Hypotheses:
                               Estimate lwr    upr
early rest - none @ 50kcal == 0  0.589   -1.759  2.936
40kcal/week - 50kcal/week == 0   2.525    0.463  4.588
lo cal - hi cal == 0            12.450   10.915 13.984
```

Summary

- Contrasts are linear combinations that sum to zero
- t-test tools are used to calculate pvalues and confidence intervals

SAS code and output for one-way ANOVA

```
DATA mice;
  INFILE 'case0501.csv' DSD FIRSTOBS=2;
  INPUT lifetime diet $;

PROC GLM DATA=mice;
  CLASS diet;
  MODEL lifetime = diet;
  LSMEANS diet / ADJUST=T;
  RUN;
```

The GLM Procedure

Dependent Variable: lifetime

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	12733.94181	2546.78836	57.10	<.0001
Error	343	15297.41532	44.59888		
Corrected Total	348	28031.35713			

SAS code and output for pairwise comparisons

The GLM Procedure
Least Squares Means

diet	lifetime LSMEAN	LSMEAN Number
N/N85	32.6912281	1
N/R40	45.1166667	2
N/R50	42.2971831	3
NP	27.4020408	4
R/R50	42.8857143	5
lopro	39.6857143	6

Least Squares Means for effect diet
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: lifetime

i/j	1	2	3	4	5	6
1		<.0001	<.0001	<.0001	<.0001	<.0001
2	<.0001		0.0166	<.0001	0.0731	<.0001
3	<.0001	0.0166		<.0001	0.6223	0.0293
4	<.0001	<.0001	<.0001		<.0001	<.0001
5	<.0001	0.0731	0.6223	<.0001		0.0117
6	<.0001	<.0001	0.0293	<.0001	0.0117	

NOTE: To ensure overall protection level, only probabilities associated with pre-planned comparisons should be used.

Statistical testing errors

Definition

A type I error occurs when a true null hypothesis is rejected.

Definition

A type II error occurs when a false null hypothesis is not rejected. Power is one minus the type II error probability.

Remark We set α to control the type I error probability. If we set $\alpha = 0.05$, then we will incorrectly reject a true null hypothesis 5% of the time.

Definition

The familywise error rate is the probability of rejecting at least one true null hypothesis.

Type I error for all pairwise comparisons of J groups

How many combinations when choosing 2 items out of J?

$$\binom{J}{2} = \frac{J!}{2!(J-2)!}.$$

If $J = 6$, then there are 15 different comparison of means. If we set $\alpha = 0.05$ as our significance level, then individually each test will only incorrectly reject 5% of the time.

If we have 15 tests and use $\alpha = 0.05$, what is the familywise error rate?

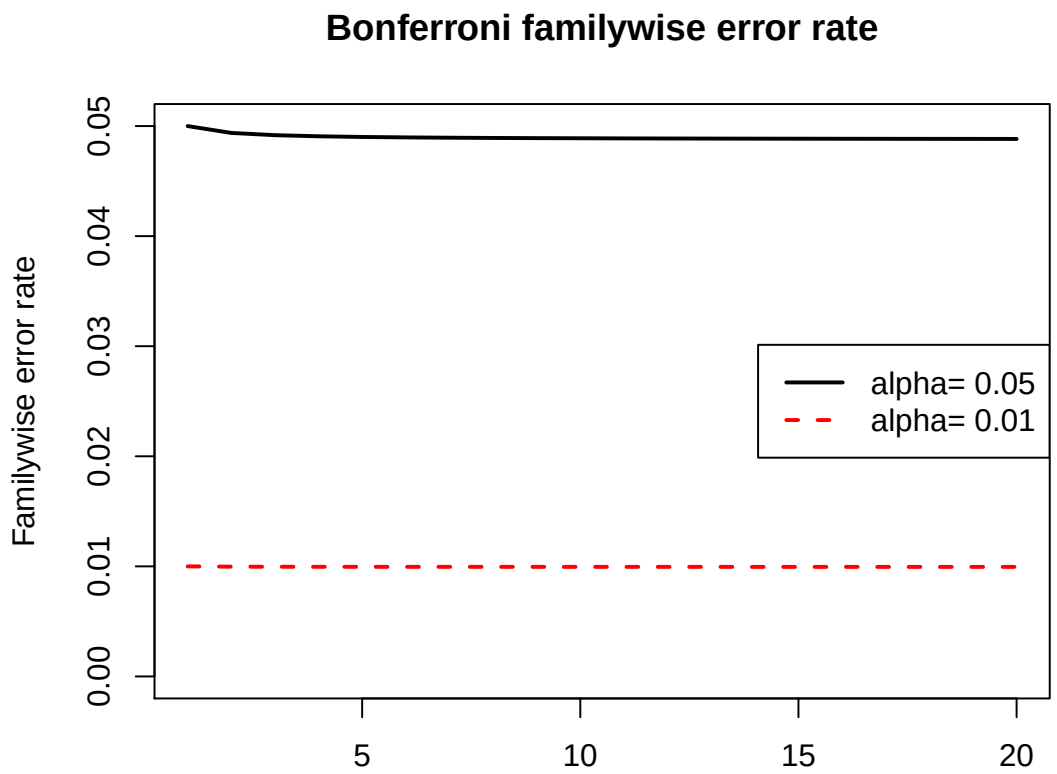
$$1 - (1 - 0.05)^{15} = 1 - (0.95)^{15} = 1 - 0.46 = 0.54$$

So there is a greater than 50% probability of falsely rejecting a true null hypothesis!

Bonferroni correction

Definition

If we do m tests and want the familywise error rate to be α, the Bonferroni correction uses α/m for each individual test. The familywise error rate, for independent tests, is $1 - (1 - \alpha/, m)^m$.



SAS code and output for pairwise comparisons

Compare the unadjusted pvalues to $\alpha/15 = 0.05/15 = 0.0033$.

Least Squares Means for effect diet
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: lifetime

i/j	1	2	3	4	5	6
1		<.0001	<.0001	<.0001	<.0001	<.0001
2	<.0001		0.0166	<.0001	0.0731	<.0001
3	<.0001	0.0166		<.0001	0.6223	0.0293
4	<.0001	<.0001	<.0001		<.0001	<.0001
5	<.0001	0.0731	0.6223	<.0001		0.0117
6	<.0001	<.0001	0.0293	<.0001	0.0117	

NOTE: To ensure overall protection level, only probabilities associated with pre-planned comparisons should be used.

Now 2-3, 3-6, and 5-6 are no longer significant.

SAS code and output for one-way ANOVA

If you use SAS to do the adjustment, compare pvalues to $\alpha = 0.05$.

```
DATA mice;
  INFILE 'case0501.csv' DSD FIRSTOBS=2;
  INPUT lifetime diet $;

PROC GLM DATA=mice;
  CLASS diet;
  MODEL lifetime = diet;
  LSMEANS diet / ADJUST=BON;
  RUN;
```

Least Squares Means for effect diet
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: lifetime

i/j	1	2	3	4	5	6
1		<.0001	<.0001	0.0009	<.0001	<.0001
2	<.0001		0.2488	<.0001	1.0000	0.0002
3	<.0001	0.2488		<.0001	1.0000	0.4402
4	0.0009	<.0001	<.0001		<.0001	<.0001
5	<.0001	1.0000	1.0000	<.0001		0.1751
6	<.0001	0.0002	0.4402	<.0001	0.1751	

Comments on the Bonferroni correction

Remark The Bonferroni correction can be used in any situation. In particular, it can be used on unadjusted pvalues reported in an article that has many tests by comparing their pvalues to α/m where m is the number of tests they perform.

Remark The Bonferroni correction is (in general) the most conservative multiple comparison adjustment we will discuss, i.e. it will lead to the least null hypothesis rejections.

Constructing multiple confidence intervals

Remark A $100(1-\alpha)\%$ confidence interval should contain the true value $100(1-\alpha)\%$ of the time.

Remark An error occurs if the confidence interval does not contain the true value.

Just like the Type I error and familywise error rate, we can ask what is the probability at least one confidence interval does not cover the true value.

The procedures we will talk about for confidence intervals have equivalent approaches for hypothesis testing (pvalues). Within these procedures we still have the equivalence between pvalues and CIs.

Constructing multiple confidence intervals

Confidence interval for the difference between group j and group j':

$$\overline{Y}_j - \overline{Y}_{j'} \pm M\, s_p \sqrt{\frac{1}{n_j} + \frac{1}{n_{j'}}}$$

where M is a multiplier that depends on the adjustment procedure:

Procedure	M	Use
LSD	$t_{n-J}(1-\alpha/2)$	After significant F-test (no adjustment)
Dunnett	multivariate t	Compare all groups to control
Tukey-Kramer	$q_{J,n-J}(1-\alpha/2)/\sqrt{2}$	All pairwise comparisons
Scheffé	$\sqrt{(J-1)F_{(J-1,n-J)}(1-\alpha)}$	All contrasts
Bonferroni	$t_{n-J}(1-\alpha/2m)$	m tests (most generic)

SAS code and output for one-way ANOVA

```
DATA mice;
  INFILE 'case0501.csv' DSD FIRSTOBS=2;
  INPUT lifetime diet $;

PROC GLM DATA=mice;
  CLASS diet;
  MODEL lifetime = diet;
  LSMEANS diet / CL ADJUST=TUKEY;
  RUN;
```

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Tukey-Kramer

Dependent Variable: lifetime

i/j	1	2	3	4	5	6
1		<.0001	<.0001	0.0008	<.0001	<.0001
2	<.0001		0.1565	<.0001	0.4684	0.0002
3	<.0001	0.1565		<.0001	0.9964	0.2460
4	0.0008	<.0001	<.0001		<.0001	<.0001
5	<.0001	0.4684	0.9964	<.0001		0.1168
6	<.0001	0.0002	0.2460	<.0001	0.1168	

SAS code and output for one-way ANOVA

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Tukey-Kramer

Least Squares Means for Effect diet

i	j	Difference Between Means	Simultaneous 95% Confidence Limits for LSMean(i)-LSMean(j)	
1	2	-12.425439	-15.965442	-8.885435
1	3	-9.605955	-13.009741	-6.202169
1	4	5.289187	1.560626	9.017749
1	5	-10.194486	-13.795557	-6.593416
1	6	-6.994486	-10.595557	-3.393416
2	3	2.819484	-0.536769	6.175736
2	4	17.714626	14.029406	21.399846
2	5	2.230952	-1.325223	5.787128
2	6	5.430952	1.874777	8.987128
3	4	14.895142	11.340571	18.449714
3	5	-0.588531	-4.009133	2.832070
3	6	2.611469	-0.809133	6.032070
4	5	-15.483673	-19.227592	-11.739755
4	6	-12.283673	-16.027592	-8.539755
5	6	3.200000	-0.416969	6.816969

False Discovery Rate

Remark Not wanting to make a single mistake is pretty conservative.

In high-throughput fields a more common multiple comparison adjustment is false discovery rate.

Definition

False discovery rate procedures try to control the expected proportion of incorrectly rejected null hypotheses.

How to incorporate multiple comparison adjustments

1. Determine what tests are going to be run (before looking at the data) or what confidence intervals are going to be constructed.
2. Determine which multiple comparison adjustment is the most relevant
3. Use/state that adjustment and interpret your results

Sulfur effect on scab disease in potatoes

The experiment was conducted to investigate the effect of sulfur on controlling scab disease in potatoes. There were seven treatments: control, plus spring and fall application of 300, 600, 1200 lbs/acre of sulfur. The response variable was percentage of the potato surface area covered with scab averaged over 100 random selected potatoes. A completely randomized design was used with 8 replications of the control and 4 replications of the other treatments.

Cochran and Cox. (1957) Experimental Design (2nd ed). pg96 and Agron. J. 80:712-718 (1988)

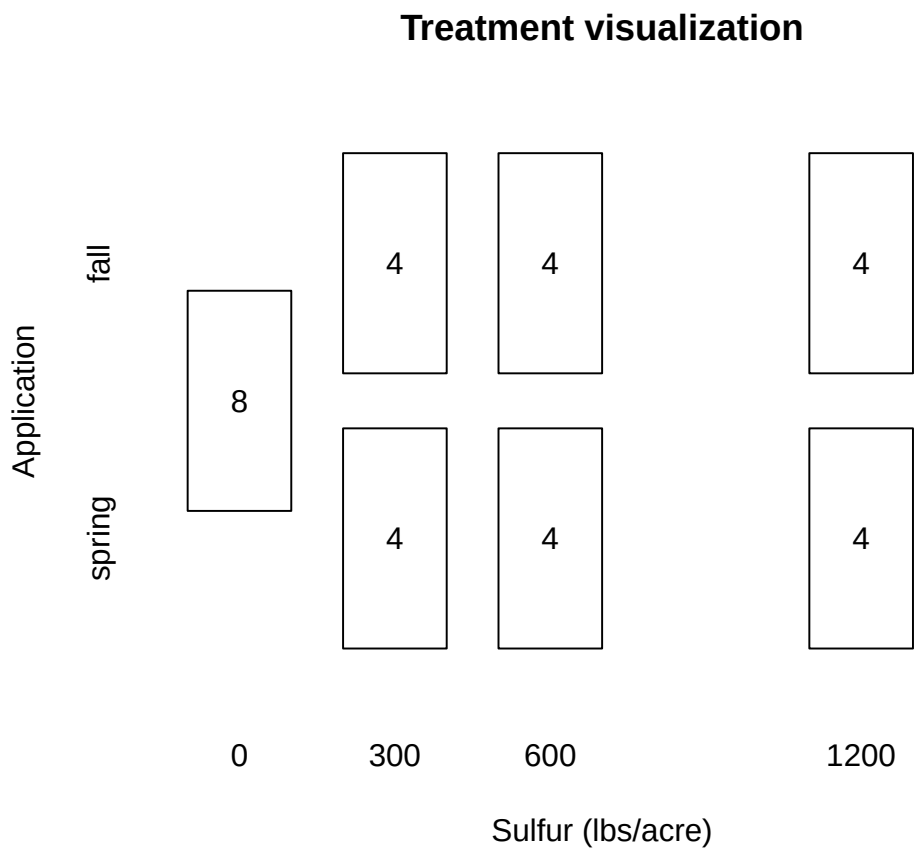
Scientific question:

- Does sulfur have any impact at all?
- Is there a difference between spring and fall?
- Is there an effect of increased sulfur (expect more sulfur causes less scab)?

Data

	inf	trt	row	col
1	9	F3	4	1
2	12	0	4	2
3	18	S6	4	3
4	10	F12	4	4
5	24	S6	4	5
6	17	S12	4	6
7	30	S3	4	7
8	16	F6	4	8
9	10	0	3	1
10	7	S3	3	2
11	4	F12	3	3
12	10	F6	3	4
13	21	S3	3	5
14	24	0	3	6
15	29	0	3	7
16	12	S6	3	8
17	9	F3	2	1
18	7	S12	2	2
19	18	F6	2	3
20	30	0	2	4
21	18	F6	2	5
22	16	S12	2	6
23	16	F3	2	7
24	4	F12	2	8
25	9	S3	1	1
26	18	0	1	2
27	17	S12	1	3
28	19	S6	1	4
29	32	0	1	5
30	5	F12	1	6

Design

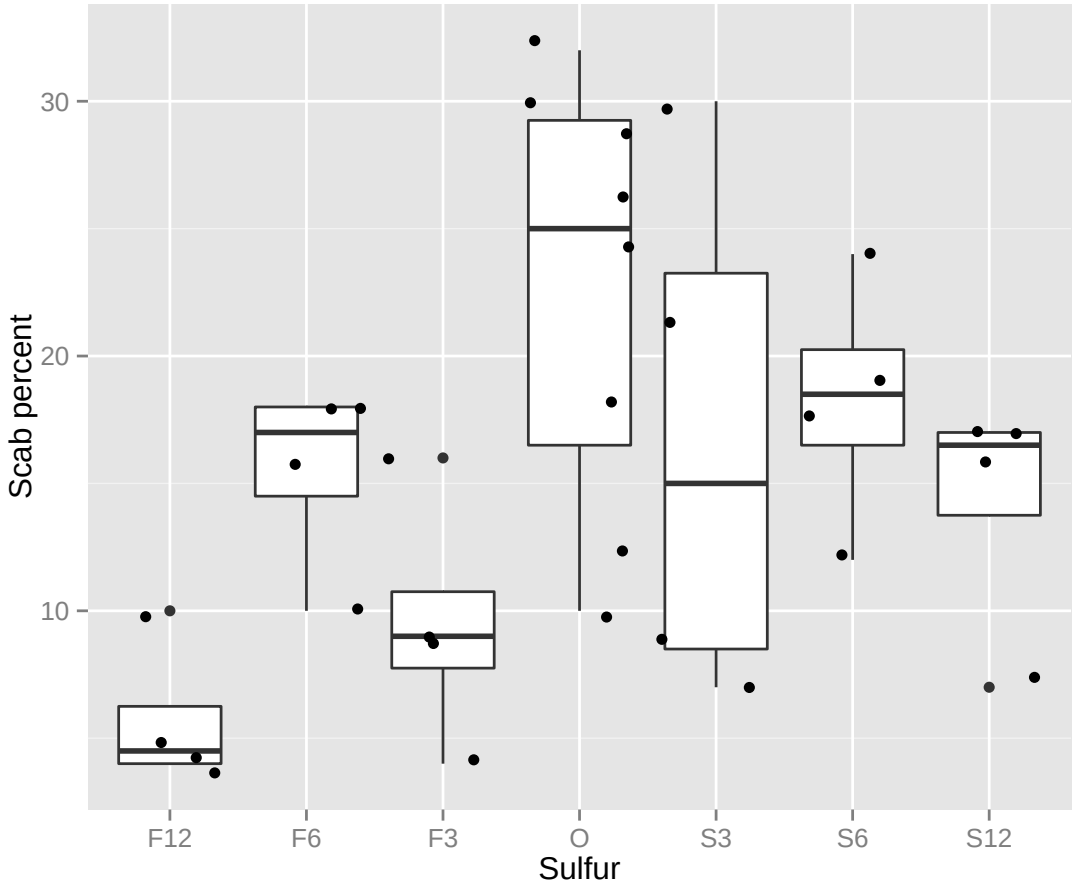

Treatment visualization
Application
fall
spring
8
4
4
4
4
4
4
0
300
600
1200
Sulfur (lbs/acre)

Design

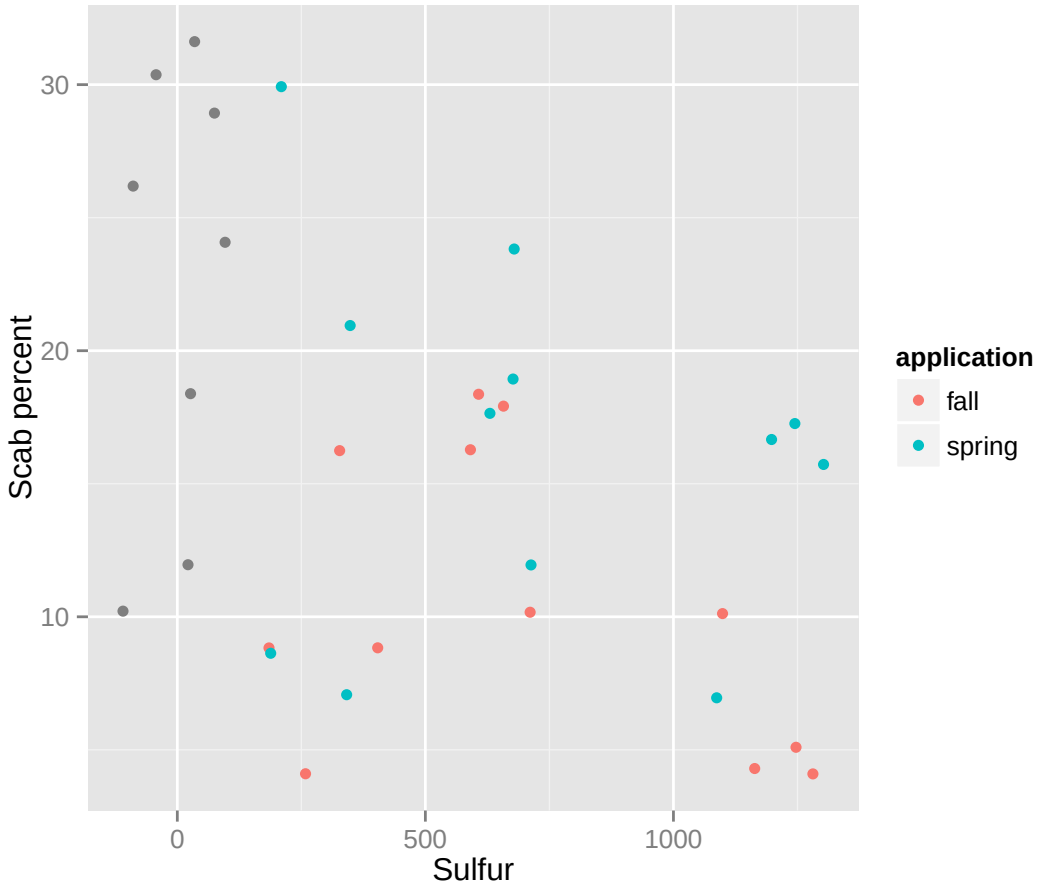
Completely randomized design
potato scab experiment

row \ col	1	2	3	4	5	6	7	8
4	F3	O	S6	F12	S6	S12	S3	F6
3	O	S3	F12	F6	S3	O	O	S6
2	F3	S12	F6	O	F6	S12	F3	F12
1	S3	O	S12	S6	O	F12	O	F3

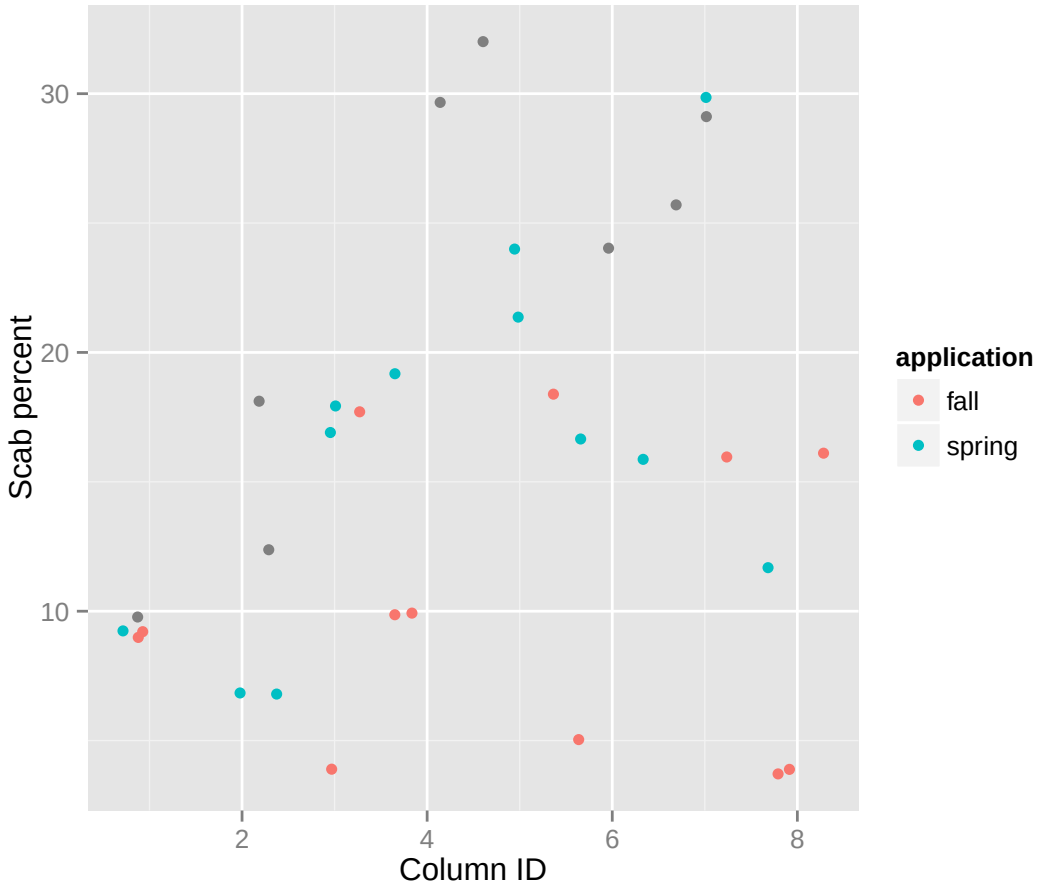
Data



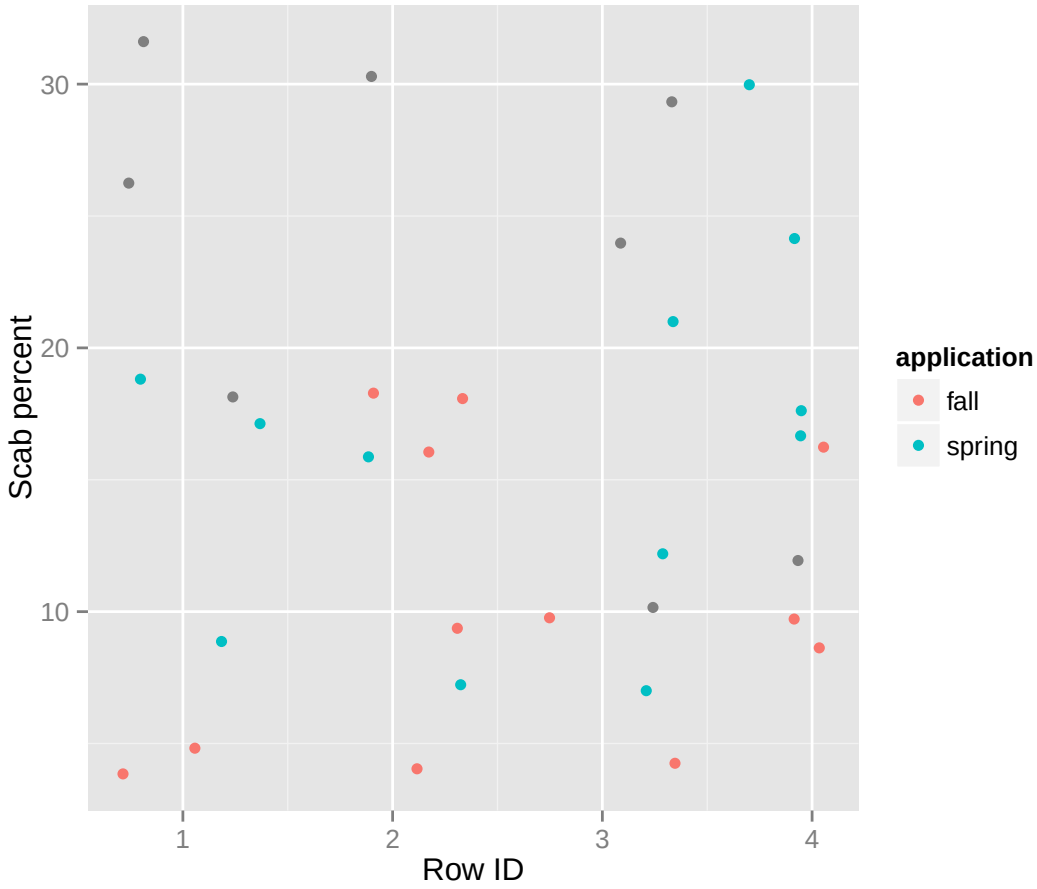
Data


30
20
10
Scab percent
0
500
1000
Sulfur
application
fall
spring

Data


Scab percent
30
20
10
2
4
6
8
Column ID
application
fall
spring

Data



Model

Y_{ij}: avg % of surface area covered with scab for plot i in treatment j for $j = 1, \ldots, 7$.

Assume $Y_{ij} \overset{ind}{\sim} N(\mu_j, \sigma^2)$.

Hypotheses:

- Difference amongst any means: One-way ANOVA F-test
- *Any effect*: Control vs sulfur
- *Fall vs spring*: Contrast comparing fall vs spring applications
- *Sulfur level*: Linear trend contrast

Control vs sulfur

$$\begin{aligned}\gamma &= \tfrac{1}{6}(\mu_{F12} + \mu_{F6} + \mu_{F3} + \mu_{S3} + \mu_{S6} + \mu_{S12}) - \mu_O \\ &= \tfrac{1}{6}(\mu_{F12} + \mu_{F6} + \mu_{F3} + \mu_{S3} + \mu_{S6} + \mu_{S12} - 6\mu_O)\end{aligned}$$

Fall vs spring contrast

- *Fall vs spring*: Contrast comparing fall vs spring applications

$$\begin{aligned}
\gamma &= \tfrac{1}{3}(\mu_{F12} + \mu_{F6} + \mu_{F3}) + 0\mu_O - \tfrac{1}{3}(\mu_{S3} + \mu_{S6} + \mu_{S12}) \\
&= \tfrac{1}{3}\mu_{F12} + \tfrac{1}{3}\mu_{F6} + \tfrac{1}{3}\mu_{F3} + 0\mu_O - \tfrac{1}{3}\mu_{S3} - \tfrac{1}{3}\mu_{S6} - \tfrac{1}{3}\mu_{S12} \\
&= \tfrac{1}{3}\left[\mu_{F12} + \mu_{F6} + \mu_{F3} + 0\mu_O - 1\mu_{S3} - 1\mu_{S6} - 1\mu_{S12}\right]
\end{aligned}$$

Sulfur level: linear trend contrasts

- The unique sulfur levels (X_i) are 0, 3, 6, and 12.
- So the linear trend contrast ($X_i - \overline{X}$) is

X_i	0	3	6	12
$X_i - \overline{X}$	$-\frac{21}{4}$	$-\frac{9}{4}$	$\frac{3}{4}$	$\frac{27}{4}$

- But 3, 6, and 12 are duplicated, so we need the average of the groups

$$\begin{aligned}
\gamma &= -\tfrac{21}{4}\mu_0 - \tfrac{9}{4}\mu_3 + \tfrac{3}{4}\mu_6 + \tfrac{27}{4}\mu_{12} \\
&= -\tfrac{21}{4}\mu_0 - \tfrac{9}{4}\left(\tfrac{\mu_{S3}+\mu_{F3}}{2}\right) + \tfrac{3}{4}\left(\tfrac{\mu_{S6}+\mu_{F6}}{2}\right) + \tfrac{27}{4}\left(\tfrac{\mu_{S12}+\mu_{F12}}{2}\right) \\
&= \tfrac{1}{8}\left[-42\mu_0 - 9\mu_{S3} - 9\mu_{F3} + 3\mu_{S6} + 3\mu_{F6} + 27\mu_{S12} + 27\mu_{F12}\right]
\end{aligned}$$

Contrasts

Trt	F12	F6	F3	O	S3	S6	S12	Div
Sulfur v control	1	1	1	-6	1	1	1	6
Fall v Spring	1	1	1	0	-1	-1	-1	3
Linear Trend	27	3	-9	-42	-9	3	27	8

SAS code

```
DATA d;
  INFILE 'potato.csv' DSD FIRSTOBS=2;
  INPUT scabp treatment $ row col;
  sulfur = 0;
  IF treatment in ("F3","S3") THEN sulfur=300;
  IF treatment in ("F6","S6") THEN sulfur=600;
  IF treatment in ("F12","S12") THEN sulfur=1200;
  application = "NA    ";
  IF treatment in ("F3","F6","F12") THEN application="fall";
  IF treatment in ("S3","S6","S12") THEN application="spring";

PROC PRINT DATA=d (OBS=10); RUN;

PROC MEANS;
  CLASS treatment;
  VAR scabp;
  RUN;
```

SAS code

Obs	scabp	treatment	row	col	sulfur	application
1	9	F3	4	1	300	fall
2	12	O	4	2	0	NA
3	18	S6	4	3	600	spring
4	10	F12	4	4	1200	fall
5	24	S6	4	5	600	spring
6	17	S12	4	6	1200	spring
7	30	S3	4	7	300	spring
8	16	F6	4	8	600	fall
9	10	O	3	1	0	NA
10	7	S3	3	2	300	spring

The MEANS Procedure

Analysis Variable : scabp

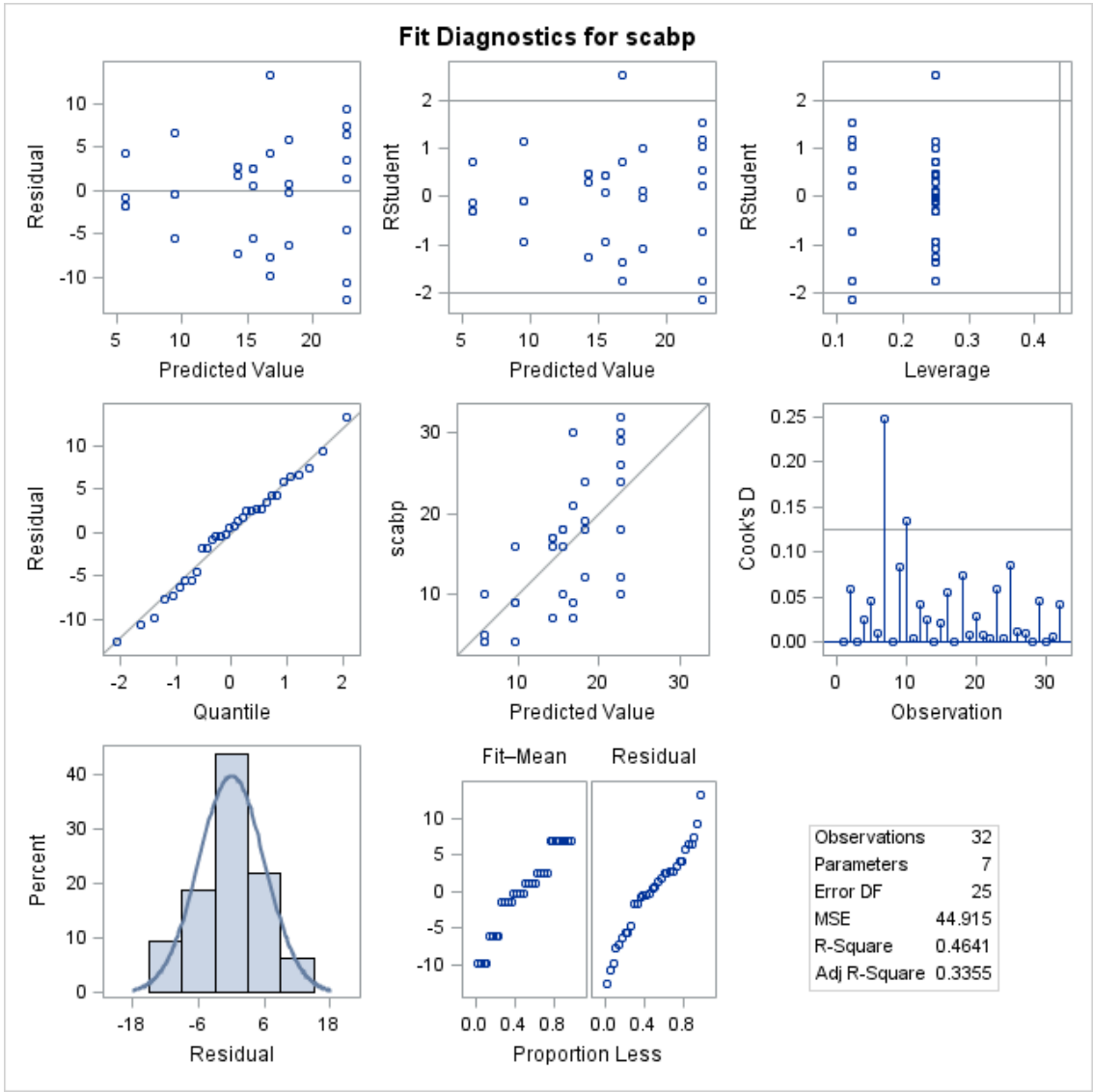
treatment	N Obs	N	Mean	Std Dev	Minimum	Maximum
F12	4	4	5.7500000	2.8722813	4.0000000	10.0000000
F3	4	4	9.5000000	4.9328829	4.0000000	16.0000000
F6	4	4	15.5000000	3.7859389	10.0000000	18.0000000
O	8	8	22.6250000	8.3655330	10.0000000	32.0000000
S12	4	4	14.2500000	4.8562674	7.0000000	17.0000000
S3	4	4	16.7500000	10.7819293	7.0000000	30.0000000
S6	4	4	18.2500000	4.9244289	12.0000000	24.0000000

SAS code

```
PROC GLM DATA=d PLOTS=(DIAGNOSTICS RESIDUALS);
  CLASS treatment;
  MODEL scabp = treatment / CLPARM;
  LSMEANS treatment / CL;
  ESTIMATE 'sulfur - control' treatment 1 1 1 -6 1 1 1 / DIVISOR=6;
  ESTIMATE 'fall - spring' treatment 1 1 1 0 -1 -1 -1 / DIVISOR=3;
  ESTIMATE 'linear trend'  treatment 27 -9 3 -42 27 -9 3 / DIVISOR=8;
  OUTPUT OUT=dres P=predicted R=residuals;
  RUN;

PROC GPLOT DATA=dres;
  PLOT residuals*predicted;
  PLOT residuals*sulfur;
  PLOT residuals*application;
  PLOT residuals*row;
  PLOT residuals*col;
  RUN;
```

Diagnostics



SAS output

```
                          The GLM Procedure

                    Class Level Information

Class          Levels    Values

treatment           7    F12 F3 F6 O S12 S3 S6

    Number of Observations Read          32
    Number of Observations Used          32


Dependent Variable: scabp

                                   Sum of
Source                     DF       Squares     Mean Square    F Value    Pr > F
Model                       6    972.343750      162.057292       3.61    0.0103
Error                      25   1122.875000       44.915000
Corrected Total            31   2095.218750

                  R-Square     Coeff Var      Root MSE    scabp Mean
                  0.464077      42.80633      6.701865      15.65625
```

SAS output

treatment	scabp LSMEAN	95% Confidence Limits	
F12	5.750000	-1.151375	12.651375
F3	9.500000	2.598625	16.401375
F6	15.500000	8.598625	22.401375
O	22.625000	17.744991	27.505009
S12	14.250000	7.348625	21.151375
S3	16.750000	9.848625	23.651375
S6	18.250000	11.348625	25.151375

The GLM Procedure

Dependent Variable: scabp

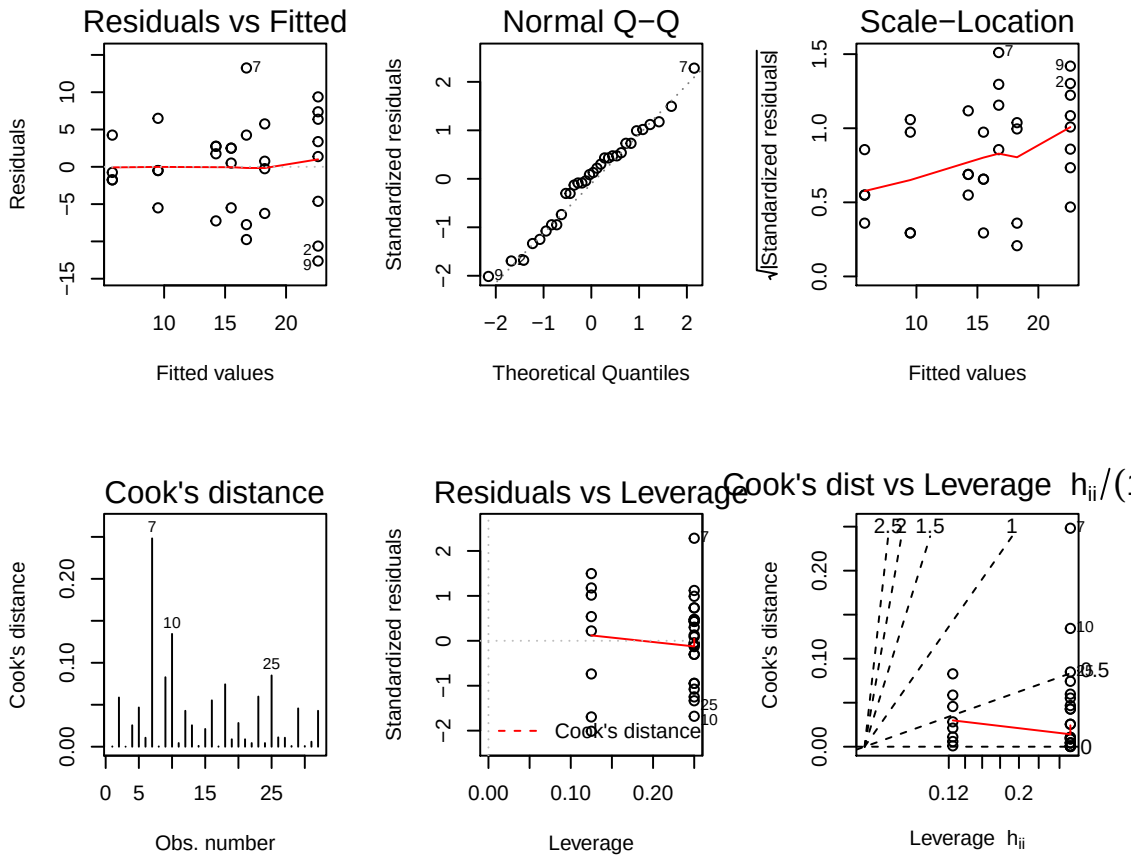
Parameter	Estimate	Standard Error	t Value	Pr > |t|	95% Confidence Limits	
sulfur - control	-9.2916667	2.7360251	-3.40	0.0023	-14.9266158	-3.6567175
fall - spring	-6.1666667	2.7360251	-2.25	0.0332	-11.8016158	-0.5317175
linear trend	-68.1562500	21.0269359	-3.24	0.0034	-111.4620350	-24.8504650

```
library(multcomp)
K = rbind("sulfur - control" = c(1, 1, 1, -6, 1, 1, 1)/6,
          "fall - spring"    = c(1,1,1,0,-1,-1,-1)/3,
          "linear trend"     = c(27,3,-9,-42,-9,3,27)/8)
m = lm(inf~trt,d)
anova(m)


Analysis of Variance Table

Response: inf
          Df Sum Sq Mean Sq F value Pr(>F)
trt        6    972   162.1    3.61   0.01 *
Residuals 25   1123    44.9
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
par(mfrow=c(2,3))
plot(m,1:6)
```



```
g = glht(lm(inf~trt-1,d), linfct=K) # notice the -1 in the model
summary(g, test=adjusted(type="none")) # unadjusted pvalues
```

```
 Simultaneous Tests for General Linear Hypotheses

Fit: lm(formula = inf ~ trt - 1, data = d)

Linear Hypotheses:
                      Estimate Std. Error t value Pr(>|t|)
sulfur - control == 0    -9.29       2.74   -3.40   0.0023 **
fall - spring == 0       -6.17       2.74   -2.25   0.0332 *
linear trend == 0       -68.16      21.03   -3.24   0.0034 **
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
(Adjusted p values reported -- none method)
```

```
confint(g, calpha=univariate_calpha()) # unadjusted confidence intervals
```

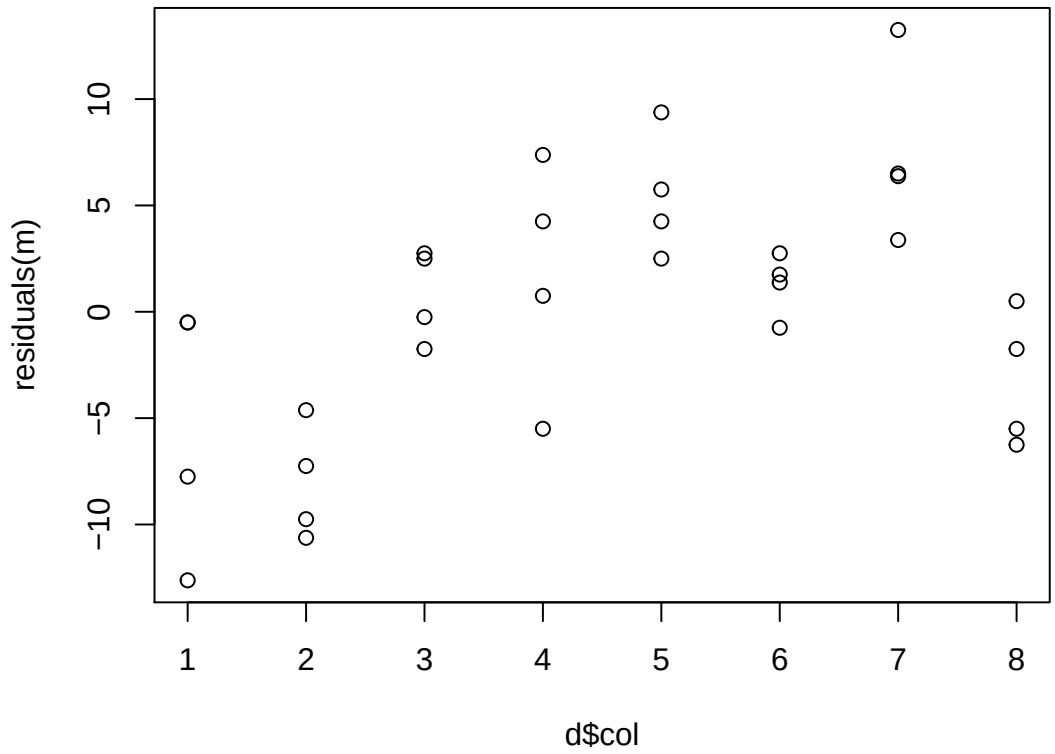
```
 Simultaneous Confidence Intervals

Fit: lm(formula = inf ~ trt - 1, data = d)

Quantile = 2.06
95% confidence level

Linear Hypotheses:
                      Estimate lwr      upr
sulfur - control == 0   -9.292  -14.927   -3.657
fall - spring == 0      -6.167  -11.802   -0.532
linear trend == 0      -68.156 -111.462  -24.850
```

```
plot(d$col,residuals(m))
```



Summary

For this particular data analysis

- Significant differences in means between the groups (ANOVA $F_{6,25} = 3.61$ p=0.01)
- Sulfur had a significant impact on scab (p=0.002)
- Fall was better than spring (p=0.03, 95% CI (0.53, 11.8))
- Linear trend in sulfur was significant(p=0.003)

- Concerned about spatial correlation among columns
- Consider a transformation of the response
  - CI for F12 (-1.2, 12.7)
  - Non-constant variance (residuals vs predicted, sulfur, application)