Biology 370L: Gall Formation on Red-bay

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Species are often very particular about the way they interact. In this lab, we will explore the interaction between a plant and a gall-forming insect called *Trioza magnoliae* (the "jumping plant louse"), a member of the family Psyllidae in the order Homoptera (which also includes the more familiar family Aphididae).

A "gall" is any abnormal swelling or malformation of plant tissue. Gall formation can be caused by insects, fungi, bacteria, or viruses. In many cases, a parasite forms galls only on certain plants, and even then only on certain parts of the plants.

Red-bay (*Persea palustris*) is a common and easily recognizable shrub of the coastal plain:

- Leaves ovate, leathery and evergreen
- Midrib of older leaves with brownish hairs
- Crushed leaves with bay-leaf-like fragrance (substitute young leaves for bay leaves in spaghetti sauce!)
- Leaves often with galls formed by an insect (a "midge")

Here we will try to answer some basic questions about where *Trioza* chooses to form a gall, as well as learn (and re-learn) a couple of basic statistical procedures.

Questions

We'll ask two broad questions. The first question relates to <u>whether</u> the insect chooses certain host plants to lay its eggs. The second question relates to <u>where</u> on the host plant the insect chooses to lay its eggs.

1. Is the proportion of infested leaves related to the size of the plant?

   The size of an organism can be measured in several ways. In our case, we'll measure stem diameter, stem height, and number of leaves. Thus, we will examine three different relationships. We wish to determine if there is a significant relationship between the proportion of leaves infested and all of the following variables:

   •**Stem basal diameter**-diameter of each separate stem at ground level (in centimeters)
   •**Stem height**-height of the longest stem (in centimeters)
   •**Total number of leaves** on the stem

2. Do infested leaves differ in size from uninfested leaves?

   Two primary factors affect the answer to this question. The first factor relates to how females choose leaves, whereas the second relates to the condition of the leaves after infestation.

   A) Mothers want the best for their offspring. The insect could be very selective as to which leaves she lays her eggs on. Should infested leaves be larger or shorter than uninfested leaves? _____

   B) Although females choose a particular leaf based on certain criteria (see above), the gall infestation can influence the size of the leaf. After the galls are formed, do you expect the leaves to stay the same size? Do you think the infested leaves will be larger or smaller than uninfested leaves? _____

Procedures

Work in groups of 2. Each group needs to find 4 red-bay plants along a height gradient. Try to sample one plant from each of the four height classes: <0.5 m tall, > 0.5 m < 1 m, >1 m < 1.5 m, > 1.5 m < 2 m.

Question 1. For each plant, record the data on basal diameter, stem height, and number of leaves (total <u>and</u> infested). We'll let the computer calculate the proportion of total leaves that are infested.

Question 2. On each plant, randomly select 2 infested and 2 uninfested leaves on the plant. Measure the length of each leaf from the base of the blade to the tip of the blade, and record the data on the sheet provided.

Upon returning to the lab, record your "Plant Sizes" data on the board so everyone can get all the data. Enter all data into a Microsoft Excel spreadsheet exactly as it appears on the data sheets. Note that you can use a formula to calculate the proportion of leaves infested.

For the "Leaf Lengths" data, wait until we're done analyzing the Plant Sizes data. Then put all your data on the board. In Excel, enter the data in just two columns (Uninfested and Infested).

Data Analyses

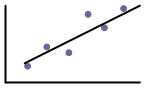
1. Is the proportion of infested leaves related to the size of the plant?

We wish to determine if there is a significant "relationship" between the proportion of leaves infested and each of the following variables: basal diameter, height, and total number of leaves. Statistically speaking, a "relationship" between two variables implies that one variable is the cause of the other. That is, one variable is "independent," and the other is "dependent" on the first. Of these two variables, which is independent and which is dependent?

proportion of infested leaves _____ basal diameter _____

Determining the presence and nature of a relationship like this is a "**regression**" problem. In regression, the objectives are 3-fold:

1. to determine if there is a significant relationship between the two variables, and if so,
2. to determine how "tight" or how "noisy" that relationship is, and
3. to estimate the parameters (slope and y-intercept) of the equation that expresses the relationship.

Think of regression as "fitting a line" to a series of points in X-Y space: 

To perform a regression in Excel, your data need to be in columns like this:

H11 =

	A	B	C	D	E	F	G
1	Diameter	Height	# of Leaves	# Infested	Proportion Infested		
2	1.3	128	77	6			
3	1.1	154	52	14			
4	0.9	67	18	3			
5	1.8	179	94	51			
6	1.6	161	49	17			
7							

Note that the "Proportion Infested" column (Column E) is empty. You will need to fill that column with formulas. For example, in cell E2 you will need to enter the formula: =d2/c2 to obtain the correct proportion. Once you have a single correct formula in E2, you can copy and paste that formula to all other cells in Column E.

The first relationship you will examine is between Diameter (Column A) and Proportion Infested (Column E). To run a regression analysis on these two variables, follow these steps:

1. On the "Tools" menu, choose "Data Analysis…".
2. In the dialog box, scroll down to "Regression".
3. Click once in the "Input Y Range:" box, and select all values in Column E (including the header in Row 1).
4. Click once in the "Input X Range:" box, and select all values in Column A (including the header in Row 1).
5. Make sure the "Labels" box is checked. This is because you selected the headers in addition to the actual data.
6. Click "Ok". This will create a new sheet containing the results of a linear regression.

Give the computer a few seconds to complete the analyses.

When you run a regression in Excel, it will produce tables like this:

Regression Statistics	
Multiple R	0.381
R Square	0.145
Adjusted R Square	0.038
Standard Error	0.636
Observations	10.000

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1.000	0.549	0.549	1.358	0.277
Residual	8.000	3.231	0.404		
Total	9.000	3.780			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	2.800	0.859	3.259	0.012	0.819	4.782	0.819	4.782
variable	-0.450	0.386	-1.165	0.277	-1.339	0.440	-1.339	0.440

- The ANOVA table indicates whether there is a significant relationship between the two variables. If "*Significance F*" is less than 0.05, then you have a significant relationship. In the above case, there is not one.
- The "tightness" or "noisiness" of the relationship (if any) is indicated by the value of "R Square." Values close to 1 indicate a very close fit to the regression line, while values close to 0 indicate a large amount of "scatter" around the line.
- The parameters of the equation are contained in the "Coefficients" column for "Intercept" and your variable of interest.

NOTE: If you do not have a significant relationship, then values of R Square and the Coefficients are irrelevant.

Because this is linear regression, you can use the Coefficients to construct a linear equation that expressed the relationship between the two variables.

By the way, what is the general equation for a line? _____

2. Do infested leaves differ in size from uninfested leaves?

This is a straightforward t-test, which you have done before (where you compared lengths of pine needles from two species). You want to compare mean leaf length between two groups: uninfested and infested.

To do a t-test, your will need all data from the entire class, so put that on the board so everyone can enter it into a spreadsheet. Your data will need to be arranged in two adjacent columns.

Once you have all the leaf length data in a spreadsheet, go to the "Tools" menu again and choose "Data Analysis…". This time, choose "t-test: Two-Sample Assuming Equal Variances". In the statistical results table that Excel produces, the appropriate p-value corresponds to “P (two-tailed)”.

Report:
Finally, write-up these results in the standard report format with your group. Be sure to address both questions in your report. Also, include at least one graph: a scatter plot of red bay size vs. % infestation. Show all significant relationships in separate graphs. If no significant relationship is found, then show the ‘best’ one.

Plant Sizes

Plant number	Stem Basal Diameter (cm)	Stem Height (cm)	Total # of Leaves	# of Leaves Infested
1				
2				
3				
4				

Leaf Lengths (cm)

Plant #1	
Uninfested	Infested

Plant #2	
Uninfested	Infested

Plant #3	
Uninfested	Infested

Plant #4	
Uninfested	Infested