Biology 3460 - Plant Physiology - Lab Exercise 1
Statistics and Scientific Writing

Objectives:
This lab is intended to:
(1) provide an introduction to basic principles of experimental design, and
(2) provide some experience with data collection and analysis.
(3) demonstrate the use of Excel as a tool for statistical analysis.
(4) indicate the elements needed for proper organization and completion of a scientific paper.

The scientific process requires mathematics as a foundation. The lab activities that we complete over the course of the semester will generate numerical data. Fundamental exercises that we complete in this lab exercise should assist you in the analysis and interpretation of those data sets. Experimental outcomes can be influenced by environmental factors AND also by chance events. For this reason, it is essential that you have some familiarity with probability and statistics, which can help with analysis and interpretation of data.
Once data have been collected and analyzed it is important to be able to share that data with the scientific community. Scientific writing involves providing a clear and concise description of experimentation and any results obtained and conclusions drawn. As a student in biology it is important that you are able to write about the experiments you perform, following the standards of the given field. In this lab you will learn both how to perform various statistical tests and what to include in scientific reports prepared about your experiments.

The Scientific Process and Data Analysis
All of you are probably familiar with the steps involved in the scientific method. This methodology is a progression of steps beginning with an information-gathering phase (observations), generation of questions based on those observations, the development of a hypothesis, predictions about experimental outcomes based on the hypothesis, the design of an experiment to test the hypothesis, collection and analysis of data, and making conclusions about the original hypothesis based on the collected data.
Depending on the type of observations being made and the data collected, there are different methods for analysis of the data.

Part A. Observation and Experimentation
Imagine that you want to know the length of your index fingers. You could come up with the hypothesis that your right and left index fingers are the same length. You could test this hypothesis by measuring your fingers. Is this really an experiment? If no, why not? If this is not an experiment, what is it?

Once you measured your fingers you could make a conclusion about your hypothesis. The conclusion regarding the results is unequivocal because you have asked a discrete question with a measurable answer. Are there any **sources of error**?

Most investigations not only provide answers, but more questions as well. Is this rather simple observation of one individual meaningful? Can the results of this study be generalized to the entire population?

After measuring your own index finger lengths you may wonder if everyone has similar results. We may wish to test the null hypothesis that members of the human population have index fingers of equal lengths. Since it is not practical to go out and measure the hands of all humans across the globe, we must take a sample. We hope that our sample is representative of the population (a **random** sample).
We could use all the people in this lab for our sample. Is this a random sample of the population? Explain your answer.

We must also hope that our sample is sufficiently large. Suppose you collected the index finger lengths of all of your classmates. You have collected lots of data. Have you done an experiment? If not, why not? If this is not an experiment, what is it?

Part B. Experimental Example – Pea Seedling Growth Investigation

Observations: Pea seedlings growing in a sunny part of your garden have much shorter, greener epicotyls than pea seedlings growing in the shade of a rhubard plant.

Question: Is the influence of light and shade responsible for the differences in shoot length?

Alternative Hypothesis (H_a): Greater amounts of light lead to shorter epicotyls.
Null Hypothesis (H_o): The amount of light has no effect on epicotyl length.

Predictions: If the amount of light has an effect on epicotyl length, pea seedlings grown in full light should have shorter epicotyls than those grown in the dark.

Experiment: Pea seeds (*Pisum sativum*) were imbibed in 7 mL of distilled water for 6 days at room temperature. One group was incubated in full light and the other in complete darkness.

The following sections of the lab exercise will help us analyze the data obtained from this experiment.

Part C. Descriptive Statistics

Once you have collected data of any type from a sample, you will likely see that there is variation in the measured data across the sample. In science it is necessary to include assessments of variation by computing **descriptive statistics**, values such as the **mean** that summarize the central tendency and **standard deviation** or **standard error** that summarize the spread of data (dispersion) around the mean. Descriptive statistics alone will <u>NOT</u> permit us to directly compare the means and draw conclusions.

Using the data set provided by your lab instructor calculate the mean epicotyl length of light-grown and dark-grown pea seedlings and the standard deviations of the samples.

Most calculators will determine the mean and standard deviation for you. We will be using the computer program Excel for much of the remainder of this lab exercise, because it is an efficient tool for calculation of many statistical tests and for creation of graphical information. If you do not know how to calculate the mean and standard deviation in Excel refer to Appendix 1 for instructions and formulae.

Part D. Inferential Statistics

Inferential statistics are mathematical methods that have been developed for statistical testing of data that give estimates of how much error is involved in the analysis. These tests fall into two categories: **parametric** and **nonparametric** tests. Parametric tests make the assumption that samples are normally distributed. Graphically, we can determine if our data are normally distributed by plotting a histogram of the frequency of given dependent variable measures (y - axis) versus some equal ranges of the dependent variable values (x-axis). The plot should yield a bell-shaped curve if the distribution is normal. Furthermore, about 68% of the data should fall within $\pm$ one standard deviation of the mean, about 95% of the data should fall within $\pm$ two standard deviations of the mean, and about 98% of the data should fall within $\pm$ three standard deviations of the mean.

Use Excel to create frequency distribution graphs for light and dark-grown pea seedling epicotyl lengths. To do this, choose the Histogram option in the Data Analysis tool pack. Your lab instructor will walk you through the process of choosing the appropriate input range, bin range, and output range.

To determine if the data are normally distributed find the range from the mean - s.d. to mean + s.d. then calculate what percentage of the total number of values falls within this range. Is the value within 5% of 68%?
Next find the range from the mean – 2s.d. to the mean + 2s.d. then calculate what percentage of the total number of values falls within this range. Is the value within 5% of 95%?
Are the data normally distributed?

Part D1. Student's t-Test

Many statistical tests are available to compare the means of two groups. The Student's t-Test is one of the most frequently used parametric tests for comparing two means of **independent** samples.

There are two different formulae for calculating the t statistic depending upon whether the variances of the groups being tested are equal or unequal. The F-Test permits you to test the hypothesis that the variances are equal (the same). Use the equation below to calculate the F statistic to determine if the variances are equal or unequal.

$$F = \frac{s_1^2}{s_2^2}$$

where, s_1^2 = larger variance (s.d.$_1$ squared), and s_2^2 = smaller variance (s.d.$_2$ squared)

Once you have calculated the F statistic, you must compare it to a table of critical values (Table 1). You will need to know the degrees of freedom (df) for the numerator and denominator (numerator df = n_1-1; denominator df = n_2-1).

If the F statistic that you calculated is greater than the critical value, then the hypothesis that the variances are the same is rejected (we conclude the variances are unequal). However, if the calculated F statistic is less than the critical value, then the hypothesis that the variances are the same is not rejected (we have no evidence the variances are different).

If we conclude the variances are equal based on the results of the F Test, then use the Student's t Test: assume equal variance formula or Excel (see Appendix 1) to calculate the t statistic. If the variances are unequal based on the results of the F Test, then use the Student's t Test: assuming unequal variance formula or Excel (see Appendix 1) to calculate the t statistic.

By calculating the t statistic, we are testing the null hypothesis that the means of the two groups are the same. After you have calculated the t statistic and the degrees of freedom (df), you must compare your calculated value to the critical values (Table 2) to estimate the corresponding p (probability) value. The p value is determined by using the row defined by the degrees of freedom and the column defined by the level of acceptable error.
Compare your calculated t statistic value to the critical value in the 0.05 column.
If your calculated t value is greater than the critical value, the hypothesis that the means are the same is rejected (the means are significantly different between the two samples). If your calculated t value is less than the critical value of t, then the hypothesis cannot be rejected. Note that when you use the Data Analysis Toolpak in Excel it will report the exact p value for you. If it is lower than 0.05 there is a significant difference between the means.

Analysis:

Mean epicotyl length of light-grown seedlings ± s.d. = __________________ mm

Mean epicotyl length of dark-grown seedlings ± s.d. = __________________ mm

Perform a t-test to determine whether there is any significant difference between the two means.

t: ______________ df: _________ p: ________________

Conclusion:
Based on the t-test, do you reject or not reject your alternative hypothesis? Make an appropriate concluding statement about the results of the experiment.

Part D2. Kruskal-Wallis Test
Sometimes, especially when our treatments have small sample sizes, the data do not fit a normal distribution. When our data fail to demonstrate a normal distribution, we must carry out a nonparametric test such as the **Kruskal-Wallis Test** to determine if two means are statistically different. This test allows us to investigate the null hypothesis that the two population means are the same, without assuming that the populations have a normal

distribution. The Kruskal-Wallis Test can also be used to analyze data that consist of more than two samples.

Assign a rank to all of the epicotyl lengths on a scale from 1 (shortest) to 20 (longest) and place an X in the third column if the length corresponds to a seed from the dark treatment.

Add all ranks for the dark-grown seeds: Dark-grown length rank sum (R_1) ________

Add all ranks for the light-grown seeds: Light-grown length rank sum (R_2) ________

If there were no difference between the means of the samples, the rank sums should be identical. If the sums are not identical, are they different enough to reject the null hypothesis of equality? To determine this, we must calculate the H statistic and compare that value to a table of critical values (Table 3). Calculate H using the following formula:

$$H = \frac{12}{N\,(N+1)} \left(\frac{R_1^2}{n_1} + \frac{R_2^2}{n_2} \right) - 3\,(N+1)$$

N = total number of experimental observations
R_1 = sum of ranks from trmt 1
n_1 = sample number from trmt 1
R_2 = sum of ranks from trmt 2
n_2 = sample number from trmt 2

Compare the calculated H statistic to the critical value. The χ^2 distribution with k-1 (where k = number of treatments) degrees of freedom closely approximates the H distribution, so we will use the χ^2 table of critical values for comparison. As with the t-test we accept a 5% probability of rejecting the null hypothesis when it is really true (i.e. use an alpha level of 0.05).
If the calculated H statistic is equal to or smaller than the listed critical value, with k-1 degrees of freedom, then the means are not significantly different. However, if the calculated H statistic is larger than the critical value, then the means ARE significantly different (we reject the null hypothesis). Excel does not have a shortcut for the Kruskal-Wallis test, but you can use Excel to make your calculations more efficient. Your lab instruction will provide an example of how to do this.

Use the Kruskal-Wallis test to determine whether there is any significant difference between the two means.

H: _____________ df: _________ p: ________________

Conclusion:
Based on the Kruskal-Wallis test, do you reject or not reject your alternative hypothesis? Make an appropriate concluding statement about the results of the experiment.

Part D3. Linear Regression: Temperature and Seed Germination Study

Observations: While peering over the fence and into your neighbor's garden, you notice that she has a black poly sheet across the surface of the garden. Several days later, the black poly is removed and you gasp as you see little bean seedlings dotting the garden soil. Glancing at your barren garden you suspect that the extra heat trapped by the black plastic hastened the germination process in your neighbour's bean garden.

Question: Does temperature influence germinating bean seed weight?

Hypothesis (H_a): As temperature of incubation increases, bean seeds weight should increase (because they should grow faster at higher temperatures).

Prediction: If incubation temperature is related to growth and seed weight in the germinating bean seeds, then beans that are incubated at higher temperatures should have greater weights than those seeds incubated at lower temperatures.

Experiment: A sample of 25 bean seeds was divided into five groups of 5 seeds each. Each group of seeds was placed in 10 mL of water and subjected to one of five temperature treatments, 5, 15, 23, 30, and 40°C for 30 h. Seeds were then individually blotted and weighed for each of the treatments. The collected data can be found in the spreadsheet provided by your instructor.

Analysis:
Unlike the previous studies in which we examined differences in variance (F-Test) and means (t-Test and Kruskal-Wallis Test), in this study we want to determine whether or not there is a relationship between our variables, the temperature treatments and the seed weights. We will use a parametric test, the **simple linear regression**, to do this. This test assumes a causal relationship between variables and allows prediction of one variable if the other is known. Another type of statistical test, a **correlation**, can also be used to examine the relationship between two factors without making any assumptions about cause and effect.

Graph the collected data using Excel. Be sure to choose the XY scatter chart type, since we are working with continuous data. Your instructor will demonstrate the appropriate steps for creation of a graph in Excel.

A simple linear regression tests the null hypothesis that the slope of the line, b (from the equation $Y = a + bX$), is equal to 0, i.e. $b = 0$.

Appendix 1 walks you through the calculations required to do a linear regression analysis by hand, but we will use Excel's analysis toolpak to complete the test for us.

Conclusion: Based on a simple linear regression analysis, is the hypothesis, 'Bean seeds incubated at high temperatures will weigh more' is supported or not supported? Make an appropriate concluding statement about the results of the experiment.

Part E. Writing Scientific Papers

Effectively communicating scientific results is just as important as designing and carrying out experiments. Scientific findings are most often communicated as oral presentations at conferences or as published papers in scientific journals. A scientific paper consists of many important components and is written following strict guidelines depending on the journal in which it is to be published. You should model your scientific papers after the format of the Canadian Journal of Botany. Refer to a recent copy of this journal for examples of style and format.

TITLE

The title is a short description of the investigation, with enough information to show the scope of the study. Sometimes the title is a statement of the main result of the investigation. The title should be sufficiently descriptive of the variables manipulated, the response, and the organism (or part thereof) observed.

ABSTRACT

This is a single paragraph that summarizes the paper. <u>It will include all of the main elements of the paper</u>; an introduction, a description of the purpose of the investigation, the main method(s) used, the significant results (not all), discussion of the results and conclusion(s) reached.

INTRODUCTION

An introduction sets up the experiment. First, the background to the investigation should be described, with appropriate key literature cited (primary literature should be included). Secondly, the purpose of the investigation should be clearly outlined. If a particular hypothesis is being tested, this should be concisely stated. The introduction should be written in the present tense.

MATERIALS AND METHODS

In this section you should describe the experimental design (controls, treatments, randomization, statistical procedures, etc.), experimental apparatus, and methodology used. This section should contain enough information so that a scientifically knowledgeable reader would be able to replicate the investigation. In the case of lab reports, you may cite the lab manual, and provide a brief description of **key elements** of the methods. Reproduction of the details from the lab manual is **NOT** required. It is generally assumed that the reader will be familiar with the general methodology, and the amount of detail needed then becomes a matter of judgment based on experience. Reference to published papers is made for details of basic procedures. Since the methods section is a description of what you have already done, it should be written in the <u>past</u> <u>tense</u>.

For the description of plant material, give the genus name and species name (remember to italicize), and cultivar if applicable. Describe the growing conditions (e.g. light quality and photoperiod, temperature, growing medium, etc.), the age of the plants, the number of plants used, etc. Equipment used and the sources of any unusual chemicals are also indicated. It is often useful to use subheadings in this section for different parts of the experiment. These subheadings may then be carried through in the Results and Discussion sections.

RESULTS

Within this section, results are summarized and described. Raw data are never included, only summarized data (descriptive and inferential statistics). Results should be summarized and presented in tables or figures, unless they are simple and can be easily described with text. Figures often offer the most easily understood representation of the data - the reader can see at a glance the key elements of the results. Tables often require more work on the reader's part to interpret. They do not represent trends in data as well as figures and are best reserved for situations where the actual numbers need to be seen. Both figures and tables should contain enough information (in figure captions, axis descriptions and legends; or in table captions, column and row headings, and footnotes) so that they can be understood *without* reference to the text in the paper.
The results section is the place to indicate results that differ statistically. For inferential statistics, the test name, the calculated statistic value, degrees of freedom, and p-value must be reported [e.g.: The growth retardant treatment (paclobutrazol) significantly reduced the height of the canola plants relative to the control (t-Test; $t = 4.96$, $df = 20$, $p < 0.05$).] The statistic critical value listed in the table of critical values should not be reported.

Do NOT present the same data twice (in both figures and tables); use one format or the other but not both.

Indicate the important features (trends or patterns) of the results **in the text** of the Results section, by referring to the tables and figures. For example, make a statement such as 'Reduction in available oxygen and application of ethylene antagonists increased the rate of ethylene production in sorghum root tips (Fig. 1)'. Do not give a verbatim summary of all of the data points that appear in your figure or table. Do not include results in figures or tables that you do not describe in the text.

Do not provide explanation or interpretation of results in the Results section. (Note: some journals treat the Results and Discussion sections as one section but in this course we will use two distinct sections). Like the Methods section, the Results section is also written in the past tense.

DISCUSSION

In this section you interpret, integrate, and explain the results. Do not simply re-present the results without explaining why those results (the trends) were seen. You will receive **no credit** for the discussion portion of your scientific paper if you repeat the trends that were observed and do not explain why these trends occurred. You must discuss your results in terms of the objective of the investigation. Why were these results seen? What physiological basis is there for an explanation? If a hypothesis was being tested, state the outcome. Draw conclusions. Explain your results in terms of present knowledge (the results obtained by other investigators, as outlined in the Introduction; cite these). If the results are different or conflict with what was expected based on current knowledge, suggest explanations. What were possible sources of error? What additional experiments could be performed to clarify points of uncertainty or to investigate the subject further?

Be sure to provide a general conclusion or “take-home message” at the end of your Discussion section rather than just stopping when you have discussed all aspects of the experiment. The last thing the reader reads should leave him/her with a clear indication of what came of the presented work and why the study was important.

LITERATURE CITED

References cited in the text are listed here alphabetically in the form required by the journal. Make sure that all of the citations in the text are included here, and that the list does not include references that are not cited in the text. This section is not a 'papers read' section. Refer to Appendix 2 for information on how to make citations throughout the paper and formatting of the Literature Cited section.

APPENDIX 1. Microsoft Excel™ and Simple Statistics

This document is designed to introduce the use of Excel to do some simple calculations.

Cells and Ranges

Data are generally entered into columns on a spreadsheet. Cells are indexed by row (1 to n) and column (A to ZZ). The upper left cell is A1; a cell below it to the right might be B3. A range of cells is indexed by the cell range separated by a colon starting with the upper-left cell to lower-right cell. For example, the cells A1, A2, A3, B1, B2, and B3 would be included in the following A1:B3.

Formulae

Cells can hold data, but they can also hold a formula to direct a calculation. Formulae can be created manually, but Excel contains some built in functions as well.

A formula starts with an equal sign, =, and then may include cell references, functions, and complex expressions. If you know the correct formula it can be directly typed in following the equal sign. If you are unsure of the available functions, you can peruse a list of available functions found by going to the Insert menu bar, down to Function, and then selecting the appropriate formula.

If cells A1 and A2 contain numbers and the formula =A1+A2 is typed into cell A3, the data in A1 and A2 are added together and the sum shown in cell A3. If further changes are made to the data in either cell A1 or A2, the sum is automatically recalculated.

Descriptive Statistics

The following formulae can also be used to calculate the mean and standard deviation:

$$\text{Mean } (\overline{X}) = \frac{\Sigma x_i}{n} \qquad \text{Standard deviation (s.d.)} = \sqrt{\frac{(\Sigma (x_i^2)) - \frac{(\Sigma x_i)^2}{n}}{n - 1}}$$

Note: When calculating a <u>sample</u> standard deviation, n-1, is used as the denominator (as shown above in the formula). However when determining the <u>population</u> standard deviation, n represents the denominator. If you use a calculator be sure to use the sample standard deviation function.

Using Excel:
Consider the following example: A column of cells (range=A1:A10) contains one data set consisting of 10 values.
To calculate the **mean**, go to cell A12 and type the formula =AVERAGE(A1:A10) and press the return key. The computed mean will be place in A12.
To calculate the **standard deviation**, go to cell A13 and type the formula =STDEV(A1:A10) and press the return key. The standard deviation will appear in A13.
To calculate **N**, go to cell A14 and type the formula =COUNT(A1:A10) and press return. Only cells containing numerical values are tallied and the value placed in cell A14. Blank cells will not be counted.

Inferential Statistics

Excel also has a data analysis tool package that may or may not be installed with the version of Excel installed on your computer. This package can be found under the Tools menu bar, and is relatively user friendly, but interpretation of the data presented is sometimes not clear to users. Toolkit (see page 13 for web address) has some additional information on the use of Excel for specific tests.

Student's t Test - assuming equal variance formula: (or choose appropriate test from Data Analysis toolpak in Excel)

$$t = \frac{\left|\bar{X}_1 - \bar{X}_2\right|}{\sqrt{\frac{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2}{(n_1 + n_2 - 2)}}\sqrt{\frac{1}{n_1} + \frac{1}{n_2}}}$$

$$\boxed{df = n_1 + n_2 - 2}$$

Student's t Test - assuming unequal variance formula: (or choose appropriate test from Data Analysis toolpak in Excel)

$$t = \frac{\left|\bar{X}_1 - \bar{X}_2\right|}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}}$$

$$\boxed{df = \frac{\left(\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}\right)^2}{\frac{\left(\frac{s_1^2}{n_1}\right)^2}{n_1 - 1} + \frac{\left(\frac{s_2^2}{n_2}\right)^2}{n_2 - 1}}}$$

Linear Regression:

Use of a table like that below is helpful in the calculation of the various parameters needed for calculation of b. Remember that there are as many X values as there are Y values.

Parameter	Calculation	Value
ΣX	Sum all X values	
$\bar{X}$	Mean of all X values	
ΣX^2	Square each X, then sum	
ΣY	Sum all Y values	
$\bar{Y}$	Mean of all Y values	
ΣY^2	Square each Y, then sum	
ΣXY	Multiply each XY pair, then sum	

The above values are used to calculate the **slope**, **b**, using the following formula:

$$b = \frac{\Sigma XY - \frac{(\Sigma X)(\Sigma Y)}{n}}{\Sigma X^2 - \frac{(\Sigma X)^2}{n}}$$

If the slope (b) has a value of 0, there is no relationship between X and Y.
Knowing the value of the slope, b, we can determine the **Y intercept**, **a**, using the formula:

$$a = \bar{Y} - b\bar{X}$$

By substituting the slope and Y intercept values into the equation, Y = a + bX, we now have an equation that can be used to fit a line to the data set. In Excel you can use the Add Trendline option to place the regression line on your graph. This line however, does not tell us statistically whether or not there is a relationship between the variables; we must calculate the variance due to regression (V_r) and the variance due to residuals (V_E). The remaining steps lead to the calculation of the F statistic.

Calculate SS_T (total sum of squares of Y values) and SS_r (sum of squares due to regression) as follows:

$$SS_T = \Sigma Y^2 - \frac{(\Sigma Y)^2}{n} \qquad SS_r = b\left[\Sigma XY - \frac{(\Sigma X)(\Sigma Y)}{n}\right]$$

The residual sum of the squares, SS_E, is calculated using the following: $SS_E = SS_T - SS_r$

To calculate the variance due to regression (V_r), sometimes called the regression mean square, and the variance due to residuals (V_E), sometimes referred to as the residual mean square, SS_r and SS_E are divided by the appropriate degrees of freedom. In the case of SS_r, the degrees of freedom are always 1, making $SS_r = V_r$. Calculate V_r and V_E using the formulae below:

$$V_r = \frac{SS_r}{1} \qquad V_E = \frac{SS_E}{n - 2}$$

Finally, the calculation of the F statistic:

$$F = \frac{V_r}{V_E} \qquad \text{(numerator df = 1, denominator df = n - 2)}$$

Compare the calculated F statistic to the critical value in Table 4. To pinpoint the critical value in the table you must know the degrees of freedom in the numerator and denominator; refer to the box above for the appropriate equation. If the calculated F statistic value is greater than the table critical value, the null hypothesis (slope is equal to 0) is rejected, there is a relationship between seed weight and incubation temperature. However, if the calculated value is less than the critical value in the table, the null hypothesis is supported and we conclude that there is no linear relationship between the two variables.

FINAL NOTE:
Over the course of the semester, you will be completing a number of experiments associated with the lab portion of Biology 3460. You will be expected to analyze your data using **both descriptive** and **inferential statistics**. For further information about scientific methodology and statistics, consult the following web site on the University of Lethbridge Biological Sciences web page:

http://home.uleth.ca/bio/toolkit/

Microsoft Excel ™ is a good spreadsheet program available to you on the Macintosh and PC computers in the Student Computer Labs. There are also many other complete statistical packages that are available at large, but only SPSS is available on the computers in the student labs. You have been provided the basic statistical tools in the preceding exercises. If you wish to use statistical computer packages, you are encouraged to do so independently. Using computerized statistical packages for data analysis is beyond the scope of this class and there is no technical support for these packages in this lab.

APPENDIX 2. Formatting in Scientific Papers

Tables and Figures:

The figure caption should be included **below** the figure. Axes should include the variable name, and units [e.g.: Time (days)]. The table caption should appear at the top of the table. See the examples below.

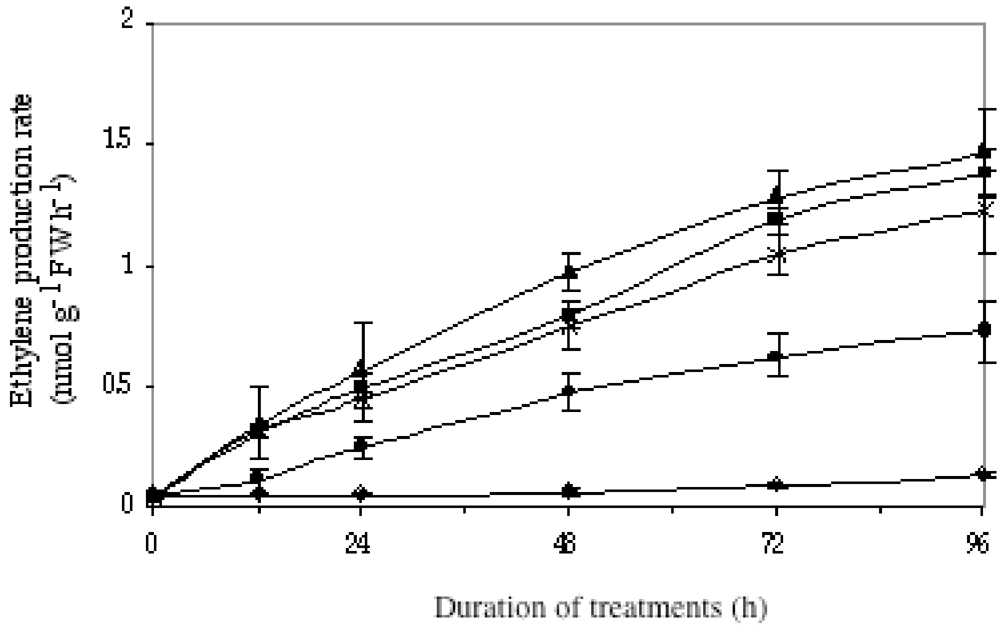

Figure 1. The effects of antagonists [normoxic (21% O2) - ◆ ; hypoxic (4% O2) - ■ ; 4% O2 + EGTA - ▲ ; 4% O2 + K-252a - ✕ ; 4% O2 + caffeine - ●] on the rate of ethylene production by freshly excised sorghum root tips. Treatments with intact plants began at the start of the time course. The data plotted represent the means ± s.e. (n=8). FW = fresh weight.

Table 1. The effect of exogenous growth regulator treatments on the height of greenhouse grown *Brassica napus* cv. Vigor and *B. rapa* cv. Shrimpy plants as measured from the soil to the shoot tip. The values represent the means ± s.e., n = 25.

Canola Cultivar	**Plant Heights Following Growth Regulator Application (cm)**		
	Paclobutrazol	**Control**	**GA_3**
B. napus cv. Vigor	37.7 ± 3.2	52.7 ± 3.7	64.9 ± 5.1
B. rapa cv. Shrimpy	27.7 ± 2.6	29.4 ± 3.3	49.7 ± 4.7

Literature Citations:
There are many ways (styles) to arrange and punctuate the required citation information, and no matter what format is adopted, there is basic information that must be included in **ALL** full citations.

<u>Journal articles:</u>
Format:
Author(s). Year. Article title. Journal title volume number: page numbers.

Example:
Loreti, E., Alpi, A. and Perata, P. 2000. Glucose and disaccharide-sensing mechanisms modulate the expression of a-amylase in barley embryos. Plant Physiology 123: 939-48.

<u>Books:</u>
Format:
Author(s). Year of publication. Book title. Publisher, publisher location.

Example:
Fosket, D.E. 1994. Plant development: a molecular approach. Academic Press, New York.

<u>Edited book with chapters by different authors:</u>
Format:
Author(s) of chapter. Year of publication. Title of chapter. In: Title of book (editors names, eds). Publisher, publisher location.

Example:
Stitt, M. 1997. Chloroplast cytosol interactions. In: Plant metabolism (Dennis, D.T., Turpin, D.H., Lefebvre, D.D. and Layzell, D.B., eds.). Addison Wesley Longman, Inc., Essex, England.

Citations within the body text:
It is common practice in scientific writings to use the author-year format for references that are cited in the text. The footnote manner of referencing that is commonly used in other disciplines is not used. For example, in text the references are cited as:

The *BRl1* gene may play a critical role in brassinosteroid perception (Clouse *et al*., 1996). [*et al*. is short hand notation that mean 'and others' and is used only if there are more than two authors of the paper]

Or, alternatively:

Clouse et al. (1996) showed that the *BRl1* gene …

For your scientific papers you are required to provide at least one (1) paper from the **primary literature** (i.e. refereed journal articles). Choose your references wisely, making sure that they are relevant to the topic being discussed, and use them appropriately in your paper. There will be no required number of citations for each report but using several citations is often better in terms of explaining and supporting your work.

A note about using Web references:
The World Wide Web can be useful in quickly finding lots of information on a given topic. Use of the Web as an aid in better understanding topics for which textbook or lab background information is not sufficient for you can be very helpful. Often times seeing the same information presented in different ways helps solidify ideas.
However, for all of the experiments you will be writing about in Biology 3460 there are excellent reference materials in your textbook, other texts, and in the primary literature. A general web page is never as good a reference as a published text or journal article, as there is usually no accountability for the information published to the internet and it can thus contain many inaccuracies. Use of web page references will be penalized whenever supporting information can be obtained from a more reputable source.

“Doing research on the Web is like using a library assembled piecemeal by packrats and vandalized nightly.” – Roger Ebert

Table 1. Table (two-tailed) of critical values to be used with the F Test of variance equality (at the 0.05 α level).

	Degrees of freedom in the numerator									
Degrees of freedom in denominator	1	2	3	4	5	6	7	8	9	10
1	647.79	799.48	854.15	899.59	921.83	937.11	948.20	956.64	963.27	968.63
2	38.506	39.000	39.166	39.248	39.298	39.331	39.356	39.373	39.387	39.398
3	17.443	16.044	15.439	15.101	14.885	14.735	14.624	14.540	14.473	14.419
4	12.218	10.649	9.979	9.604	9.364	9.197	9.074	8.980	8.905	8.844
5	10.007	8.434	7.764	7.388	7.146	6.978	6.853	6.757	6.681	6.619
6	8.813	7.260	6.599	6.227	5.988	5.820	5.695	5.600	5.523	5.461
7	8.073	6.542	5.890	5.523	5.285	5.119	4.995	4.899	4.823	4.761
8	7.571	6.059	5.416	5.053	4.817	4.652	4.529	4.433	4.357	4.295
9	7.209	5.715	5.078	4.718	4.484	4.320	4.197	4.102	4.026	3.964
10	6.937	5.456	4.826	4.468	4.236	4.072	3.950	5.855	3.779	3.717
11	6.724	5.256	4.630	4.275	4.044	3.881	3.759	3.664	3.588	3.526
12	6.554	5.096	4.474	4.121	3.891	3.728	3.607	3.512	3.436	3.374
13	6.414	4.965	4.347	3.996	3.767	3.604	3.483	3.388	3.312	3.250
14	6.298	4.857	4.242	3.892	3.663	3.501	3.380	3.285	3.209	3.147
15	6.200	4.765	4.153	3.804	3.576	3.415	3.293	3.199	3.123	3.060
16	6.115	4.687	4.077	3.729	3.502	3.341	3.219	3.125	3.049	2.986
17	6.042	4.619	4.011	3.665	3.438	3.277	3.156	3.061	2.985	2.922
18	5.978	4.560	3.954	3.608	3.382	3.221	3.100	3.005	2.929	2.866
19	5.922	4.508	3.903	2.559	3.333	3.172	3.051	2.956	2.880	2.817
20	5.871	4.461	3.859	3.515	3.289	3.128	3.007	2.913	2.837	2.774
21	5.827	4.420	3.819	3.475	3.250	3.090	2.969	2.874	2.798	2.735
22	5.786	4.383	3.783	3.440	3.215	3.055	2.934	2.839	2.763	2.700
23	5.750	4.349	3.750	3.408	3.183	3.023	2.902	2.808	2.731	2.668
24	5.717	4.319	3.721	3.379	3.155	2.995	2.874	2.779	2.703	2.640
25	5.686	4.291	3.694	3.353	3.129	2.969	2.848	2.753	2.677	2.613
30	5.568	4.182	3.589	3.250	3.026	2.867	2.746	2.651	2.575	2.511
50	5.340	3.975	3.390	3.054	2.833	2.674	2.553	2.458	2.371	2.317
75	5.232	3.876	3.296	2.962	2.741	2.582	2.461	2.366	2.289	2.224
100	5.179	3.828	3.250	2.917	2.696	2.537	2.417	2.321	2.244	2.179

Table 2. Table (two-tailed) of critical values to be used with the Student's t Test (at the 0.5, 0.1, 0.05, 0.001, and 0.001 α levels).

Degrees of Freedom	**t critical value**				
	0.5	0.1	0.05	0.01	0.001
1	1.000	6.314	12.706	63.656	636.578
2	0.816	2.920	4.303	9.925	31.600
3	0.765	2.353	3.182	5.841	12.924
4	0.741	2.132	2.776	4.604	8.610
5	0.727	2.015	2.571	4.032	6.869
6	0.718	1.943	2.447	3.707	5.959
7	0.711	1.895	2.365	3.499	5.408
8	0.706	1.860	2.306	3.355	5.041
9	0.703	1.833	2.262	3.250	4.781
10	0.700	1.812	2.228	3.169	4.587
11	0.697	1.796	2.201	3.106	4.437
12	0.695	1.782	2.179	3.055	4.318
13	0.694	1.771	2.160	3.012	4.221
14	0.692	1.761	2.145	2.977	4.140
15	0.691	1.753	2.131	2.947	4.073
16	0.690	1.746	2.120	2.921	4.015
17	0.689	1.740	2.110	2.898	3.965
18	0.688	1.734	2.101	2.878	3.922
19	0.688	1.729	2.093	2.861	3.883
20	0.687	1.725	2.086	2.845	3.850
21	0.686	1.721	2.080	2.831	3.819
22	0.686	1.717	2.074	2.819	3.792
23	0.685	1.714	2.069	2.807	3.768
24	0.685	1.711	2.064	2.797	3.745
25	0.684	1.708	2.060	2.787	3.725
26	0.684	1.706	2.056	2.779	3.707
27	0.684	1.703	2.052	2.771	3.689
28	0.683	1.701	2.048	2.763	3.674
29	0.683	1.699	2.045	2.756	3.660
30	0.683	1.697	2.042	2.750	3.646
50	0.679	1.676	2.009	2.678	3.496
75	0.678	1.665	1.992	2.643	3.425
100	0.677	1.660	1.984	2.626	3.390

Table 3. Table of critical values to be used with the Kruskal-Wallis Test (at the 0.5, 0.1, 0.05, 0.01, and 0.001 α levels).

Degrees of Freedom	2 critical value				
	0.5	0.1	0.05	0.01	0.001
1	0.455	2.706	3.841	6.635	10.827
2	1.386	4.605	5.991	9.210	13.815
3	2.366	6.251	7.815	11.345	16.266
4	3.357	7.779	9.488	13.277	18.466
5	4.351	9.236	11.070	15.086	20.515
6	5.348	10.645	12.592	16.812	22.457
7	6.346	12.017	14.067	18.475	24.321
8	7.344	13.362	15.507	20.090	26.124
9	8.343	14.684	16.919	21.666	27.877
10	9.342	15.987	18.307	23.209	29.588
11	10.341	17.275	19.675	24.725	31.264
12	11.340	18.549	21.026	26.217	32.909
13	12.340	19.812	22.362	27.688	34.527
14	13.339	21.064	23.685	29.141	36.124
15	14.339	22.307	24.996	30.578	37.698
16	15.338	23.542	26.296	32.000	39.252
17	16.338	24.769	27.587	33.409	40.791
18	17.338	25.989	28.869	34.805	42.312
19	18.338	27.204	30.144	36.191	43.819
20	19.337	28.412	31.410	37.566	45.314
21	20.337	29.615	32.671	38.932	46.796
22	21.337	30.813	33.924	40.289	48.268
23	22.337	32.007	35.172	41.638	49.728
24	23.337	33.196	36.415	42.980	51.179
25	24.337	34.382	37.652	44.314	52.619
26	25.336	35.563	38.885	45.642	54.051
27	26.336	36.741	40.113	46.963	55.475
28	27.336	37.916	41.337	48.278	56.892
29	28.336	39.087	42.557	49.588	58.301
30	29.336	40.256	43.773	50.892	59.702
40	39.335	51.805	55.758	63.691	73.403
50	49.335	63.167	67.505	76.154	86.660

Table 4. Table (one-tailed) of critical values for F to be used with ANOVA or simple linear regression (at the 0.05 α level).

Degrees of freedom in denominator	Degrees of freedom in the numerator												
	1	2	3	4	5	6	7	8	9	10	20	40	120
1	161.	199.	215.	224.	230.	234.	236.	238.	240.	241.	248.	251.	253.
2	18.1	19.0	19.1	19.2	19.3	19.3	19.3	19.3	19.3	19.4	19.4	19.4	19.4
3	10.1	9.55	9.28	9.12	9.01	8.94	8.89	8.85	8.81	8.79	8.66	8.59	8.55
4	7.71	6.94	6.59	6.39	6.26	6.16	6.09	6.04	6.00	5.96	5.80	5.72	5.66
5	6.61	5.79	5.41	5.19	5.05	4.95	4.88	4.82	4.77	4.74	4.56	4.46	4.40
6	5.99	5.14	4.76	4.53	4.39	4.28	4.21	4.15	4.10	4.06	3.87	3.77	3.70
7	5.59	4.74	4.35	4.12	3.97	3.87	3.79	3.73	3.68	3.64	3.44	3.34	3.27
8	5.32	4.46	4.07	3.84	3.69	3.58	3.50	3.44	3.39	3.35	3.15	3.04	2.97
9	5.12	4.26	3.86	3.63	3.48	3.37	3.29	3.23	3.18	3.14	2.94	2.83	2.75
10	4.96	4.10	3.71	3.48	3.33	3.22	3.14	3.07	3.02	2.98	2.77	2.66	2.58
11	4.84	3.98	3.59	3.36	3.20	3.09	3.01	2.95	2.90	2.85	2.65	2.53	2.45
12	4.75	3.89	3.49	3.26	3.11	3.00	2.91	2.85	2.80	2.75	2.54	2.43	2.34
13	4.67	3.81	3.41	3.18	3.03	2.92	2.83	2.77	2.71	2.67	2.46	2.34	2.25
14	4.60	3.74	3.34	3.11	2.96	2.85	2.76	2.70	2.65	2.60	2.39	2.27	2.18
15	4.54	3.68	3.29	3.06	2.90	2.79	2.71	2.64	2.59	2.54	2.33	2.20	2.11
16	4.49	3.63	3.24	3.01	2.85	2.74	2.66	2.59	2.54	2.49	2.28	2.15	2.06
17	4.45	3.59	3.20	2.96	2.81	2.70	2.61	2.55	2.49	2.45	2.23	2.10	2.01
18	4.41	3.55	3.16	2.93	2.77	2.66	2.58	2.51	2.46	2.41	2.19	2.06	1.97
19	4.38	3.52	3.13	2.90	2.74	2.63	2.54	2.48	2.42	2.38	2.16	2.03	1.93
20	4.35	3.49	3.10	2.87	2.71	2.60	2.51	2.45	2.39	2.35	2.12	1.99	1.90
21	4.32	3.47	3.07	2.84	2.68	2.57	2.49	2.42	2.37	2.32	2.10	1.96	1.87
22	4.30	3.44	3.05	2.82	2.66	2.55	2.46	2.40	2.34	2.30	2.07	1.94	1.84
23	4.28	3.42	3.03	2.80	2.64	2.53	2.44	2.37	2.32	2.27	2.05	1.91	1.81
24	4.26	3.40	3.01	2.78	2.62	2.51	2.42	2.36	2.30	2.25	2.03	1.89	1.79
25	4.24	3.39	2.99	2.76	2.60	2.49	2.40	2.34	2.28	2.24	2.01	1.87	1.77
26	4.23	3.37	2.98	2.74	2.59	2.47	2.39	2.32	2.27	2.22	1.99	1.85	1.75
27	4.21	3.35	2.96	2.73	2.57	2.46	2.37	2.31	2.25	2.20	1.97	1.84	1.73
28	4.20	3.34	2.95	2.71	2.56	2.45	2.36	2.29	2.24	2.19	1.96	1.82	1.71
29	4.18	3.33	2.93	2.70	2.55	2.43	2.35	2.28	2.22	2.18	1.94	1.81	1.70
30	4.17	3.32	2.92	2.69	2.53	2.42	2.33	2.27	2.21	2.16	1.93	1.79	1.68
40	4.08	3.23	2.84	2.61	2.45	2.34	2.25	2.18	2.12	2.08	1.84	1.69	1.58
60	4.00	3.15	2.76	2.53	2.37	2.25	2.17	2.1	2.04	1.99	1.75	1.59	1.47
120	3.92	3.07	2.68	2.45	2.29	2.18	2.09	2.02	1.96	1.91	1.66	1.50	1.35