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Statistics in its best -  
revealing unexpected effects

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**WOW Factor!**

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**General linear models (not Generalized linear model)**

- Traditionally, authors tend to separate t-tests, ANOVA, Regression and Analysis of Covariance (ANCOVAs). However, because they share the same calculations (and theories and assumptions), we often classify these methods under the category of **General Linear Models**.
- *General Linear Models* (unlike Generalized linear models) assume that response variables are normally distributed.

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**General linear models**

Linear Model	Common name
$Y = \mu + X$	Simple linear regression
$Y = \mu + A_1$	One-factorial (one-way) ANOVA
$Y = \mu + A_1 + A_2 + A_1 \times A_2$	Two-factorial (two-way) ANOVA
$Y = \mu + A_1 + X + (A_1 \times X)$	Analysis of Covariance (ANCOVA)
$Y = \mu + X_1 + X_2 + X_3$	Multiple regression
$Y = \mu + A_1 + g + A_1 \times g$	Mixed model ANOVA
$Y_1 + Y_2$ $= \mu + A_1 + A_2 + A_1 \times A_2$	Multivariate ANOVA (MANOVA)

Y (response) is a continuous variable  
 X (predictor) is a continuous variable  
 A represents categorical predictors (factors)  
 g represents groups of data (more on this later)

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$Y = \mu + A_1 + X_1 + (A_1 \times X_1)$  Analysis of Covariance (ANCOVA)

- Test for differences in slopes among groups (treatments).
- Adjust for the effects of a covariate  $X_1$  (continuous) in an ANOVA design (response variable  $Y$  and a categorical variable  $A_1$ ).
- $\mu$  the grand mean (i.e., mean of the response across all observations independent of their groups).

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**The effects of grazers on the performance of individuals and populations of scarlet gilia, *Ipomopsis aggregata***

Authors: Joy Bergelson, Michael J. Crawley

*I. aggregata* exhibits considerable powers of regrowth following removal of its primary shoot by herbivores, but we found no evidence of overcompensation (i.e. of significantly higher plant performance where plants were exposed to ungulate herbivory) in a comparison between individuals on grazed and ungrazed sides of enclosure fences




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### Study design and data

- 40 plants (*Ipomopsis*) were allocated to a grazing factor (two levels: grazed or ungrazed):

- 1) Grazed plants were exposed to grazers for the first two weeks of stem elongation (initial plant size measured as diameter of the rootstock top).
- 2) After two weeks, fence was built to prevent grazing.
- 3) At the end of the growing season, fruit production (dry weight in mg was recorded for each plant.
- 4) Initial plant size (diameter of the *rootstock* top, i.e., root size) was thought to influence fruit production and it was also measured.

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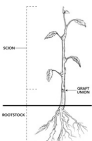
### Data structure




Fruit production (dry weight in mg).

Grazing (Yes / No).

Initial plant size (diameter of the rootstock top).



	B	C	D
	Fruit	Grazing	Root
	58.77	Ungrazed	6.225
	60.98	Ungrazed	6.487
	14.73	Ungrazed	4.919
	19.28	Ungrazed	5.13
	34.25	Ungrazed	5.417
	35.53	Ungrazed	5.359
	87.73	Ungrazed	7.614
	63.21	Ungrazed	6.952
	24.25	Ungrazed	4.975
	64.34	Ungrazed	6.93
	52.92	Ungrazed	6.388
	23.35	Ungrazed	5.851
	53.61	Ungrazed	6.013
	54.86	Ungrazed	5.938
	64.81	Ungrazed	6.264
	75.24	Ungrazed	7.181
	80.64	Ungrazed	7.001
	18.89	Ungrazed	4.426
	75.49	Ungrazed	7.302
	46.73	Ungrazed	5.836
	116.05	Grazed	10.253
	38.94	Grazed	6.968
	60.77	Grazed	8.001
	88.37	Grazed	9.039
	70.11	Grazed	8.91
	14.95	Grazed	6.506
	29.7	Grazed	7.891
	80.31	Grazed	8.988
	83.35	Grazed	8.975
	105.07	Grazed	9.844
	79.79	Grazed	8.508
	50.08	Grazed	7.354
	78.28	Grazed	8.643
	41.48	Grazed	7.916
	98.47	Grazed	9.351
	40.15	Grazed	7.066
	52.26	Grazed	8.158
	46.64	Grazed	7.382
	71.01	Grazed	8.515
	44.03	Grazed	6.43



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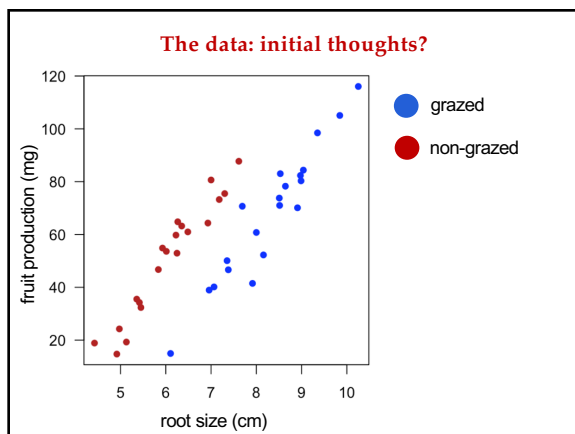
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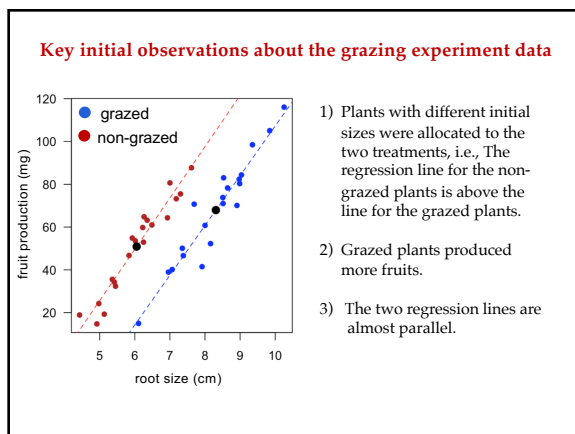
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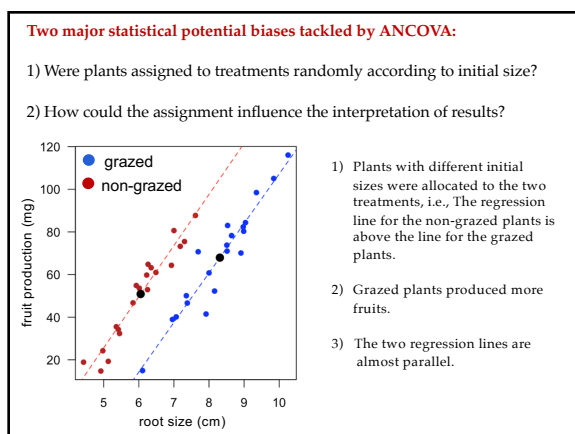
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Let's start with a simple ANOVA (a two-sample  $t$  test could have been used as well;  $t^2 = F$ ) comparing the fruit production as a function of grazing (i.e., grazed, non-grazed).

```
> anova(lm(Fruit~Grazing))
Analysis of Variance Table

Response: Fruit
Df Sum Sq Mean Sq F value Pr(>F)
Grazing 1 2910.4 2910.44 5.3086 0.02678 *
Residuals 38 20833.4 548.25
```

What's the conclusion? {

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Let's start with a simple ANOVA (a two-sample  $t$  test could have been used as well;  $t^2 = F$ ) comparing the fruit production as a function of grazing (i.e., grazed, non-grazed).

```
> anova(lm(Fruit~Grazing))
Analysis of Variance Table

Response: Fruit
Df Sum Sq Mean Sq F value Pr(>F)
Grazing 1 2910.4 2910.44 5.3086 0.02678 *
Residuals 38 20833.4 548.25
```

What's the conclusion? {

**Greater fruit production under grazing!**

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Greater fruit production under grazing! **Can this conclusion be justified given that the initial root sizes in grazed plants are larger than non-grazed plants?**

```
> anova(lm(Root~Grazing))
Analysis of Variance Table

Response: Root
Df Sum Sq Mean Sq F value Pr(>F)
Grazing 1 50.918 50.918 56.087 5.411e-09 ***
Residuals 38 34.498 0.908
```

```
> anova(lm(Fruit~Grazing))
Analysis of Variance Table

Response: Fruit
Df Sum Sq Mean Sq F value Pr(>F)
Grazing 1 2910.4 2910.44 5.3086 0.02678 *
Residuals 38 20833.4 548.25
```

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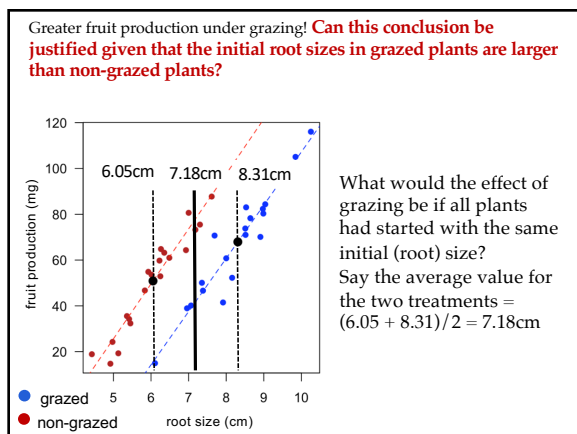
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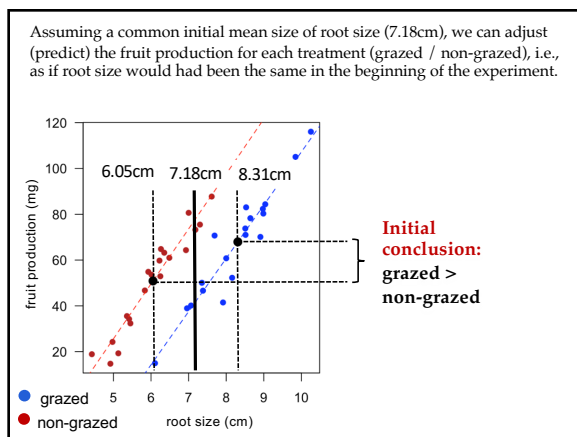
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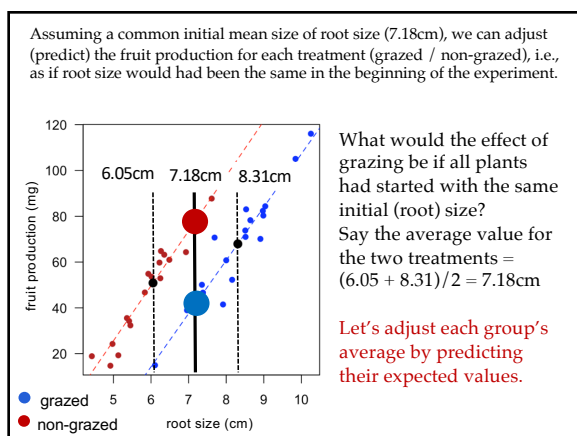
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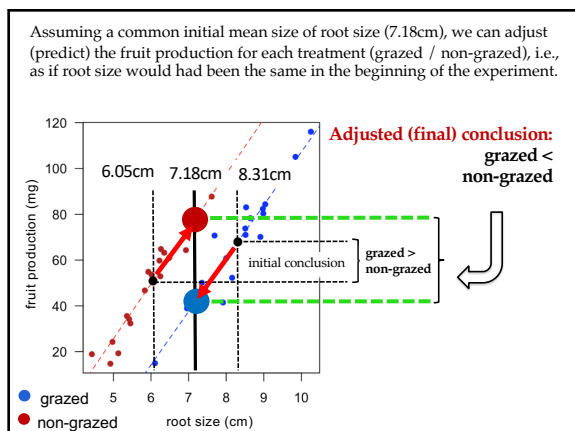
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Statistics in its best -  
revealing “unexpected” effects

**Initial conclusion:** grazed > non-grazed.

**Adjusted (final) conclusion:** grazed < non-grazed.

— THE —  
WOW Factor!

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**When can we use ANCOVA to adjust for a continuous predictor (here initial plant size)?**

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$Y = \mu + A_1 + X_1 + (A_1 \times X_1)$  Analysis of Covariance (ANCOVA)

- It "combines" ANOVA and regression into one analysis (we know they are the same models).
- As such, it includes at least one categorical predictor (factor, e.g., Grazing) and one continuous predictor (e.g., initial root size).
- The goal of an ANCOVA (in general) is to test for the effect of a categorical predictor while adjusting (controlling) for the effect of a continuous predictor.
- The continuous predictor is called covariate.

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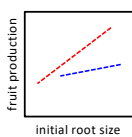
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22

Analysis of Covariance (ANCOVA) - possible model outcomes  
Which cases allow to control (adjust) for a covariate (root size)?



● grazed  
● non-grazed

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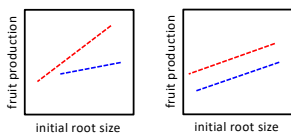
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23

Analysis of Covariance (ANCOVA) - possible model outcomes  
Which cases allow to control (adjust) for a covariate (root size)?



● grazed  
● non-grazed

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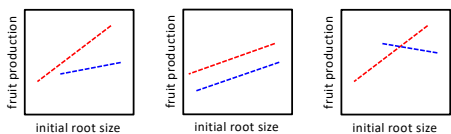
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Analysis of Covariance (ANCOVA) - possible model outcomes  
Which cases allow to control (adjust) for a covariate (root size)?



● grazed  
● non-grazed

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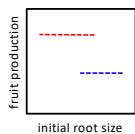
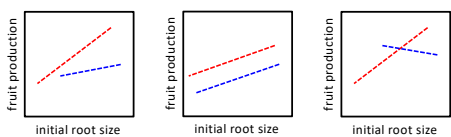
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Analysis of Covariance (ANCOVA) - possible model outcomes  
Which cases allow to control (adjust) for a covariate (root size)?



● grazed  
● non-grazed

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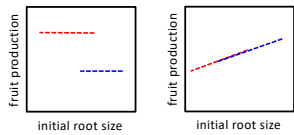
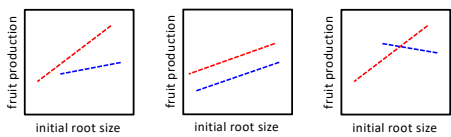
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Analysis of Covariance (ANCOVA) - possible model outcomes  
Which cases allow to control (adjust) for a covariate (root size)?



● grazed  
● non-grazed

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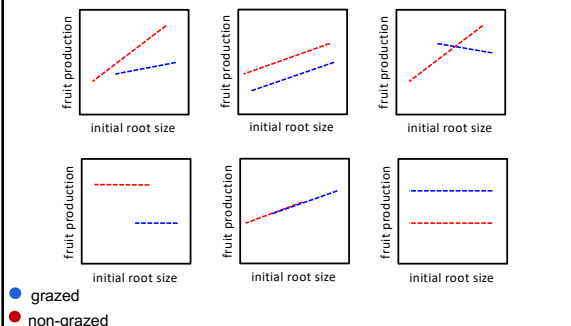
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Analysis of Covariance (ANCOVA) - possible model outcomes  
Which cases allow to control (adjust) for a covariate (root size)?



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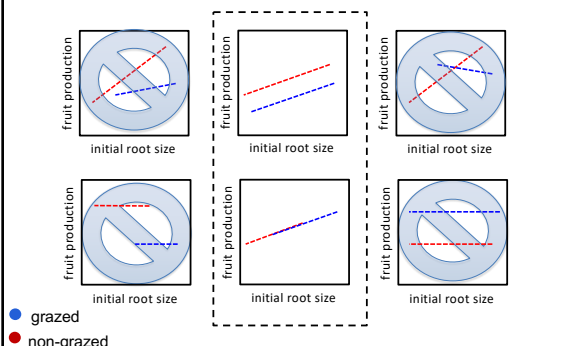
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Analysis of Covariance (ANCOVA) - possible model outcomes  
Which cases allow to control (adjust) for a covariate (root size)?



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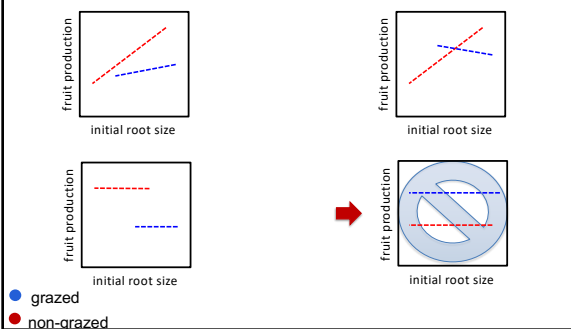
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No need for adjustments as initial root size do not differ between grazed and non-grazed treatments



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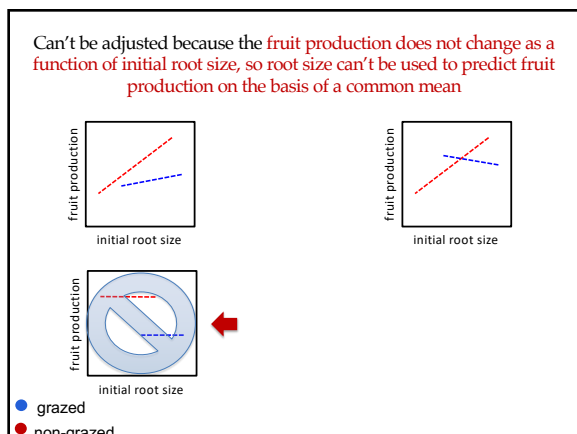
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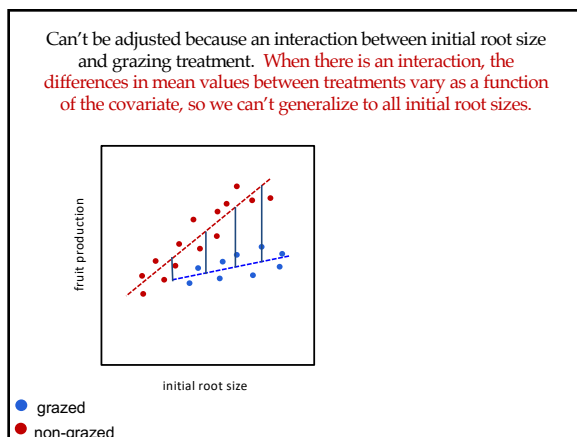
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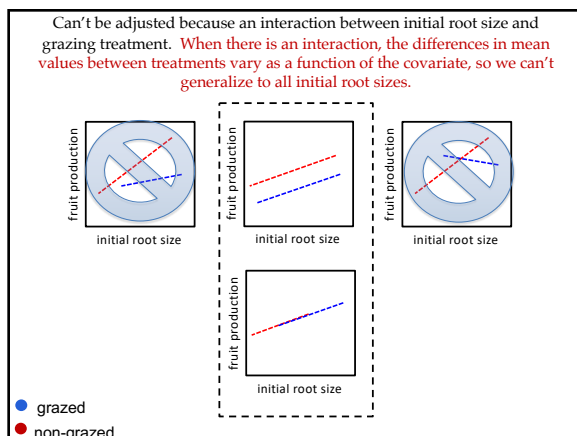
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There are approaches for the more complex cases when slopes differ between groups or when the response (Y) does not depend on the covariate (initial root size) – next lecture.

● grazed  
● non-grazed

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A common (equal) slope (parallel curves) between groups implies that mean differences between groups in their response (fruit production) are the same regardless of the value of the covariate (initial root size).

● grazed  
● non-grazed

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**When can ANCOVA adjustments be used? Statistical assessments**

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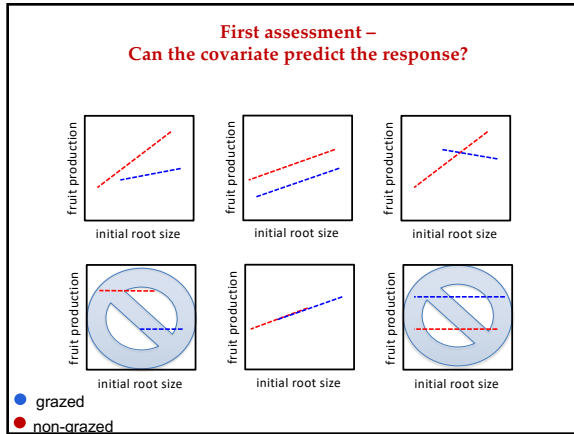
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**First assessment –  
Can the covariate predict the response?**

**H<sub>0</sub>:** The slope of the regression of fruit production on initial root size is zero ( $\beta = 0$ ).

**H<sub>A</sub>:** The slope of the regression of fruit production on initial root size is not zero ( $\beta \neq 0$ ).

```
> anova(lm(Fruit ~ Root))
```

Analysis of Variance Table

Response: Fruit

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Root	1	16795.0	16795.0	91.844	1.099e-11 ***
Residuals	38	6948.8	182.9		

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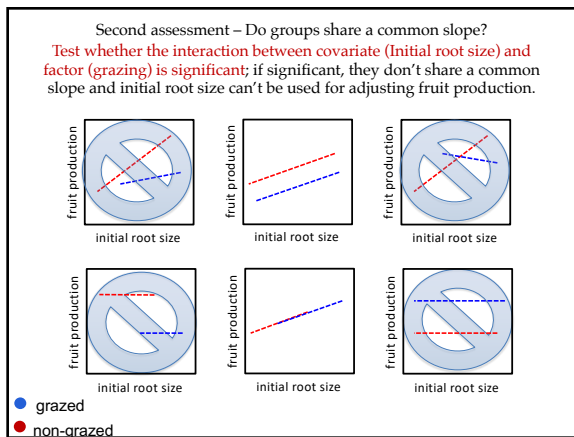
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**Second assessment – Do groups share a common slope?**  
 Test whether the interaction between covariate (Initial root size) and factor (grazing) is significant.

**H<sub>0</sub>:** There is no interaction between grazing treatment and initial root size (i.e., grazing/no-grazing (groups) do not differ in their slopes).

**H<sub>A</sub>:** There is an interaction between grazing treatment and initial root size (i.e., grazing/no-grazing differ in their slopes).

```
> anova(lm(Fruit ~ Root*Grazing))
```

Analysis of Variance Table

Response: Fruit

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Root	1	16795.0	16795.0	359.9681	< 2.2e-16 ***
Grazing	1	5264.4	5264.4	112.8316	1.209e-12 ***
Root:Grazing	1	4.8	4.8	0.1031	0.75
Residuals	36	1679.6	46.7		

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**Second assessment – Do groups share a common slope?**  
 Test whether the interaction between covariate (Initial root size) and factor (grazing) is significant.

**H<sub>0</sub>:** There is no interaction between grazing treatment and initial root size (i.e., grazing/no-grazing (groups) do not differ in their slopes).

**H<sub>A</sub>:** There is an interaction between grazing treatment and initial root size (i.e., grazing/no-grazing differ in their slopes).

Note that testing for differences in slopes (Y on X) between groups (e.g., grazed versus non-grazed), i.e., testing the interaction between the categorical (groups) and X, is interesting in itself.

In the problem analysed here we don't want to have them different but in other cases we may (e.g., allometric differences).

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**Second assessment – Do groups share a common slope?**  
 Test whether the interaction between covariate (Initial root size) and factor (grazing) is significant.

Remember: when there is an interaction, then the differences in mean values between treatments vary as a function of the covariate, so we can't generalize to all initial root sizes (more on this later).

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**So far, we have:**

Covariate can predict the response

```
> anova(lm(Fruit ~ Root))
Analysis of Variance Table

Response: Fruit
      Df Sum Sq Mean Sq F value    Pr(>F)
Root    1 16795.0 16795.0   91.844 1.099e-11 ***
Residuals 38  6948.8   182.9
---

```

Groups share a common slope

```
> anova(lm(Fruit ~ Root*Grazing))
Analysis of Variance Table

Response: Fruit
      Df Sum Sq Mean Sq F value    Pr(>F)
Root    1 16795.0 16795.0 359.9681 < 2.2e-16 ***
Grazing  1  5264.4  5264.4 112.8316 1.209e-12 ***
Root:Grazing 1    4.8    4.8  0.1031    0.75
Residuals 36 1679.6    46.7
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```

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Now we can test for differences in adjusted means; but before that:

**Critical statistical issues underlying  
General Linear Models  
(including ANCOVAs)**

**Lecture 10 -  
a pedagogical guide  
(Type I and III sum-of-square)**

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