SC/BIOL 2010 4.0 - PLANT BIOLOGY January 2016

Lab Manual Contents

This lab manual belongs to:

_____

My lab section: _____ Day: _____ Time: _____

My TA: _____

Course information

Overview

Plant Biology introduces you to the field of botany. Botany is all about photosynthetic organisms, which include the plants that you see around you. But, members of the Plant Kingdom are not the only autotrophs. Algae and some prokaryotes are also autotrophs.

But, you won't only be learning about autotrophs in BIOL 2010. While the Animals course covers only one kingdom, Animalia, this course, and other Plant Biology or Botany courses cover much more: 3 kingdoms, including the heterotrophic fungi, plus the prokaryotes, Archaea and Bacteria.

Thanks to new data from the field of molecular systematics, these traditional groupings are changing and evolving, and we will cover the new classifications here, too.

In Plant Biology you will learn about the biodiversity, evolution, and ecology of Plants, Algae, Fungi, Protists (which is everything that doesn't fit into one of the other 4 kingdoms), and Prokaryotes. You will also learn about the different kinds of sexual reproduction -- the 3 main life-cycles, along with some physiology, genetics, and the importance of plants to people.

The laboratories illustrate and support key aspects of the lectures.

Course Director	**Dawn R. Bazely**, Room 206 LB 416 736-2100 ext. 2019 Email: dbazely@yorku.ca Website: http://moodle.yorku.ca/
Laboratory Coordinator	**Ryan Scott** Email: rwscott@yorku.ca, Office hours by appointment only
Lectures	MWF 9:30 - 10:30 p.m. LSB 103 (Life Sciences Building)
Website	http://moodle.yorku.ca/ See also http://www.yorku.ca/plants (this is Roger Lew's website with some great information and material)
Laboratories	M-R 2:30 pm- 5:30 pm 118 LB (Lumbers Building) M-T 6:30 pm - 9:30 pm 118 LB (Lumbers Building)
Mark Distribution	Two Term Tests 25% total (10% for lowest mark, 15% for highest)[1] Final Exam 35% Laboratory 40% in total – grade breakdown is on p.1
Textbook	Raven's *Biology of Plants* (8th ed.) – Evert& Eichhorn. 2012.

[1] In the event of an absence from a term test, please provide a letter stating that you were absent, and acknowledging that the weight of the missed term test (15%) will be added to the weight of the final exam.

Laboratory Schedule
Also, please see the MOODLE Google Calendar– important dates will be added to it

Week	Scheduled labs
1. Jan 4 – 8	Lab 0 – Scientific Art: Draw fruit & vegetables
2. Jan 11 – 15	Lab 1 – Photosynthesis
3. Jan 18 – 22	Lab 2 –Algae AND Lab 3- Fern life cycle*
4. Jan 25 – 29	Lab 4 – Fungi – experiments with SCOBYS
5. Feb 1 – 5	Lab 5 – Bryophytes
6. Feb 8 – 9	Lab 6 – Seedless Vascular Plants
7. Feb 15 – 19	READING WEEK – measure your SCOBYs
8. Feb 22 – 26	Lab 7 – Gymnosperms
9. Feb 29 – Mar 4	Lab 8 – Pollen Identification
10. Mar 7 – 11	Lab 8 –Pollen Identification and Pollen Tube Growth
11. Mar 14 – 18	Lab 9 –Angiosperm Anatomy Project Week I **
12. Mar 21 – 25	Lab 9 – Angiosperm Anatomy Project Week II
13. Mar 28 – Apr 1	Make-up (may be used for Lab Quiz)

*Note – Lab 3 and Lab 4 Fern life cycle lab extend across several weeks.
** **You must obtain a flowering plant for study in the Angiosperm Project.**

Safety Practices

1. **Absolutely no eating or drinking is permitted in laboratories**. (This includes bottled water!)
2. Safety glasses are **required** for many of the labs.
3. It is suggested that latex gloves be worn when handling preserved or hazardous material. Disposable gloves will be provided when necessary and they should be put in the appropriate garbage bin at the end of each laboratory session.
4. Laboratory rooms are to be left clean and tidy and all waste must be discarded in the appropriate labeled containers.
5. Lab coats should be worn in all laboratories.

Laboratory mark distribution: worth 40% of your final mark

Note: Dates &times of lab quizzes & lecture quizzes to be announced in Moodle Calendar

LABORATORY	ASSESSMENT TYPE	VALUE
Lab 0 Fruit and Vegetable Anatomy	Technical drawing - will be covered in lab quiz	quiz
Lab 1 Photosynthesis	Lab Write-up	5%
Lab 2 Algae	Drawing – will be covered in lab quiz	quiz
Lab 3 Fern Life Cycles	Lab Write-up	10%
Lab 4 Fungi -	Lab Write-ups	5%
Lab 5 Bryophytes -	Microscope use - will be covered in lab quiz	quiz
Lab 6 Seedless Vascular Plants -	Drawing – will be covered in lab quiz	quiz
Lab 7 Gymnosperms	ID – will be covered in lab quiz	quiz
Lab 8 Pollen Identification and Pollen Tube Growth	ID and pollen tube growth will be covered in lab quiz	quiz
Lab 9 Angiosperm Anatomy Project: parts 1 & 2		10%
2 Lab quizzes (10% total)	1. Algae, Fungi	5%
	2. Bryophytes, Seedless Vascular Plants, Gymnosperms	5%

In natural science the elements of truth ought to be confirmed by observation[1].

Carolus Linnaeus in Philosophia Botanica (1751)

The foundational learning objective of these lab exercises is to hone your observational skills

[1] *In scientia Naturali*
Principia veritatis
Observationibus confirmari debent.

General Information

Introduction

These laboratories introduce you to a range of organisms that in the past were all recognized as "plants". Today, many of these organisms are known to have little relationship to the kingdom Plantae (some being closer relatives of animals than plants!) but we have included them in this course because of tradition. Even so, they are fascinating and intrinsically interesting - as are the true plants to which this course is mainly devoted. The laboratory exercises are designed to support material introduced in lecture, and provide students with the opportunity to work with actual specimens.

The student's attitude to laboratory study is an important factor in determining how well the session will go. If the student is interested in learning, is willing to put in time and effort, and take ownership of their learning, it will be a rewarding and enjoyable experience. Many of the laboratory exercises are self-directed, and students have found that their performance in the lab component is correlated with the time and effort put into the lab.

Laboratory Administration

The laboratory sessions are under the general supervision of the laboratory coordinator. This is the person you should see about any questions or problems associated with the administration of the lab or about any other situation that you cannot resolve with your demonstrator.

There are only a few rules that are necessary for the smooth functioning of the lab.

1. You will not be excused from labs except for valid documented reasons. If you miss a session you might be able to make it up, but you must see the laboratory coordinator, for written permission if such an arrangement becomes necessary. A student may change their lab day only if space is available at another time.
2. Follow standard laboratory safety precautions (lab coat, safety glasses, gloves [page 1]) as indicated in certain specific lab exercises, or as instructed by your demonstrator.
3. Always consider others and leave the room at least as neat as you found it. Do not wait for your demonstrator to ask you to clean up; take the initiative to do this yourself and make sure that your microscope lenses and stage are perfectly clean and that equipment is returned to its proper storage place.

   Additionally, always make sure that you dispose of paper, tissue, utensils, etc. appropriately in the correctly labeled container - paper, sharps, glass, etc.

Laboratory Procedure

1. Prompt and regular attendance at the laboratory sessions is essential to allow adequate time to complete the activities.

2. Always prepare for the lab exercise by reading through the protocol and then come to the lab with questions about anything that you do not understand.

3. Please feel free to consult with your lab colleague and to exchange information about the lab exercise with other students and with your instructor and demonstrator. However, do not expect them to be able or willing to answer everything you ask. Some questions may be unanswerable. Additionally, we expect you to think independently and to do your lab work mostly on your own.

4. Please ensure you hand in your assignments on time! Not only is this is a good work habit, but the late penalties will encourage you to do so.

Laboratory Assessment

It is important to arrive at the lab session prepared, having read the appropriate lab manual content. In some labs, there will be a pop quiz at the beginning of the lab. (If you have prepared for the lab, you should not have a problem with the pop quizzes!)

For those laboratories devoted to the study of diversity of organisms (labs 2 through 6), your knowledge of this material will be assessed in the lab write-ups, drawings, and quizzes. During the labs dealing with diversity (2-6) you should concentrate on observation, including drawings, diagrams and note-taking about the organisms you are studying. Take advantage of the opportunity to practice your drawing (dealt with below under Techniques). You may be expected to identify organisms using a key, so make sure that you are familiar with the use of keys prior to the lab exam.

Labs 8-9 and 10-11 are not to be written up as "formal" lab reports but they should have an Introduction, a Methods and a Results section. The Results section should describe your findings and your interpretation of your results. More details are provided in the lab protocol.

The laboratories form a very important part of the course, and are worth a total of 40% of your final grade.

A student who is not satisfied with the grading of a laboratory submission should first speak with the TA. If satisfaction is not achieved, the student should submit the work to the lab coordinator for independent reappraisal, including a note (signed by the TA) attesting that the work has been discussed with the TA. This **MUST** be done within **14 days** of the work being returned to the student. ***Please note:*** *reappraisal may result in the original grade being raised, lowered or confirmed.*

Techniques

There are several techniques that you will need to learn if you are to do well in this course.

a) The Microscope. Review the use of the microscope and make sure that you know the correct way to operate both the compound and the dissecting microscope.

Operation of the Microscope: Kohler Illumination

1) Move the condenser upwards until it is a few millimeters below the stage.
2) Use the 10x objective to do this procedure.
3) Place a slide on the stage and focus on the edge of it.
4) Close the field diaphragm (it is on the base of the microscope) until there is a tiny circle of light. You may need to close the condenser diaphragm (using the lever on the side of the condenser) to see the circle of light.
5) Focus the condenser (using the small knob to the left of the stage). Focus until the circle of light has a sharp outline.
6) Open the field diaphragm a small amount, then use the two keys on the front of the condenser to center the circle of light in the field of view.
7) Open the field diaphragm until the light fills the entire field of view.
8) The microscope should now be ready for use. You may have to focus and center the condensor as you move from one objective to another.

b) Preparing a wet mount. Using a pipette, place a few drops of your sample on a cleaned glass slide. Then place a cover slip over the liquid, being careful to put one edge of the cover slip down in the liquid first. Then let the other end down slowly so that you drive out most of the air bubbles. It often helps to lower the cover slip with forceps. (It also helps to learn to recognize air bubbles so that you do not spend valuable time looking at and drawing them!) Sometimes you might want to stain a specimen already mounted on a slide. You can do this by placing as drop of the preferred stain at an edge of the cover slip and then by drawing the stain across the specimen droplet by placing absorbent paper at the opposite side of the cover slip.

c) Drawings - There are a number of very good reasons for making drawings as part of a biology laboratory exercise. Probably the most important reason is that before you can make a useful drawing you must observe your specimen closely and accurately. Secondly, your drawings will form an important record of what you have seen and will be invaluable as study aids. Drawings are infinitely preferable to photographs, as a drawing can be a composite representation of your cumulative experience with your specimen, whereas a photograph represents the appearance of the specimen at a single moment in time.

Although we will not be marking your drawings every week, you may be required to submit your drawings on occasion for grading and feedback. Also, you will be given marks for quality of your drawings in the lab write-ups.

Many students will insist that as they lack artistic ability, they are unable to execute worthwhile drawings. This is not so, as artistic impression is not what is required. Drawings are a way of

encouraging you to observe things closely and they should be used as a record of your understanding of the material. Considerable time and effort should be spent on your laboratory drawings and accuracy and neatness in their execution are essential. If you take care with your work and follow our suggestions your drawing technique will develop quickly[2].

i) A drawing should be a complete and accurate representation and should communicate your understanding of the specimen. Therefore do not make idealized drawings. Draw what you actually see, not what you imagine should be there. (Observe the general appearance of your specimen, then identify the most significant features.)

ii) A drawing should be large enough to represent all the details without crowding. Drawings are rarely too large; they are often too small. Show only as much detail as is necessary for an understanding of the specimen. i.e. it is unnecessary to reproduce in detail the entire contents of a microscope field.

iii) Use a sharp, hard (2H) pencil on hard, smooth, unlined paper. Do NOT use pencil crayons. Use a clean eraser.

iv) Use simple narrow lines. Represent depth, if necessary, by stippling (making dots close together) - **not** by shading. **Never** colour your drawings.

v) Leave plenty of margin around your drawing for labels. Label lines should be drawn with a ruler, should usually be parallel to the top and bottom of the page (and certainly never crossed), and should end at a uniform distance from the edge of the page. Usually all labels are on one side of the drawing.

vi) Every drawing should be accurately and fully titled.

vii) The magnification of every drawing should be calculated and indicated at the bottom right hand corner, e.g. mag = 25 X.

d) Magnification

$$\text{Magnification} = \frac{\text{Size of object drawn}}{\text{Real size of object}}$$

Make sure that all your measurements are in the same units.

To estimate the real size of an object seen under the microscope, measure (with a ruler) the diameter of the field of view of the objective that you are using. Then decide the size of the object by the fraction of this diameter that it occupies.

[2] With the advent of modern technology (cell phones with built-in cameras etc.), taking a photo is easy. But please remember that when you draw, you are honing your observational skills in a way that isn't possible with a photograph. We don't discourage taking pictures, but don't rely on them! How you see and observe, and note distinguishing characteristics is something you will retain in your mind (with practice). This does not happen with a photograph. The pedagogical terms are **passive viewing** (photograph) and **active viewing** (observation, drawing, and notes). Be **active**.

The following distances are a rough guide:

Objective 10X 1500 μm diameter
Objective 40X 350 μm diameter
Objective 100X 150 μm diameter etc.

$$\text{Remember } 1\mu\text{m (micron)} = \frac{1}{1000} \text{ mm } (= 0.001 \text{ mm})$$

For a more accurate measurement of the real size of your object you should use an eyepiece micrometer. For every microscope that you use, please follow the procedure listed below

1. Notice the uncalibrated eyepiece micrometer inside either the left or right eyepiece of the microscope.
2. Place the stage micrometer on the microscope stage and focus on the scale gradations. Each millimeter is divided in hundreds. Therefore, each small gradation is (i.e. each 1/100 gradation) 10μ long (0.01 mm).
3. Superimpose the eyepiece micrometer scale on the stage micrometer scale. Then calibrate the eyepiece micrometer by counting the number of eyepiece divisions that are needed to span one stage micrometer division. That number of eyepiece micrometer divisions then spans a distance of 10μ at that power.
4. Repeat for every power on the microscope. Use this scale to calculate the real size of your object for the equation given above.

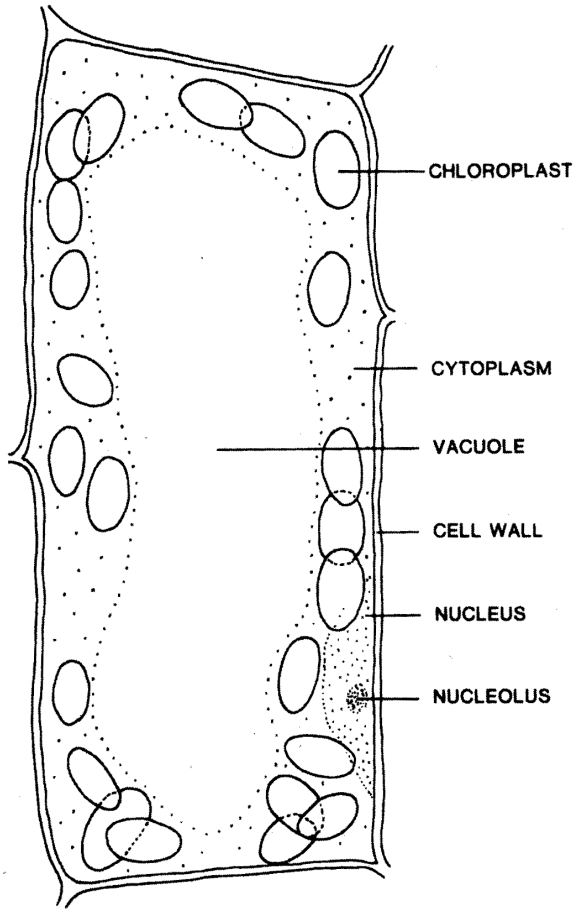
Example of a laboratory drawing

NAME _____

LAB SECTION NO. _____

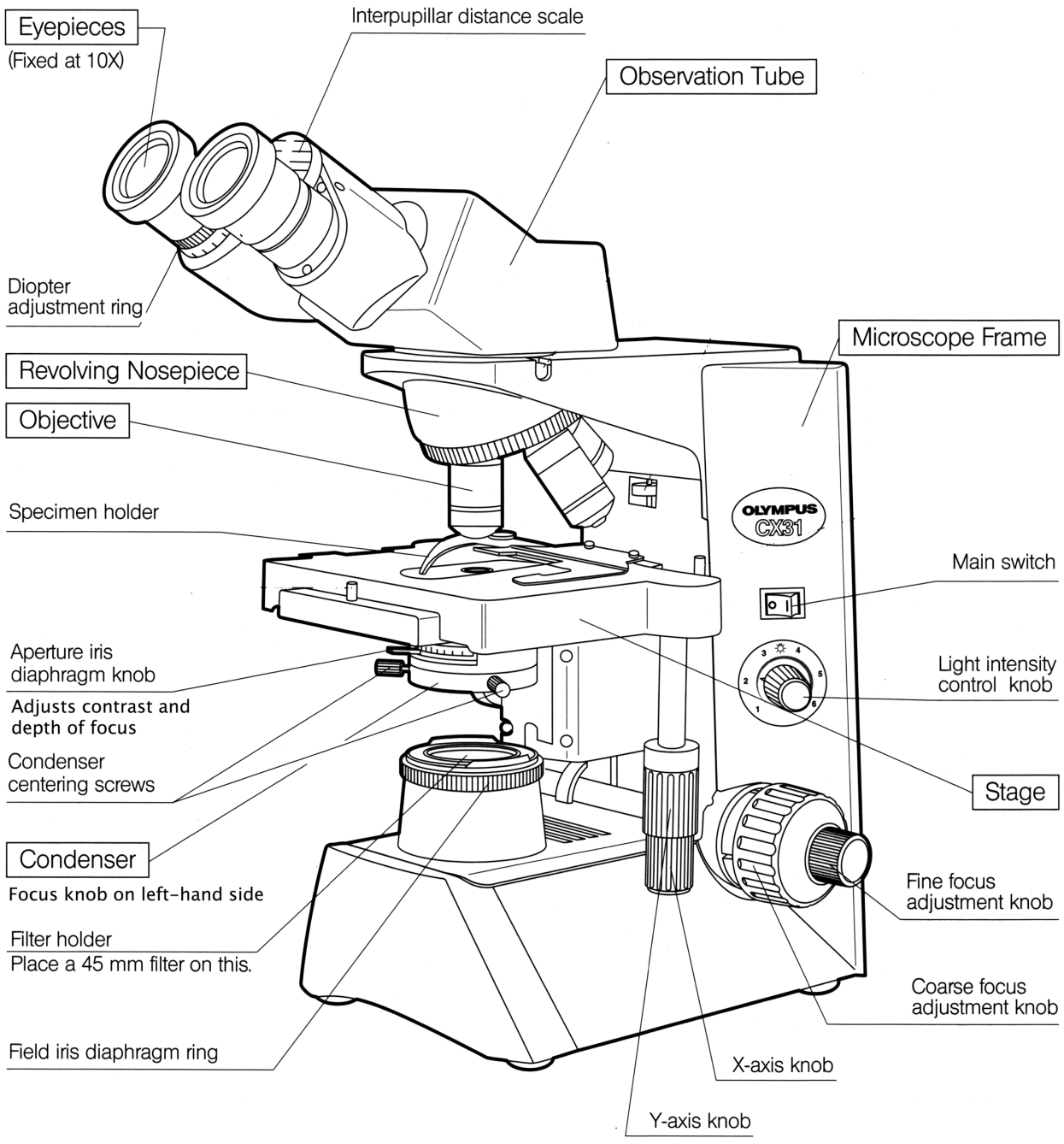
DATE _____

***ELODEA* CELL**



Magnification: **2,500×**

Microscope Schematic: Parts of the microscope are shown below.



Data Analysis[3]

The major objective of the Plant Biology Labs is to develop your observational skills. But in addition, in some exercises you will be collecting quantitative data. For these lab exercises, you may be required to analyze data. The following is a brief description of the analytical and statistical analyses you may be required to do for your lab write-ups.

Data Distributions. One of the most useful analytical tools used to explore the nature of data is the "stem and leaf" diagram (Tukey, 1977). Suppose we have a set of measurements of O_2 production from the Photosynthesis lab. These are data compiled from all lab groups for a particular light treatment (*please be advised that the data are fictitious and may bear no similarity to your 'real' data*).

In ml g^{-1} h^{-1}: 0.5, 0.6, 0.8, 1.8, 0.4, 0.7, 0.7

To create a stem and leaf, we usually sort them first, from lowest to highest value:

0.4, 0.5, 0.6, 0.7, 0.7, 0.8, 1.8

create the **stem**:

```
0 (0.4 to 0.6)
0 (0.7 to 0.9)
1 (1.0 to 1.2)
1 (1.3 to 1.5)
1 (1.6 to 1.8)
```

and add the data to the right (the leaf):

```
0 456
0 778
1
1
1 8
```

Notice that most of the data are clustered between 0.4 and 0.8. One datum is not: 1.8.

Central Values. So, how do we estimate the central value of the data? We can use the **median**. This is the halfway point, where half the data are smaller and half the data are larger. For the sorted data, the median is 0.7 (the 4th ranked of the seven values, sorted from smallest to highest). More generally, once your data are sorted, the median is the n^{th} datum calculated from 0.5(1+N), equal to 4 in our case. If the sample size is an even number, you take the mean of the two middle values to obtain the median.

We can also use the **mean**, which is the sum of all the data, divided by the number of data (sample size, N). The general formula is:

$$\bar{x} = \frac{\sum_{n=1}^{n=N} x_n}{N}$$ where x_n are the data, N is the sample size, and $\bar{x}$ is the mean.

For this data:

$$\frac{(0.4 + 0.5 + 0.6 + 0.7 + 0.7 + 0.8 + 1.8)}{7}$$

equals a mean of 0.79

[3] Developed by Roger Lew, Sapna Sharma and Christopher Luszczek.

Data Spread (Variability). It is often useful to estimate how variable the data are. This can be variability due to experimental error, or variability that exists naturally in the population being analyzed. For our data, the extremes —0.4 and 1.8— already give us an idea, but to measure more precisely how clustered the data are, **hinges** are useful. These are the values halfway between the median and the extremes. For our data, the hinges are 0.55 and 0.75. That is, most of the data are clustered between 0.55 and 0.75. More generally, the hinge values can be determined by first determining the ranked value equal to the median (in our case the 4th value, from 0.5(1+7), then the rank of the hinge values is calculated from 0.5(1 + 4) = 2.5. You can check the ranking procedure with the following example (pre-sorted):

$$0.3, 0.5, 0.5, 0.6, 0.7, 0.8, 1.0, 1.8$$

The median is 0.5(1 + 8) = 4.5th rank: 0.65. The hinges are 0.5(1 + 4.5) = 2.75th rank. Rounded to the 2.5th rank (that is, between the 2nd and 3^{d} rank), the hinges are 0.5 and 0.9.

For this data set, the stem and leaf diagram (with median M and hinges H shown) looks like this:

```
0   3
0 H 55
0 M 67
0 H 8
1   0
1
1
1
1   8
```

A more complex estimate of the spread is the **standard deviation**. This is an estimate of population variance, but be careful, this is only true for so-called "Normal" distributions. The formula for the calculation of the standard deviation (squared, s^2) is:

$$s^2 = \frac{\sum_{n=1}^{n=N}(x_n - \overline{x})^2}{N-1}$$ where x_n are the data, N is the sample size, and $\overline{x}$ is the mean.

This can be simplified somewhat:

$$s^2 = \frac{\sum_{n=1}^{n=N} x_n^2 - \frac{\left[\sum_{n=1}^{n=N} x_n\right]^2}{N}}{N-1}$$

Although many wouldn't consider this much of a simplification, it does make the calculation easier! Now, most calculators can calculate the standard deviation (*s*) automatically. But, should your batteries run out, the calculation for our data is:

$$\sum x^2 = (0.4)^2 + (0.5)^2 + (0.6)^2 + (0.7)^2 + (0.7)^2 + (0.8)^2 + (1.8)^2$$

equal to 5.63. and

$$\left[\sum x\right]^2 = [0.4 + 0.5 + 0.6 + 0.7 + 0.7 + 0.8 + 1.8]^2$$

equal to 30.25, divided by 7 (our sample size) is equal to 4.32. Now we can take these values (5.63 and 4.32) and calculate s^2 = [(5.63-4.32) / 6] = 0.22. Taking the square root: the standard deviation, *s* = 0.47.

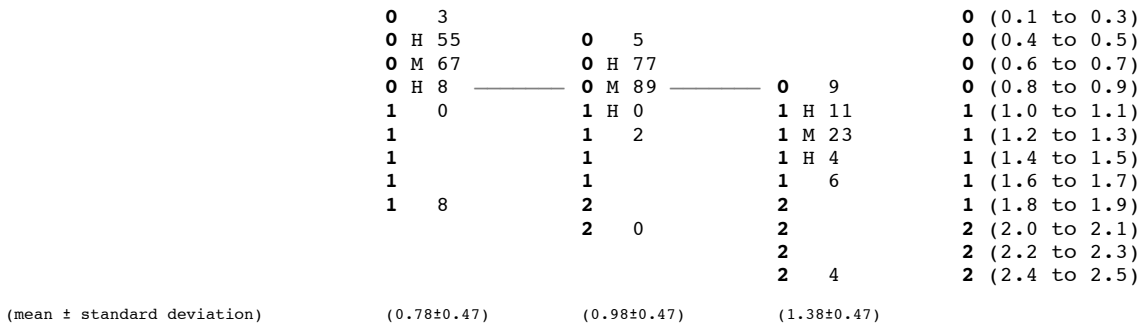
So, the mean ± standard deviation is: 0.79 ± 0.47 (n = 7). Note that it is very helpful to indicate the sample size.

For the purposes of Plant Biology lab exercises, the mean ± standard deviation would be considered useful summary statistics. Stem and leafs are more useful in exploratory analysis. For example, in our data, that 1.8 really falls outside the clustered distribution. Why? Is it experimental error? A new discovery?

Evaluating Differences —graphical comparators. Much of statistical analysis is devoted to comparisons of two (or more) datasets to determine if the datasets are different. Evaluating statistical differences is complex: In scientific work, t-tests and ANOVA (Analysis of Variance) are the traditional techniques. These techniques can fail when the sample number is small, when the populations are not "Normal" distributions, or when the data spreads of the two (or more) datasets are different.

The hinges of the stem and leaf provide us with a natural measure of the spread of the data. If two datasets are different, then the hinges are unlikely to overlap. But be warned, unlike the traditional techniques (t-tests and ANOVA), the 'non-overlap' does *not* translate into a statistical metric, it is *only* a graphical comparator.

For example, the first two datasets are *not* different, the first and third *are*:



Similarly, for the summary statistics of mean ± standard deviation (shown above), if the ranges of the two datasets (from the mean *minus* the standard deviation to the mean *plus* standard deviation) do not overlap, than it is likely that the results are different. Using this criterion, the first and third datasets are *not* different. This may be because the standard deviation is affected by the outlying value, while the hinge values are not.

For either approach, the comparisons are neither quantitative nor definitive. Even so, the simple techniques of comparing data spreads to evaluate differences are easy to perform, and, offer a robust approximation that will help you as you consider how different your data are.

Evaluating Differences —statistical tests. Biologists often rely upon a more formal comparative technique —the t–test— to evaluate differences quantitatively. This is especially useful for ecologists, who often work with large datasets with inherent variability. But it is also important for bench scientists who want to test well-defined hypotheses. For example, do two genetic strains of an organism grow at the same rate (or not)? Does a phytochemical cure cancer? In these examples, the *null* hypotheses would be that the strains grow at the same rate, and cancer rates are the same with or without treatment with the phytochemical. The t-test provides a way to test whether differences are significant. As implied above when explaining graphical comparators, the t-test statistic is complex: it assumes a normal distribution, and readily fails when samples sizes are small. But it is a common statistical test that is traditionally used by biologists, so students should understand and be able to use it.

In a formal way, the t-test compares two means, say $\bar{X}_A$ and $\bar{X}_a$. The simplest way to do this is to subtract one from the other $\bar{X}_A - \bar{X}_a$. But, this doesn't account for the variability (spread) in the data. To account for variability, we can divide by the standard deviation: $\frac{\bar{X}_A - \bar{X}_a}{s}$ to normalize to the variability. This tells us how many standard deviations there are between $\bar{X}_A$ and $\bar{X}_a$. But the equation has to be cast in a more complete form that accounts for the variability of *both* means, and, the sample size: $t = \frac{\bar{X}_A - \bar{X}_a}{\sqrt{\frac{1}{2}(s^2_{X_A} + s^2_{X_a})} \cdot \sqrt{\frac{2}{n}}}$. This is simpler than it looks! To compare two data sets, we compare the means ($\bar{X}_A$ and $\bar{X}_a$) but must also account for the variability of both samples ($\sqrt{\frac{1}{2}(s^2_{X_A} + s^2_{X_a})}$ accounts for both standard deviations) and how much sampling we have done ($\sqrt{\frac{2}{n}}$, n is the sample size). Now, the larger t is, the greater the difference between the two samples, and the more likely the samples are different. The equation states that the greater the difference in $\bar{X}_A$ and $\bar{X}_a$, the larger t will be, that small standard deviations will make t larger (since they are in the denominator), and that the larger the sample size, the larger t will be. A graphical explanation should help (figure 1).

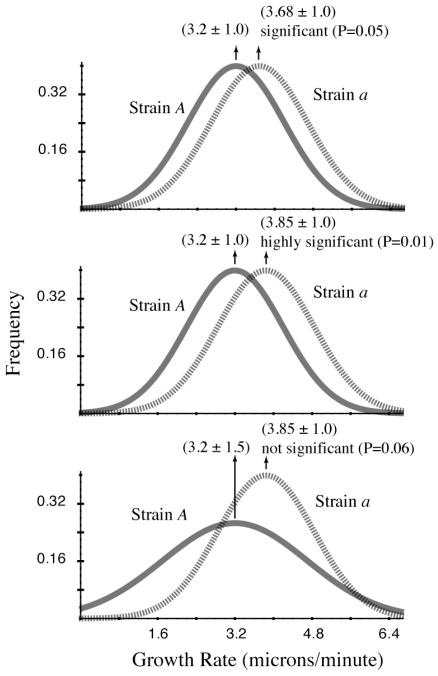


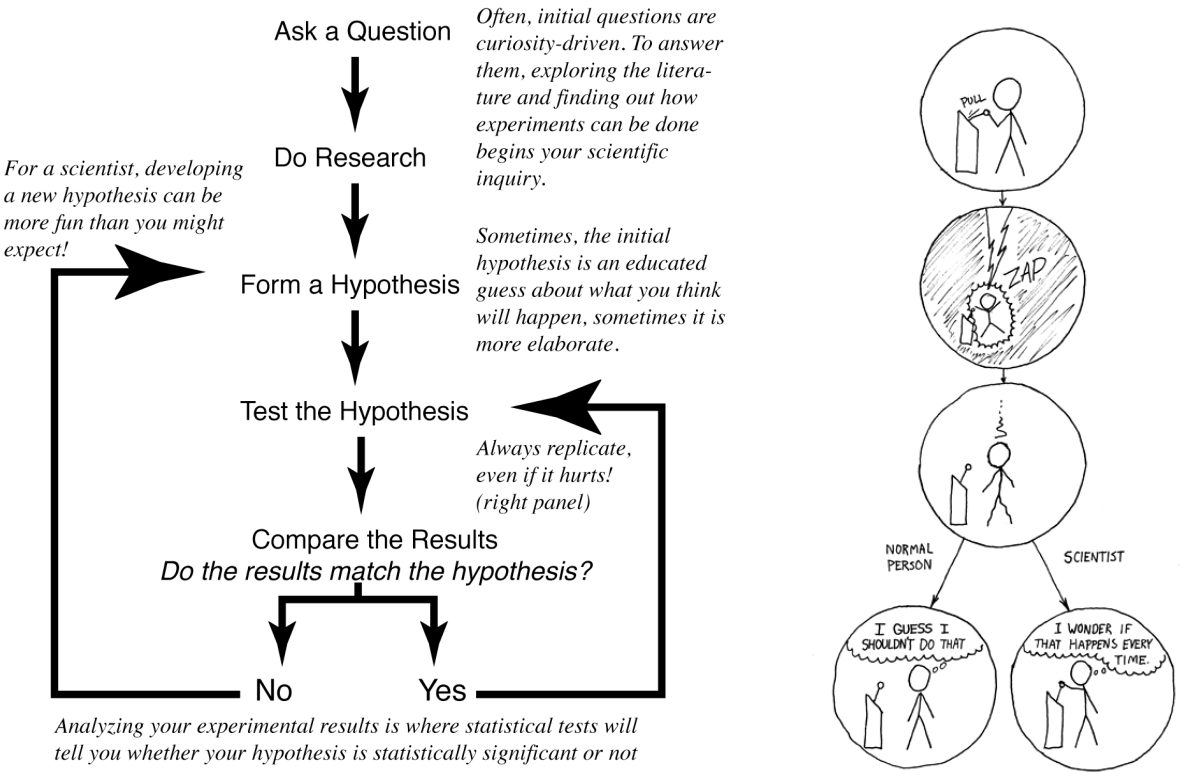
Fig. 1. Graphical Explanation of the t-test. The curves are normal distributions for the growth rates of two strains: A (3.2 microns/minute) and a (3.68 and 3.85 microns/minute). Are the growth rates of the two strains different? The probabilities were calculated from t assuming a sample size of 20. 3.68 is significantly faster (**top panel**), and 3.85 even more so (**middle panel**). But if we increase the variability of the growth rate of strain A from 1 to 1.5, neither of the a strains grows significantly faster (**bottom panel**). In other words, significant differences depend on how much faster, and how much variability there is in your data.

The value of t tells you how different the two samples are. But this is usually cast in the form of a probability (which lies between 0 to 1). To convert to a probability, you have two options. You can use a software program —SPSS is one example, even excel can do it— or a table of t values (Table I) to calculate the probability.

Evaluating Differences —constructing a hypothesis. When using statistical tests of differences, it is absolutely crucial to construct a formal hypothesis. This is especially true when working with natural populations —including clinical research on humans— because of the

inherent population variability that exists. A carefully constructed hypothesis minimizes the risk of research that can't be replicated. An overview of hypothesis testing is shown in figure 2.

Fig. 1. Graphical Explanation Hypothesis Testing. The left diagram explains the process. Your TAs will provide you with a more formal explanation. The right diagram explains the importance of replicating your experimental results, courtesy of Randall Munroe at xkcd (http://xkcd.com/242/).



t–Test Recipe. There are at least 7 steps that one can follow to perform a statistical test. We will review each of the steps using a hypothetical example and you will perform the same procedure on your data.

1. What is the appropriate statistical test to use? There are numerous statistical tests that one can perform. However, you need to be aware of the type of data that you have and what your null hypothesis is to help you identify which statistical test is appropriate for your data.

You will use a t-test when you have continuous data ($-\infty$ to ∞) and you would like to compare the means of two populations. For example, you could be interested in knowing the difference in heights, weights, or growth rates of different genetic strains.

Example. The example that we will follow for this exercise is comparing growth rates of different mating types of the *Neurospora crassa* fungus. The data are as follows (and are hypothetical):

Growth rates of mating type A (cm h^{-1})	6	8	7
Growth rates of mating type a (cm h^{-1})	2	1	3

2. What is the statistical significance (P) at which I will evaluate my data? The P value at which you test statistical significance is a pre-determined value. In biology, the convention to follow is

to use an P value of 0.05 which suggests that there is only one chance in twenty that a situation was a coincidence.

3. What are your statistical hypotheses?

A. Null hypothesis: The null hypothesis usually states that there is **NO** difference between the two groups, such that the mean of group 1 is equal to the mean of group 2. If you are doing an experiment and you are looking at the effect of a treatment on the group of individuals, the null hypothesis would state that there is no effect of the treatment on the means of the individuals in the study before and after the treatment. So, for the fungal growth rates, the null hypothesis would be that there are no differences in the growth rates of A and a mating type strains.

What would be the null hypothesis for the following research questions:
- Will tomato plants grown with or without sunlight be the same height?
- Does attending class have an influence on student performance on exams?

B. Alternate hypothesis: The alternate hypothesis usually states that there is **A** difference between the two groups, such that the mean of group 1 is **not** equal to the mean of group 2. If you are doing an experiment and you are looking at the effect of a treatment on the group of individuals, the alternate hypothesis would state that there is an effect of the treatment on the means of the individuals in the study before and after the treatment. So, for the fungal growth rates, the null hypothesis would be that there are differences in the growth rates of A and a mating type strains.

What would be the alternate hypothesis for the following research questions:
- Will tomato plants grown with or without sunlight be the same height?
- Does attending class have an influence on student performance on exams?

4. What is the calculated t-statistic? To calculate the t-statistic, you will need to know the mean ($\overline{X}$), variance (S^2), and sample sizes (n) of the two groups:

$$t = \frac{\overline{X}_A - \overline{X}_a}{\sqrt{\frac{1}{2}(s^2_{X_A} + s^2_{X_a}) \cdot \sqrt{\frac{2}{n}}}} = \frac{\overline{X}_A - \overline{X}_a}{\sqrt{(\frac{s^2_A}{n_A} + \frac{s^2_a}{n_a})}}$$

Remember that the formula for the mean is: $\overline{x} = \frac{\sum_{n=1}^{n=N} x_n}{N}$ and for the variance is $s^2 = \frac{\sum_{n=1}^{n=N} (x_n - \overline{x})^2}{N-1}$

Example: Let's try this out with our hypothetical dataset.

First, let's calculate the values we will need for mating type A:

1. Mean of mating type A = (6 + 8 + 7)/3 = 21/3 = 7
2. Variance of mating type A = $((6-7)^2 + (8-7)^2 + (7-7)^2)/3-1 = (1 + 1 + 0)/2 = 2/2 = 1$
3. n = 3

We do the same for mating type a.

1. Mean of mating type a = (2 + 1 + 3)/3 = 2
2. Variance of mating type a = $((2-2)^2 + (1-2)^2 + (3-2)^2)/3-1 = (0 + 1 + 1)/2 = 2/2 =1$
3. n = 3

Now, let's put it all together into the formula to calculate the t-statistic:

$t_{calculated}$ = (7-2)/sqrt (1/3+ 1/3)
= 5/0.81

=6.17

5. What are the degrees of freedom? It is the number of values in the final calculation that are free to vary; a measure of independence between points. The formula to calculate degrees of freedom = n–1; where n is the number of observations in the groups. In our example, we have 2 groups with three individuals each.

Degrees of freedom = 3-1 = 2

6. Compare the calculated t-statistic against the critical t-statistic (Table I).

- If $t_{calculated} > t_{critical}$ —Reject the Null hypothesis (that is, no difference in growth rates).
- If $t_{calculated} < t_{critical}$ —Accept the Null hypothesis.

Example: Our t-calculated value is: 6.17
Based on 2 degrees of freedom and a significance level of 0.05, the t-critical value is 4.30 (Table I).

Since t-calculated is greater than t-critical, we reject our null hypothesis.

7. What is the biological interpretation of your statistical analysis? Here, we relate the rejection or acceptance of the null hypothesis back to our study. If we accept the null hypothesis, we can say that there is no statistical difference between the means of the two groups. If we reject the null hypothesis, we would state that there is a statistical difference between the two groups.

Example: We rejected our null hypothesis. Therefore, there is a statistical difference in the growth rates between A and a mating types of the fungus. The growth rates of mating type A are faster than the growth rates of mating type a.

Degrees of freedom	P=0.05	P=0.025	P=0.01	P=0.005
1	12.71	25.45	63.66	127.32
2	4.30	6.20	9.92	14.09
3	3.18	4.17	5.84	7.45
4	2.78	3.50	4.60	5.60
5	2.57	3.16	4.03	4.77
6	2.45	2.97	3.71	4.32
7	2.36	2.84	3.50	4.03
8	2.31	2.75	3.36	3.83
9	2.26	2.68	3.25	3.69
10	2.23	2.63	3.17	3.58
11	2.20	2.59	3.11	3.50
12	2.18	2.56	3.05	3.43
13	2.16	2.53	3.01	3.37
14	2.14	2.51	2.98	3.33
15	2.13	2.49	2.95	3.29
16	2.12	2.47	2.92	3.25
17	2.11	2.46	2.90	3.22
18	2.10	2.44	2.88	3.20
19	2.09	2.43	2.86	3.17
20	2.09	2.42	2.84	3.15
21	2.08	2.41	2.83	3.14
22	2.07	2.41	2.82	3.12
23	2.07	2.40	2.81	3.10
24	2.06	2.39	2.80	3.09
25	2.06	2.38	2.79	3.08
26	2.06	2.38	2.78	3.07
27	2.05	2.37	2.77	3.06
28	2.05	2.37	2.76	3.05
29	2.04	2.36	2.76	3.04
30	2.04	2.36	2.75	3.03
40	2.02	2.33	2.70	2.97
60	2.00	2.30	2.66	2.92
120	1.98	2.27	2.62	2.86
infinity	1.96	2.24	2.58	2.81

Table I. Critical t-statistic values. The left column lists the degrees of freedom. The columns to the right list the critical t-statistics for the probabilities at 0,05, 0.025, 0.01 and 0.001. Biologists tend to use a probability of 0.05, describing it as statistically significant.

For the *example* of mating type strains A and a, the calculated t-statistic was 6.17 and the critical t-statistic is 4.30 (the P=0.05 column for 2 degrees of freedom). Since the calculated t-statistic is greater than the critical t-statistic, the growth rates are significantly different at the 0.05 level.

Exercise 1. Follow the 7 steps to determine if there's a statistical difference in the following hypothetical dataset. The research question is: is there a statistical difference between the

heights of two trees, X and Y?

Height of Tree X (m)	Height of Tree Y (m)
1	5
2	6
3	7
4	8

1. What is the appropriate statistical test to use?
2. What is the statistical significance (α) at which I will evaluate my data?
3. What are your statistical hypotheses?
    A) Null hypothesis
    B) Alternate hypothesis
4. What is the calculated t-statistic?
5. What are the degrees of freedom?
6. Compare the calculated t-statistic against the critical t-statistic.
7. What is the biological interpretation of your statistical analysis?

Exercise 2: Follow the 7 steps to determine if there's a statistical difference in your dataset.

Summary. There are many ways to analyze data. Some, like the stem and leaf, provide you with a straightforward way to look at how your data are distributed, and even graphical comparisons of two (or more) datasets. This technique is part of an analytical technique called exploratory data analysis. Often, scientists need to use more quantitative techniques. One of these —the t-test— is very common. The analytical techniques that you may use in your lab exercises will vary from year to year. Your TAs will provide you with the additional information you need to analyze data you obtain in your experiments.

Reference: Tukey, J.W. (1977) Exploratory Data Analysis. Addison-Wesley Publishing Company. pp. 27–32.

Guidelines for Lab Reports

The following guidelines are included as a courtesy to help you in preparation of lab reports in this course. Please refer to the individual lab marking schemes for details of individual lab mark breakdowns. Your TA may provide specific instructions that should be followed for any given lab report.

Title Page

- An informative report title or title of lab experiment
- Course number and title
- Your name and ID number
- Your lab partner's name
- TA's name
- Date of report submission

Introduction

- Purpose: A single, concise statement of the major objective of the lab, i.e. what are the questions you are tying to answer.
- Background: Explain principles underlying the experiment. Include any information which may be necessary in order to understand your stated purpose of the lab.
- You may **refer** to relevant items from textbooks or journal articles, but ***do not recopy the introduction from the lab handout or other possible sources.***

Materials and Methods

- Describe the experimental design in a short paragraph in past tense – just the key concepts underlying the plan for doing the experiment.
- This section should be very short with only enough detail to make it clear what protocols you followed - just enough to allow someone else to reproduce your work from the same written sources.

Results

- Describe the observations in your experiment in past tense.
- Tables, diagrams and drawings can be placed in this section. All figures must be labeled correctly, appropriate titles must be given for each figure, and all figures should be referred to in the text of the results section. Drawings should follow the guidelines presented in the lab manual.
- Do not interpret the experimental data in this section; the discussion is where you will analyze and interpret the results.

Discussion

- In this section, you must explain, analyze, and interpret your results, being especially careful to explain any errors or problems. Present the principles, relationships, and generalizations as shown by your results. Identify any weaknesses in the experimental design or procedures, and the significance of the results. Remember, you are not restating the results, you are discussing them.
- You may be expected to answer particular questions given in the lab manual.
- This is probably the single most important part of the report, since it is here that you demonstrate that you understand and can interpret what you have done.

Conclusions

- Restate the main objective of the experiment and give a brief statement that summarizes your results.

References

- Be sure to give full citations of all references used. Web pages are not likely to be acceptable references.
- Prepare a complete alphabetized list of references that you cited within your report. The lab manual is only a beginning.
- Journal citation should be in the following format:

Author(s). Date of publication. Title of article. Journal title. Volume number: pages.
E.g.:
Robinson, C. H. 2001. Cold adaptation in Arctic and Antarctic fungi. New Phytologist. **151**: 341-353.

Referencing

In science, we place a high value on ideas – intellectual property. In any scientific writing there is a need to attribute original findings, models, and conclusions to the original author. (It does not diminish your accomplishments to build upon the existing knowledge in the field, but rather, shows your thoroughness in research.) Any time you present a statement of fact, you should back it up with a scientific reference, preferably the primary work(s). It is considered plagiarism if you present unattributed ideas as your own.

When citing references in the text of your work, provide the author(s) name and publication date after a statement that is based upon their work. If subsequent statements are logically connected and also based on the same source, the citation does not need to be duplicated.

"Common knowledge" does not need to be cited. If you are not sure whether something is common knowledge, it is worthwhile to do some investigating.

The use of direct quotes is not common in scientific writing, and should be avoided.

Be sure that you do not include original phrases (and in some cases, entire sentences) from the your sources. It is important to summarize by putting things in your own words – and this is more than changing a word or two! Even following the order of particular sentences in a paragraph can be unacceptable, if you are presenting the same information. This is something I particularly want to highlight, because it is considered plagiarism, a serious academic offense. It does take some practice to learn to summarize appropriately.

Many people are unaware of what constitutes plagiarism. If you would like more information about referencing, please check out the expanded referencing guide on the course website: http://www.yorku.ca/plants/referencing.html

LABORATORY EXERCISES
Laboratory 0 – Drawing Fruit and Vegetable Scientific Style: Anatomy Project[4]

We will watch Episode 1 of the BBC documentary, How to Grow a Planet
See http://tvo.org/video/documentaries/how-to-grow-a-planet/episode-1

NOTE: **IF YOU HAVE YOUR DISSECTING KIT, THIS IS GOOD BUT WE WILL PROVIDE ALTERNATIVES (A LAB COAT IS RECOMMENDED)**

"The main goal of botanical illustration is not art, but scientific accuracy. It must portray a plant with the precision and level of detail for it to be recognized and distinguished from another species.

Although photography and perhaps particularly microscopic photography, may help inform botanical work, there is certainly still a need for botanical illustration because it can represent clearly what may not easily be seen in a photograph. Outline drawings for example, distinguish elements that cannot easily be made out using reflected light alone. Also, the composition of the image can be manipulated more fully in illustration, and features displayed together which may not easily be shown simultaneously in nature." From
https://www.bgci.org/resources/botanical_illustration/

Objective

To explore the anatomy of a fruit or vegetable, research its economic importance and its nutritive value.

Method

We will provide you with a selection of fruit and vegetables.

- First, make a drawing of your entire fruit or vegetable so that you can show what its grosser morphological features are like.
- Next, make 3 different cross-sections and draw and label them – include scale. The main types of cross-sections are: transverse, latitudinal and tangential (http://www.ucmp.berkeley.edu/IB181/VPL/Ana/Ana2.html)
- Finally, make diagrams from microscope mounts of peels

The botanical definition of a fruit is "a mature, ripened ovary (or group of ovaries), containing the seeds, together with any adjacent parts that may be fused with it at maturity" (Raven et al., 2005[5]).

Vegetable is not a botanical term. Dictionary definitions will describe a vegetable as a plant or part of a plant used as food. Vegetables arise from a diverse array of anatomical structures of the

[4] Special thanks to Chris Luszczek, Ahmed Hamam and Debbie Freele for their assistance developing the lab exercise.
[5] Raven PH, Evert RF, Eichhorn SE (2005) Biology of Plants. 7th edition. WH Freeman and Company. Glossary (page G-9).

plant. Food storage by plants can use corms, bulbs or tubers (amongst other things). A corm is a fleshy food-storing underground stem (sometimes with small, thin leaf structures). A bulb is also a modified stem, with food-containing leaves. Tubers are short fleshy underground stems. Potatoes are an example of a tuber, which can develop from stolons (slender stems that grow along the soil surface) or rhizomes (underground stems). Sweet potato is an example of a tuberous root. Carrots and beets are taproots. Many of the foods that we would describe as vegetables are in fact seeds or fruits from a botanical perspective. A key to the diversity of fruits is provided at the end of the exercise.

During your lab period you should cut freehand sections of the various parts of the fruit or vegetable. Be judicious in your selection. You should prepare diagrams and/or accurate drawings to illustrate the overall organization and details of specific cell types characteristic of each part of the fruit or vegetable. Remember that all drawings and diagrams must contain an accurate indication of scale (i.e. magnification). Your illustrations must be clearly labeled. All of the cell types should be given their correct names. You can check the characteristics of each cell type, and thus the correct name, from your textbook. The following techniques may help you analyze your plant when used in conjunction with freehand sections.

- Epidermal peels —many fruits have a waxy coating (to minimize drying out) with well-defined features. Peel the epidermis by hand and mount on the microscope to observe these structures if they are present. Transverse sections will reveal the nature of the structural 'coat' on the fruit (or vegetable) surface.
- Ruthenium Red —pectin should stain pink to red when stained with Ruthenium Red (0.05% in H_2O), thus showing pectin-rich tissue (often found in the cell walls).
- Iodine —mount fresh sections in iodine solution (0.5% in 5% aqueous KI) for 2 min., rinse with H_2O and mount in H_2O. Starch stains deep blue (long chain molecules) or red-brown (short chain molecules). Good for possible storage tissue (decrease the staining time for tissues that are very rich in starch).
- Sudan IV —fix sections in 10% formalin for 10 min., then replace formalin with 50% ethanol for about 1 min. Stain in Sudan IV (saturated in 70% ethanol) for 30 min. Rinse in 50% ethanol and mount in 75% glycerol. Stains fats and oils red, good for possible oil storage tissue.
- Toluidine blue 0 (TBO) —mount fresh sections in TBO (0.05% in H_2O) for 10 sec. to 1 min., rinse in H_2O for 1 min and mount in H_2O. Lignified walls (e.g. xylem) stain navy to greenish blue, phloem vessels stain purple and phloem fibers stain sky-blue.

At the end of the lab your demonstrator MUST initial your diagrams and notes.

<u>Lab report – there is none, but you must complete and show your TA 5 different diagrams</u>

At the end of lab you must show your TA 5 correctly titled labeled diagrams of an uncut fruit or vegetable and 3 different cross-sections plus a diagram of a microscopic view.

You will be expected to know how to correctly draw and label live material for the lab quizzes.

Fruit Key[6]

Dry and dehiscent[7], monocarpellary[8] (without persistent septum)
- dehiscent along 1 edge —**follicle**— milkweed, magnolia
- dehiscent along 2 edges (Legume Family) —**legume**— peanuts, all beans

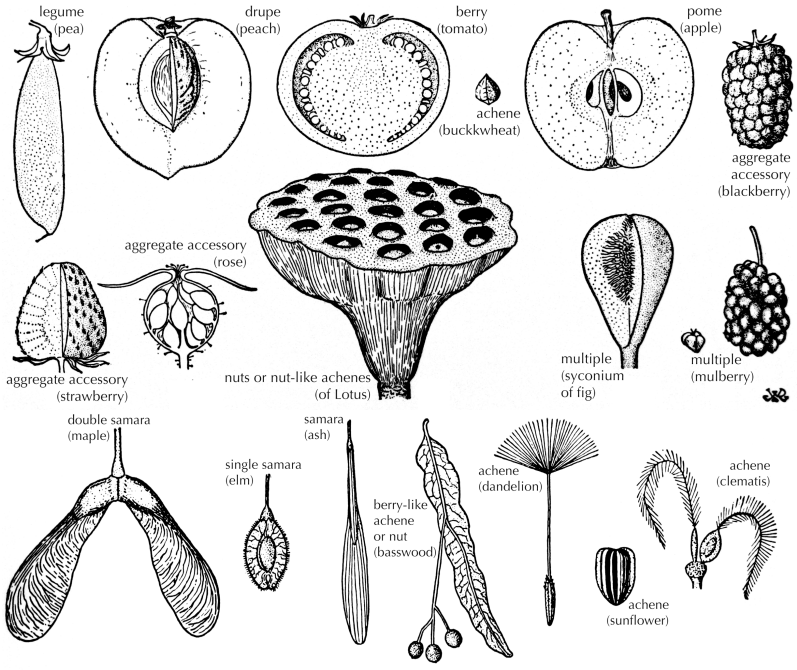
Dry and dehiscent, bi-pluri-carpellary (with persistent septum)
- derived from several fused carpels, opening by slits, pores or a cap —**capsule**— cotton, poppy
- long and thin, dehiscent by two valves —**silique**— mustard
- short and broad silique —**silicle**— shepherd's purse

Dry and indehiscent
- Winged —**samara**— ash, maple, tulip tree
- Wingless:
  - With a thin shell (pericarp)
    - pericarp fused entirely to seed coat (Grass Family) —**caryopsis (grain)**— all cereals
    - pericarp not fused entirely to seed coat —**achene**— sunflower
  - large, with a hard shell (pericarp) —**nut**— acorn, macadamia

Fleshy and indehiscent
- Heterogenous in texture, having
  - One seed enclosed within a bony endocarp —**drupe**— peach, cherry, plum, olive, coconut
  - Papery or cartilaginous carpels in an inferior ovary[9] —**pome**— apple, pear
  - A hard or firm rind (exterior) and soft interior
    - From an inferior ovary —**pepo**— cucmber, watermelon, pumpkin, squash
    - From a superior ovary —**hesperidium**— all citrus fruits, noted for their leathery skin with oils
- Homogeneous, fleshy throughout —**berry**— tomato, grape, coffee

Compound fruit
- Derived from more than one pistil —**aggregates**— strawberry, rose hip, raspberry

The examples of fruits come from Transeau, Sampson and Tiffany (1953) Textbook of Botany.



[6] Source: modified from Asa Gray (1879) Gray's Botanical Textbook (Sixth Edition). Volume 1: Structural botany or organography on the basis of morphology to which is added the principles of taxonomy and phytography. Ivison, Blakeman and Company. pp. 304.

[7] dehiscent: seeds are released by the opening of a pore, slit or valve.

[8] monocarpellary: derived from a single carpel, which may contain multiple ovaries (and thus many seeds). The presence of a single or multiple carpels can be deduced from the persistence of septa separating the carpels.

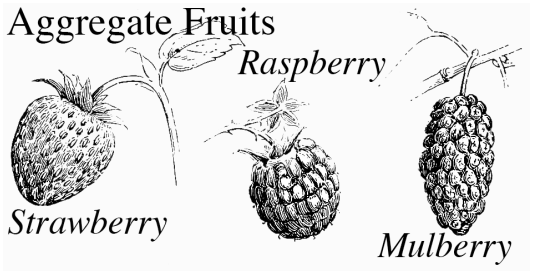
[9] Inferior (and superior ovary) refers to the location of the ovary within the flower, subtending the flower, (or above it).

Examples of Fruits and Vegetables for Dissection

This list is by no means exhaustive, but gives examples of commonly available fruits and vegetables in winter, in Toronto.

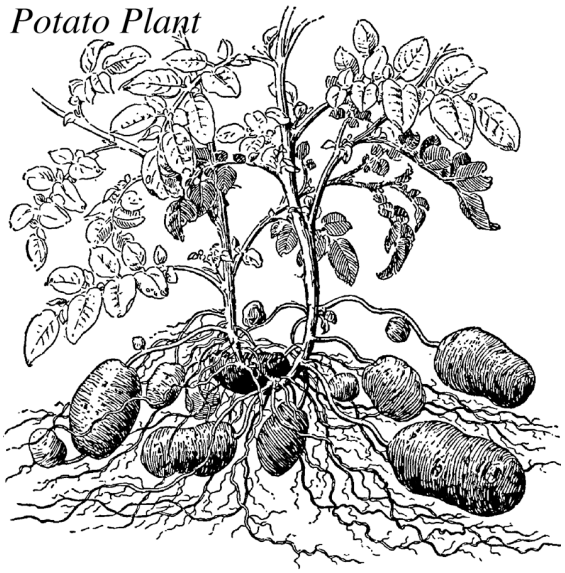
Fruits

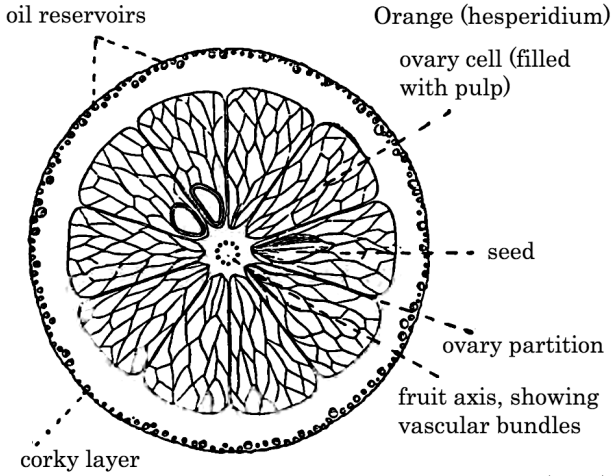
drupe— peach, cherry, plum, olive, coconut, avocado
true berries— tomato, grape, coffee, eggplant, pomegranate
hesperidium— all citrus fruits
pepo— cucmber, watermelon, pumpkin, squash, zucchini
pome— apple, pear
aggregates— strawberry, rose hip, raspberry
multiple fruits— pineapple, fig
nut— macadamia, chestnuts
caryopsis (grain)— all cereals
achene— sunflower
legume— peanuts, all beans



Vegetables

corms— water chestnuts, taro
tubers— potato, sweet potato, ginger
bulbs— onions, leeks, garlic
taproot— carrots, turnips, radish, beet
petioles— celery, rhubarb
leaves— cabbage, spinach
leaf buds— Brussels sprout





Bergen: High School Botany (1904)