

TOMATO PLANT GROWTH

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PURPOSE

We will learn how differences in two environmental variables impact growth of tomato seedlings. We will further examine how these two factors interact in their influence on growth.

DESCRIPTION

Tomatoes are easily grown from seeds even in winter. By placing plants in a south-facing window, they may be kept warm and provided with a broad range of light wavelengths, both of which are critical for successful growth. Tomatoes grow tall, so height can be measured weekly to provide data on growth rate as well as to serve as an indicator of general plant robustness. Plants are fragile, so they require care in handling when performing experimental manipulations. We will manipulate water and fertilizer levels.

THEORY

In nature and in agriculture, multiple environmental influences impact plants and animals. Understanding how such influences - or factors - operate involves teasing apart their effects by developing an appropriate experimental design that permits analysis of not only factor effects but also whether factors interact. An **interaction** may be defined as the failure of a response (e.g. growth rate) to one factor (e.g. water) to be the same at differing levels of a second factor (e.g. fertilizer). We will use the advanced statistical technique called **factorial analysis of variance** to examine effects of individual factors as well as to uncover the presence of interactions.

METHODS

We will plant seeds in plastic seed starting trays filled with potting soil. Saturate the soil with water, with the thumb make a light depression in the center of each tray cell, place two seeds in each depression and cover seeds with several mm of soil. Plant 25 cells, cover them with a clear plastic dome to prevent evaporation and retain warmth, and place the cells on an electrically heated seed-starting mat.

When the seeds germinate in about a week, remove the clear cover, thin each cell to the strongest seedling and place the seedlings in a south-facing window, preferably with a grow-light kept 10 cm above the tops of the plants. Let the seedlings grow until true leaves appear, watering them to saturation once weekly. At this point, for a minimum of five weeks, subject the plants to experimental treatments. The two water treatments are 50 vs. 100 ml of water and the two fertilizer treatments are 1 tbs. liquid fertilizer (e.g. Miracle-gro)/ gallon water and no fertilizer. Hence, five plants will receive 50 ml of 1 tbs. fertilizer and 50 ml of plain water, five will receive 50 ml of 1 tbs. fertilizer and no additional water, five will receive no fertilizer and 100 ml water and five will receive no fertilizer and 50 ml of water. The five additional plants can be used to replace any plants that die before treatments start. The treatments with no fertilizer are a **control** for the effects of fertilizer.

We will perform a two-factor factorial analysis (called a 2 x 2 design), with water and fertilization being the factors (**treatments**) entered into the design. Statistical tests permit us to compare a **null hypothesis** (H_0) of no difference among treatments to an **alternate hypothesis** (H_1) of statistical differences occurring among treatments.

We will measure the effects that these treatments have on growth as well as whether an interaction between treatments occurs. By having five observations for each combination of water and fertilizer treatments, we will also account for individual variation, or **error**. The data that we will enter into the analysis of variance will be the final height measurements made on each plant

The factorial analysis of variance that we will perform is configured like that of the following example. Use this example to prepare tables of **treatment groups** and **treatment totals**. The example compares the effects of phosphorus and potassium supplements on clover aboveground biomass (g):

TREATMENT GROUPS					
Phosphorus: Potassium:	High P		Low P		Total
	High K	Low K	High K	Low K	
	8.53	17.53	39.14	32.00	
	20.53	21.07	26.20	23.80	
	12.53	20.80	31.33	28.87	
	14.00	17.33	45.80	25.06	
	10.80	20.07	40.20	29.33	
Sum	66.39	96.80	182.67	139.06	484.92
Mean	13.28	19.36	36.53	27.81	
Sum of each observation ²	963.88	1887.02	6913.63	3912.17	13676.70
No. of observations/group:	5				
No. of P treatments:	2				
No. of K treatments:	2				

TREATMENT TOTALS			
Potassium	Phosphorus		Total
	High	Low	
High	66.39	182.67	249.06
Low	96.80	139.06	235.86
Total	163.19	321.73	484.92

- a. Calculate a correction factor (= intercept term):

$$CF = \text{total of sums}^2 / (\text{no. obs.} \times \text{no. each treatment type})$$

$$= 484.92^2 / (5 \times 2 \times 2) = 11757.37$$

- b. Calculate the total sum of squares:

$$TSS = \text{total sum of each obs.}^2 - CF$$

$$= 13676.70 - 11757.37 = 1919.33$$

- c. Calculate the treatment (in this case, clover varieties) sum of squares:

$$Tr SS = (\text{sum}^2 \text{ of each variety added together} / \text{no. obs.}) - CF$$

$$= (66.39^2 + 96.80^2 + 182.67^2 + 139.06^2) / 5 - 11757.37 = 1539.41$$

- d. Calculate an error sum of squares (the amount of data variation left over after that due to treatments and interactions is accounted for):

$$\begin{aligned} \text{ESS} &= \text{TSS} - \text{TrSS} \\ &= 1919.33 - 1539.41 = 379.92 \end{aligned}$$

e. From the table of treatment totals, compute the sum of squares for the separate treatments:

$$\text{SSP, SSK} = \text{total of the square of each P sum} / (\text{no. obs.} \times \text{no. treatments}) - \text{CF}$$

$$\text{SSP} = 163.19^2 + 321.73^2 / (5 \times 2) - 11757.37 = 1256.75$$

$$\text{SSK} = 249.06^2 + 235.86^2 / (5 \times 2) - 11757.37 = 8.71$$

f. Compute the interaction between effects of P and K:

$$\text{SSP,K} = \text{TrSS} - \text{SSP} - \text{SSK}$$

$$\text{SSP,K} = 1539.41 - 1256.75 - 8.71 = 273.95$$

g. Prepare an analysis of variance (ANOVA) table in which mean squares are computed by dividing sums of squares by their degrees of freedom and F tests are computed by dividing mean squares by the error mean square:

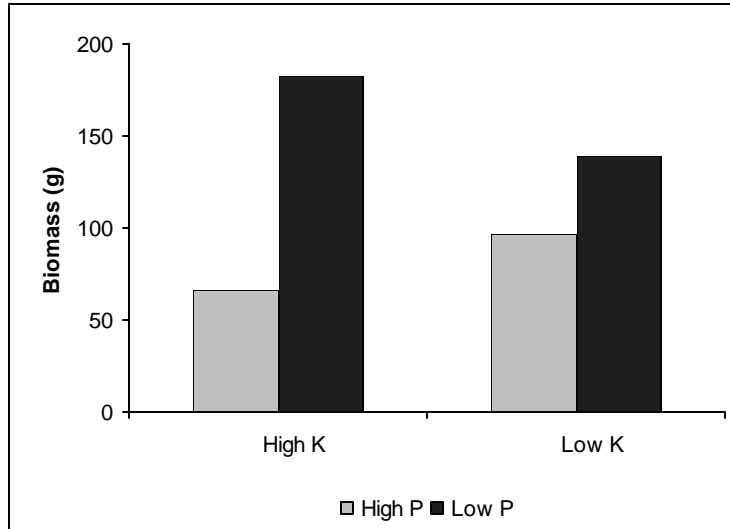
ANOVA				
Source of variation	degrees of freedom (df)	Sum of squares (SS)	Mean square (MS) (sum of squares/df)	F test (Treatment MS/Error MS)
P treatment	P treatments - 1 = 1	1256.75	1256.75	**52.91
K treatment	K treatments - 1 = 1	8.71	8.71	0.37
P x K interaction	(P - 1)(K - 1) = 1	273.95	273.95	**11.53
Error	P x K x (no. obs. - 1) = 16	379.92	23.75	
Total	Total obs. - 1 = 19	1919.33		

The F values for P and the P x K interaction are greater than 8.53, the 1% probability (P) value (indicated in the table with a double asterisk) for 1 and 16 degrees of freedom (obtained from a table of F values; P values may be found in a statistics text or on the internet), that differences observed are due to chance. Hence, we conclude that means for P significantly differ from each other and there is a significant P x K interaction. There is no significant difference between K treatments, however.

ANALYSIS

Graphing data is always the first step in any analysis.

- 1) First plot for each of the four treatments the measurements on plant size for each date of measurement. Keep in mind that the slopes ($\Delta y / \Delta x = \text{change in height/time}$) of curves generated represent the rates of growth. Compare qualitatively (without statistics) the curves and their slopes with respect to the treatments. Are the shapes of the curves different? Are their slopes different?
- 2) Also plot the data from the treatment totals table like the following from the above example:



This graph relates directly to the statistical tests performed. In this case, the graph demonstrates that high phosphorus increases clover biomass at low potassium levels but low phosphorus decreases biomass at low potassium levels. Hence, there is an interaction between the effects of phosphorus and potassium on biomass.

INTERPRETATION

Based on the graphs and the statistical tests, evaluate 1) how growth **rates** compare for the four treatments and for the control, 2) whether significant differences exist between the treatments and 3) whether significant interactions occurred. Based on the data, which treatment combination was most effective at promoting plant growth?

The control was a partial one. Effects due to which of the two factors were controlled and effects due to which were not? Explain. How did the control plants compare in growth to the treated plants?