Chapter 1

Orientation to Plant-Biology Lab

Why study plants? An introduction to plants

Plants sustain life on earth by trapping light energy and converting it into stable chemical energy. Photosynthetic organisms appeared about 3.5 billion years ago (the first human-like primates appeared a mere 2 million years ago)[1]. Plants have evolved into biochemically complex organisms that dominate the landscape.

Human civilization has long depended on plant life for sustenance. We have exploited plant resources in several ways, primarily for food and fiber through agriculture and for energy through fossil deposits. In order to utilize plant resources, we must gain a better understanding of how plants work. This understanding began at the dawn of civilization, and today we are in the midst of an exciting era in plant biology.

Scientists in fields ranging from taxonomy to biochemistry and molecular biology are elucidating the diversity and complexity of plant life. In the last several decades, we have gained a significant understanding of various plant processes. Our basic understanding has served as the key step into the new era in plant biology, in which we can design plants that use natural resources efficiently, are resistant to various pests, and are more productive. These products of plant biotechnology (which is itself a product of the basic plant sciences) promise to be the tools that would help us effectively manage our critical resources (land and water), while still fulfilling the needs of the growing human population.

This lab course is designed to introduce the student to the variety and complexity of plant life, and to plant interactions with other living organisms. We will take an observational as well as an experimental approach to understand plants. First, we will study seed plants particularly as they relate to mankind. Second, we will study briefly the biology of non-seed plants, such as ferns. Third, we will explore organisms that were traditionally considered plants, but are now classified into other kingdoms (some protists and fungi)[2]. In the course of the semester, we will also conduct experiments to study plant growth and development, and plant interactions with other organisms. These experiments are designed to teach students to think critically, to design and execute experiments to answer specific questions, and to communicate the results effectively.

Doing good science

Good scientists have a common set of qualities that are acquired from experience and practice.

Good observational skills. Good science stems from making keen observations. In many lab exercises, you will be expected to observe several slides and specimens and make drawings. Carefully observe each slide or specimen. Note the orientation and proportions of various

[1] Raven *et al.* 7e is required for the laboratory and it should be consulted to flesh out these intentionally brief notes. (It is also required for BOT 3015, regardless of the instructor or semester. Students in BOT 3015 who wish to take BOT 3015L later are asked to retain the textbook.)
[2] Students currently taking BOT 3015 or who have taken Outlaw's version of BOT 3015 will have his class notes. Others may download relevant passages from http://www.southernmatters.com/ For sake of economy, there is minimum redundancy between these notes and the lecture notes. Here, for example, you should refer to the class notes for modern classification schemes. Note that Powerpoint presentations are available; the user name is <guest> and the password is <plantsarecool>.

structures, especially on slides. Make neat (not necessarily artistic) and scientifically correct drawings in pencil. Drawings must be of precisely what is seen, not what is supposedly seen. Instructors will check during the exercise and compare drawings with what is under the microscope. Analyze parts in relation to their names; this analysis will help you remember them better. Keep your eyes open and your curiosity alive. Note anything unusual that you may observe, even if you are not asked to label it.

Good problem-solving skills. Good science is all about solving problems. This skill is especially important when you design experiments. First, identify the question(s) you seek to answer. Second, design an experiment or experiments that will test your ideas. Include required controls in your experiment. Finally, if the results are inconclusive, devise a new plan.

Good organizational skills. There is no substitute for being neat and organized in experimentation and analysis. Have your tools and materials ready before you start an experiment, so that you do not flounder during an important step. Outline procedures before you start, and check each off as you follow them (an example will be given later).

While analyzing data, list additional questions that you could answer from the experiments that you conducted. Organize your data, and select the most appropriate representation of the data (A table? A line graph? A bar graph? What should the axes on your graphs represent? &c.).

Good record-keeping skills (maintaining a lab notebook). Though underappreciated by novices, good record-keeping is imperative to doing good science. In BOT 3015L, the importance of the notebook is emphasized. Thus, each student will maintain a notebook, and record his/her observations (notes/drawings) during each lab period. In addition, all questions should be answered in the notebook in complete sentences. The first pages of each notebook are reserved for a table of contents.

Broadly, two types of labs occur in this course:

1. Observational labs. Entries in your notebook before the laboratory are not required. After examining specimens/slides, observations made during lab will be recorded (according to the manual and drawing list). Drawings should be in pencil on unlined, right-hand pages of your notebook and about ½ the size of the paper. Include a clear description of what is drawn, including such things as scientific and/or common name of specimen, what type of section it is (e.g. cross section, longitudinal section, whole mount, etc.), magnification (total (eyepiece x objective)), what type of tissue (e.g. leaf, root tip, etc.), of objective of looking at the specimen (e.g. visualize mitosis, vascular system, comparing dicot and monocot root, etc.). Always include any procedure that was followed to obtain or visualize the sample. All drawings should be drawn as seen in the microscope. Use close and accurate observation. Label drawings with arrow-free pointers and write in normal orientation (horizontal plane and right side up). Notice shape, size, contents, patterns, and movement. Parts of the specimen should be drawn to scale and a scale bar should be included when possible. An example of a good drawing can be found at http://www.zoology.ubc.ca/courses/bio332/Images/amoeba1.jpg. Note that although the details are not drawn throughout the whole specimen, there is detail as seen in the microscope and notes describe where and what detail is not drawn. All of the parts are drawn, although repetitive parts do not need to be drawn. Now compare to http://www.zoology.ubc.ca/courses/bio332/Images/amoeba2.jpg.

2. Experimental labs. Experimental procedures should be entered into your lab notebook before the lab.

a. The first pages of each notebook are reserved for a table of contents.

b. The right-hand pages are used to record protocol, solution preparation, methods, and results (*e.g.* drawings and observations).

c. The left-hand pages are for planning and interpretation. There, summarize a previous experiment (e.g., with a graph and a short statement), outline the next experiment, make a note of literature citations, record calculations, etc.

d. Protocols should be recorded before the experiment on the right-hand pages. The system to ensure that protocols are followed in this course is to place a check by each task as it is accomplished. A statement such as "experiment like pg 10" is inadequate. A typical protocol can be recorded as shown on the following:

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13 June 2005

Treatment of *Brassica rapa* plants with GA

Time: 10:05 am

_√__ Measure plant heights

1. _20_ mm
2. _40_ mm

_√_ Apply 20 µl 100 µM GA to 1st leaf of plants 1, 2 and 3.

___ Apply 20 µl 10 µM GA to 1st leaf of plants 4, 5 and 6.

____ ……..etc.…….

Even now, your experiment is not over. Analyze your data. Discuss your interpretation with the TA. Write out your protocol for the next experiment.

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Good writing and communication skills (writing a scientific report). This essential skill is learned by practice. A scientist must effectively communicate his results and their implications in a clear, precise, and scientific manner. A substantial cost is associated with creating new knowledge that merits publication in a journal. In brief, present your work carefully if you expect it to be read. Only work distilled to essentials can be published. (In other words, a scientific report is not a diary.) Understanding the importance and costs of communicating science puts one well on his way to creating a good report. Of course, the best way to learn to write is to read, and then write for the purpose at hand.

In summary, make your point in the most straightforward manner. There is no room for personal style, rambling, or inaccuracies in scientific writing.

1. Organization of a long lab report (GA report).

   a. Title. Thousands and thousands of people will read your title. How many will be stimulated to read your paper? Make every word count. Here's a bad title: "Effect of temperature on plants." Here is a good title: "The temperature threshold for dormancy induction in *Malus* seedlings." Remember that generally articles are not even retrieved by people; computers do it on the basis of key words, words in the title, author, or citations. "Effects of..." or "Studies on..." are meaningless expressions. On the other hand, "dormancy" will alert the searcher right away. No one will retrieve the first title because there is little chance that the study will be relevant to the researcher.

   b. ii. Abstract. If the title is crafted well, readers might be attracted to the abstract, which is a summary of the essential pieces of information and conclusions reached. Brevity is of the essence. For example, methodological details, unless important to the conclusion, should be omitted. References to previous work usually should be omitted. A reader will use the abstract to determine quickly whether the full document has relevancy to his or her interest. The task is to put into 150-200 words the major elements of a scientific paper, viz., (a) the question investigated and why, (b) the general methods employed, (c) the essence of the results, and (d) the principal conclusions.

   c. Introduction. The purpose of the introduction is to state the nature and the scope of the problem. Why was it interesting and worthwhile to do? You should provide scientific background; the reader in your target audience should not have to consult other work for a general understanding of your paper. What will the data permit the reader to conclude? Chart the course that will follow; the introduction is a kind of map—scientists are busy, make it easy for them.

   d. Materials and methods (written in past tense). Full details that permit a competent colleague to reproduce your work are required. [Plant used, materials used, description of experimental set-up, all methods in detail, (details of what you did and when, what special care you took in what step, etc.)]

      The Materials and methods, and the Results are the cornerstones of a scientific paper. It is fine to reference other work that is readily available or common knowledge. Everyone will know "according to the method of Lowry et al. (1951)," but for most methods, even if you reference, you will want to give the reader a notion of the approach, e.g., "Potassium was determined by flame photometry (Doe and Doe, 2005)." Note the method of inserting a citation within the text.

   e. Results. This is the new knowledge, your contribution. It is not a place to rehash methods. It is not a place to express opinions. Predigest your data. The reader is not interested in the results on the day that you broke your measuring cylinder. It is your responsibility to edit your work—no one has the interest or time to go through the hundreds of measurements you may have made. Include a representative set experiments or, if appropriate, statistical representations, such as the mean and associated errors, and other statistical analyses. Remember that the other parts of the report are included as support for the results. Use a written section to describe the results of the experiment and to support and refer to any graphical representations. For figures and tables, include a written legend that describes what is being represented.

f. Discussion. The discussion is perhaps the hardest part to write, as it requires "the grand vision." What do the data mean? How do your results and all other results provide a more complete interpretation of how the world works? Your task here is to discuss, not simply to restate, the results. Do your data fit with those of Jones et al. (2004)? Are your results internally inconsistent? Point to the strengths and weaknesses of your work. Are there alternative interpretations? When you finish writing the discussion, which you should do last, decide whether to throw the paper away or try to get it published.

g. Literature. Research starts with the literature, and it is fitting that the report should end with it. Restrict the number of references to the minimum. No one cares how many papers you have read. For generally accepted ideas (*e.g.* mass action), use no reference. For accepted ideas more or less peripheral to the central issue of your work, provide a reference to the secondary literature (reviews). Then, the choice is harder. Do not simply cite a paper because it conveniently supports your work. Do what is right, fair, and accurate.

   Cite a paper in the reference list as follows: Doe JJ, Smith SM 2005 Process methods for large-scale production of plant antibodies. J Expt Bot 5: 25-34.

   In summary, your lab reports should be set up like a research article in a scientific journal. Although journal styles vary somewhat—the Abstract might be called a Summary or the Methods may be placed after the Discussion—all papers have in principle the elements listed above. The quality of your reports and the explanation of results are as important as your results themselves. In other words, despite your best efforts, you may not obtain interpretable results. In this class, a report is required; in the real world, those results are not of interest, but may form the basis for further investigations. Remember, an investigator is not out to prove a point. She or he develops a testable hypothesis, executes a research plan, and decides whether the results support the hypothesis or invalidate it.

2. Organization of a short lab report (Two reports).

Two short experiments will be conducted in addition to the GA experiment. The short experiments will be conducted in pairs or small groups depending on time and material constraints. At the end of each short experiment, you will obtain data, either in the form of numbers or observations. Although the experiments are performed collaboratively, reports are products of each student.

Each short report should be 1-2 pages long (single-spaced, 12-point font), prepared professionally (word processing and spreadsheet applications), and contain the following sections:

a. Title. Limit to one sentence, preferably reflecting a result. E.g., "Malate accumulation in the guard-cell apoplast modulates stomatal aperture size."

b. Goal of the experiment. Limit to one or two sentences. E.g., "Exogenous malate activates the guard-cell anion channel, which is the cardinal event in stomatal closure. Here, I determined the *in vitro* malate concentrations required to diminish aperture size and compared that with *in planta* malate concentrations in the guard-cell apoplast."

c. Scientific background. Limit to about seven sentences. What is the importance of this experiment? What is already known about the various components of the system in question? What particular aspects of this system are you interested in? Why? E.g., "Carbon dioxide for photosynthesis is acquired by plants from the atmosphere through stomata. However, water vapor is lost through stomata, too. Therefore, the aperture size must be adjusted to permit photosynthesis, but avoid deleterious water loss. Drought induces malate concentration increase in the leaf apoplast and malate induces stomatal closure. Here, we report that under certain conditions, sufficient malate accumulates around guard cells themselves to diminish aperture size, which was previously unknown."

d. Hypothesis. Limit to one or two sentences. The hypothesis is one of the most critical elements of scientific inquiry. Formulation of a hypothesis requires a comprehensive knowledge of the area of inquiry, the ability to distinguish important from unimportant questions, and the conceptual skills to devise a question that can be tested with proposed approaches and which yield an unambiguous answer. The hypothesis and goal(s) obviously overlap. Demonstrate your rationale for the hypothesis.

e. Materials and methods. Limit to about seven sentences. Briefly describe in past tense how the experiment was conducted. Although it is tempting to think that this section is *pro forma*, in fact, this section requires a great deal of judgment concerning the appropriate amount of detail. Consider, for example, the present case. Do you simply report the concentration of malate used, or is it appropriate to report the pH of the solution, the counter ion of malate, the source (if malate is often contaminated with a stomatal effector), the temperature (if the effect of malate is temperature sensitive), the time of day (if there are diurnal patterns in stomatal sensitivity to malate), the precise growth conditions (as it is known that sensitivity to stomatal effectors is related to plant history) . . . and the list could go forever. Obviously, choking a report with trivia is not desirable, but the elements required for interpretation are. In a word, judgment.

f. Results. Present your numerical data, as appropriate. Use proper units, and label the data clearly. Of course, tables and figures must include a detailed, written legend. Consult any scientific journal for a guide. The written section of the results should describe and refer to the tables and figures.

g. Discussion. Limit to about five sentences. Do your data fit your hypothesis? What do the results tell you in terms of the functioning of the system? What is your final conclusion? What subsequent studies are of interest?

Crop investigation

The top food crops in the world are, in approximate order, wheat, rice, maize, potato, and barley and account for about 70% of the total calories consumed by humans worldwide (3). Our lives are sustained by agriculture. Agriculture became the primary means of procuring food about 10,000 years ago (less than 1% of human existence); until then, humans were hunters and gatherers (1). The domestication of crops impacted human population, history, and society. With the introduction of agriculture, humans became sedentary and families grew (maybe because sources of food were more reliable or to help maintain the farm). In addition, sedentary life allowed a few people to produce food for the forming villages, while others could focus on increasing human knowledge and invention resulting in increases in population. The numbers below demonstrate population growth prior to agriculture through today (4).

10, 500 years ago 5 million people worldwide

↓ 8000 years

~130 million people

↓ 2150 years

~500 million people

↓ 300 years

~2500 million (2.5 billion)

↓ 50 years

~6000 million (~6 billion)

Although it is debatable how much of the growth in population is due to agriculture, it is certain that agriculture has and will be responsible for the sustenance of the population. Of all proteins consumed by humans worldwide, plants contribute ~70% and animals ~30% (4). Many of the more than six billion people worldwide are starving and malnourished; however, this seems to be due to problems in food distribution rather than production (1). Unfortunately, economics supercede need in food-distribution decisions within and across countries. Advances in agriculture to sustain our growing population include irrigation techniques; protection from pests; genetic improvements resulting in better yield, quantity, and quality; domestication of new crops; and genetic engineering (4) (transfer of one or a few foreign genes into plant cells (for perspective, one model plant for genetic studies, *Arabidopsis thaliana*, has ~25,700 genes)).

In addition to human population, domestication impacts ecology and landscapes. For example, after crop domestication, animal domestication followed. Large herds of grazing animals destroyed many pastures containing domesticated and native plants in the Near East (Lebanon, Syria, Turkey, Iran, Iraq) resulting in desert formation (4). Of the total land area of Earth, 11% is used for crops, 24% for pastures, and 31% is forest (1).

By cultivating plants, humans have greatly impacted and directed the evolution and characteristics of crop plants by deciding which plants grow where, protecting useful plants from diseases, and helping to evolve new species and characteristics of species that would not survive without human intervention (1). Some may refer to domestication as "directed evolution" by intentional selection instead of natural selection. For example, natural selection on maize would favor seeds (kernels) dispersing, but intentional selection for agriculture favors seeds remaining on the cob (similar for cereal crops such as wheat). Other characteristics that farmers often select for include loss of dormancy, loss of fruit production for those crops that humans use for roots or other vegetative tissues (e.g. potatoes), loss of seeds for those crops that humans use for fruits (e.g. bananas), and increased size of the organ of interest (e.g. compare the corn cob of wild corn with that you see in the supermarket) (1). As an example of the degree to which human selection can impact plant characteristics, all of the following have been domesticated from the same species, *Brassica oleracea*: cabbage, kale, kohlrabi, Brussels sprouts, cauliflower, and broccoli (3).

In addition to food, plants are cultivated for many purposes including oil, pesticides, perfumes, shelter, fuel, cloth, tools, and drugs. Interestingly, until about 150 years ago, botany was a branch of medicine. Today, ¼ of the prescriptions written in the USA contain at least one product that has been derived from a plant; plants are used even more commonly in other countries. Plants have been and are used to treat every system of the human body (2). Take a look around; there are few objects around you that are not derived from plants.

Bibliography:
1. Chrispeels, MJ and Sadava, DE. *Plants, Genes, and Agriculture.* Boston: Jones & Bartlett, Inc., 1994.
2. Lewis, WH. *Medical Botany.* New Jersey: Wiley & Sons, Inc., 2003.
3. Outlaw WH Jr BOT 3015 Class Workbook
4. Raven, PH, Evert, RF, and Eichhorn, SE. *Biology of Plants 7th edition*. New York: W.H. Freeman and Company, 2005.

Research and presentation

Now, you will be investigating, in groups of two or three, one of the crops listed below.

maize	banana	okra	flax
wheat	yam	barley	black pepper
soybean	rice	cacao	
peanut	sweet potato	coffee	

In your notebook (or on a sheet of paper that will be incorporated into your notebook), answer the following questions using complete sentences. Each student is responsible for answering all questions, but the research and presentation are group efforts. As references, utilize texts provided by the instructor and the Internet. *Plants, genes, and agriculture*, cited above, can be found as an electronic book (ebook) through the FSU library online catalog. Include citations indicating from which reference the information came and a list of references cited. (One way to do this is to number the references and then cite elements of information using the number as in the introduction above.) Finally, each group will give a short oral presentation (~three minutes) about the answers to the questions below to the rest of the class. All group members should present some information and information should be presented in a logical order. Please, also introduce yourself during the presentation.

Questions

1. What is/are common names for your crop?
2. What is the genus for your crop?

3. What parts of the plant are used by humans and for what purpose(s)?
4. From what region of the world did the crop come?
5. For about how long has your crop been used by humans?
6. What countries grow and/or export the crop today?
7. What are some interesting facts about this crop that you found?