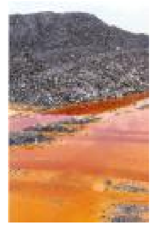
Two-Way Analysis of Variance (ANOVA) with Interactions



Example *: Helsel (1983) examined the impact of mining and rock type on water quality as measured by iron concentration levels in watershed runoff :*

> ***Rock*** *: S=Sandstone, L=Limestone*
> ***Mine*** *: U=Unmined, R=Reclaimed, A=Abandoned*
> ***Iron*** *: Concentration in mg/L*
> ***logs*** *: log (Iron concentration)*

In this instance it is better to analyze the logarithms of the concentrations (it is almost ALWAYS necessary to take logs of concentration data!!!)

Comparing Differences in means due to Two Factors

- **Two-way ANOVA compares means in groups of two different factors.**
- Two-way ANOVA also considers **interactions** between the two different factors.

An **interaction** effect is a **non-additive effect** : that is, something unexpected happens for particular combinations of levels of different factors.

Example *: Laundry . . . .*

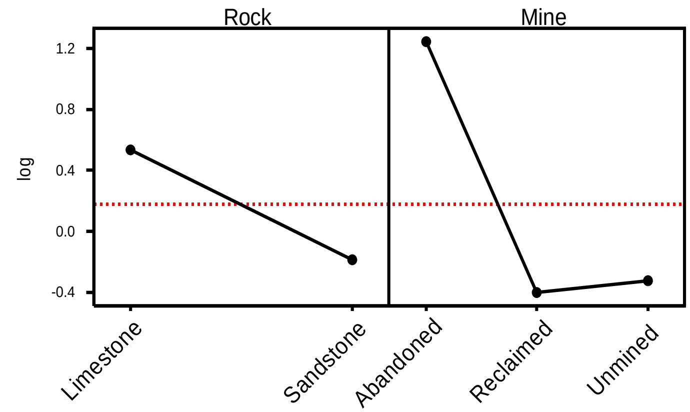
Main Effects Plots

- This is a plot of the **MEAN** value in each group. *Notice that the plotted values are identical to those in the ALL row and column in the means table.*

Main Effects Plots in MINITAB : Use `Stat` → `ANOVA` → `Main Effects Plots.`

Main Effects Plots in SPSS : Use `Analyze` → `Compare Means` → `One Way ANOVA.` Click on `Options`, choose `MEANS PLOT.`

This plot shows:



- *Mean log(iron conc.) are lower in sandstone than in limestone*
- *Mean log(iron conc.) is higher in abandoned mines than in unmined or reclaimed areas.*
- *The dotted line is the overall mean log(iron) level.*

Interactions : Interactions Plot

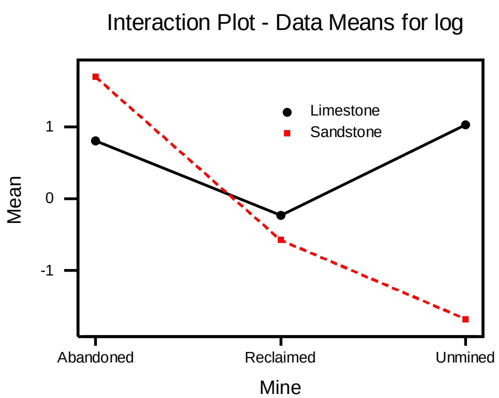
- In the above plots, <u>the means in groups of one factor are calculated ignoring the effect of other factors.</u> *Mean limestone log(iron conc) levels are calculated based on values over all mine types.*

- However, it may be for some mine types that log(iron conc) levels are higher for limestone, while for other mine types, log(iron conc) levels may be lower for limestone.

- To see this visually, make an **Interaction plot**.

Interaction Plots in MINITAB : Use `Stat` → `ANOVA` → `Interaction Plots.` It doesn't matter which variable you list first (although plots will be different)

Interaction Plots in SPSS : Use `Analyze` → `General Linear Model`→ `Univariate.` Choose `Plots`, then enter one variable for Horizontal Axis and one variable for Separate Lines. Then click ADD

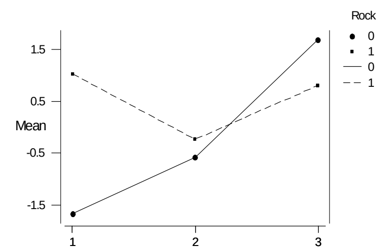


This plot shows that limestone values are lower than sandstone values for abandoned mines; the effect is reversed in unmined areas.

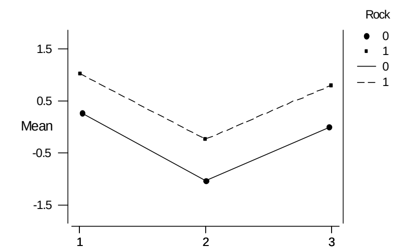
In a two factor interaction plot, if the lines denoting group means do <u>not</u> move in a parallel fashion, it is likely that there is an interaction between the factors.

If the lines for two different groups in one factor do move in a parallel fashion, it suggests that there is a fixed difference between groups at all levels of the other factor **(i.e. no interaction).**

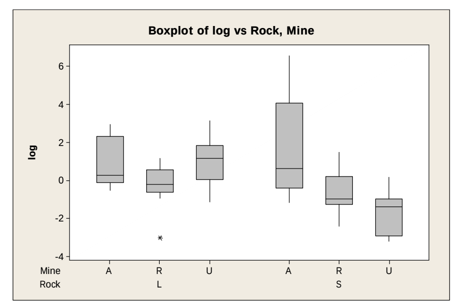
Interaction



No Interaction



You can also make boxplots by treatment group combination (just list both factors when making boxplots)



The Two-Way ANOVA Model

For the mine data, our model is:

$$Y_{ijk} = \text{rock effect}_i + \text{mine effect}_j + \text{interaction}_{ij} + \varepsilon_{ijk}$$

In symbolic (Greek!) notation, this is sometimes written as

$$Y_{ijk} = \mu + \tau_i + \gamma_j + \tau\gamma_{ij} + \varepsilon_{ijk}$$

where

ε_{ijk} =(the errors) **come from a** $N(0,\sigma)$ **distribution**

i indexes the 'row' factor (i.e. i=1 corresponds to sandstone, i=2 is limestone

j indexes the 'column' factor (i.e. j=1 for abandoned, j=2 reclaimed, etc.)

k indexes observations within a level of factor combinations (i.e. k =1 for the first observation from a sandstone reclaimed mine, etc)

As in One-Way ANOVA, assume that means may be different, **but σ is the same for all groups.**

Notation *(The 'DOT' notation)*

Dot(.) = sum over this index
Bar (¯)= average in this group

Notation	Interpretation	*Example (Mine)*
y_{ijk}	Individual observations	$y_{111} = -1.6$ *(first observation in sand unmined area)*
$\bar{y}_{i..}$	The **MEAN** of the observations in each **row** group (average over the j and k indices)	$\bar{y}_{Lime..} = 0.53$ *(mean of all observations in limestone)*
$\bar{y}_{.j.}$	The **MEAN** of the observations in each **column** group (average over the i and k indices)	$\bar{y}_{.Aband.} = 1.3$*(mean of all observations at abandoned mines)*
$\bar{y}_{ij.}$	The **MEAN** of all observations for each combination of row and column groups (average over the k index)	$\bar{y}_{\text{Lime Unmined}.} = 1.0$ *(m (mean of all observations in unmined limestone)*
$\bar{y}_{...}$	The **MEAN** of **all observations** (average over i , j, k)	$\bar{y}_{...} = 0.17$ *, mean of all the data.*

Using the same mathematical trick as last time, we can write observations as

$$y_{ijk} = \bar{y}_{...} + (\bar{y}_{i..} - \bar{y}_{...}) + (\bar{y}_{.j.} - \bar{y}_{...})$$
$$+ (\bar{y}_{ij.} - \bar{y}_{i..} - \bar{y}_{.j.} + \bar{y}_{...}) + (y_{ijk} - \bar{y}_{ij.})$$

observation = overall mean + row factor effect + column factor effect + interaction effect + residuals/errors

Rearranging, squaring, and adding, we get

$$\sum_{i,j,k}\left(y_{ijk} - \bar{y}_{...}\right)^2 = \sum_{i,j,k}\left(\bar{y}_{i..} - \bar{y}_{...}\right)^2 + \sum_{i,j,k}\left(\bar{y}_{.j.} - \bar{y}_{...}\right)^2$$
$$+ \sum_{i,j,k}\left(\bar{y}_{ij.} - \bar{y}_{i..} - \bar{y}_{.j.} + \bar{y}_{...}\right)^2 + \sum_{i,j,k}\left(y_{ijk} - \bar{y}_{ij.}\right)^2$$

Give names to the pieces : this where we **ANALYZE THE VARIANCE!!**

$$SST = SSA + SSB + SSAB + SSE$$

Total Sum of Squares =
Sum of Squares due to Factor A
+ Sum of Squares due to Factor B
+ Sum of Squares due to interaction
+ Sum of Squares due to Errors

DEGREES OF FREEDOM

Each variation term again has an associated number of degrees of freedom

Total:	*N-1*	(*N*=78 obs. total in mine data)
Factor A:	*I-1*	(*I*=2 types of rock)
Factor B:	*J-1*	(*J*=3 types of use)
Interaction:	*(I-1)*(J-1)*	
Error	*N-1-(I-1)-(J-1)-(IJ-J-I+1) = N-IJ*	

Hypothesis Tests For the Importance of Each Factor in the Model : F-Tests!

- Measure the amount of variation explained by each factor relative to the variation associated with the errors.
- **If the F-statistic is large, reject the hypothesis that that particular factor is not significant**

Test the Significance of This Factor	Degrees of Freedom	Sum of Squares = Variation due to this factor	Mean Square = Sum of squares/d.f.	F-statistic
Factor A	*I-1*	*SSA*	*MSA=SSA/(I-1)*	*F=MSA/MSE*
Factor B	*J-1*	*SSB*	*MSB=SSB/(J-1)*	*F=MSB/MSE*
Interaction	*(I-1)(J-1)*	*SSAB*	*MSAB= SSAB/(I-1)(J-1)*	*F=MSAB/MSE*
Error	*N-IJ*	*SSE*	*MSE=SSE/(n-IJ)*	
Total	*N-1*	*SST*		

Two-Way ANOVA in MINITAB : Use `Stat → ANOVA → Two-Way`.

NOTE : This only works if you have a BALANCED DESIGN. A **balanced design** has the **same number of observations in for every combination of treatment factors** *(i.e. for alcohol data, we have 79 observations for each combination of treatment factors – i.e. 79 females in sororities, etc.)*

Two-Way ANOVA in SPSS : Use `Analyze → General Linear Model→ Univariate.` Enter Dependent variable, list two categorical variables in `Fixed Factors.`

Two-Way ANOVA in MINITAB : Use `Stat → ANOVA → Two-Way.`

NOTE : This only works if you have a BALANCED DESIGN. A **balanced design** has the **same number of observations in for every combination of treatment factors** *(i.e. for mine data, we have 26 observations for each combination of treatment factors – i.e. 26 observations of unmined sandstone areas).*

Two-way ANOVA: log versus Rock, Mine

Source	DF	SS	MS	F	P
Rock	1	10.166	10.1665	4.64	0.035
Mine	2	45.140	22.5701	10.30	0.000
Interaction	2	43.438	21.7190	9.91	0.000
Error	72	157.844	2.1923		
Total	77	256.589			


```
S = 1.481   R-Sq = 38.48%   R-Sq(adj) = 34.21%

                      Individual 95% CIs For Mean Based on
                      Pooled StDev
Rock        Mean      ---+---------+---------+---------+------
L       0.536154                    (---------*--------)
S      -0.185897      (--------*---------)
                      ---+---------+---------+---------+------
                      -0.50      0.00      0.50      1.00

                      Individual 95% CIs For Mean Based on
                      Pooled StDev
Mine       Mean       --+---------+---------+---------+-------
A       1.25000                             (-------*------)
R      -0.40192       (------*------)
U      -0.32269        (------*------)
                      --+---------+---------+---------+-------
                     -0.80      0.00      0.80      1.60
```

- **These results indicate that, mine type, rock type, and the interaction of mine and rock type are all significant predictors of log(iron) concentration. Rock by itself is of borderline significance.**

Rule : if you have a significant interaction effect, you ALWAYS LEAVE THE MAIN EFFECT IN THE MODEL!

Reason – the interaction effect quantifies **departures from the additive main effects** (i.e. calculate interactions after accounting for main effects – see equation for sum of squares . . .)

Comparing Means in Combinations of Groups

- Every **TWO-Way ANOVA** problem can be turned into a **ONE-Way ANOVA** problem with $r*c$ groups

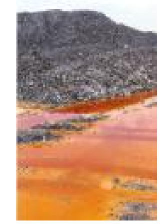
Two-Way ANOVA		One-Way ANOVA
Row Factor (2 levels)	**Column Factor** (3 levels)	**Combined Factor** (6 levels)
Limestone Sandstone	Unmined Reclaimed Abandoned	Lime - Unmined Lime – Reclaimed Lime - Abandoned Sand - Unmined Sand – Reclaimed Sand - Abandoned

- This allows you to compare combination of group means using **multiple comparison techniques from One-Way ANOVA.**

Make Combined Variable in MINITAB : Use `Data` → `Concatenate.`

Make Combined Variable in SPSS : Use `Transform` → `Compute Variable.` Then use the `CONCAT` function. Note this only works for two string variables, so have to make string variables first.

Example : Mine Data. *Use Tukey Comparisons to compare means for all combinations of groups.*



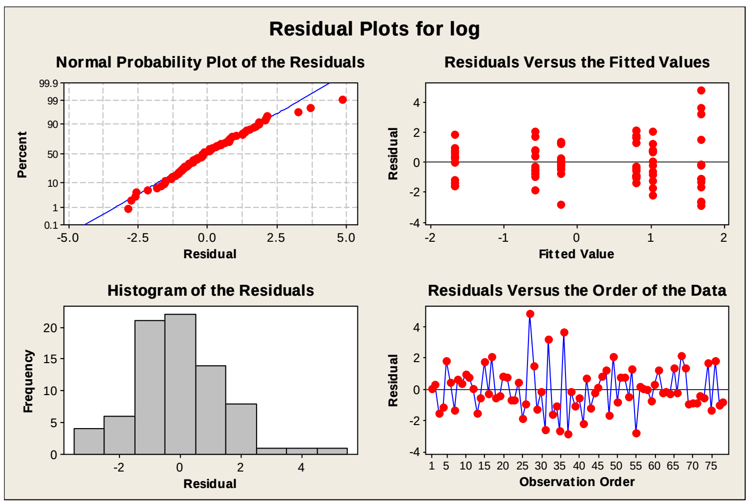
```
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Combined
Individual confidence level = 99.54%

Combined = LA subtracted from:

Combined   Lower  Center   Upper  -------+---------+---------+---------+--
LR        -2.734  -1.034   0.666          (-----*----)
LU        -1.474   0.226   1.926              (-----*----)
SA        -0.811   0.889   2.589                (-----*-----)
SR        -3.081  -1.381   0.319         (----*-----)
SU        -4.182  -2.482  -0.782     (-----*----)
                                  -------+---------+---------+---------+--
                                      -3.0       0.0       3.0       6.0
Combined = LR subtracted from:

Combined   Lower  Center  Upper  -------+---------+---------+---------+--
LU        -0.440   1.260  2.960                 (----*-----)
SA         0.223   1.923  3.623                   (----*-----)
SR        -2.047  -0.347  1.353           (-----*-----)
SU        -3.149  -1.448  0.252       (----*-----)
                                 -------+---------+---------+---------+--
                                     -3.0       0.0       3.0       6.0
Combined = LU subtracted from:

Combined   Lower  Center   Upper  -------+---------+---------+---------+--
SA        -1.037   0.663   2.363                (----*-----)
SR        -3.307  -1.607   0.093        (-----*----)
SU        -4.409  -2.708  -1.008    (-----*-----)
                                  -------+---------+---------+---------+--
                                      -3.0       0.0       3.0       6.0
Combined = SA subtracted from:

Combined   Lower  Center   Upper  -------+---------+---------+---------+--
SR        -3.970  -2.270  -0.570      (----*-----)
SU        -5.072  -3.372  -1.671  (-----*----)
                                  -------+---------+---------+---------+--
                                      -3.0       0.0       3.0       6.0
Combined = SR subtracted from:

Combined   Lower  Center  Upper  -------+---------+---------+---------+--
SU        -2.802  -1.102  0.599          (----*-----)
                                 -------+---------+---------+---------+--
                                     -3.0       0.0       3.0       6.0
```

Checking the Model Assumptions

As with regression and with one-way ANOVA, it is important to check the model assumptions in two-way ANOVA. This is accomplished using **Residual Plots**

Residual Plots for Two-Way ANOVA in MINITAB : Use `Stat` → `ANOVA` → `Two-Way`, choose `graphs` and choose `Four in One.`

Residual Plots for Two-Way ANOVA in SPSS : Use `Analyze` → `General Linear Model` → `Univariate`, choose `Options`, click on `Residual Plot` and `Spread vs. Level Plot.` This doesn't give quite when MINITAB gives, but it's still helpful. Optionally, you can choose SAVE and click on `Unstandardized Predicted Values` and `Residuals`. Then use these to create residuals plots on your own.



Oak Drought Experiment: Helen Mills

Dr. Berlyn and Helen performed an acute drought greenhouse experiment on a low-elevation oak that lives in hot, dry environments (*Quercus laceyi*) and a high-elevation oak that lives in cooler, wetter environments (*Quercus sideroxyla*) in the Sierra del Carmen, Mexico.

20 seedlings of each species (L or S) were randomly assigned to either a drought or control treatment (D or C).

I.E.

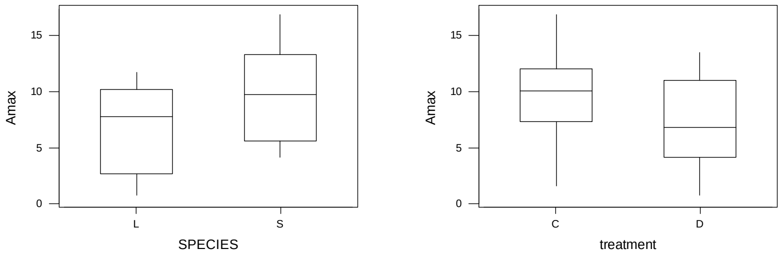
- 10 LC seedlings
- 10 SC seedlings
- 10 LD seedlings
- 10 SD seedlings

Control seedlings were watered to saturation every 3 days during the course of the experiment.

Quercus sideroxyla *Quercus laceyi*

Drought seedlings were withheld water from the onset of the experiment.

After 4 weeks, we measured photosynthesis (A_{max}) on all of the seedlings to determine whether there were species -and/or treatment – level differences in seedling performance, and whether or not there was a species-treatment interaction effect.

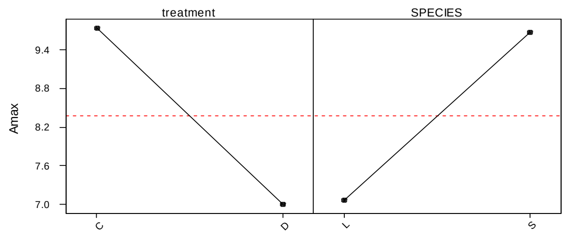


Variance looks pretty evenly distributed…….

Make Main Effects Plot:

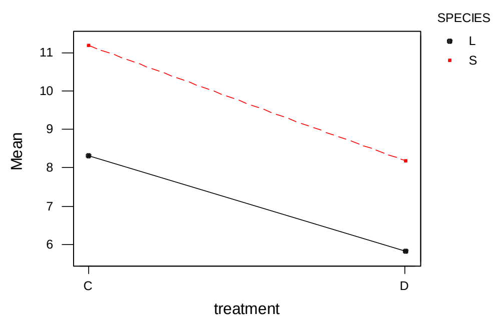
This suggests that there are treatment- and species-level differences

Main Effects Plot - Data Means for Amax



BUT…the interaction plot looks parallel, suggesting the absence of any interaction effect.

Interaction Plot - Data Means for Amax



Now let's run the two-way ANOVA to look for species, treatment, and interaction effects:

Two-way Analysis of Variance

```
Analysis of Variance for Amax
Source         DF        SS        MS        F        P
SPECIES         1      54.5      54.5     3.55    0.030
treatmen        1      60.0      60.0     3.92    0.058
Interaction     1       0.6       0.6     0.04    0.851
Error          28     429.3      15.3
Total          31     544.4

                        Individual 95% CI
SPECIES        Mean  ---------+---------+---------+---------+--
L              7.07   (-----------*-----------)
S              9.68                  (-----------*-----------)
                     ---------+---------+---------+---------+--
                          6.40      8.00      9.60     11.20

                        Individual 95% CI
treatmen       Mean  ------+---------+---------+---------+-----
C              9.74                (---------*---------)
D              7.00   (---------*---------)
                     ------+---------+---------+---------+-----
                        6.00      8.00     10.00     12.00
```

There are significant treatment and species-level effects, but n**o treatment-species interaction effects.** This is called an **ADDITIVE MODEL : NO INTERACTION EFFECTS!.**

Additive Model in MINITAB : Use `Stat` → `ANOVA` → `Two Way` and click on the box `Fit Additive Model.`

Conclusions :

Photosynthesis

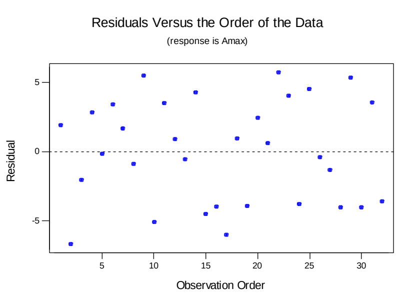
- *Q.* sideroxyla has lower photosynthesis(A_{max}) than *Q. laceyi*
- I.E. higher elevation oaks from cool-wet environments have higher photosynthesis than lower elevation oaks from hotter-drier environments.
- This indicates that these species have developed ecophysiological adaptations that promote their success under different growing conditions

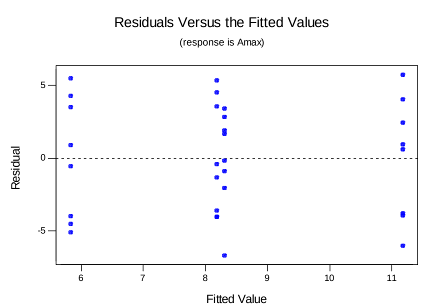
Drought

- Drought causes a significant drop in photosynthesis (Amax) for both species.

Lack of an interaction effect indicates that species did not respond differently to treatments…..i.e. species are both very drought tolerant regardless of their distributions in hot-dry or wet-cool environments.

Check Residuals:





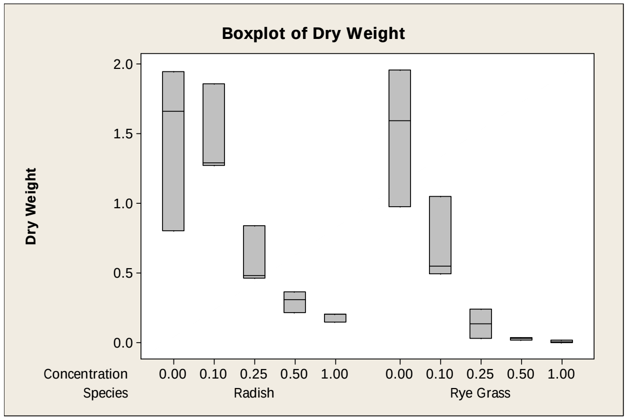
<u>Example :</u> (LAST TIME!) Round-Up™.
Several Yale Forestry students tested Round-Up™'s claim that after killing every plant it touches, it deteriorates into 'harmless components in the soil' after several weeks. Five levels of Round-Up™ weed killer were tested on soil (0% to 100% of recommended concentration). Rye grass seeds <u>and</u> radish seeds were planted, harvested, and dried after 3 weeks.

Radish	
Concentration (% of Full Strength)	Dry Weight
0.00	0.8019
	1.9457
	1.6644
0.10	1.8613
	1.2914
	1.2735
0.25	0.4617
	0.4858
	0.838
0.50	0.365
	0.3118
	0.2169
1.00	0.2064
	0.2032
	0.1471

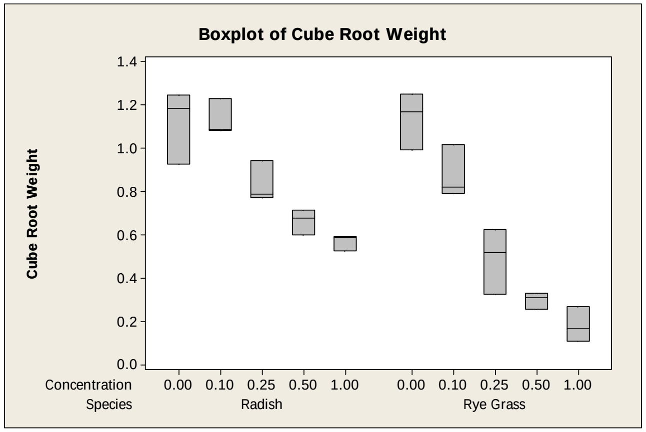
Rye Grass	
Concentration (% of Full Strength)	Dry Weight
0.00	1.5963
	0.9778
	1.9583
0.10	0.5504
	0.4977
	1.0528
0.25	0.1396
	0.2444
	0.0343
0.50	0.0171
	0.029
	0.0354
1.00	0.0045
	0.0013
	0.0195

In One-Way ANOVA, we saw that we needed to transform the data to **stabilize the variance** (i.e. make the variance similar in each category).

Untransformed data (variances <u>unequal)</u> :

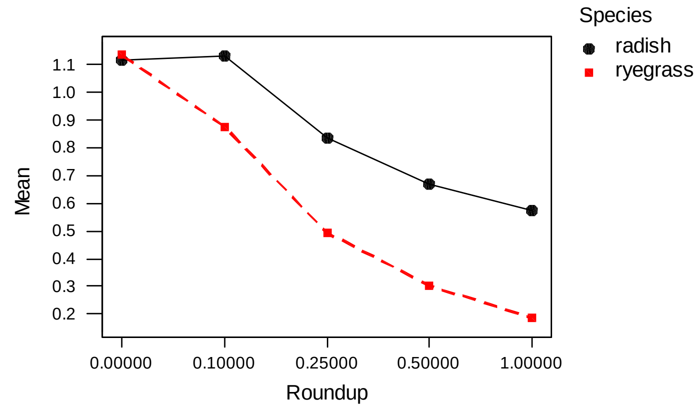


***Remember :** last time we argued that volume = (height)^3, OR Height = cube root (volume). **So – take cube roots!** Not perfect, but better.*



Make Interaction Plot :

This suggests there is an interaction between Species and Round-Up : Mean response is similar when no Round-up, but ryegrass means are lower as Round-up level increases.



Perform Two-Way ANOVA :

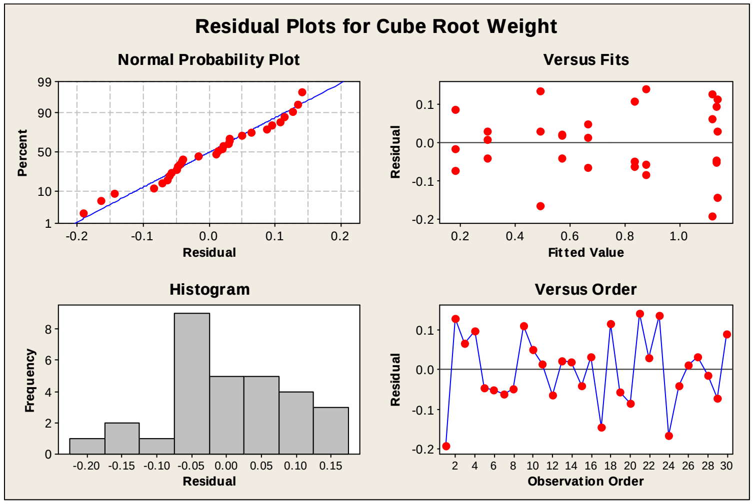
Output from MINITAB :

```
Analysis of Variance for CubeRoot
Source         DF        SS        MS        F        P
Species         1    0.5348    0.5348    48.17    0.000
Roundup         4    2.5345    0.6336    57.07    0.000
Interaction     4    0.1659    0.0415     3.74    0.020
Error          20    0.2220    0.0111
Total          29    3.4572


                   Individual 95% CI
Species      Mean  ------+---------+---------+---------+-----
radish      0.865                            (-----*----)
```

```
ryegrass        0.598   (-----*-----)
                        ------+---------+---------+---------+-----
                          0.600     0.700     0.800     0.900

                        Individual 95% CI
Roundup          Mean   ---------+---------+---------+---------+--
0.00000         1.128                                (--*---)
0.10000         1.005                           (--*---)
0.25000         0.664               (---*--)
0.50000         0.484        (--*---)
1.00000         0.378    (--*---)
                        ---------+---------+---------+---------+--
                             0.500     0.750     1.000     1.250
```

This suggests that there are differences between mean Round-Up Levels, differences between mean species levels, and a significant interaction.

Check Residuals :



<u>NOTE</u> : In MINITAB, the commands `Stat` → `ANOVA` → `Two Way` only works if your data is **balanced** : i.e. you have the same number of observations for every combination of treatment factors (here, there were three observations for each species : Roundup combination). If your data is **unbalanced**, use the commands `Stat` → `ANOVA` → `General Linear Model.` To specify an interaction, use Species*Roundup. Output is the same as Two-Way.

NOW : what to do when we have an **unbalanced design?** That is, what if there are **different numbers of observations in for each combination of treatment groups?**

Use a Generalized Linear Model

(tune in next time . . . .)