Zoology Field Experiment

Hypothesis: This study is designed to evaluate the hypothesis that _____

plants will show less feeding damage than _____ plants, given that

the former are aromatic and thus protected by chemical defenses.

Background: We will examine plants of 3 species to see which is more heavily damaged by insect feeding. We will be using two techniques: Random field sampling and computerized image analysis.

Procedure: At the field station divide into groups of 4. Each group will need 3 large plastic bags, a pruner, a clipboard and a table of random numbers (below).

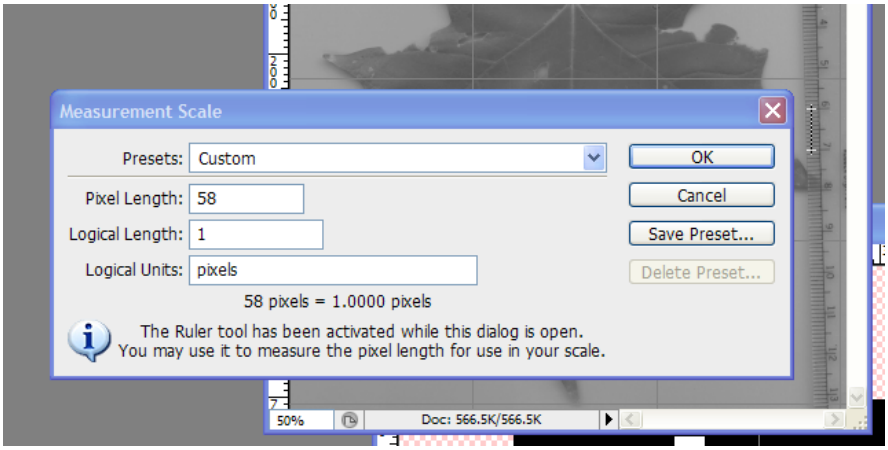
7	5	5	6	3	3	1	6	2	6	0	2	6
6	5	3	4	0	2	6	3	1	2	7	2	0
1	3	7	6	3	7	3	5	6	5	7	2	3
7	6	3	2	3	7	4	6	5	2	1	7	6
7	0	6	4	3	7	7	5	2	3	1	5	3
6	2	4	2	2	1	0	6	1	3	5	0	4
2	1	3	0	2	1	1	5	6	4	6	0	5
3	1	4	0	7	4	1	7	2	3	4	4	3
1	3	7	4	2	1	6	0	6	7	3	3	7
0	1	7	6	3	1	0	2	5	1	2	5	4
0	5	5	5	5	6	0	3	2	2	1	4	2
1	7	3	7	3	2	5	0	4	2	5	6	3
2	0	3	2	2	7	5	6	3	1	4	0	6
6	1	7	1	2	7	0	7	7	0	7	1	1
7	0	1	4	3	0	5	1	1	0	1	6	5
5	6	2	4	6	4	7	3	2	6	5	7	0
7	5	0	7	0	7	6	7	0	5	1	1	2
5	7	4	3	1	1	2	4	3	4	5	6	0
3	2	3	2	0	4	6	1	3	4	1	1	5
7	0	1	2	5	2	1	1	5	3	6	0	6
4	4	7	4	6	2	3	5	7	1	6	7	5
1	2	4	7	2	7	4	7	6	5	3	5	4
3	7	5	2	7	5	3	5	5	2	7	6	7
5	7	6	7	7	5	7	1	0	1	2	0	6
1	1	7	3	1	0	1	1	7	1	1	7	0
4	6	6	3	6	7	2	0	0	5	5	5	3
7	2	6	3	1	2	2	4	7	2	3	4	2
5	4	6	5	5	2	5	2	3	2	4	4	6
2	1	2	1	1	2	2	1	4	0	1	4	1
4	3	1	3	6	4	5	7	1	1	0	7	5
2	0	4	1	0	7	3	4	4	6	7	6	6
7	0	0	7	3	5	5	0	1	7	3	2	1
3	6	6	6	2	3	0	3	6	1	5	4	4
2	7	6	0	3	6	5	2	2	5	1	5	3

To take samples:

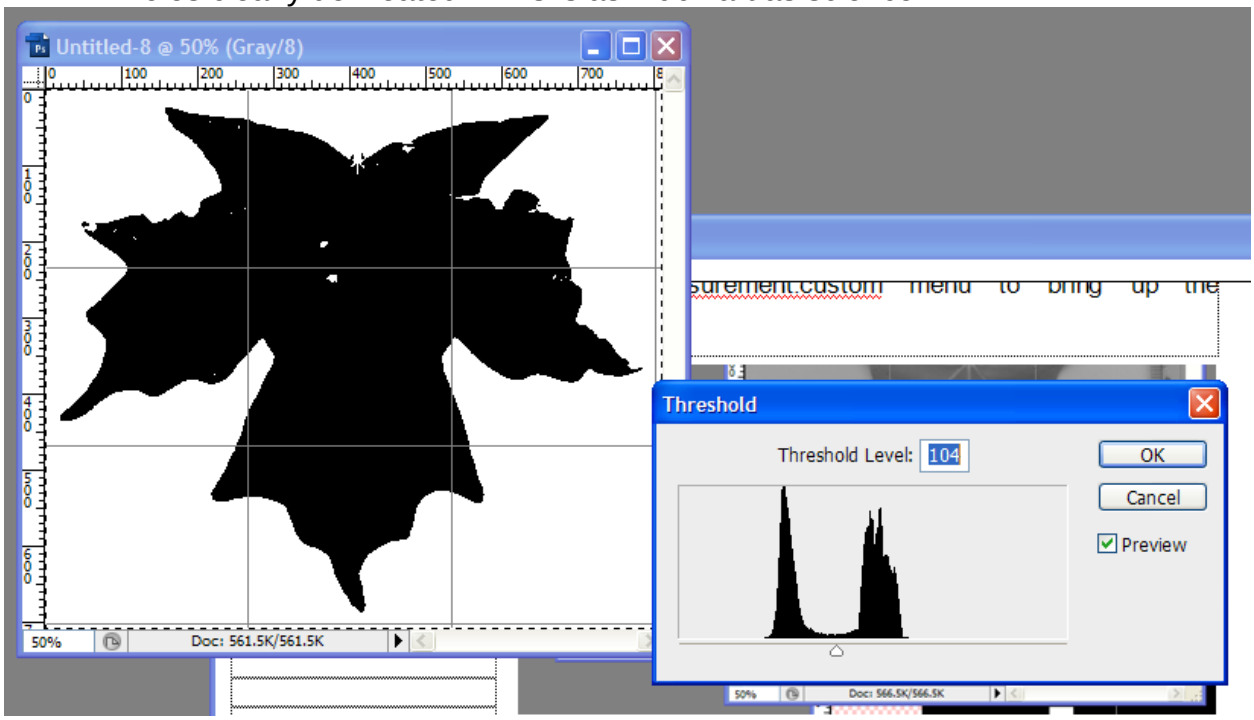
1. Determine who the oldest person in your group is.
2. Take the number of the month in which that person was born. Starting at the upper left of the table, count that many numbers to the right and use that number. If you go past the end of a line, resume counting at the left of the line below. If you reach the end of the sheet, start over at the top.
3. Find a tree of one of the species you are studying. Starting from the ground, count up the number of branches indicated by the number you chose in step 2. If the number is a 0, go on to the next number. If the number of the branch chosen is out of reach (or there aren't that many branches) go on to the next number. If branches are opposite – start counting from the bottom with the right side branch being counted first.
4. Once you have selected a branch, remove 3 leaves (or leaflets on compound leaves). Choose only green leaves – but do not exclude or choose leaves on the basis of leaf damage. Place these in a plastic bag. Keep track of the number of leaves in the bag. If there are less than 3 leaves on the branch take them all. If there are more than 3, start at the base one time and the tip the next and in the middle the next time.
5. Move to a tree of one of the other species you are studying. Using the next random number in the table, collect another sample of leaves.
6. Continue until you have at least 15 of each species of leaf.
7. Back at the lab, label the samples and place them in the freezer as directed.

Data Analysis: We will use a scanner and Photoshop software to determine the area of the leaves.

1. Place a leaf on the scanner with a ruler close to it.
2. Open Adobe Photoshop and use the File:Import menu item to start the scanner.
3. Scan the leaf as a grayscale image.
4. Crop the resulting image as small as possible, leaving a portion of the ruler visible.
5. Use the analysis:set measurement:custom menu to bring up the Measurement Scale Dialog:

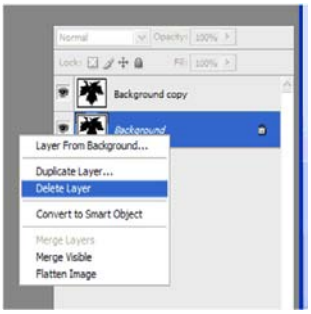


6. In the dialog, click and drag on the image of the ruler to mark off one centimeter. As you can see in the image to the right, in this case 1 centimeter = 58 pixels. WRITE THIS NUMBER DOWN!
7. Use the image:adjustments:threshold menu item. Adjust the slider (see below) in the dialog box until the leaf appears solid black, the background white, and the holes clearly delineated. This is as much art as science.

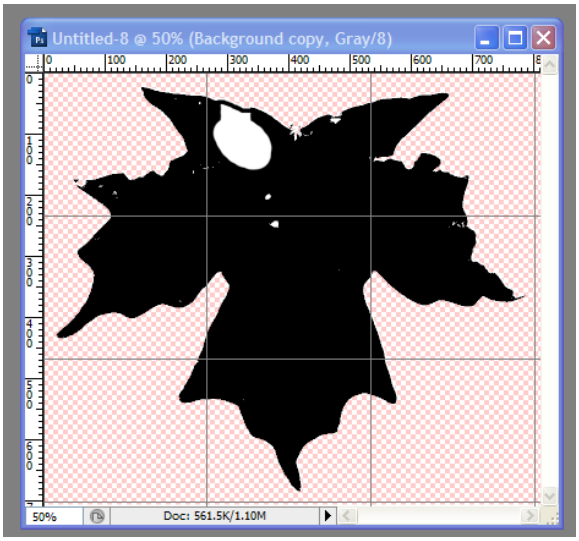


8. The leaf is now black and white. Use the brush tool  to draw a black line to close off any insect holes that reach the edge of the leaf. Try to draw the line to approximate where the edge of the leaf would be.

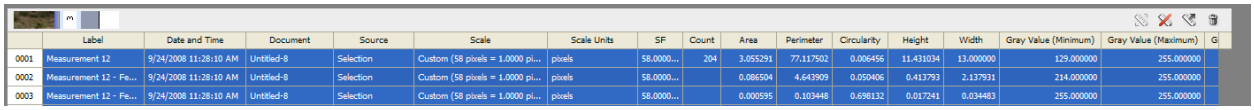
9. Use the eraser tool to erase whatever is left of the ruler. The eraser cam be made very large to facilitate this.
10. The next step is to delete the background. Use the Layer:Duplicate Layer menu to make a copy of the background. Then, select the background layer and delete it (right)



11. Now, use the Magic wand tool. Set the tolerangnce to 0 and be sure the "contiguous" box is checked, and that the single selection mode(leftmost icon next to tolerance box) is selected. Click once on the background to select it, then press the delete key to remove the background. It should look like this:



12. Finally, you are ready to analyze the image. Go back to the Magic Wand and UNCHECK the contiguous box. Now, click on one of the white spaces (where the insects fed).
13. Use the Analysis:Set Measurement Scale:Custom menu to confirm that your scale is still set to the value you found in step 6.
14. With all of the white areas thus selected, use the Analysis:Record Measurements menu to open up a box which will look like this:



	Label	Date and Time	Document	Source	Scale	Scale Units	SF	Count	Area	Perimeter	Circularity	Height	Width	Gray Value (Minimum)	Gray Value (Maximum)	G
0001	Measurement 12	9/24/2008 11:28:10 AM	Untitled-8	Selection	Custom (58 pixels = 1.0000 pi...	pixels	58.0000...	204	3.055291	77.117502	0.006456	11.431034	13.000000	129.000000	255.000000	
0002	Measurement 12 - Fe...	9/24/2008 11:28:10 AM	Untitled-8	Selection	Custom (58 pixels = 1.0000 pi...	pixels	58.0000...		0.086504	4.643909	0.050406	0.413793	2.137931	214.000000	255.000000	
0003	Measurement 12 - Fe...	9/24/2008 11:28:10 AM	Untitled-8	Selection	Custom (58 pixels = 1.0000 pi...	pixels	58.0000...		0.000595	0.103448	0.698132	0.017241	0.034483	255.000000	255.000000	

15. The top line of the box should say something like Measurement 1 (2, 3, whatever). This gives the sum total of all the white areas on the leaf – where the insect has been feeding. The remaining lines give the data for each individual white spot on the leaf. In the example above, there were 204 such spots, most of them very tine. Go into Excel and record the area of the total line (the first line) (see image below).

16. Use the trash can icon on the measurement table to delete all the measurements. Use the magic wand again to select the BLACK areas this time, and again record the measurements. The table now gives you the leaf area. Record that number in Excel as well.
17. Repeat steps 7-16 for every leaf. When you are done, add columns to your excel file to calculate total leaf area and percent eaten:

Species: Maple

	Feeding area	Leaf area	Total Leaf Area	Percent Eaten
Leaf 1	3.06	68.28	71.34	4.29
Leaf 2	4.96	57.97	62.94	7.89
Leaf 3	6.24	52.65	58.89	10.60
Leaf 4	1.43	69.76	71.18	2.00
Leaf 5	4.16	54.61	58.77	7.08
Leaf 6	6.01	12.41	18.42	32.63
Leaf 7	11.17	26.03	37.20	30.04
Leaf 8	9.92	25.78	35.70	27.79
Leaf 9	8.29	12.00	20.29	40.86
Leaf 10	14.18	42.15	56.33	25.17
Totals	69.43	421.62		
Average			49.11	18.84

Species: Oak

	Feeding area	Leaf area	Total Leaf Area	Percent Eaten
Leaf 1	5.16	38.28	43.44	11.88
Leaf 2	4.14	39.64	43.78	9.45
Leaf 3	3.00	76.84	79.83	3.75
Leaf 4	13.10	91.81	104.91	12.49
Leaf 5	9.35	30.92	40.28	23.22
Leaf 6	4.83	89.75	94.58	5.11
Leaf 7	6.55	19.06	25.61	25.59
Leaf 8	3.84	100.60	104.44	3.68
Leaf 9	2.59	84.65	87.24	2.97
Leaf 10	3.89	58.03	61.92	6.29
Totals	56.46	629.58		
Average			68.60	10.44

Each student will have to analyze 11 or 12 leaves. When all of the data is in the Excel spreadsheet, make sure each student has a copy of the file. **From here on out you are on your own!**

With the data in hand, you are now to write a lab report. It should have all the sections and follow all the guidelines for a 105 lab report (as modified by these instructions).

- The introduction should address the two species studied (relationships, distribution, uses, special facts, etc.), reported presence/absence of chemical defenses, reported insect herbivores, any previous work done along these lines. This section needs to be documented PRIMARILY with primary and secondary sources; a few tertiary sources of a scientific nature may be used. The weaker the sources, the weaker the grade!

- For the methods, write up what you actually did (as opposed to what we planned to do). Don't forget 3rd person/passive voice.
- The results sections should include at least some rudimentary statistical analysis (i.e. what simple statistical test can you use to see if the two means of percent area consumed are different?). If you have not had statistics, let me know and/or see the online statistics document.
- The explanation of conclusions should back up your results with other evidence gleaned from primary and secondary sources. Be sure to discuss and results from the literature which do not agree with your results.
- Future experiments should include some basic proposals to investigate new questions that arise from your results.
- The Literature Cited should include all the sources you cited, be in the correct format, and have enough sources to document your work.
- Don't forget that in-text citations should have author, year and page number.
- For each citation, print out the page and highlight the text you paraphrased. If it isn't obvious, add the page #, author and year in ink so I know which page came from which source.
- Overall length? Let it find its own length. 5 pages (double-spaced, typed) is too short; 12 pages is getting long.
- If you go to the writing center, take these instructions and inform the tutor that the report has to follow the 105 guidelines (which they should have).
- Submit a copy to turnitin.com by midnight on the date the paper is due.

Some questions to consider:

Why did we calculate % leaf damage rather than just sticking with the absolute area?
What sources of bias did we have?
What were the controls in this study? How does this type of study lend or not lend itself to the use of controls?
How would you make this study more experimental?
What would you like to measure to help interpret your results?
What else might explain differences in feeding damage between plants? Did we control for those explanations adequately? If not, how could we have done so?
What sources of error were there in the experiment?

Note: you don't have to include any of this in your report, but being able to answer them will strengthen your report.