Guide to Lab Exercises in Concepts of Botany, 4th Ed. (Spring)

BotanY

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Guide to Lab Exercises in Concepts of Botany

Introduction to Botany

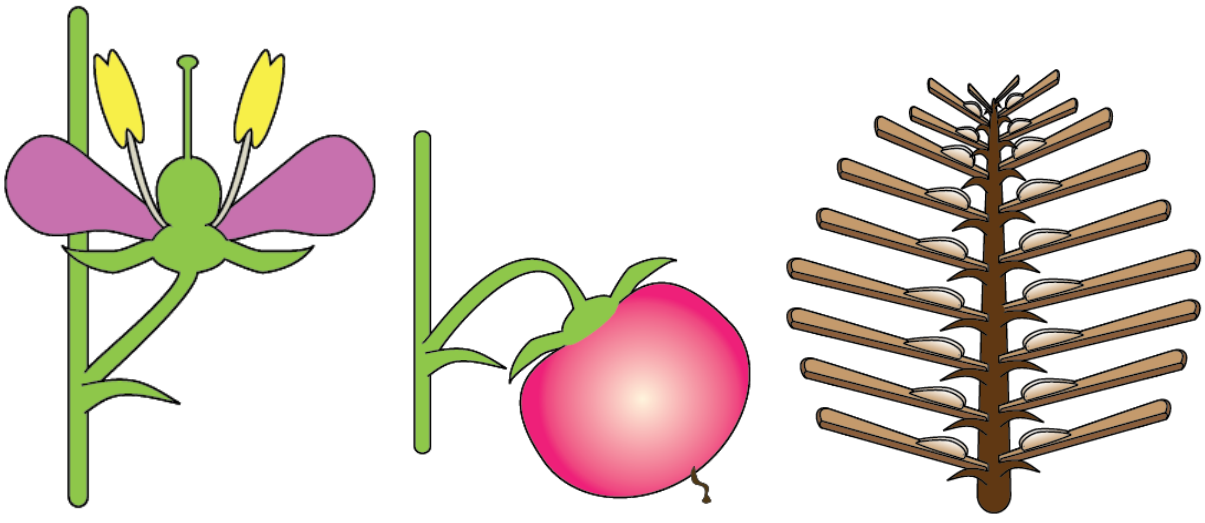
Today you will be introduced to botany by way of introductory material in each of the subdisciplines that are covered in our advanced botany courses at MU, which form the spine of the Botany Option within the Biology major. The outline of this lab is as follows:

I. Today's Lab
- **A.** Plant Systematics,
- **B.** Plant Developmental Biology & Horticultural Science,
- **C.** Plant Physiology & Biochemistry.**,**
- **D.** References,
- **E.** Glossary,
- **F.** Credits.

II. Summary Questions & Problem Set

In the process, you will learn foundational skills, concepts, knowledge and of resources to sustain you throughout the semester. In particular,

1. You will learn how plants are classified and of the basics regarding scientific botanical nomenclature;
2. You will review the aspects of the plant cell that distinguishes plants from other organisms;
3. You will connect important uses of plants by humans to their cellular structures and life history traits;
4. You will familiarize yourself with the basic characteristics of and distinctions between the four major groups of plants;
5. You will sow seeds of various plant species for observations of plant development and morphology later in the semester; and
6. You will be introduced to some of the ways in which botanists apply simple math to control for complexities of plant structure during experiments.



I. Today's Lab

A. Plant Systematics

Plant systematics (or systematic botany) is the subdiscipline of botany that involves the discovery and documentation of plant diversity, plant evolution, and the construction of systems of classification (taxonomies) that reflect this knowledge.

1. The Taxonomic Hierarchy (working in groups of 4)

***a. Introduction*.** Review Table A1, which gives you the taxonomic classification of two important plant species, common bean and corn. The taxa (singular taxon) are ordered from top to bottom in order of most to least inclusive taxonomic ranks. Study Table A1 and then answer questions that follow.

Table A1. The taxonomic hierarchy, exemplified for two species of angiosperms: *Phaseolus vulgaris* (common bean) and *Zea mays* (corn). Taxonomy consistent with your Raven Biology of Plants (Evert & Eichhorn 2013).

Rank	**Example Classification of *Phaseolus vulgaris* (common name; included species)**	**Example Classification of *Zea mays* (common name; included species)**
Domain	Eukaryota (eukaryotes; >1.4 million)	Eukaryota (eukaryotes; >1.4 million)
Kingdom	Plantae (plants; 336,750)	Plantae (plants; 336,750)
Phylum	Anthophyta (angiosperms; 300,000)	Anthophyta (angiosperms; 300,000)
Class	Eudicotyledonae (eudicots; 200,000)	Monocotyledonae (monocots; 90,000)
Order	Fabales (legumes and allied families; 2,929)	Poales (grasses and allied families; 22,000)
Family	Fabaceae (bean or legume family; 19,100)	Poaceae (grass family; 10,000)
Genus	*Phaseolus* (beans; 128)	*Zea* (teosintes; 5)
Species	*Phaseolus vulgaris* (common bean; 1)	*Zea mays* (corn; 1)

b. Questions (Using Table A1 and the internet).

1.) **On the nature of scientific names for species:**

Note that the names of taxa from domain on down to genus consist of just one word (i.e., they are uninomials). The taxonomic rank of species, however, is special. With common bean as an example, notice how a species name consists of two parts, the genus name "*Phaseolus*", and the specific epithet "*vulgaris*". Whereas other species may share the genus name ("*phaseolus*" is Latin for "bean" and there are many species of bean), and yet other species in other genera may share the same specific epithet ("*vulgaris*" merely means "common" in Latin), the two parts together comprise a unique species name.

Thus, the scientific names of species are said to be which of the following?

a.) uninomials b.) binomials c.) trinomials d.) polynomials

For fun: Which of the above "–nomial" terms above applies best to our system of naming persons in the United States and most Western cultures? Hint: start by thinking of your own full name.

2.) **On the nature of common names:**

a.) What are the common names in Table A1 for *Phaseolus vulgaris* and *Zea mays*? Use the scientific name of each species and the **Encyclopedia of Life** (www.eol.org) to find the Brief Summary at each species's EOL page to determine if there are other common names for each species and, if so, write what these are.

b.) Type "corn (disambiguation)" into **Wikipedia** (www.wikipedia.org) to see if the word "corn" always refers to plants of the species *Zea mays*. Alternatively, do so in a dictionary. Does corn always refer to just one plant species? Explain.

3.) **Subspecific taxa:**

Below the species level some plant species are subdivided into subspecies (abbreviated ssp. or subsp.) and/or varieties (abbreviated var.). Find the taxonomy sidebar of the **EOL.org** species page for *P. vulgaris* and *Z. mays* and tell me which if any species is divided into subspecific taxa. Write their names here.

4.) **What is a genus?**

A genus (plural *genera*) is a group of relatively closely related, similar species typically easily distinguished from other such groups (i.e., from other genera).

a.) How many other species are in the genus *Phaseolus*? How many in *Zea*?

b.) Use the Encyclopedia of Life (www.eol.org) to name at least one other species in each genus.

5.) **How are scientific names written?**

a.) All scientific names are written with a capital first letter. However, Table A1 shows that only some taxa are italicized. Taxa at which ranks in Table A1 are italicized? Answer this and you have the general rule to follow: taxa at these ranks and only these ranks are italicized or underlined when written (e.g., in reports, etc.).

b.) Notice how the specific epithet is not capitalized, whereas the genus name is. This is a good rule to follow when you write species names. Now that you know the rules, go back to Question 3 and be sure you've written species names correctly.

2. Taxonomic Characters (working in groups of 4)

Systematists place smaller taxa (e.g., species) into larger taxa (e.g., the kingdom) based on shared characters (characteristics) that they discover through morphological, anatomical or molecular studies.

a. Members of the plant kingdom share certain distinctive cellular characters. In BIOL 101 you spent an entire lab making wet mounts of various plant and animals cells and, supplemented by lecture, you learned about the characteristics that plants share with animals and fungi as eukaryotes and which were unique to plants. See how much you remember from 101 by completing Table A2.

1.) Complete Table A2 by placing a check mark in the cell under each of the three major kingdoms of multicellular organisms if that organelle or structure is present in at least some cell types in members of the kingdom.

Table A2. Organelles or cell structures that are more or less discernible with the compound light microscope.

	Plantae	**Animalia**	**Fungi**
Aleuroplast			
Amyloplast			
Cell wall (cellulosic)			
Cell wall (chitonous)			
Chloroplast			
Chromoplast			
Elaioplast			
Large central vacuole			
Leucoplast			
Mitochondrion			
Nucleolus			
Nucleus			
Plasma membrane			
Plastid			

2.) **Ethnobotany of cell structures unique to plants.** Ethnobotany is often seen as a subdiscipline of or at least a companion science to systematics. Here we review plant cell structure from an ethnobotanical perspective: i.e., how they translate into the many benefits that only plants provide for humans. At your bench is a set of printed color figures with captions that match the questions and text below. Study these figures and be sure you can discern the cellular structures visible in them, you have seen most of these before during the Cell & Microscopy lab in BIOL 101. If you remember the function of the plant organelles and structures from Table A2, then you will be able to provide the names of the plant organelle/structures from Table A2 that are responsible for the human uses described.

Fig 1.a. What is the abundant organelle found in this micrograph of a leaf from the aquatic plant *Elodea densa* (**A**, close up in **B**) that makes plants green and gives the world its food and free oxygen (O_2) while, at the same time, removing the greenhouse gas CO_2 from the atmosphere (**C**)?

Name this organelle:

Fig 1.b. This micrograph from plasmolyzed epidermal cells of a red onion (**A**) show two of these (one and two) structures from two adjacent cells coming together. This is the primary plant structure that is responsible for the fiber in our diet (**B**). Fiber is plant carbohydrate that we cannot digest (i.e., we do not have the proper enzymes to do so). Soluble fiber, which dissolves in water, helps your body lower glucose levels and cholesterol in the blood. Insoluble fiber, which does not dissolve in water, helps food move through your digestive system, thereby promoting regularity and preventing constipation.

Name this structure:

Fig 1.c. **A**, Many medicines and recreational drugs from plants accumulate and are stored in plant cells in this organelle (from a Transmission Electron Micrograph). **B**, Taxol®, for example, is a chemotherapeutic chemical from yews (of the genus *Taxus*) that works by inhibiting cancer cell division by disrupting the mitotic spindle.

Name this organelle:

Fig 1.d. **A**, Water-soluble pigments such as red, purple or blue anthocyanins from the red onion epidermis (**A**), beets, cherries (**B**) or red or purple grapes are stored in this organelle. **B**, Anthocyanins are powerful antioxidants with numerous reported health benefits.

Name this organelle:

Fig 1.e. **A**, Starch, which is naturally white and is a concentrated source of calories, comes from which organelle? **B**, Under the microscope, you can test for the presence of starch in a cell by applying a drop of potassium iodide to the tissue under the cover slip: any starch will react with the KI/I_2 by turning purple, as seen in this light micrograph from potato tuber tissue.

Name this organelle:

Fig 1.f. **A**, As many fleshy fruits ripen, lipid-soluble pigments are made to signal ripeness to potential animal seed dispersers. **B**, As seen in this light micrograph from tissue of a red bell pepper, these pigments are made and stored in which organelles?

Name this organelle:

Fig 1.g. While all seeds have these, the protein-rich seeds of members of the legume family, Fabaceae, such as soybeans (**A**) and peanuts (**B**) are exceptionally rich in this type of organelle.

Name this organelle:

Fig 1.h. Carotenoids like beta-carotene are important antioxidants and vitamin-A precursors in the human body. Carotenoids are synthesized and stored in which organelle type?

Name this organelle:

Fig 1.i. **A**, various types of cells (dead) that one would expect to find in paper. Which cell structure of such cells persists after death of the cell and provides the structural material of paper? **B**, Now pause for a moment to think of the importance of paper to humanity.

Name this structure:

Fig 1.j. These organelles provide the oil we extract in the production of edible oils such as olive oil, peanut oil, etc.

Name this organelle:

Fig 1.k. Wood is made of plant cells and the structural strength of wood is due to lignification of which cell structure? **A**, pieces of lumber as extracted from a tree stem. **B**, a scanning electron micrograph of wood from pine, courtesy of Robert Farrell.

Name this structure:

b. The Major Groups of Plants (working in groups of 2-4)

The major groups of plants within the kingdom include those in Table A3. They can be distinguished based upon the nature of their reproductive structures and, generally, vegetative structures (Table A3). Study this table.

Table A3. The four major groups in the Plant Kingdom, their appearance (origin) in the fossil record, number of known species, gross vegetative structure, and nature of the final reproductive/dispersal structure in extant species.

Common Botanical Name	**Common Synonyms**	**Origin (Ma)***	**Number of known species**	**Reproductive/Dispersal Structure Generalization**	**Gross Vegetative Structure**
Angiosperms	flowering plants	c. 150	c. 300,000	Seed(s) enclosed in a fruit that develops from a flower.	Broadleaf herbs, shrubs or trees; vascular tissue present.
Gymnosperms	cycads, conifers, or ginkgo	c. 300	c. 750	Naked seed(s) aggregated on but not enclosed in cones or cone-$_{like}$ structures.	Needle-, scale- or broad- leaved shrubs or trees; vascular tissue present.
Pteridophytes	ferns & fern allies, seedless vascular plants: including horsetails, scouring-rushes, lycophytes, clubmosses & quillworts	c. 400	c. 13,000	Spores produced in small cone-like structures or in clusters on undersides of leaves.	Minute or broad-leaf herbs; vascular tissue present.
Bryophytes	mosses, liverworts, or hornworts	c. 450	c. 23,000	Spores produced in small, stalked, solitary capsules.	Small, thalloid or minute-leafy herbs; vascular tissue absent.

*Listed in megaannum (Ma) or "million years ago". From fossil record evidence summarized by Kenrick & Crane (1997).

1.) Bryophytes (consult Table A3 and the specimens at the Bryophyte Station).
Bryophytes were the first plants to appear in the fossil record about 450 Ma (Table A3) and branched off of the plant phylogeny before the evolutionary origin of vascular tissue, seeds, cones, flowers or fruits. Their approximately 23,000 **extant** species still lack these features and so are considered **primitive** in these respects.

Study the specimens of moss and liverwort in the lab as representatives of bryophytes, then answer the following questions:

a) How would you describe their stature?

b) How do you think their small stature relates to their lack of vasculature?

c) How does their lack of vasculature relate to where they grow?

2.) Pteridophytes (consult Table A3, and the Pteridophyte Station).
Pteridophytes evolved ca. 400 Ma from some bryophyte lineage with the origin of vascular tissue. Although this is a diverse group, we have representatives of the large and familiar fern family Polypodiaceae for you to study and then answer the following questions.

*a.) The most conspicuous parts of a fern plant are the leaves, since they are large and above-ground, whereas the stem (**rhizome**) is typically short and underground. Most ferns have distinctive compound (not simple) leaves too. What does "**compound leaf**" mean?*

*b.) Study the poster of the fern – figures 1 (plant), 2 (rhizome), 3 (six **sori** on underside of leaflet), 4 (**sorus** in cross-section), 5 (**sporangium**).*

c) Are ferns evolutionarily an older or young group than the gymnosperms and angiosperms?

3.) Gymnosperms (consult Table A3, and the Gymnosperm Station).
The first gymnosperms evolved at least 300 Ma with the origin of the **seed** which, in **seed plants**, replaced the **spore** as the **dispersal structure**. In gymnosperms, the seeds are borne **naked** in **cones** or cone-like structures called **strobili**. Gymnosperms are a small but diverse group.

a.) Examine the leaves of the pines, spruces, and firs it the gymnosperm station. Would you describe these as broadleaves or needle-like?

b.) In conifers like pines, the seeds are borne generally in woody, tough cones. See if you can find seeds in the representative cones.

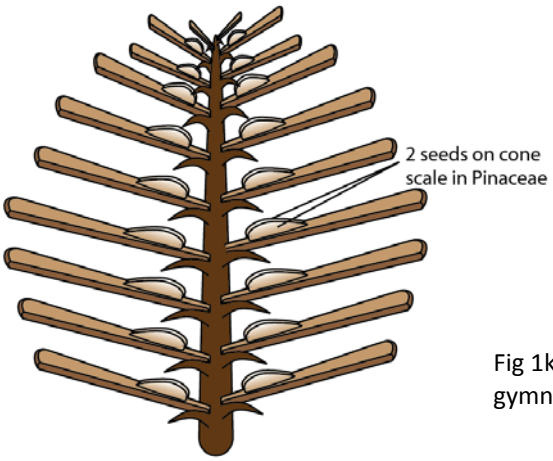


Fig 1k. The seed-bearing cone of a conifer , a type of gymnosperm.

c) Examine the herbarium and/or living specimens of cycads. How are these leaves similar to those of ferns? How are their reproductive structures different than those of ferns?

d) Place the name "Gymnosperms" in the appropriate white box label in Figure 1k.

4.) Angiosperms (consult Table A3 and the Angiosperm Station).
The first angiosperms evolved at ca. 150 Ma with the origin of the **flower** and **fruit** (Fig 1L)**.** The fruit in particular is a structure unique to angiosperms which encases the seeds at maturity. The fruit is derived from the ovary of the flower.

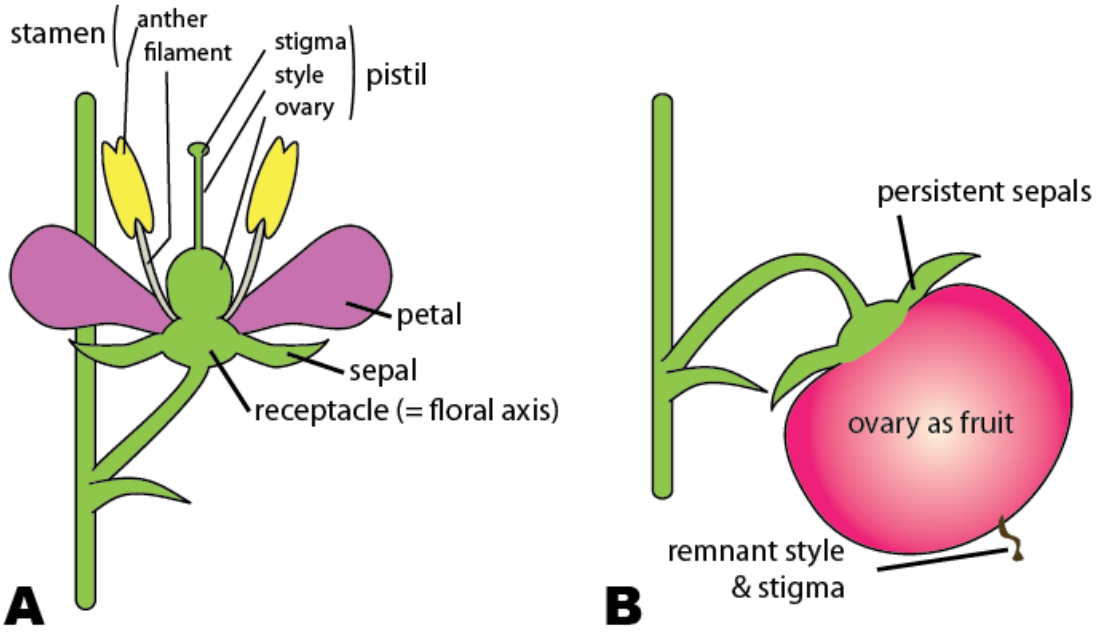


Fig 1L. **A**, Basics of flower structure. **B**, the same flower in fruit, where it is not uncommon for the sepals to persist and for the stalk to bend in holding the heavy fruit.

a.) Make a labeled drawing of the large plastic flower model at the level of detail shown in Figure 1LA.

b.) Make a study and labeled drawing of a lily or tulip flower from the florist.
What happened to the sepals in this flower?

c.)Study the magnolia herbarium specimen.
Do the plants have needle-like, scale-like or broad leaves?

Are the flowers on this inconspicuous or conspicuous?

d.) Study the oak herbarium specimen.
Do the plants have needle-like, scale-like or broad leaves?

How do the leaves compare with those of the magnolia?

Are the flowers on this inconspicuous or conspicuous?

e.) The pistil of the flower turns into a fruit. Study the tomato, lily /iris, and oak fruits present.
The tomato is a type of fruit known as a berry. Is this a fleshy or dry fruit? Is it dehiscent (opens) or indehiscent (remains closed)?

The lily or iris fruit is known as a capsule. Is this a fleshy or dry fruit? Is it dehiscent (opens) or indehiscent (remains closed)?

The acorn on the oak specimen is a type of fruit known as a nut. Is this a fleshy or dry fruit? Is it dehiscent (opens) or indehiscent (remains closed)?

B. Plant Developmental Biology & Horticultural Science

Broadly defined, horticulture is the subdiscipline of botany that concerns the theory and practice of plant cultivation and propagation, as well the development of new cultivars or improved horticultural techniques. Plant developmental biology is the subdiscipline of botany that concerns patterns and processes of plant development, from seed to fruit and any developmental process in between.

Multi-week Study: Cultivation and development of the radish, *Raphanus sativus* Working in groups of 4.

1. This week (Week 0) –

a. Hypothesis formulation regarding the nature of the radish.

Without using anything other than your eyes, formulate a hypothesis about whether or not the edible portion of the radish develops from root, stem or leaf. Write this down here. In the coming weeks, we will then test that hypothesis by watching radish plants grow and develop from seeds that you sow.

b. Sowing of seeds.

- Label two 3 to 4-inch pot with your group name, lab section, date and the species's scientific
- Fill both with soil to a height of 1 cm below the top.
- Place 1 seed in each of 5 evenly spaced, 1 to 1.5 cm-deep depressions in the soil created with your finger in each pot (10 seeds total: 5 in each pot). Then use your finger to bury the seeds at that 1-1.5 cm depth with surrounding soil.
- Place in the greenhouse and give them a first watering. After that, greenhouse staff will care for them.

2. Week 1 – Germination & Seedling Observations. Following your completion of the Seeds & Seedlings laboratory, do the following:

a. Determine the percent of seeds that germinated and show your work here.

b. Below, make two drawings of a typical seedling for this week. Label various parts visible using the terminology you learned in the Seedlings lab. Use adjectives to describe the color of each part.

Intact (rooted) seedling *Uprooted (entire) seedling incl. root*

c. Photograph the seedling. Save it, noting in the filename that it is a Week 1 seedling. Incorporate the photograph into a Powerpoint file and label all parts. Be prepared to present this and answers to the following questions to the class. Send the file to your instructor via email.

d. Based on your seedling structure, is the radish a dicot or monocot angiosperm? What is your evidence for this?

e. Is there any evidence in support of or against your hypothesis about what the radish (the swollen portion of the radish plant) develops from? Explain.

3. Week 2 –Seedling Observations. Following your completion of the Primary Morphology lab, do or answer the following:

a. Have any additional seeds germinated since last week? Explain.

b. Describe the phyllotaxy, complexity and blade margin of the cotyledons and any other leaves present. Below, draw and label each of the leaf types present on the radish seedling.

c. Carefully uproot one of the seedlings, remove soil from the root and photograph the seedling. Save it, noting that it is a Week 2 seedling. Incorporate the photograph into a Powerpoint file and label all parts, including the location of the shoot and root apices. Be prepared to present this and answers to this week's questions to the class. Send the file to your instructor via email.

d. Note color of various parts.

e. Does the root system appear to be that of a tap or fibrous root system?

f. Is there any evidence in support of or against your hypothesis about what the radish (the swollen portion of the radish plant) develops from? Explain.

4. Week 5 – During the Plant Modifications & Marketplace Vegetables Lab.

Following your completion of the Plant Modifications & Marketplace Vegetables lab, do the following:

a. Carefully remove one of the radish plants. Please note color of the various parts. Photograph the whole plant and save it, noting that it is a Week 5 plant. Incorporate the photograph into a Powerpoint file and label all parts, including the location of the shoot and root apices. Be prepared to present this and answers to this week's questions to the class. Send the file to your instructor via email.

b. Compare a store-bought radish and/or photograph of one to the plant of yours. Is there any evidence in support of or against your hypothesis about what the radish (the swollen portion of the radish plant) develops from? Explain.

5. Weeks8, 9 or 10 if need be – During lab.
If your observations during Week 5 were not conclusive regarding the developmental origin of the edible radish as root, stem or leaf, then revisit your radish plants to make additional observations that may be relevant to this question.

a. Carefully remove one of the radish plants. Photograph it and save it. Incorporate the photograph into a Powerpoint file and label all parts, including the location of the shoot and root apices. Be prepared to present this and answers to this week's questions to the class. Send the file to your instructor via email.

b. Compare a store-bought radish and/or photograph of one to the plant of yours. What are your conclusions regarding the development of the radish and radish plant.

C. Plant Physiology & Biochemistry

Plant physiology and the related plant biochemistry are the subdisciplines of botany that concern the processes of plant function, from the molecular or chemical basis of plant development and defense to plant nutrition. Actual physiological experiments with plant hormones, photosynthesis and water will be carried out later in the semester. For now, however, we will ask you to carry out some computational exercises related to labs we will conduct later in the semester.

1. Plant Chemicals and Recreational Drugs.

Table 1.4 lists the top five illicit, recreational drugs by global use. Use the internet to determine which of these come from plants and which do not. If the drug is from an organismal source (i.e., non-synthetic), then also provide the binomial species name. Then answer the following questions:

a.) How many of these come from plants? Animals? Fungi? Synthetics?

b.) What do the top three come from: plants, animals, fungi, or synthetics?

c.) From which group of organisms do more of the top five drugs come from: plants, animals, or fungi? Provide a biologically meaningful explanation for this.

Table 1.4. Top five most used illicit recreational drugs in 2009, according to the United Nations Office of Drugs and Crime (2010) and ranked according to their lower-end estimate of users.

Rank	Drug	Estimated Users aged 15-64	Ultimate Source (plant, fungus, animal, or totally synthetic)	Species if Organismal Source
1.	Cannabis	128,910,000 – 190,750,000		
2.	Cocaine	15,070,000 – 19,380,000		
3.	Opiates	12,840,000 – 21,880,000		
4.	Amphetamines	13,710,000 – 52,900,000		
5.	Ecstasy	10,450,000 – 25,520,000		

2. Estimating and Comparing Water Content of the Plant Body vs. the Seed. (working in groups of 4)

a. Water content (by mass) of a plant. On your benches are paper lunch bags or containers containing the dried remains of *Zea mays* plant from plants that were harvested living a few days ago and weighed before drying by your instructor. These containers have the wet weight on them. Determine the dry weight of one of these plants and use it with the fresh weight to determine the water content of a living plant.

- Record and compare the dry weight to the wet weight to estimate the percent water by weight of the living plant.
- Return all parts to the container for the next class once you are done.
- Living representatives of these plants are in the room for your reference.

b. Water content (by mass) of a seed. Seeds contain a living embryo surrounded by living nutritive tissue. They are remarkable structures which highlight a major difference between animal life and the life of a seed plant: that the seed plant embryo arrests its development, entering a state of dormancy that can last months to hundreds or thousands of years, depending upon the species and the environment. Part of this dormancy is achieved by decreasing its water content. But, by how much?

Each kernel of popcorn consists of a relative big single seed surrounded by a hard wall. It pops when the kernel is heated, converting the seed's liquid water into steam and the resulting pressure eventually ruptures the seed and the water is thus released. We can thus weigh popcorn kernels before and after popping and take the difference in weight to indicate the proportion of kernel weight that was water vs. dry mass.

- Using a clean (eating-safe) Dixie cup, weigh a quarter of Dixie cup of corn (tare away the Dixie cup weight).
- Then pop that corn in hot-air popper.
- Remove any unpopped kernels, weigh them and subtract them from the original kernel weight to produce an adjusted unpopped kernel weight.
- Weight the popped corn together.
- Calculate the percent water in unpopped popcorn kernels by weight. Use the popped corn weight and the adjusted unpopped kernel weight. Show your work.

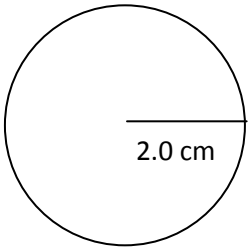
3. DUE TO PROBABLE TIME CONSTRAINTS, DO NOT DO THE FOLLOWING UNLESS INSTRUCTED TO DO SO BY YOUR INSTRUCTOR:

Morphometrics of Plants: Calculating Leaf Surface Area as an Example

Leaf surface area is an important parameter affecting a plants ability to capture light for photosynthesis, transpire or respire, and conserve water. As such, it often is an important parameter to quantify when conducting physiological experiments. The following walks you through aspects of calculating surface area generally: first of simple shapes, and then of complexly shaped organs such as leaves.

a. Area of a square or rectangle. How does one calculate the surface area of a square or rectangle? Calculate the area for the rectangle below. What are the units of area?

b. Area of a circle. How does one calculate the surface area of a circle? Calculate the area for the circle below. What are the units of area?



c. Area of a leaf. How does one calculate the surface area of a complex, organic shape such as that of a leaf blade? Take an English-ivy leaf off a live plant in the room, draw it below, add a scale bar (in cm), then calculate the area of one surface (e.g., the upper) of that leaf. (**Hint:** use the cm ruler, razor blade, and electronic balance at your desk. Start by excising a piece of the leaf of known area (e.g., 3 x 3 cm) and then weigh that piece to the nearest 0.01 g. After a few more steps, you can provide a very good estimate of that leaf's surface area.)

D. References

Evert RF, SE Eichhorn. 2013. Raven Biology of Plants, 8th Edition. WH Freeman and Co. New York, NY, USA.

Kenrick P, PR Crane. 1997. The origin and early evolution of plants on land. *Nature* 389: 33-39. United Nations Office of Drugs and Crime. 2010. *World Drug Report*. United Nations, New York.

E. Glossary

Some terms you will encounter during this lab are as follows:

- **Compound leaf** = a leaf that is divided in to 2 or more leaflets. Contrast with "simple leaf".
- **Cone** = the structure containing the seeds in coniferous gymnosperms.
- **Ethnobotany** = the study of plants in human culture.
- **Free-sporing** = free-sporing plants are those plants that lack seeds and, instead, disperse by means of spore release.
- **Homology** = similarity (i.e., sameness) of structures due to common-descent.
- **Horticulture** = see text in lab.
- **Ovule** = a structure within the ovary of a flower or in the cone or strobilus of a gymnosperm that contains the egg and thus confers female function. Ovules develop into seeds.
- **Petal** = a type of organ in a flower; in animal-pollinated flowers, the petals tend to be showy and sometimes scented in order to attract would-be pollinators.
- **Pistil** = a type of organ in a flower whose function it is to house the ovules and receive the pollen.
- **Plant biochemistry** = see text in lab.
- **Plant developmental biology** = see text in lab.
- **Plant physiology** = see text in lab.
- **Plant Systematics** = see text in lab.
- **Reproductive (syn. fertile)** = in modern usage, the part of a plant's body or life-cycle that produces spores and/or seeds (reproductive parts). Flowers, sporangia, cones, strobili, for example, are reproductive parts. Contrast with "vegetative".
- **Sepal** = a type of organ in a flower, whose typical function is to envelop and protect the flower in bud.
- **Simple leaf** = a leaf with only one, undivided blade. Contrast with "compound leaf".
- **Sporangium** (plural ***sporangia***) = a small structure in which meiosis occurs to produce spores.
- **Spore** (plural ***spores***) = a haploid, unicellular product of meiosis in plants and fungi.
- **Stamen** = a type of organ in the flower whose function is to make pollen (pollen has male function, since it will make sperm).
- **Strobilus** (plural ***strobili***) = a cone-like aggregation of modified leaves that bear sporangia.
- **Taxon** (plural ***taxa***) = a group of organisms to which a formal taxonomic name is given.
- **Thalloid** (syn. **thallose**) = a flat plant that is not differentiated into root, stem or leaf. Liverworts are common examples of this.
- **Vegetative** (syn. **sterile**) = in modern usage, the part of a plant's body or life-cycle that produces no spores or seeds (i.e., no reproductive parts). Leaves, stem and root, for example, are vegetative parts. Contrast with "reproductive".

F. Credits

This lab was developed by Christopher Hardy with contributions from Ryan Wagner on earlier versions. You may cite it as follows:

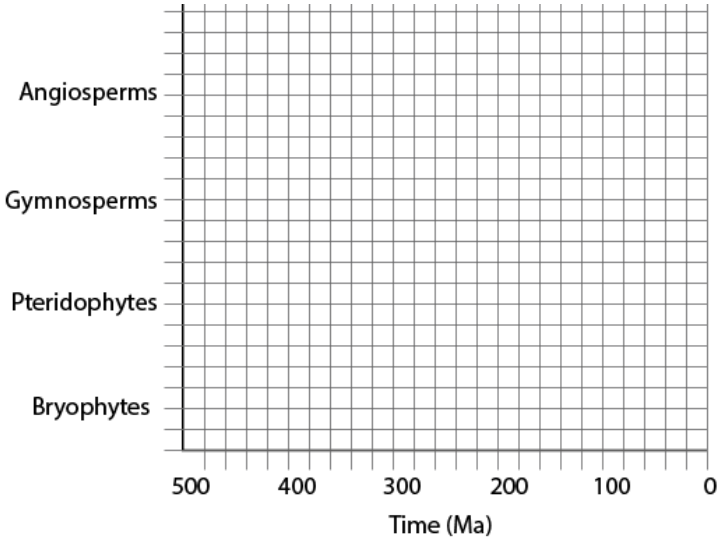
Hardy CR, RL Wagner. 2016. Introduction to botany. Pp. 1-22 in CR Hardy, RL Wagner (eds) *Guide to Lab Exercises in Concepts of Botany, 4th Edition*. Millersville, Pennsylvania, USA.

II. Summary Questions & Problem Set

Answer the following questions based on the materials and information already made available or gathered by you today (e.g., tables, figures and your answers to previous questions).

1. Create a vertical bar graph depicting the relative numbers of extant species contained in each of the four broad categories of plants in the plant kingdom. Create a caption below the figure that includes a single sentence descriptive title.

2. Create a horizontal bar graph depicting the span of time that each of the four broad categories of plants have existed. The X axis below has the units of "millions of years before present" (Ma). Create a caption below the figure that includes a single sentence descriptive title.



3. Place the names of the 4 major plant groups, Bryophytes, Pteridophytes, Gymnosperms & Angiosperms, in the appropriate box on the phylogenetic tree diagram below. What evolutionary story does this phylogenetic tree tell you?

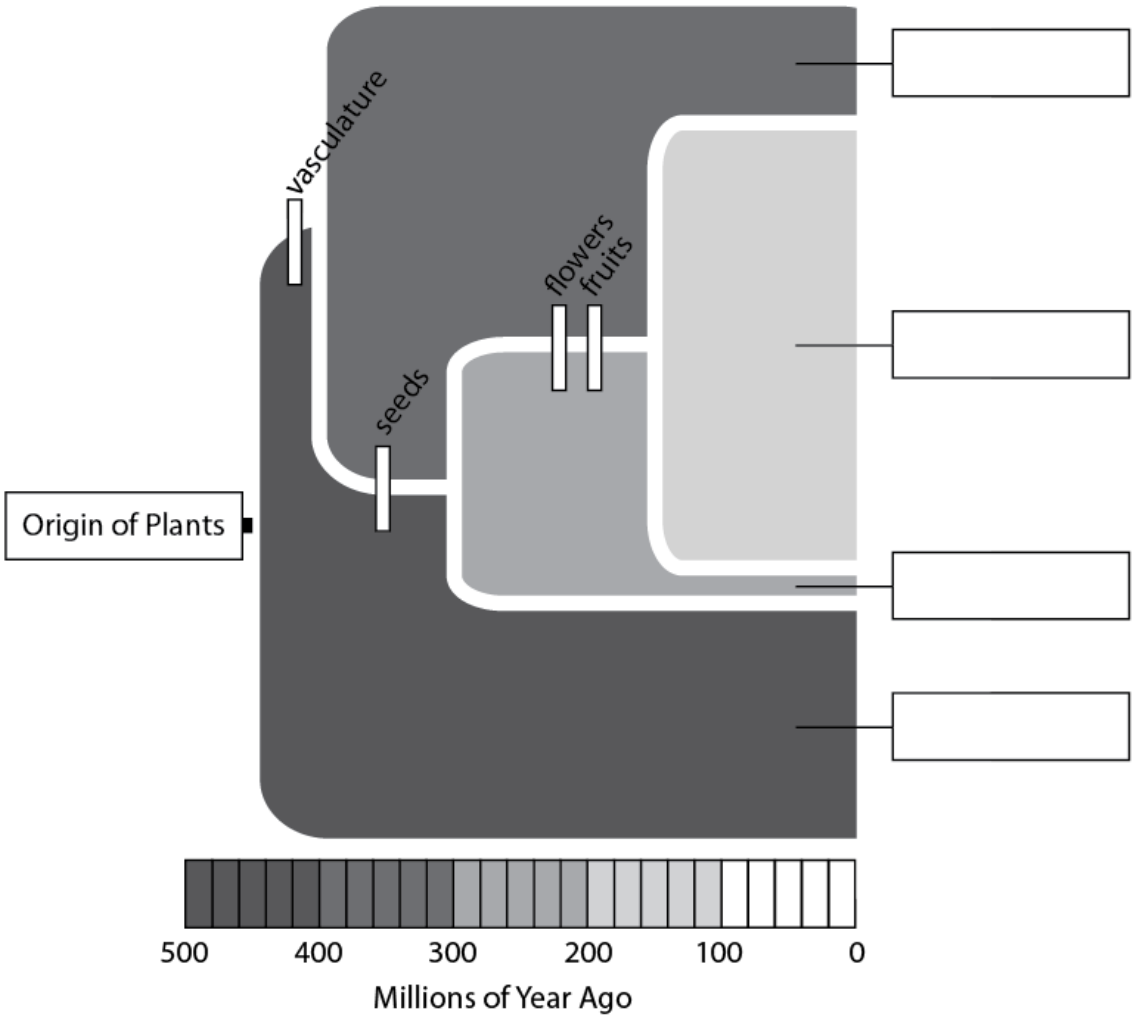


Fig 1m. Simplified cladogram depicting phylogenetic relationships and relative ages among the 4 major groups of plants: bryophytes, pteridophytes, gymnosperms and angiosperms.

4. List cellular and subcellular structures or organelles that distinguish plants from both animals and fungi.

5. List the different plastid types below and provide a brief 1-4-word description of their function.

Plastid Type	**Function**

6. Which organelle is responsible the primary amount of nutritional fiber from plants in our diets?

7. What is the name of the industrious little plant organelle which takes the greenhouse gas CO_2 out of our atmosphere?

8. Fruits are formed from the _____ of _____.

9. Seeds in conifers are produced on _____.

10. Seeds in angiosperms are produced in _____.

11. _____ rather seeds are the units of dispersal in pteridophytes.

12. _____ rather seeds are the units of dispersal in bryophytes.

13. What is the scientific species name for corn?

14. What is wrong with the way that the species scientific name for ginkgo is written below?

ginkgo bi loba

15. Why are scientific names more precise than common (i.e., vernacular) names for species? Provide an example from your research today of why this is so.

16. Of the top 3 illicit drugs used recreationally by humans, how many are plant-based?

17. Why or why wouldn't you expect plants rather than animals to be the source of many drugs?

18. Which taxon is more inclusive, the bean family or bean genus?

19. **Sequence in the taxonomic hierarchy:**
    Order the taxonomic ranks (left-most column in Table A1) from least to most inclusive, from left to right below.

****

Reminder: Be sure to also familiarize yourself with the appearance and function of plant cell structures & organelles, as well as the terms and definitions provided in the glossary.

Seeds & Seedlings

All plants start their lives as a zygote that develops into an embryo via a process called embryogenesis. In spermatophytes (seed plants), the embryo is housed in a seed: the seed consists of an embryo, accompanied by some nutritive tissue such as endosperm in angiosperms, housed in an outer, protective seed coat (Fig 1A).

The typical embryo consists of one or more cotyledons (embryonic leaves; compare Figs 1A and 1B). The cotyledons may become large and displace and assume the nutritive role of the nutritive tissue at embryonic maturity in some species (e.g., legumes; Fig 1C). Below the cotyledons are the embryo's hypocotyl (stem axis below the cotyledons) and radicle (embryonic root, with an apical meristem at its tip). Above the cotyledons is the first shoot apex, consisting of the epicotyl (stem axis above the cotyledons plus its shoot apical meristem) plus any first leaf primordia. This embryonic shoot apex is often called the plumule, particularly when conspicuous. This basic plan, as you will see today, is elaborated on to various degrees in different species

This lab will introduce you to the important structures and processes early in the life of a seed plant: the process of embryo formation, seed structure, germination, and seedling development. We use this study to also introduce you to two of humanity's most important plant families, the legumes and grasses whose seeds have provided the nutritional foundation on which our very civilization rests.

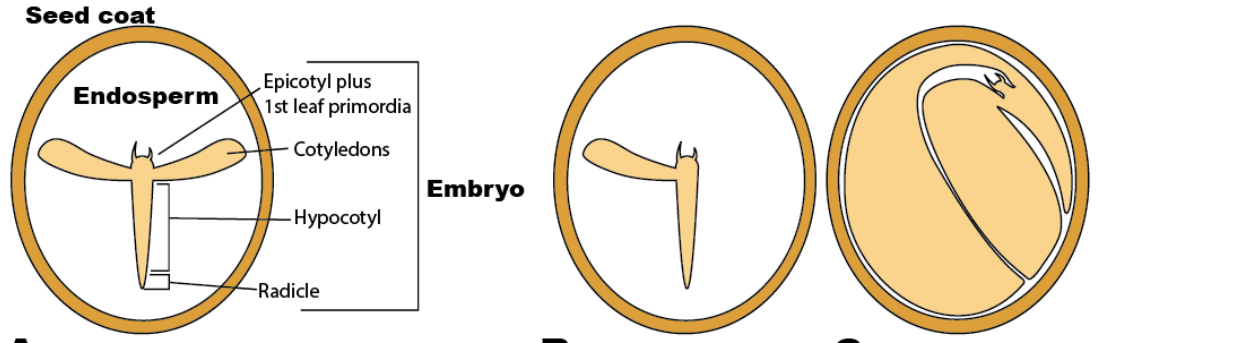


Fig 1. Stylistic representation of seeds and their embryos. Angiosperm embryos generally have either one or two cotyledons.

Objectives for this lab:

1) Learn the process of embryogenesis in a spermatophyte.
2) Use important crop plants as models for learning seed and seedling structure.
3) Learn basic systematic, morphological, and ethnobotanical information about cereal and leguminous plants and the parts we use.
4) Relate the properties and uses of different types of cereal grain flour to their chemical makeup.

Table of Contents for this lab:

I. Embryogenesis

Embryogenesis in spermatophytes is the process whereby a zygote in the ovule develops into a mature embryo in a seed (Fig A1). Unlike animals, spermatophyte embryos do not mature in a continuous process towards adulthood or maturation: rather, the plant embryo, once fully formed, will enter into a period of dormancy inside its "time capsule" the seed for a period of days, months, years or, in some cases, centuries until conditions are right for the breaking of dormancy and the resumption of development. In tropical rainforests, for example, where the growing season can be year-round, seeds may germinate immediately upon release from the fruit or strobilus. Not surprisingly, the seeds of temperate-climate plants that have formed in the autumn will typically see their embryos overwinter in the seed in the dormant state, only to resume growth following germination of the seed in the spring (Fig A2).

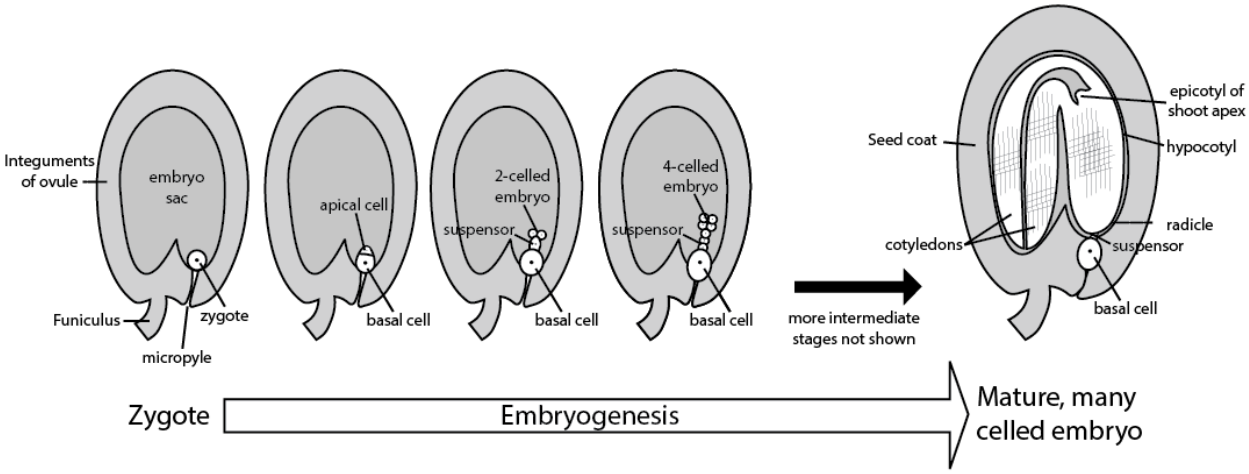


Fig A1. Seed medial longitudinal sections depicting the early stages and mature embryo formation in the shepherd's purse, *Capsella bursa-pastoris*. Multiple intermediate stages not shown. The **funiculus** is the stalk that attaches the ovule and developing seed to the fruit wall (material tissue).

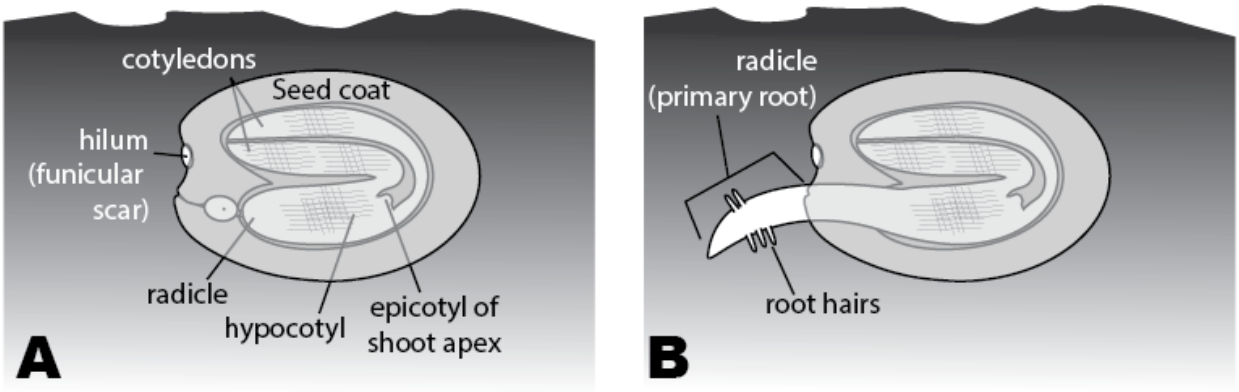


Fig A2. When the mature seed has been released from the fruit and onto or into the soil (**A**), and the right conditions have been met, germination (**B**) may occur.

Fig A3. The shepherd's-purse, Capsella bursa-pastoris of the mustard family, Brassicaceae. Illustration modified from that of Jacob Sturm (1771-1848). **a**, whole plant. **b**, single leaf from basal rosette. **c**, flower showing 4 white petals. **d**, flower in bud, side-view. **e**, flower's sepals from side, all other floral parts removed. **f**, single petal. **g**, six stamens and single pistil from side, petals and sepals removed. **h**, pistil from side. **i**, fruit. **K**, fruit longitudinally sectioned to reveal seeds inside. **l**, single seed with funiculus.

A. Embryogenesis Slide Series

Use the compound scope and prepared slides from the *Capsella* (shepherd's-purse) embryogenesis series. Each slide has longitudinal sections through a developing fruit (as in Fig A3). In each, multiple seeds will be sectioned, but only one or two seeds will be sectioned just right to see the parts of the developing embryo. You will have to search the slide for the right section.

Study Fig A1 first for orientation. Not all the stages are shown here because we want you to discover them yourself with the microscope.

Then, on the next page, attempt to locate and draw the embryo, suspensor, basal cell and the surrounding seed for the best section on each slide. As appropriate, assign each to one of the following stages, arranged below alphabetically:

- ○ Bending cotyledons
- ○ Globular
- ○ Heart
- ○ Mature embryo stage
- ○ Torpedo
- ○ Two-celled

1. Prepared slide: Precotyledon

Stage name?

What is the basal cell's size relative to other cells?

Is it possible to see the suspensor and/or basal cell? If not, why?

Approximately how many cells can you see in the embryo?

Drawing

2. Prepared slide: Early Cotyledons

Stage name?

Is it possible to see the suspensor and/or basal cell? If not, why?

Is shepherd's-purse a dicot or monocot? How can you tell?

Drawing

3. Prepared slide: Bending Cotyledons

Stage name?

Is it possible to see the suspensor and/or basal cell? If not, why?

Is shepherd's-purse a dicot or monocot? How can you tell?

Stage name?

Is it possible to see the suspensor and/or basal cell? If not, why?

Is shepherd's-purse a dicot or monocot? How can you tell?

Drawing

4. Prepared slide: Mature Embryo

Stage name?

Is it possible to see the suspensor and/or basal cell? If not, why?

Does the embryo look curved? If so, why do you think that is?

Drawing

II. From Seed to Seedling

A. Legumes

The **Legume family**, with 19,000-19,700 species (Heywood et al. 2007), is the third largest plant family (behind the sunflower and orchid families) and is known to scientists by any of two accepted taxonomic names, **Fabaceae** (fah-BAY-see-ee) or **Leguminosae** (lay-goo-meh-NO-see). Many members of the family are cultivated for their edible, protein-rich seeds or their edible entire fruit (Table B). Examples of leguminous plants cultivated for their edible (when immature) whole fruits include string beans, sugar-snap peas, and snow peas. Some also are cultivated for their oils, such as the peanut, or their nutritious seedlings ("sprouts"), such as alfalfa and various beans. Even entire plants are sometimes grown as fodder for livestock (e.g., soybeans and, as a major component of some hays, alfalfa and clover). The **pulses** comprise the subset of the family cultivated for their edible, dry seeds and include kidney beans, Lima beans, lentils, and peanuts among others.

Legumes are second, behind the grasses, in terms of importance to humanity for food. Whereas grasses in the human cuisine are valued primarily for their starch-rich grains, and only secondarily for oils and fiber, legume seeds or fruits in the human diet are valued primarily for their extremely high protein content, and secondarily for their oil content and carbohydrate content.

Table B. Some legume species and their native ranges.

Species (common name)	**Nativity, Origin of Domestication**
Arachis hypogaea (peanut)	Brazil, S America
Glycine max (soy)	E Asia
Lens culinaris (lentil)	E Mediterranean, SW Asia
Pisum sativa (pea)	E Mediterranean, SW Asia
Phaseolus lunatus (Lima bean)	Lima Peru & Vicinity, S America
Phaseolus vulgaris (common beans, Kidney beans)	Central America

1. Legume Seeds

a. Seeds come from ovules.

Seeds come from **ovules** and so it is not surprising that the shape and features of a seed can be related back to **homologous** features of the ovule. The ovule is attached to the ovary wall via its **funiculus** (Fig B1). The ovule consists of its **integuments** (2 in angiosperms, 1 in gymnosperms) that enclose the **embryo sac** containing the **egg**. The embryo sac is called as such because it is where the embryo will develop after the zygote forms from the fertilized egg. There is an opening or pore in the integument(s) called the **micropyle** near the egg of the embryo sac. Study the ovule and its parts in Fig B1 and then fill in the blanks in Fig B2 to name the surface features of a legume seed.

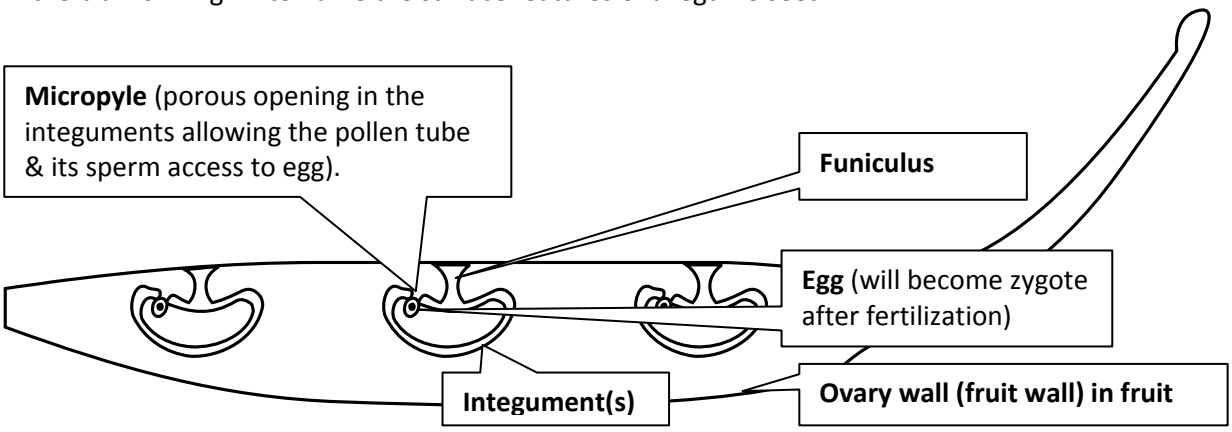


Figure B1. A longitudinal sectional view to the inside of the ovary to reveal the salient features of ovules.

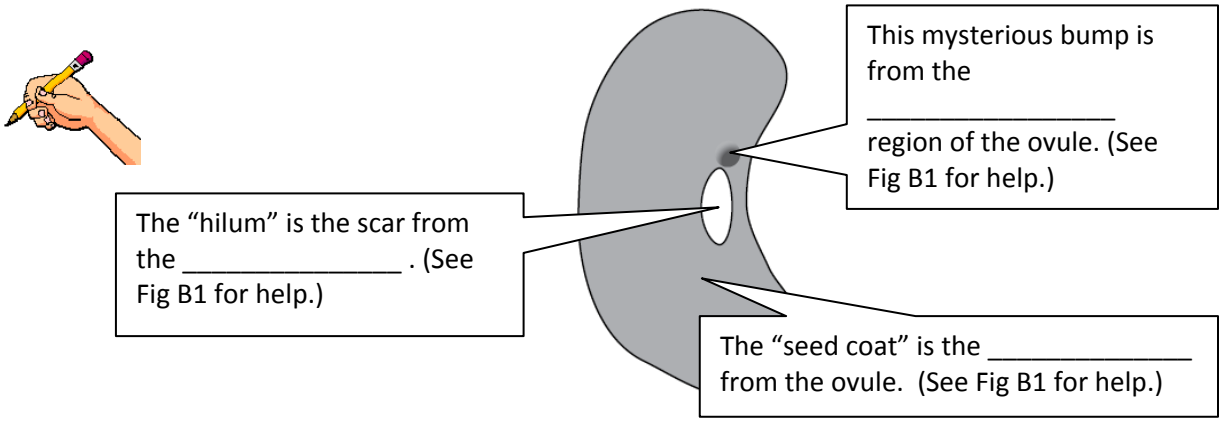


Figure B2. A typical kidney bean seed. Complete the label for these features after studying the labeled ovule depicted in Fig B1.

2. Legume Embryos as Examples of Dicot Angiosperm Embryos

a. Peanuts from the Side or Back of the Room. Wash & clean your hands. Then take a peanut fruit and crack it open, save one seed for the questions below and eat the other(s) if you like.

The saved seed: use your fingers to split the peanut embryo into its two natural "halves". One half will be one cotyledon, separated from the rest of the embryo, and the other half will be the second cotyledon with the embryo axis still attached.

Label the parts of the photograph below /next page with the following terms pertaining to embryo structure.

Seedcoat = outer cell layers of the seed; hard and protective in most seeds, papery and easily removed in the peanut.
Cotyledon = the "seed leaves", of which there are two in all legumes and dicot angiosperms, and part of the embryo; in legumes they are packed with protein, some starch and oils absorbed or produced during development from the nutrition derived from the endosperm, and they will supply nutrition to seedling upon germination.
Hypocotyl = the embryonic stem below the cotyledons.
Radicle = the embryonic root axis below the hypocotyls.
Plumule = the short shoot (including epicotyl and first true leaf primordia) above the cotyledons.
Cotyledon Scar = the scar left by a cotyledon on the rest of the embryo when you split the peanut in half and thereby tore the cotyledon off of the embryo.

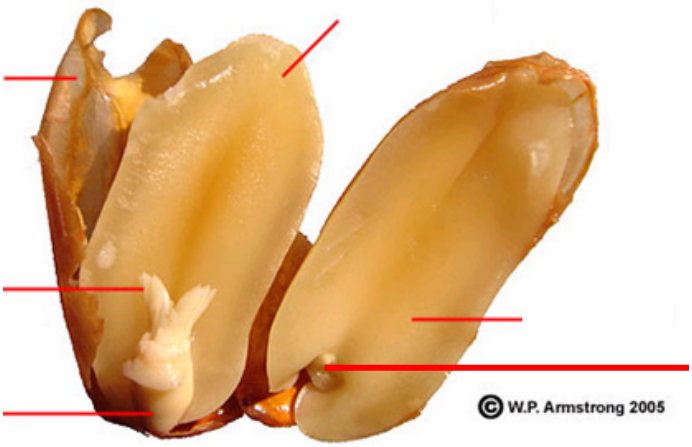


Figure B3. The inside of a peanut seed is all embryo. Label this figure.

b. Kidney Bean Embryos.

1-day Imbibed Seeds from side or back of room.

Imbibition (the taking on of water by a seed) is an important step that initiates **germination**. Examine kidney beans that have been soaked in water overnight. Tear off the seed coat, break off a cotyledon and compare and contrast these via drawings with the peanut. Draw and label them below as the peanut is above.

1) How is the seed and embryo structure in both species of legume similar?

2) Is the position of the embryo or attachment point and shape of the cotyledons different in the kidney bean relative to the peanut? How so?

3. Legume Seedlings as Examples of Dicot Seedlings.

a. Kidney (Garden) Bean Seedlings

Be sure you can identify all of the structures of a seedling. Since seedlings develop from embryos, the embryonic structures should be visible on the young seedling. Working in pairs, grab one each of 3 variously aged bean seedlings as follows: 6-day, 10-day and 14-day-old seedlings (from back of the room). Clean off dirt if any and take them back to your desk for drawing. Compare them to each other and to their embryonic forms. Use Raven's Fig 22-10 (p. 535) for help.

1) Observations & Drawings

When visible, be sure to label the

(1) seed coat
(2) 2 cotyledons,
(3) hypocotyl,
(4) radicle, and
(5) plumule (including epicotyl) on all three stages.

6-DAY 9-day 14-DAY

2) Emergence of the Seedling:

a) In the bean seedling, it is the _____ that elongates to raise the shoot out of the soil.

a) root b) hypocotyl c) epicotyl

b) How does the hook-like curvature of the hypocotyl facilitate the emergence and protection of the important shoot apical meristem through the soil?
c) On the 14-day old plant, can you still find the cotyledons on them? If so, what do they look like? If not, where'd they go?

B. Grasses

The grass family, with 10,000 species (Heywood et al. 2007), may not be the largest plant family but is globally the most economically important. It is known by scientists by any of two accepted taxonomic names, **Poaceae** (poh-EH-see-ee) or **Gramineae** (grah-MEN-ee-ee). Members of the family are widely cultivated: some for use of their whole plants or stems as fodder crops, straw, landscape ornamentals or lawn grass, or for construction materials (e.g. bamboos); the **cereals** are the subset of the family (or their grains) cultivated for their edible, starch-rich, entire fruits called grains (Table C). The cereals are prized primarily for a starch-rich tissue (endosperm) in the seed surrounding the embryo. Of secondary economic importance in the grain is the oil (e.g., corn oil) or products that can be derived from the starch such as ethanol (where the starch is converted into ethanol by a combination of enzymes and yeast) or corn syrup (where the starch had been synthetically converted into smaller saccharides such as maltose or fructose).

Table C. Cereals discussed in this lab manual and their native ranges.

Species (common name)	**Nativity, Origin of Domestication**
Avena sativa (oats)	Europe
Hordeum vulgare (barley)	SW Asia
Oryza sativa (common rice)	SE Asia
Triticum aestivum (bread wheat)	SW Asia
Zea mays (corn, maize)	Mexico
*Zizania aquatic*a (wild rice)	North America

1. Grass Fruits & Seeds.

The grain (i.e., fruit) of a grass is a particular type of fruit unique to the family called a "**caryopsis**." Only grasses have it. A caryopsis is a single-seeded, dry, **indehiscent** fruit in which the seed coat is fused to the fruit wall. **Cereal** grain species include wheat, maize (aka corn in the USA), rye, barley, oats, and rice among others.

a. Dissecting Scope and 1-day imbibed maize kernel from back/side of room.

What you commonly call the kernel of corn is actually the 1-seeded fruit (caryopsis). Here, imbibition has been allowed to occur for 1-day in order to soften the caryopsis and allow you to section it. Specifically, cut this in half lengthwise to view the parts of the fruit and seed (Fig C1). Use forceps to position for easy viewing with dissecting scope or make a thin section of the kernel if you have trouble with using the forceps to hold cut surface up for viewing with scope.

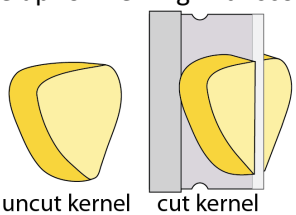


Fig C1. Cut a soft maize kernel in half longitudinally, perpendicular to broad face.

1) Draw and label the parts as follows: the (1) embryo (aka "germ"; in lower corner), (2) endosperm (most of seed), and (3) the fused seedcoat/fruit wall (aka "bran").

2) Add a drop of potassium iodide (I_2-KI) onto the cut section. This will stain starch purple.

What tissue is staining positive for starch? That is, what will be the young seedling's food source?

3) Look at an unsectioned, intact maize kernel: *Can you see the position of the embryo from the outside with the naked eye? Explain.*

2. Grass Embryos as Examples of Monocot Angiosperm Embryos

a. Compound Scope and Prepared Slide of "Zea (Maize) Embryo".

Observe this longitudinal section through a maize caryopsis with embryo, and let's add some detail to our observations above.

1.) Using Figure 22-12 in Raven's (p. 536), draw the sectioned caryopsis seen on the slide and label the parts of the fruit and embryo that are also listed below. Fill the space provided.

Parts of the embryo

1) **Cotyledon** (aka scutellum in grasses) = the single seed leaf.
2) **Coleoptile** = a sheath-like hood surrounding the plumule.
3) **Plumule** = the embryonic shoot apex containing the epicotyl, shoot apical meristem, and first non-cotyledonous leaves.
4) **Radicle** = the embryonic root.
5) **Coleorhiza** = a sheath-like cap covering the radicle.

Parts of the endosperm

Aleurone Layer = the outermost layer of the endosperm which is protein-rich, whereas most of the endosperm is starchy. During germination, the enzyme amylase is secreted by both the aleurone layer and the scutellum to break down the endosperm's starch into the sugar maltose to feed seedling development.

Draw the maize caryopsis anatomy here

3. Grass Seedlings as Examples of Monocot Seedlings.

Be sure you can identify all of the structures of a seedling. Since seedlings develop from embryos, the embryonic structures should be visible on the young seedling. Working in pairs, grab one each of 3 variously aged corn seedlings as follows: 4-day, 7-day and 12-day old seedlings (from back of the room). Clean off dirt if any and take them back to your desk for drawing. Compare them to each other and to their embryonic forms. Use Raven's Fig 22-11 (p. 536) for help.

1) Observations & Drawings

When visible, be sure to label the

(1) seed coat/fruit wall
(2) cotyledon (aka scutellum in grasses),
(3) hypocotyl/radicle,
(4) plumule (including epicotyl),
(5) coleorhiza
(6) coleoptile.

4-DAY 7-DAY 12-DAY

2) Emergence of the Seedling:

a.) In the corn seedling, it is the _____ that elongates to raise the shoot out of the soil.

a) root b) hypocotyl c) epicotyl

b.) Suggest a functional role for the coleoptile and coleorhiza (Hint: think sheathing and protection, but of what and when?).

c.) Where is the single cotyledon during germination? What is its role?

d.) Use Fig 22-11 in Raven's and your own observations of live seedlings to determine if adventitious roots form on the seedling and what happens to the primary root.

e) On the 12 day old plant, can you still find the coleoptile on it?

4. Wheat Flour vs. Flours from Other Cereals.

Two unknown flours in side/back of room, labeled “Flour A” and “Flour B”.

Put your microscopes away. You are done with them and you do not want to get them dirty in this next exercise.

- Take about 100 ml of flour each from “A” and “B” in two different 100 ml beakers back to your bench.
- Remove about 60 ml of flour and place it on your table, and add about 16 ml of water to the remaining 40 ml of flour in the beaker.
- Stir until you work it into a sticky mass.
- Coat your hands and the dough ball with the extra dry flour so you can remove the dough from the beaker and knead into a ball repeatedly without it sticking to your hands or the table.
- Once well kneaded, try to flatten each type into a small, flat pizza-like shape about 4 inches diameter.

a. Which type was more elastic?

b. Which type was easier to spread flat without it coming apart?

c. What is the identity of your unknown flours? The two are wheat and rice, yet it is only wheat that has a special protein in the endosperm called gluten. Gluten is what gives wheat dough its elastic properties and is the reason wheat is preferred for making leavened breads, cakes, etc. Explain how this works?

C. Radishes

Return to the *Introduction to Botany* lab and perform the Week 1 exercises regarding radish seedling development.

III. Further Exercises & Summary Questions

A. Interpreting Seed Morphology

Dry Legume Seeds from the back of room. Examine any one of the red or darker colored legume seeds available in beakers or dishes. The most conspicuous features common to all of these seeds are their **seed coat** (which comes in a variety of colors), and two other structures identified as "unknowns" in Fig B2. After having completed the labeling in Fig B2, be sure you can find these structures on these real seeds.

B. Summary Questions

1. What is a cereal and which lab page did you find the definition?

2. What is a pulse and on which page did you find that definition?

3. What is the technical definition of a legume fruit and on which page...?

4. Which, the cereals or the pulses, are the most important to humanity in terms of total calories consumed?

5. Which had more abundant starch: cereals or pulses?

6. Which has more abundant protein: cereals or pulses?

7. How does the legume seed differ from the grass (maize) seed with respect to endosperm at maturity?

8. How does the legume embryo differ from the grass embryo with respect to cotyledon number and presence or absence of the coleoptile & coleorhiza?

9. How does the legume compare and contrast with the caryopsis? Complete the table below.

Table D1. Comparison of legumes and caryopses.

Fruit Type	***Dry or Fleshy @ Maturity ?***	***Seediness*** *(1 or more than 1?)*	***Seed Coat Fusion*** *(fused to fruit wall or free from fruit wall)*	***Dehiscence*** *(dehiscent or indehiscent)*
Legume *(bean family)*				
Caryopsis *(grass family)*				

10. Can you draw and arrange, in sequence the stages of embryogenesis in *Capsella?*

11. Can you draw and label a legume seed & embryo, a corn caryopsis & embryo?

12. Which embryonic structure is the first to emerge from the seed upon germination?

IV. References

Heywood VH, RK Brummitt, A Culham, O Seberg. 2007. *Flowering Plant Families of the World*. Firefly Books: Ontario, Canada.

Thomé OW. 1885. *Flora von Deutschland, Österreich und der Schweiz.* Gera, Germany.

V. Credits

This lab was developed by Christopher Hardy. You may cite it as....

Hardy CR. 2016. Seeds and seedlings. Pp. 23-42 in CR Hardy, RL Wagner (eds) *Guide to Exercises in Concepts of Botany, 4th Edition*. Millersville, Pennsylvania, USA.

VI. Glossary

Bran = the vernacular for the hard wall of a cereal grain, formed by the fusion of the seed coat and fruit wall.

Caryopsis = the 1-seeded fruit of a grass (family Poaceae) that is dry and indehiscent at maturity and has the seed's coat fused to the fruit wall.

Cotyledon = the "seed leaves", of which there are two in all legumes and dicot angiosperms, and part of the embryo; in legumes they are packed with protein, some starch and oils absorbed or produced during development from the nutrition derived from the endosperm, and they will supply nutrition to seedling upon germination.

Cotyledon Scar = the scar left by a cotyledon on the rest of the embryo when you split the peanut in half and thereby tore the cotyledon off of the embryo.

Dehiscent = a structure such as a fruit that naturally opens are maturity, releasing its contents.

Dormancy = the phase of a seed plant's life when, as an embryo in a seed, it remains alive but does not grow. This period will end with germination.

Embryo = the baby plant inside a seed.

Embryogenesis = formation of the embryo of a plant from a zygote.

Endosperm = the nutritive tissue of an angiosperm seed.

Epicotyl = the primordial stem above the cotyledon(s) on an seed plant embryo.

Funiculus = the stalk attaching an ovule to the ovary or fruit wall.

Germ = the vernacular for embryo in a cereal grain.

Germination = the breaking of a seed's (embryo's) dormancy; visually marked by the emergence of the radicle.

Hilum = the scar on a seed that is left by funiculus.

Hypocotyl = the embryonic stem below the cotyledons.

Imbibition = the hydration of a seed that is necessary to precede germination.

Indehiscent = a structure such as a fruit that does not open at maturity.

Legume = the fruit type of plants in the bean family, Fabaceae, that is derived from a simple pistil and, at maturity, is dry and dehisces along two sutures.

Ovule = the structure in an ovary that contains the egg. Upon fertilization, the ovule will develop into the seed.

Fruit wall = the fruit wall; derived from the wall of the flower's ovary.

Pedicel = the stalk of a flower.

Plumule = the short shoot (including epicotyl and first true leaf primordia) above the cotyledons.

Radicle = the embryonic root axis below the hypocotyls.

Seedcoat = outer cell layers of the seed; hard and protective in most seeds, papery and easily removed in the peanut.

Spermatophyte = a plant (or species) that produces seeds as part of its lifecycle: an angiosperm or gymnosperm. Synonymous with "seed plant."

Zygote = the single cell formed following fertilization.

Primary Morphology

Morphology is study of the form and structure of organisms. The word comes from the Greek *morphé*, for "form", and *lógos*, for "study". Morphology can also be used in a slightly different, yet related sense, as the actual structure, form or appearance of an organism. An example of this latter use is given by way of a sample quiz question: "Inspect the plant on your desk and provide a written description of the morphology of the stem and leaves." The correct answer to this would be to describe (in technical detail of course) what the plant's stems and leaves look like. In this lab today, we will restrict our attention to angiosperms, since they comprise the bulk of both plant species and plant biomass in the terrestrial ecosystems in which we live.

Table of Contents for this lab:

Note: Your instructor may ask you to make observations of the radish seedlings you planted in the first lab, particularly the young root and root hairs.

A. Basic Shoot Study

The vegetative shoot consists of the two organs, stem and leaf. Each leaf is attached to the stem at a node, an axillary bud is formed in the axil of each leaf, and the stretch of stem between two successive nodes is called an internode. All cells and organs of the primary plant body ultimately arise from the shoot apical meristem. The tip of a shoot that contains the apical meristem and leaf primordia and axillary bud primordia is called the shoot apex. Typically the shoot apex is very short because the internodes between leaf primordia have not yet elongated.

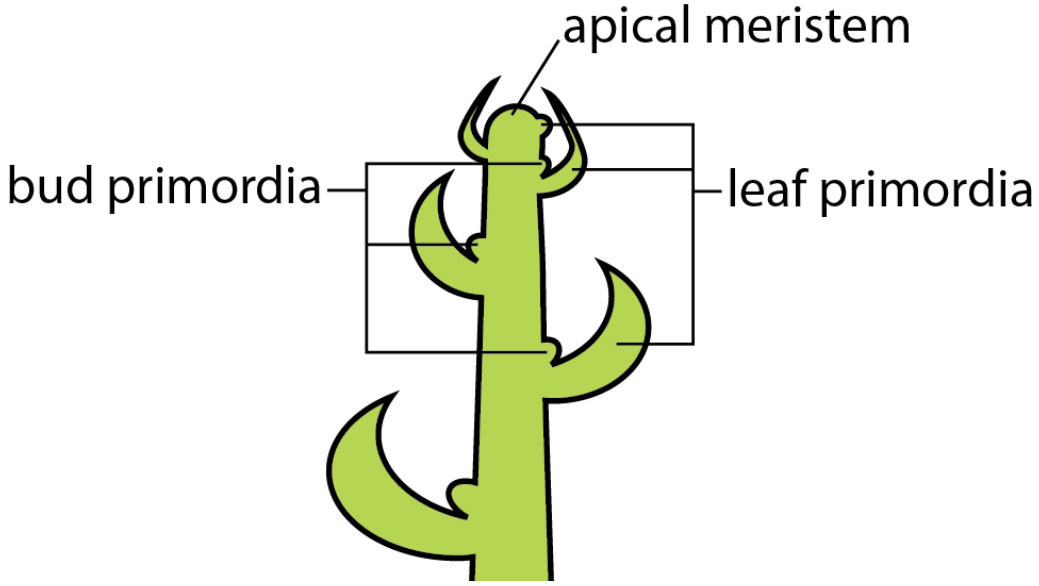
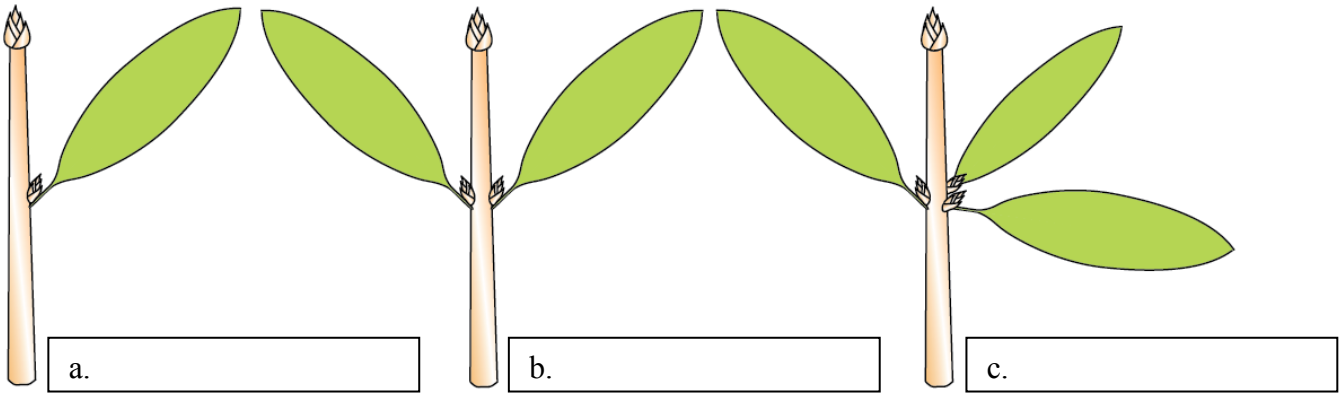


Figure above: A stylized, expanded diagram of a shoot apex.

1. Phyllotaxy & Leaf Arrangment.

Leaves can be arranged on a stem in one of three ways. Alternate phyllotaxy is where there is just one leaf per node, opposite phyllotaxy is two leaves per node, and whorled phyllotaxy is where there are 3 or more leaves per node.

a-c. Label the 3 drawings below as either alternate, opposite, or whorled, as appropriate.



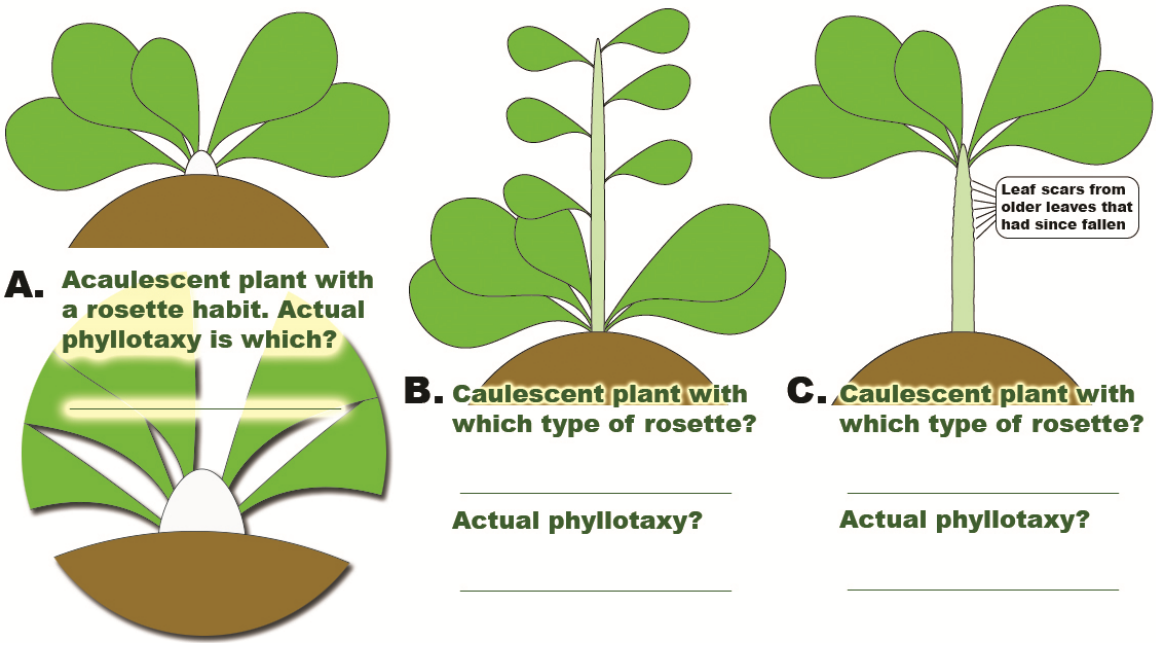
d. The relationship between the number of leaves at a node and the number of axillary buds.

1) *If there are two leaves per node, how many axillary buds will you find associated with these leaves at a given node?*

2) *How many axillary buds if there are five leaves per node?*

e. Rosettes are clusters of leaves arising from nodes separated by very short internodes.

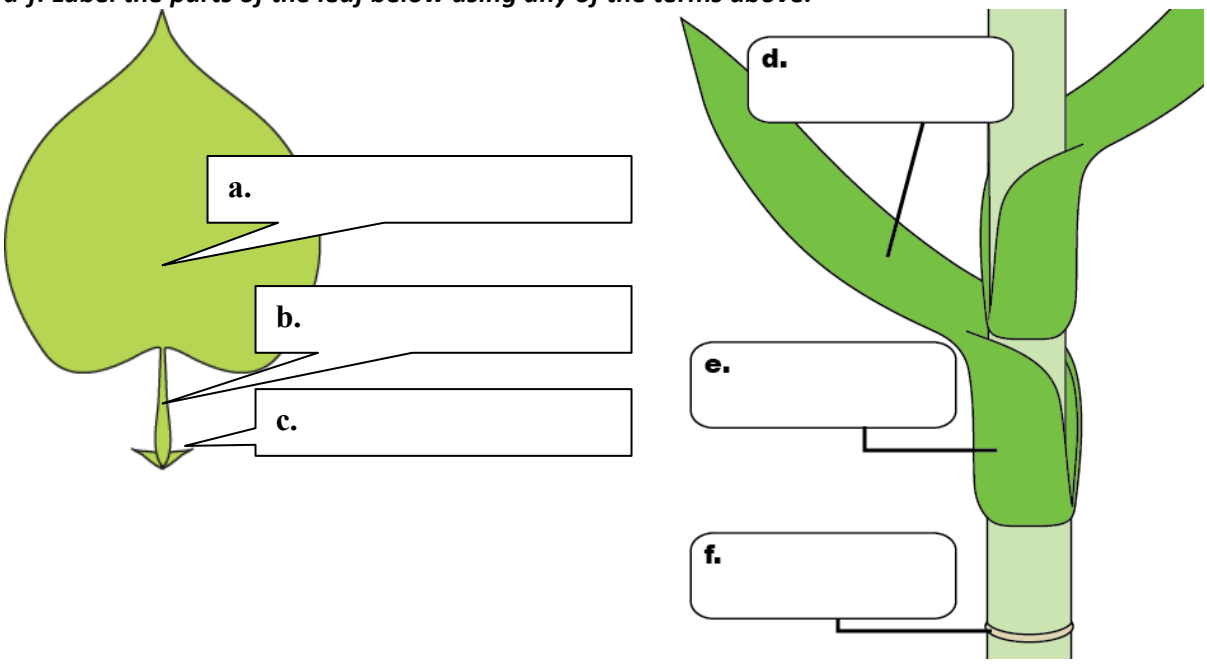
If the plant is caulescent (i.e., has a well developed above-ground stem, like most plants), then one can distinguish between basal rosettes and terminal rosettes. If the rosette is on an acaulescent plant, then the plant as a whole is said to have a rosette habit and the term "terminal" vs. "basal" are not typically applied. Answer the following questions, A-C.



2. Parts of a Typical Leaf.

Many leaves are differentiated into a blade (flattened, expanded portion) and petiole (i.e., "stalk"; a petiolate leaf has a petiole, a sessile leaf lacks one). Stipules (a small pair of appendages) may also be present at the base of a leaf or seemingly on the stem at the node – the stipules are always considered part of the leaf, however (the leaf is stipulate when present, exstipulate when lacking). Whereas most generally attach to the stem on one side, the leaves of many monocot angiosperms (e.g., grasses, lilies, orchids, aloes) lack a true petiole and have a sheathing leaf base (i.e., the leaf base sheathes the stem for some distance) that is attached to the stem in a circular manner, completely around the node. In this latter case of circular leaf bases, one would find that when such leaves fall off, they leaf circular leaf scars.

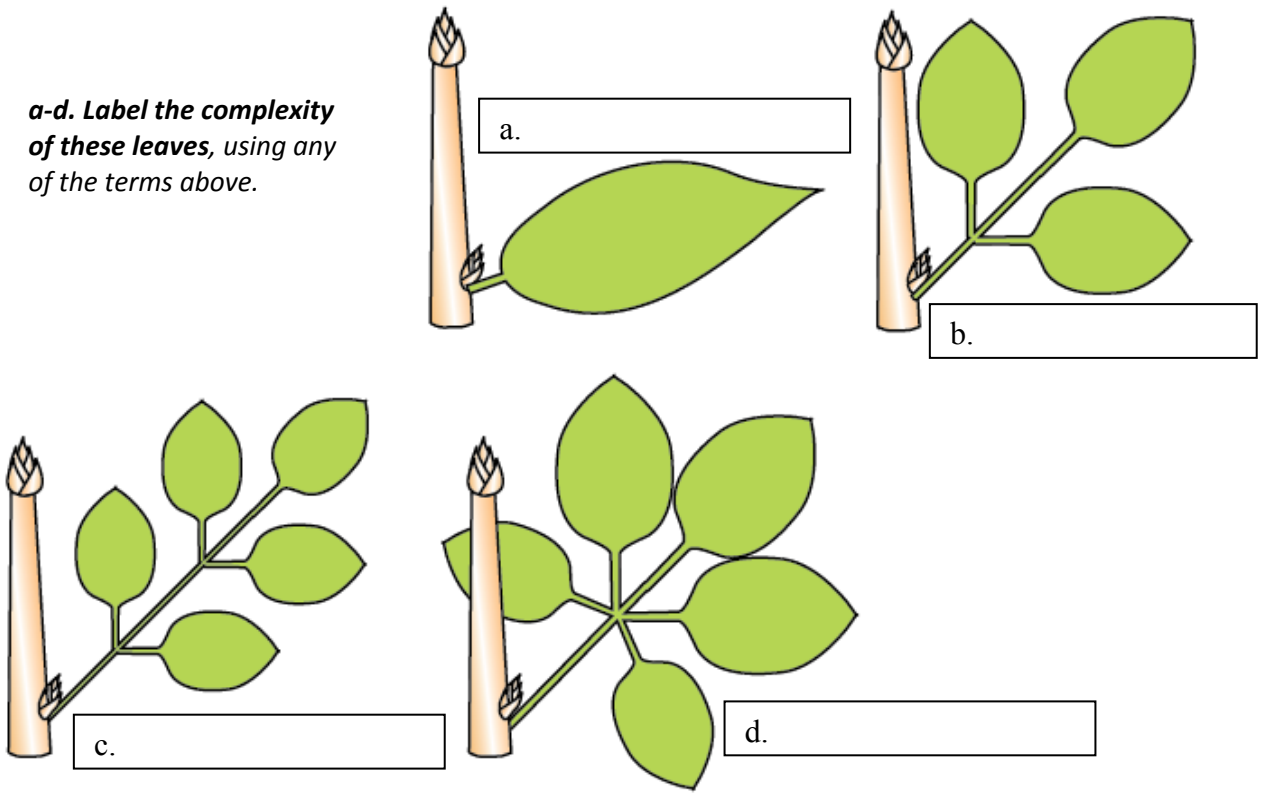
a-f. Label the parts of the leaf below using any of the terms above.



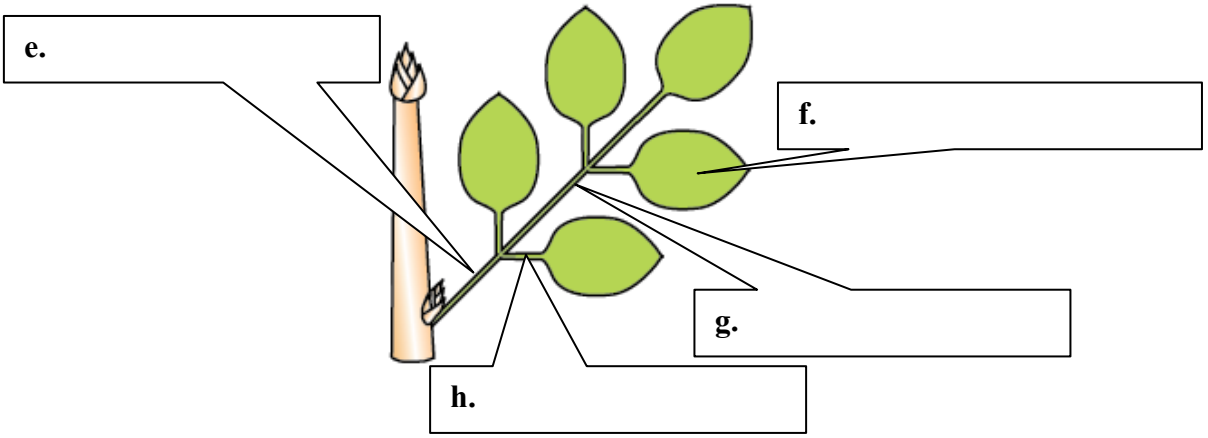
3. Leaf Complexity.

Leaves may either be simple (one blade) or compound (subdivided into more than one blade called leaflets). Compound leaves may be trifoliate (divided into 3 leaflets), pinnate (>3 leaflets arranged laterally along one central axis called a rachis), or palmate (>3 leaflets all radiating from a single point). Compound leaves may be once, twice, or more pinnately or palmately compound (e.g., 2-pinnate, also called bipinnate). Compound leaves are typically petiolate, and the petiole is the leaf stalk between the stem and the first leaflet. After the petiole, the main leaf axis distal to the first leaflet of a pinnately compound leaf is called the rachis. A secondary or higher order rachis on a bipinnate or higher-pinnate leaf is called a rachilla (plural rachillae). The stalk (if any) of a leaflet in a compound leaf is called a petiolule.

***a-d. Label the complexity of these leaves**, using any of the terms above.*

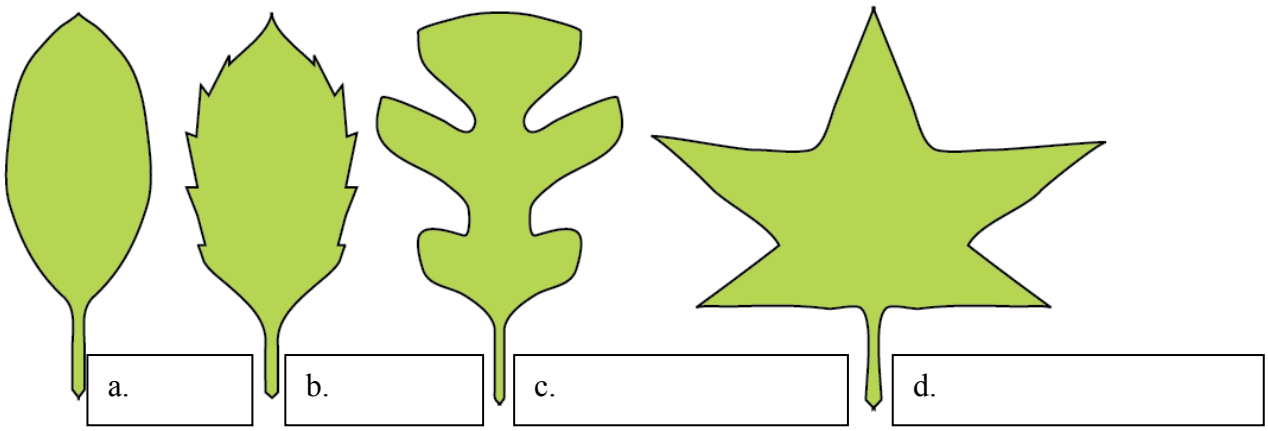


***e-h. Label the parts of the compound leaf below**, using any of the terms above.*



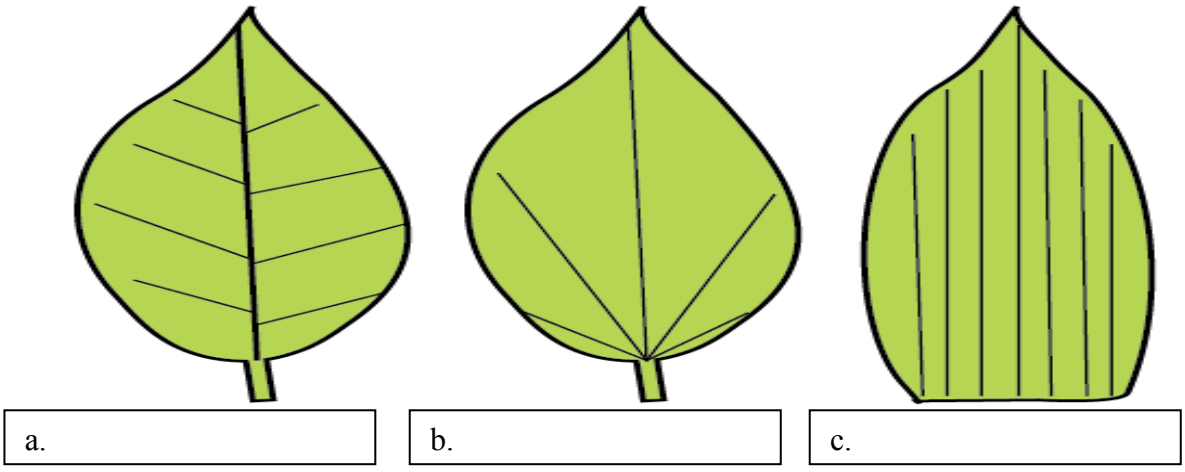
i. Describe the phyllotaxy of the plant above in e-h. _____

4. Leaf/Blade Margins.
Blade margins may be entire (no teeth or lobes), toothed, or lobed. Lobed margins may be pinnately lobed (lobes arranged laterally along an imaginary central axis), or palmately lobed (lobes radiating from more or less a single point).



5. Venation.
The *main* or *primary* vascular bundles (i.e., plant "veins") of leaves may be arranged in various patterns. Pinnate venation has a central midvein with lateral secondary veins, palmate venation is where there are multiple main veins radiating from a single point at or near the blade base, and parallel venation is where the main veins run more or less parallel to each other. The secondary or tertiary veins off of the primary veins of the pinnate and palmate types also often appear to be reticulate, and so sometimes they are more generally referred to as "reticulate venation" or "net venation" in contrast to "parallel venation".

a-c. Label the diagrams below appropriately.



6. Live Shoot Practice Quiz.
Move around the room and examine the leaves of the plant specimens provided and determine the phyllotaxy, stalking, stipule presence, complexity, margin, and venation of each. Record your observations of the leaf morphology below:

Plant Name	**Phyllotaxy** (alternate, opposite, whorled, or basal or terminal rosette)	**Stalking** (petiolate or sessile)	**Stipules** (stipulate or exstipulate)	**Complexity** (simple, trifoliate, 1-pinnate, 2-pinnate, palmate)	**Margin** (entire, toothed, pinnately lobed, or palmately lobed)	**Venation** (pinnate, palmate, parallel)
a.						
b.						
c.						
d.						
e.						
f.						
g.						
h.						
i.						
j.						
k.						

More Space for Additional Specimens if Available						
Plant Name	**Phyllotaxy** (alternate, opposite, whorled, or basal or terminal rosette)	**Stalking** (petiolate or sessile)	**Stipules** (stipulate or exstipulate)	**Complexity** (simple, trifoliate, 1-pinnate, 2-pinnate, palmate)	**Margin** (entire, toothed, pinnately lobed, or palmately lobed)	**Venation** (pinnate, palmate, parallel)

7. Functions of Leaf

The primary function of the typical leaf is photosynthesis, a function/process which requires light capture and extensive gas exchange with the environment. Explain how the morphology of the leaf facilitates this.

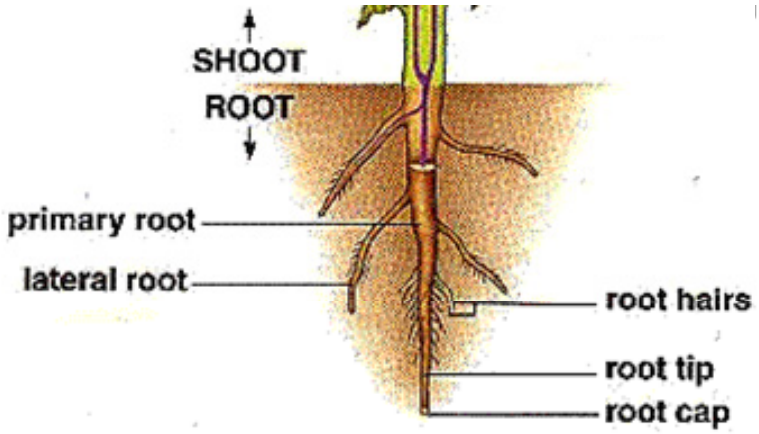
8. Functions of Stem

The primary function of the typical stem is not photosynthesis, but rather support and elevation of the leaves in a position for optimal photosynthesis. Furthermore, the stem is the conduit for conduction of water plus minerals from the roots to the leaves, and sugars from the leaf to the roots. Explain how the shape and form of the typical stem facilitates at least some of these functions.

B. Basic Root Study

1. Structure

Unlike the shoot, the root has no nodes or internodes. A single root consists simply of a cylindrical axis with a root apex (commonly called root tip) consisting of the root apical meristem, juvenile cells derived from the apical meristem, and root cap distally. This root apex is not very long (e.g., ≤1 cm). Just proximal to the apex is the root hair zone, which can be 1-4 cm in length. Root hairs are unicellular, filament-like protuberances from the surface of the root. They greatly increase the surface area through which water and minerals can be absorbed. Root hairs have a limited lifespan and soon wither away as more are produced in younger, more distal tissue that has developed and is maturing. More proximal, then, to the root hair zone along a root's axis is older root that is devoid of root hairs, contains lateral or secondary roots, and may become woody in shrubs and trees.



a. Working in pairs, obtain a germinated radish seedling that you had planted in your first lab and examine it. Use a dissecting scope for close observations at the tip. Draw the root and label the root hairs and the root cap.

What is the function of the root hairs?

Do the root hairs extend to the very tip? Explain why they do or why they don't.

Can you discern the root cap?

In many species, the primary root usually develops as a prominent taproot, which may give rise to lateral roots. This system, in which the primary root is dominant in size, is called a taproot system. In other species, the primary root or any single root is not dominant and the system is more homogeneous in appearance. This is called a fibrous root system. There are other species having systems with characteristics of both.

b. Label the drawings below as appropriate.

1.

2.

c. Visit the living or dried herbarium specimens and describe each type below as either tap-, fibrous-, or intermediate in their root system morphology.

Plant name	***Tap-, Fibrous-, or Intermediate Root System***	***Speculate on the adaptive value of this particular root system***

2. Functions

The root has the primary functions of **ABSORPTION** of water plus minerals, **ANCHORAGE** to the soil / substrate, and **STORAGE** of food (typically in the form of starch). Of course, the vascular system of the root must also **CONDUCT** (transport) the water plus minerals it absorbs to the shoot. In the spring, the sugars stored in the root must also be conducted (transported) to the shoot to fuel the growth of new leaves.

The distribution of functions is primarily as follows:

- the root apical meristem provides new cells to the growing root;
- the root cap protects the root apical meristem and secretes a lubricant (mucigel) that also hosts beneficial (nitrogen-fixing) bacteria;
- the root hair zone absorbs most of the water and minerals; it also aids anchorage;
- the older root proximal to the root hair zone gives rise to lateral roots, and its continued function is largely that of anchorage and food storage.

3. Adventitious Roots

Adventitious roots are roots that arise from sections of stem tissue, rather than from other root tissue. In many plants, whenever a stem lays horizontal, along the ground, adventitious roots will form on the stem that is in contact with the soil. In many vines, adventitious roots form on the stem that is in contact with the tree or other substrate that is being climbed by the vine.

When these adventitious roots do not go into the soil for whatever reason, they are sometimes called aerial roots. When they function to help support the stem or prop it up from the ground, they may be called prop roots.

a. Observe the adventitious roots of living *Hedera* (English ivy) specimens in the room.

1) *Do they arise from the internodes or nodes?*

2) *These plants typically grow as climbing vines. How do adventitious roots facilitate this type of growth form?*

b. Observe the adventitious roots of the living *Anthurium* (anthurium, flame-lily) and/or *Philodendron* (philodendron) specimens in the room.

1) *What structures anchor these plants to their host trees as they climb to the rainforest canopy?*

2) *What do you think the diamond-shaped scars on the stem are?*

c. Observe the adventitious roots of the dried *Zea* (maize, corn) plant in the room.

1) *What do these roots do for the plant?*

2) *What would you call them?*

C. Radish Morphology at 2 Weeks

Return to the *Introduction to Botany* lab and perform the Week 2 exercises regarding radish development.

D. Further Exercises

1. Visit the coconut plant in the stairwell area between Roddy and Caputo Halls. Apply your accumulated taxonomic knowledge from lab 1 and your morphological knowledge from this lab to answer the following.

What is the scientific name for this species:

What is the scientific and common names for the family that it is in:

Is the plant a bryophyte, pteridophyte, gymnosperm, or angiosperm?

Use your book or the "Introduction to Botany" lab to give me the phylum and class to which the coconut palm belongs:

Describe the general habit of the plant:

1) Phyllotaxy (apply all terms that apply):

2) Leaf stalking:

3) Leaf complexity:

4) Blade margins:

5) Blade venation:

6*) Does the leaf have a rachis?*

2. Trichomes (living or herbarium specimen materials)
Trichomes are plant hairs. Organs that lack them are said to be glabrous, whereas organs that have them are said to be pubescent. Where present, they arise from the epidermis of the shoot organs as multicellular protuberances that range from being uniseriate (one, unbranched cell file) to branched. Some are even glandular – usually secreting substances from a bulbous distal cell that is sticky to the touch.

Use the dissecting scopes at the trichomes station to make a study of the leaves and/or stems with trichomes present. Try to draw a single trichome from each specimen, noting the form of the trichome.

3. Details of alternate- and opposite-leaved plants.

a. Spiral vs. distichous alternate phyllotaxy. By definition, alternate phyllotaxy is where there is one leaf per node. However, alternate leaves may be either spirally arranged (spiral) or distichously arranged (distichous) along the stem. Look up these terms in the Glossary (Appendix 1) if need be.

Find examples of spiral vs. distichous, alternate phyllotaxy from among the plants at the stations referenced in Exercise 6 of Part A above.

Plants with Spiral Alternate Phyllotaxy	**Plants with Distichous Alternate Phyllotaxy**

Which form of alternate did you find to be more common? *This reflects the norm in nature. Provide a biological/ecological explanation for this being the norm.*

b. Decussate vs. distichous opposite phyllotaxy. Most opposite leaves may be arranged in a decussate pattern, where the pair of leaves at the higher node is rotated 90 degrees relative to the pair at the preceding lower node. Alternatively, one could hypothetically have all the pairs arranged in the same single plane – a distichous, opposite pattern.

Find the plants with either of these two types in the room and list below.

Plants with Opposite, Decussate Phyllotaxy	**Plants with Opposite, Distichous Phyllotaxy**

Which form of opposite did you find to be more common? *This reflects the norm in nature. Provide a biological/ecological explanation for this being the norm.*

4. Ask your instructor if sufficient material is available and, if so, make your own adventitious roots using a purple-heart plant (*Tradescantia pallida*) or other plant indicated by your instructor.
Take a stem cutting that has at least three leaves along the stem. Place the cutting in a cup containing moist vermiculite, peat, or a beaker of water so that the cut end plus about two nodes are covered by the wet medium. Label the cup/cutting with your name and the date. Keep them watered in weeks to come until roots form. Then take them home and start growing your own.

a. For Consideration

1) *When the cutting has finally rooted, where did the adventitious roots arise from? Nodes, internodes or both?*

5. Comparative Morphology of the Shoot (living material)
Find the plants of *Tradescantia pallida* (purple-heart, setcreasea) and *Tradescantia spathacea* (rhoeo or moses-in-the-cradle) in the room.

a. Note that they are in the same genus, are closely related, and therefore share things in common such as precise phyllotaxy, leaf venation, blade margin, coloration (partially) and leaf base form, etc. Describe these similarities below.

b. Despite their close relationship, however, their growth forms (habits) look very different due to what?

c. Draw a section of mature stem in both species that contains 3 nodes: side by side.

d. Peel back one leaf on each plant: locate the axillary bud(s). How many axillary buds do you find associated with one leaf at a given node?

e. How does internode length change from the base to the tip?

6. Go outside with clippers and cut off a 10-15 cm length of shoot on a broadleaf, evergreen tree or shrub (e.g. holly).

a. Draw this and label the vegetative organs using terms learned in this lab.

b. Describe the morphology of this shoot using terms and concepts from this lab.

E. Credits

This lab was developed by Christopher Hardy. You may cite it as follows:
Hardy CR. 2016. Primary morphology. Pp. 43-60 in CR Hardy, RL Wagner (eds) *Guide to Exercises in Concepts of Botany, 4th ed*. Millersville, Pennsylvania, USA.

F. Glossary of some of the terms used in this lab

Acaulescent = literally "stemless", referring to the habit of a plant wholly without or at least without a conspicuous above-ground stem. Contrast with Caulescent.
Alternate Phyllotaxy = one leaf per node.
Apical Meristem = a localized region of perpetual cell division; the mound of perpetually young, meristematic cells just beneath the root cap in the root and above the youngest leaf primordium in the shoot.
Axillary Bud = a bud in the axil of a leaf, with the potential to develop into a lateral shoot system (a branch) or, in angiosperms for example, a flower, depending upon the position in the stem and the time of year.
Caulescent = literally "stemmed", referring to the habit of a plant with a well developed above-ground stem. Contrast with Acaulescent.
Distal = towards the end or tip of an axis or organ. Opposite of proximal.
Distichous Phyllotaxy = a type of alternate phyllotaxy where the leaves occur on one side or the other of a stem, but always in the same imaginary plane. Less common generally, but very common in grasses.
Glabrous = a surface lacking trichomes.
Habit = the growth form of a plant; e.g. herbaceous, woody, tree, shrub, rosette, etc.
Internode = the stretch of stem between two successive nodes.
Leaf Scar = the scar on a stem where a leaf had once been attached.
Leaf Sheath = the base of a leaf that ensheaths the stem to which it is attached.
Node = the point of leaf attachment on a stem.
Opposite Phyllotaxy = two leaves per node.
Phyllotaxy = the arrangement of leaves on a stem.
Primordium (plural, primordia) = a very young, embryonic organ such as that of a leaf or axillary bud.
Proximal = towards the base of an axis, organ, or appendage. Opposite of distal.
Pubescent = a surface possessing trichomes.
Root Apex = the tip of a root that includes the root cap, root apical meristem, and juvenile cells derived from this.
Shoot Apex = the tip of a stem that includes the shoot apical meristem, juvenile cells derived from this, and leaf and axillary bud primordia.
Spiral Phyllotaxy = a type of alternate phyllotaxy in which the leaves seem to follow a phyllotactic spiral around and up the stem. The most common type of alternate phyllotaxy.
Trichome = a plant hair.
Whorled Phyllotaxy = 3 or more leaves per node.

Primary Anatomy

Anatomy is the study of the internal structure of organisms. An anatomical study may range from the level of the cell, to tissues, to whole tissue systems in plants. The word "anatomy" comes from the Greek *anatomia*, which is derived from *ana* (separate, apart from) and *temnein* (to cut open). Anatomy can also be used in a slightly different, yet related sense as a noun - as the actual internal structure of an organism or organ. An example of this latter use is given by way of a sample quiz question: "Use your microscope and the prepared slides of the spruce needle to detail the anatomy of a spruce needle." The correct answer to this would be to describe (in technical detail of course) what the cellular and histological structure of the needle looked like. In this lab today, we will restrict our attention to seedplants (gymnosperms and angiosperms), since they comprise the bulk of both plant species and plant biomass in the terrestrial ecosystems in which we live.

Table of Contents for today's lab:

A. Tissues & Tissue Systems

Plants have three tissue systems: dermal, ground, and vascular tissue systems. In the primary plant body, the dermal system consists of the tissue called the epidermis; the vascular system consists of vascular tissues typically arranged in fascicles or bundles (i.e., vascular bundles); and the ground tissue system consists of all other tissues between the vascular and dermal tissues.

1. Dermal Tissue System Basics

The dermal tissue system comprises the external cell layer of the plant and has a function similar to our skin in that it provides a physical separation from the environment. Only a single tissue makes up the dermal tissue: the epidermis. In the root this tissue is simple, consisting primarily of one cell type. In the shoot, this tissue is generally complex in that various cell types can be present, such as guard cells of stomatal complexes, the cells of trichomes, etc.

a. Study the upper (adaxial) and lower (abaxial) epidermis of a rhoeo (*Tradescantia spathacea*) leaf by making an epidermal peel and wet mount of each.

During examination, wear gloves or simply wash your hands before touching your face or eyes since crystals in this plant irritate some people.

1) Start with the abaxial epidermis, which is the purple and lower one in this case. *Draw and label one stoma and its stomatal complex of guard cells and 4 subsidiary cells surrounding it. Subsidiary cells are part of the stomatal complex in some species and may help to regulate the opening and closing of the stoma. Note how the subsidiary cells are a different shape and appearance than the guard cells and surrounding regular epidermal cells.*

Notice the frequency of stomata: abundant, rare or absent?

Draw a couple of regular epidermal cells. Contrast the shape of a rhoeo epidermal cell from that of the onion epidermis seen earlier or in a previous lab.

2) Move to the adaxial (upper) epidermis, which is green in this case.
Contrast the frequency of stomata here with that in the lower epidermis. Are they abundant, rare or absent on the lower surface?

2. Vascular Tissue System Basics

The vascular tissue has the role of long distance transport of nutrients and water throughout the plant. There are two different types of vascular tissue, xylem and phloem, both of which are complex tissues.

XYLEM (Use Figure 1 in this packet, figures in your text or on posters in the lab to help with this)

TRACHEIDS – Water-conducting cells found in all vascular plants. Cells dead at maturity, with secondarily-thickened and lignified, pitted walls and tapered ends. They also function in support. In sections stained with both blue and red, these tend to stain red due to lignin content.

VESSEL ELEMENTS – Water-conducting cells found only in angiosperms. Cells dead at maturity and variously secondarily-thickened and lignified; broad empty, tube-like cells connected end-to-end to form **VESSELS**. The end plate of a vessel element is called a **PERFORATION PLATE**. These cells also function in support. In sections stained with blue and red, these tend to stain red due to lignin content.

XYLEM FIBERS--Very long cells with very thick, secondarily thickened, lignified walls; dead at maturity. Provide support. In sections stained with blue and red, these tend to stain red when heavily lignified.

Xylem PARENCHYMA—Relatively unspecialized, thin-walled cells. Rather more abundant in the ground tissue. Living at maturity. Stain blue.

PHLOEM (Use Figure 1 in this packet, figures in your text or on posters in the lab to help with this)

SIEVE CELLS -- The sugar-conducting cells of pteridophytes and gymnosperms. Living at maturity. In sections stained with blue and red, these tend to stain blue. Porous patches in the walls between two adjacent sieve cells if called a **SIEVE AREAS**.

SIEVE TUBE MEMBERS – The sugar-conducting cells of angiosperms. Living at maturity. Tube-shaped cells connected end-to-end in **SIEVE TUBES**. In sections stained with blue and red, these tend to stain blue. Sieve areas are present on these, but most phloem fluid flows vertically from member to member through **SIEVE PLATES**.

COMPANION CELLS—Parenchyma cells associated with and facilitating the function of sieve tube members. Stain blue.

PHLOEM FIBERS—fibers sometimes form amidst or next to the sieve tube members in some species. Similar in appearance to fibers of the xylem or ground tissue sclerenchyma. Stain red if heavily lignified.

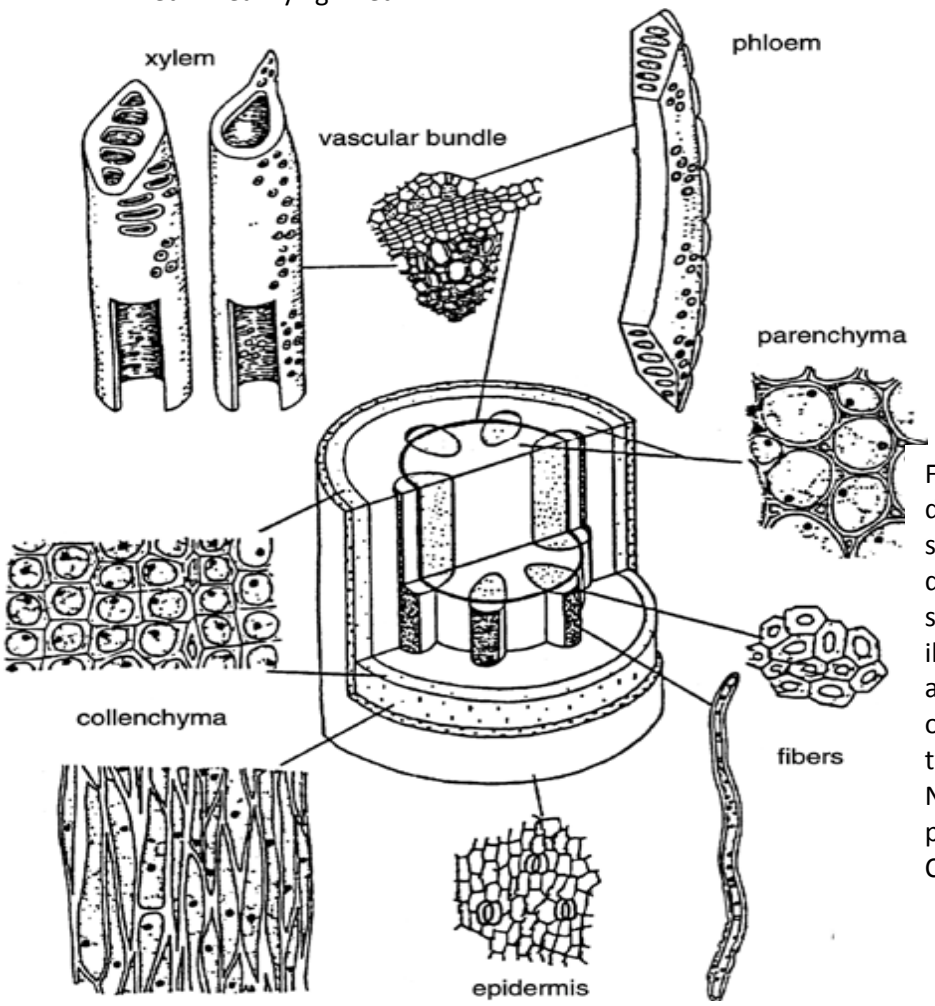


Fig. 1. Idealized three-dimensional diagram of sections through a typical dicot stem (with no secondary growth), illustrating the locations and general morphologies of various cell and tissue types. (Adapted from Niklas, *Plant Biomechanics*, p. 267, University of Chicago Press, 1992).

a. Xylem Observations: Oak or Magnolia Maceration (prepared slide).

A maceration is where the middle lamellas holding together the cells of a tissue are digested away to separate the cells. You should be able to find all four types of xylem cells in this prepared slide of an oak or magnolia xylem maceration.

b. Phloem Observations: *Cucurbita* (pumpkin) longitudinal section (prepared slide).
This shows phloem of a vascular bundle in long section in particularly exquisite detail. Locate sieve tubes and their respective sieve tube members. Also note where these elements meet at sieve plates.

3. Ground Tissue System Basics

The bulk of the plant body is filled with ground tissue, of which the most common and generalized type of tissue is parenchyma. Two other types of tissues, collenchyma and sclerenchyma, may also occur in the ground tissue, but are not as common as parenchyma here.

PARENCHYMA. Living at functional maturity. A simple tissue consisting thin-walled, nonspecialized cells functioning in photosynthesis, storage, or secretion (Fig. 5). When blue and red stains are used to stain a section, the parenchyma cell walls stain blue.

COLLENCHYMA. Living at functional maturity. A simple tissue consisting of elongate cells with unevenly thickened, flexible primary cell walls especially rich in pectin. Collenchyma functions in flexible support and, when present, is usually is found beneath the epidermis in petioles, leaf midribs, or stems (Fig. 1).

SCLERENCHYMA. Dead at functional maturity. A simple tissue. Cells with very thick, secondary, usually lignified and rigid, cell walls. Their cell walls function in rigid support and protection. Sclerenchyma cells can be elongate and fibrous (fibers)(Fig. 1), occurring in networks or strands, or the cells can be of irregular shapes forming isolated or nests of stone cells (sclereids). When blue and red stains are used to stain a section, any sclerenchyma cells stain red if they are heavily lignified, and they usually are.

a. Have you ever noticed that a celery petiole has elastic strands that can be peeled away from just beneath the abaxial epidermis? These strands consist of one of the following three types of tissue: *parenchyma, collenchyma, or sclerenchyma*. Find out which one by using the compound microscope to study a cross-section wet mount of the celery petiole**.** Draw several cells and take note of their cell walls.

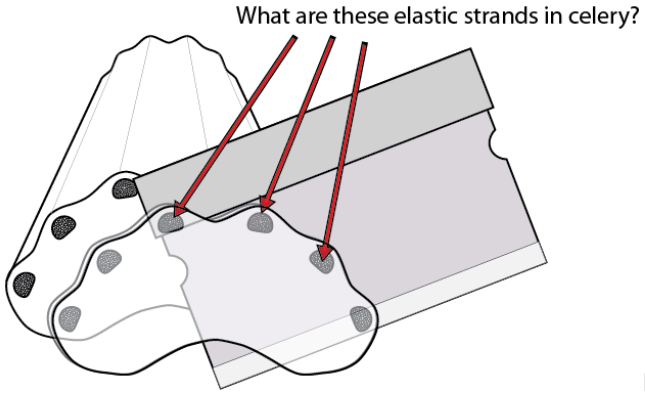


Fig. 2.

b. **In the celery:** All other tissue besides these elastic _____ bundles and the vascular bundles you can see more internally is parenchyma tissue - filling the space between these bundles and the epidermis.

Notice the following about parenchyma: thin cell walls, substantial intercellular space.

c. Sclerenchyma cells can sometimes be seen associated with vascular tissue of some species or the ground tissues of other species. In some plants like pear, the fruit ground tissue contains clusters of sclereids (known commonly as stone cells) amongst the parenchyma. These give the fruit a gritty texture. Make a wet mount of **pear fruit** tissue and identify, draw the stone cells.

d. Examine the cross-section of a *Linum* (flax) stem. Just outside the phloem or just beneath the epidermis is sclerenchyma consisting of fibers. They are the very thick-walled cells, although the walls have not been heavily lignified. These fibers are what linen and canvas are made from.

B. Comparative Anatomy of Roots, Stems, and Leaves

1. Stem Cross-Section.

In the stem, the vascular tissues occur in fascicles or bundles called vascular bundles. Collectively, the bundles in a stem are referred to as a stele. Dicots and gymnosperms have a eustele, where the bundles appear as a ring in a stem cross-section (aka transverse section). Monocots have an atactostele, where the bundles appear scattered in a stem cross-section. The ground tissue between the outermost bundles and the epidermis is called cortex. Both stelar types have it. The ground tissue in the center of a eustele is called pith.

a-d. Label the cross-section diagrams below with the appropriate terms described above.

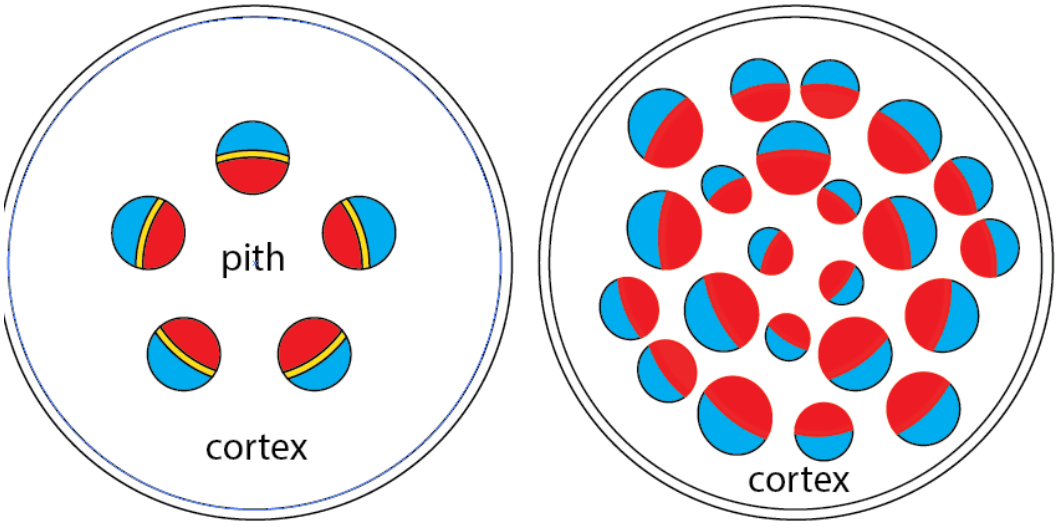


Fig. 3.

a. Stele Type:	b. Stele Type:
c.Plant Groups:	d.Plant Group:

e. Use compound microscope to study the two stem cross-sections on the prepared slide "Typical Monocot and Dicot Stem."

1) Which one is the dicot, and which is the monocot?

2) Draw a single vascular bundle from the monocot.

2a) Is the xylem half of the bundle towards to center or periphery of the stem?

2b) In the monocot bundle there are 2 or 3 vessel elements that are very wide and stand in stark contrast to any tracheids or parenchyma cells in the bundle.

Label these vessel elements and any tracheids and parenchyma in your drawing.

2c) In the phloem half, you should be able to see sieve tube members (large-diameter and lacking nuclei) adjacent to their companion cells (small-diameter, nucleate).

Label these in sieve tube members and companion cells in your drawing.

2d) Note that in the monocot there is a sheath of sclerenchyma cells surrounding a bundle and that these cells have stained red on account of their lignification. Do not confuse these sclerified cells with vessels or tracheids, since the sclerified cells do not conduct water.

3) Locate a vascular bundle in the dicot stem.

3a) Depending on which slide you have, you may note also that the dicot bundles each have very thick-walled, narrow-lumened (narrow interiored), lignified fibers capping the phloem half o the bundle (see below).

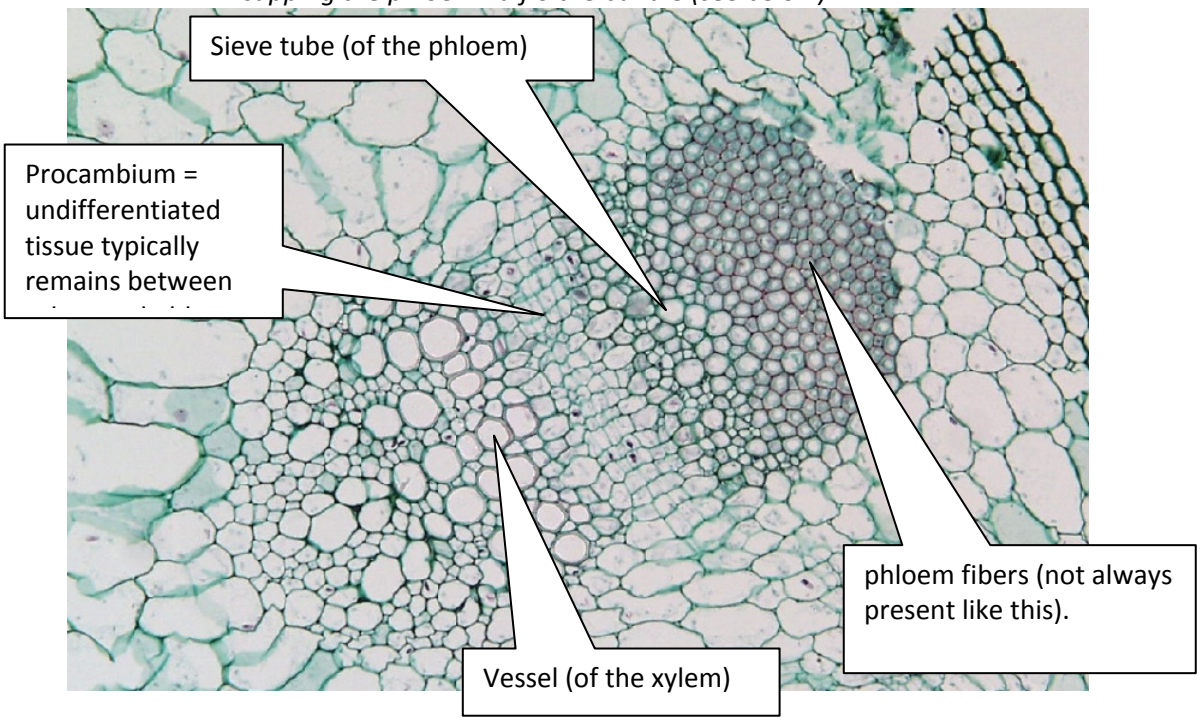


Fig 4. *Helianthus* (sunflower) vascular bundle, close-up. The blue stain is picking up the cellulose of the primary cell wall. The red stain is picking up the lignified cell walls.

3b) Note also that the dicot bundles have a narrow band of procambium between the phloem and xylem. The procambium remains undifferentiated and has potential to become meristematic again.

3c)Look at the ground tissue of the cortex just to the inside of the epidermis. Which of the following best describes this tissue:

Parenchyma, Collenchyma or Sclerenchyma?

2. Leaf Cross-Section.

In the Leaf, the vascular tissues occur in vascular bundles as they do in the stem, but the leaf is flat and so they cannot be arranged as in the stem. Rather than cortex and pith, the ground tissue parenchyma surrounding the bundles is called mesophyll and it is here where the chloroplasts reside.

a. Examine a prepared slide of a typical dicot leaf cross-section. Perform the following tasks.

1) *Locate the epidermis, mesophyll, and vascular bundles. How is the arrangement of these tissue systems in the leaf different than that in a typical dicot stem?*

2) Can you identify the thickened midrib portion of the leaf? What is this for? Notice that the vascular bundle seen in the midrib is larger than those throughout the lamina.

3) Can you see stomata in the epidermis? Hint: these should be greater in frequency on the lower (abaxial) surface. Make a high-mag drawing of a stoma plus adjacent guard cells and other epidermal cells. Label the guard cells.

Was this stoma open or closed when the leaf was sectioned?

4) Is the mesophyll more or less densely packed immediately internal to the stoma?

How does this tissue density facilitate gas exchange?

5) Identify a vascular bundle. Is the xylem on the top (adaxial) or bottom (abaxial) side of the bundle? How does this relate to its position in a stem bundle?

6) The mesophyll is differentiated into <u>spongy</u> (less dense, lots of intercellular spaces) and <u>palisade</u> (densely packed, columnar cells) mesophylls. The cells of both are rich in chloroplasts, although the palisade cells may be richer. Note also that the palisade mesophyll occurs beneath the top (adaxial) surface only.

How does the density and distribution of palisade mesophyll relate to sunlight capture and photosynthesis?

How does the density and distribution of spongy mesophyll relate to the need for gas exchange and distribution for photosynthesis in the leaf?

3. Root Cross-Section.

The vascular tissue of the root does not occur in bundles, but in a central region called the vascular cylinder. Between the vascular cylinder and the epidermis is ground tissue called the cortex. Monocot roots are distinctive in that there is a pith (patch of parenchyma) in the center of the root.

a. Examine the prepared slide(s) of typical dicot and monocot roots. Compare and contrast the two and, based on the diagrams below and on posters in the room, determine which is which. The diagrams below are abstractions and generalizations from reality: What you see under your microscope will vary.

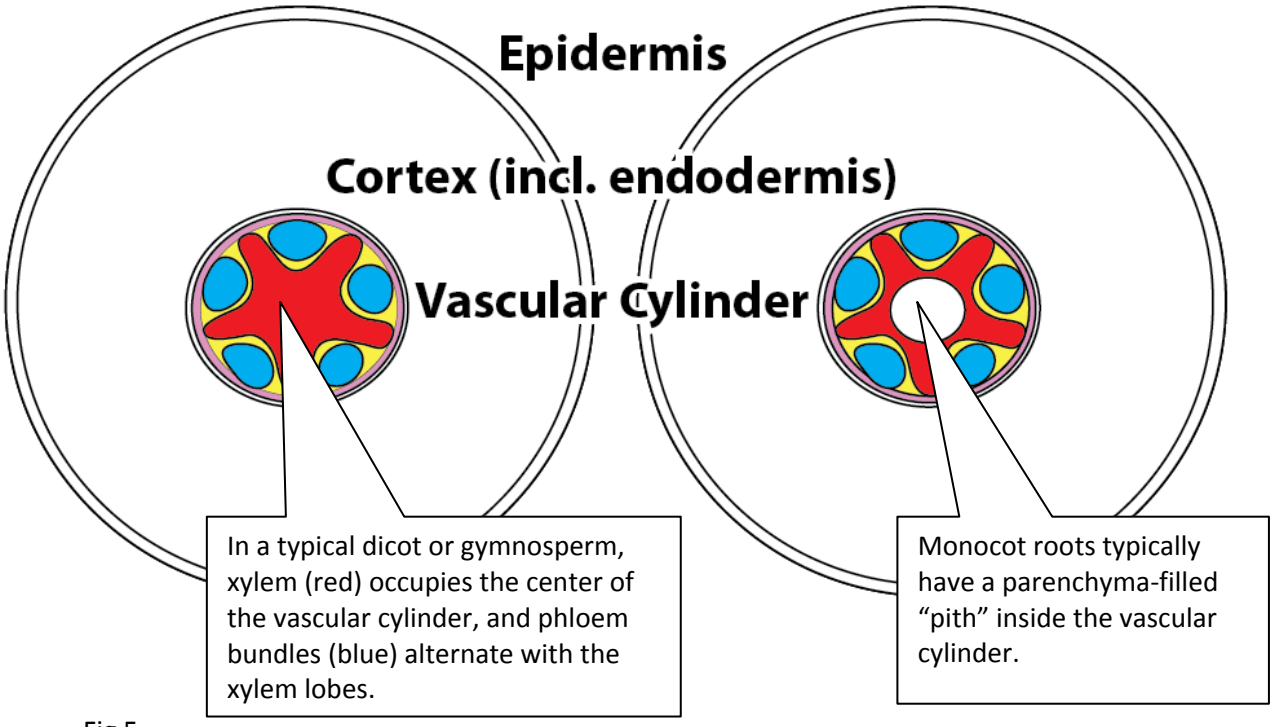


Fig 5.

1. Look in the cortex (cortical cells) of either root. Do you see evidence of a starch storing organelle? What is this organelle called?

2. Inspect the vascular cylinder and innermost layer of the cortex of the dicot carefully in more detail Make a drawing. Find the following structures and label them in your drawing. Use your posters in the room or figures in your textbook to help.

(1) XYLEM,

(2) PHLOEM,

(3) PROCAMBIUM = undifferentiated region between the primary xylem and primary phloem that remains meristematic,

(4) ENDODERMIS = partially suberized (SUBERIN is a typically red-staining, water-repellant substance), thick-walled cells older roots; innermost layer of the cortex. Some endodermal cells remain thin-walled & retain their Casparian strips for a while.

(5) PERICYCLE = thin-walled parenchyma cell layer just inside the endodermis; this is the outermost layer of the vascular cylinder. Can continue to divide to form lateral roots & part of vascular cambium upon initiation of secondary growth.

(6) PASSAGE CELLS = cells of the endodermis opposite the xylem lobes, which are not thick-walled or heavily suberized.

C. The Root and Shoot Apices

A shoot apex consists of a shoot apical meristem (SAM), juvenile derivative cells and leaf primordia at the tip of a shoot. A root apex consists of a root cap, root apical meristem (RAM) and juvenile derivative cells at the tip of a root. A SAM is a mound of initials and their immediate, still meristematic derivative cells above the youngest leaf primordium. A RAM is a small population of initials and their immediate, still meristematic derivative cells just proximal to the root cap.

Just proximal to each type of apical meristem, the derivative, juvenile tissue differentiates into 3 meristematic regions called the primary meristems, which will give rise to the 3 tissue systems (Table 3.1).

Table 3.1. The three primary meristems that organize in the shoot and root apices just proximal to the apical meristems.

Primary Meristem	Definition
Protoderm	Outer cell layer whose cells, although still meristematic, will give rise to and eventually differentiate into the dermal tissue system.
Procambium.	Central (root) or strands (shoot) of cytoplasmically dense (thus heavily staining) meristematic cells that will give rise to the vascular tissue system.
Ground Meristem	All other meristematic tissue other than the protoderm and procambium, which will give rise to the ground tissue system.

1. Root Apex (in longitudinal section).

Using the prepared onion (*Allium*) root tip longitudinal section slide, *draw and detail enough of the root apical region to include the following regions* (see also root models or posters in the room for help).

(1) ROOT CAP
(2) ROOT APICAL MERISTEM
(3) PROTODERM,
(4) GROUND MERISTEM,
(5) PROCAMBIUM.

Can you infer the existence of mitotic activity in any of the cells when they were living?

Where do you see the most amount of mitotic activity?

How does the meristem contribute to the growth of the plant?

What is the function of the root cap?

2. Shoot Apex (in longitudinal section).

Examine the prepared slide of a *Coleus* shoot apex.

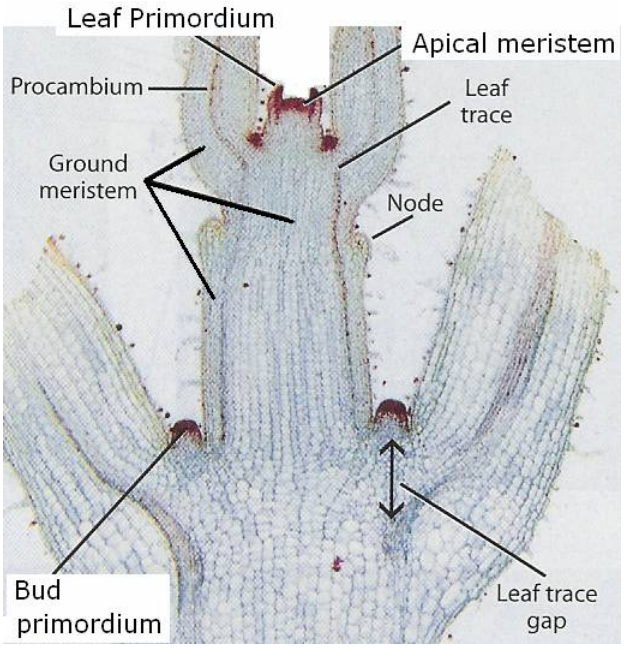


Fig. 6. The shoot apex in the mint relative *Coleus*.

a. Under the microscope, can you identify all of the structures labeled in figure 6?

b. How many nodes are portrayed in the picture above, and on the stem mounted on the microscope slide?

c. How are the bud primordia in Fig. 6 similar to the apical meristem at the tip of the primary stem?

d. What primary meristem is portrayed by the label "leaf trace" in the figure above? Protoderm, procambium, or ground meristem? Explain what "leaf trace"means.

e. What is the phyllotaxy (i.e. opposite,alternate or whorled) of the Coleus plant? How can you tell?

D. Further Exercises

1. Origin of Lateral Roots

Whereas stem branches arise exogenously from axillary buds, root branches (lateral roots) are said to arise endogenously. Closely examine the prepared slide of the ***Salix*** branch root origin and *DRAW your observations below*.

Label the tissues within the primary root cross section and the tissue regions of the lateral root.

Based on your observations, as well as a dissection of the word, what does endogenous mean?

What outer cell layer of the vascular cylinder does the lateral root arise from?

Does the vascular tissue appear to be continuous from the primary root to the lateral root?

What tissues of the primary root are penetrated by the developing lateral root?

2. Contrast a typical grass leaf anatomy (here, *Zea mays* or corn) with that of the typical dicot leaf.

The leaves of monocots, like grasses, have a slightly different organization but still contain all of the primary tissues one would expect.

Observe the cross-section of a Zea mays leaf (prepared slide). Draw and label the cross-section with the typical leaf tissues and cells. Include the specialized features.

How is the ground tissue organized differently from the dicot leaf?

What layer of mesophyll is missing?

Suggest a reason for the missing layer.

Distinctive to many grasses are fat, inflated looking BULLIFORM CELLS in the epidermis. Find these and suggest an adaptive function (hint: they loose water and shrink during water stress).

3. Variation in Cell Shape and Composition of the Epidermis

Observe a prepared slide of *Sedum* epidermis (w.m.) and compare it to what you saw with you onion peel in the first section and the rhoeo (*Tradescantia spathacea*). This section is similar to those in that it represents a surface view of an epidermis.

Draw and label one stoma and its stomatal complex of two guard cells surrounding it.

Contrast the shape of a guard cell from that of the surrounding epidermal cells.

Contrast the shape of a sedum epidermal cell from that of the onion and rhoeo epidermises seen earlier.

In total, how many different cell types can you see on the sedum epidermis?

4. Root Growth and Maturation

Examine the Onion (*Allium*) root tip longitudinal section (l.s.) slide and on your drawing in the roots section above, label the Regions of CELL DIVISION, CELL ELONGATION, and MATURATION if present (there are root hairs forming in the Region of Maturation). Then complete this table to the best of your ability:

Zone/Region	relative cell length (short, intermediate, long)	frequency of mitotic cells (low, medium, high)	presence of mature xylem or phloem cells? (yes/no)	presence of root hairs? (yes/no)
3. Region of maturation				
2. Region of cell elongation				
1. Region of cell division				

Can you identify the files of cells in the root?

What primary tissue system gives rise to the root hairs?

5. Morphological Plasticity: Sun vs. Shade Leaves

Water availability is not the only environmental factor that influences the development of the leaf. Changes in the basic organization can also be seen based on the INTENSITY of light the leaves develop in. In other words, leaves that develop in full sunlight and leaves that develop in shade have differences in their anatomy. Interestingly, these types of leaves can be found on the same plant.

Observe the prepared slide: *Sambucus* (elderberry) sun vs. shade leaf cross-section. Compare the sun and the shade leaves under the microscope, and fill in the table below.

	Sun	Shade
Thickness of cuticle (thin, thick)		
Amount of palisade mesophyll; (organized in single or multiple layers)		
Chloroplasts per unit area (density is High or Low)		
Leaf thickness (thin or thick)		

Consider what this tells you about the function of those leaves.

Why might the differences be beneficial for leaves growing in the shade or in full sun?

What part of a plant would you expect to find either of these leaves in?

6. Leaf Adaptations in Relation to Water in the Environment

a. Mesomorphic Leaves (typical leaf anatomy)

Review your observations of the typical dicot leaf. Make a note of how the PALISADE PARENCHYMA and the SPONGY PARENCHYMA are arranged.

How many mesophyll cell layers do you typically see for this type of leaf?

Also note the appearance of the epidermis and the ground tissues.

b. Xeromorphic (arid leaf anatomy)

Observe and DRAW one of the xeromorphic leaf examples: *Nerium oleander* (oleander) or *Ammophila* (beach grass) in cross-section. These types of leaves are specially adapted to growth in harsh, arid or otherwise dessicating environments such as a sandy beach with salt spray.

How do these leaves differ from Lilac?

Label your drawing with the typical tissues, cells.
Label the STOMATAL CRYPTS and CUTICLE.
What purpose do these structures have?

Identify and label the trichomes and stomata.
Where are they found?

How is the cuticle different from Lilac?

How does the Epidermis differ?

Is there any change in the basic organization of the ground or vascular tissues?

c. Hydromorphic Leaves (water leaf anatomy)

Observe and DRAW the hydromorphic leaf example: *Nymphaea odorata*, "water-lily" in cross-section. This type of leaf is adapted for aquatic habitats and often is submerged in water.

Draw the cross-section of the water lily.

Label all of the typical tissues and cells.

Label the intercellular spaces.

How does this type leaf differ from the previous two?

How would the organization of the ground tissue help this type of plant survive?

Do you see any SCLEREIDS in the mesophyll?

What might their purpose be?

How does the mesophyll tissue differ from xerophytes?

Are there any differences in the organization of the Ground and vascular tissues compared to mesophytes?

E. Glossary of some terms used in this lab

Abaxial (surface of leaf) = the surface of the leaf that was facing away from the stem axis when the leaf was a primordium. Typically this is the lower surface of the mature leaf. Derived from the Latin *ab* (meaning "from") and *axial* (meaning "of the axis").

Adaxial (surface of leaf) = the surface of the leaf that was facing towards the stem axis when the leaf was a primordium. Typically this is the upper surface of the mature leaf. Derived from the Latin *ad* (meaning "to" or "towards") and *axial* (meaning "of the axis").

Complex Tissue = a tissue composed of more than one cell type.

Cytoplasmic streaming = active cycling of the cytoplasm in a living cell for the function of quickly distributing essential substances from one region to another in the cell.

Guard Cells = the two living cells that surround a stoma. Changes in the turgor of guard cells regulate stomatal opening.

Lumen = The space bounded by the plant cell wall.

Simple Tissue = a tissue composed of one cell type.

Stoma (plural, stomata) = the pore in an epidermis that is bounded by two guard cells, through which gases are exchanged. The stoma can be opened or closed by the action of the guard cells.

F. Credits

This lab was developed by Christopher Hardy with contributions from Ryan Wagner. You may cite it as.... Hardy CR, RL Wagner. 2016. Primary anatomy. Pp. 61-84 in CR Hardy, RL Wagner (eds) *Guide to Exercises in Concepts of Botany, 4th ed*. Millersville, Pennsylvania, USA.

Wood, Cork, & Bamboo

Beyond food, your life is built upon the woody parts of plants which provide us with the materials for the construction of shelters, flooring and art, as well as the corky parts used in construction, bulletin boards, baseball bats and wine bottling. In this lab we will make a study of these materials, from their anatomy and natural occurrence in plants, to the various ways in which they are used.

Objectives for this lab:

1) Understand how secondary growth develops.
2) Understand the anatomy of secondary parts.
3) Understand the anatomical basis for annual rings.
4) Relate the anatomy of wood to the "grain" of wood, and the way that lumber is cut and used.
5) Understand the basis for the different properties and use of wood and cork.
6) Compare and contrast wood and bamboo, and the products made from them.

Table of Contents for this lab:

A. Wood

Our understanding of the strengths and limitations of wood as a natural product requires a basic understanding of the anatomy and development of wood. The following exercises will accomplish this task and ask you to apply your new-found knowledge.

1. Development of the vascular cambium.

Within the first year of a stem's life, the procambium between the xylem and phloem of each vascular bundle (the line bisecting each bundle, below left) again becomes meristematic. This meristematic activity then spreads to the parenchyma between the bundles to complete the formation of the vascular cambium (the circle connecting the bundles, below right). Subsequent action of the vascular cambium results in concentric rings of secondary xylem to the inside and secondary phloem to the outside.

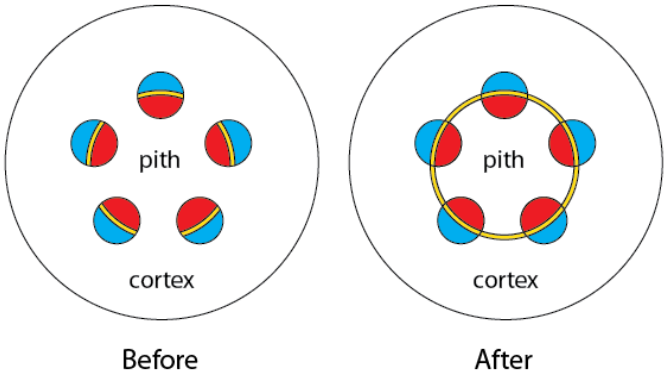


Figure above: Cross-sections of a stem with 5 vascular bundles before and after the formation of the vascular cambium.

a. Prepared slide & Compound Scope: *Medicago* Young & Mature Stem c.s. OR Three Stem Stages c.s.. Examine this slide which has either two or three stem transverse sections (also called cross-sections, abbreviated as *c.s.* or *x-section*) on it, each of a different age. Determine which section represents the youngest stage and which the oldest. Often, the youngest is the smallest but size is not always a reliable indicator: rather, the degree to which the vascular cambium is formed is the best indicator of age.

Draw the youngest stem where the bundles are separate because the vascular cambium has not yet formed.

Now draw a stem where there is evidence periclinal divisions that are in the process of forming the vascular cambium.

1) Can you find evidence of the first periclinal divisions in the parenchyma between the bundles? What does this evidence look like? **Note:** It is primarily periclinal cell division of cambial cells that add girth to the plant. In order for the expanding cambium to keep up with the increasing girth of the stem, occasional anticlinal cell division adds cells to the circumference of the cambium.

2) In which section have the bundles been united by the vascular cambium?

3) What will happen to the phloem and the xylem halves of the primary vascular bundles as secondary growth continues?

a. they will stay together b. the phloem half will be pushed out.

2. Anatomy of 1-yr and 3-yr old woody stems

(with compound light microscope).

a. Prepared slide: *Tilia* 1-yr old stem x-section. This basswood (*Tilia*) stem was 1 year old when cut. Examine it for the following tissues in this sequence from the center to periphery and answer the questions:

Pith – the center of the stem retains the original pith of the primary stem.

1) What type of tissue is present here (parenchyma, collenchyma, or sclerenchyma)?

Xylem – there is one ring of secondary xylem (wood) that has formed here. Note the large-diameter cells lacking cytoplasm and nuclei (vessel elements, assembled into vessels), the narrower cells without cytoplasm or nuclei (which are either tracheids or fibers), and the thin-walled cells with cytoplasm and nuclei (these are parenchyma cells). All mature vessel elements, tracheids, and fibers will have relatively thick walls because of secondary thickening and will typically stain red in our preparations because of lignin deposition. Only the parenchyma cells will not stain very red because they are not lignified.

2) *Name two primary functions of secondary xylem.*

Vascular cambium – locate the position of the vascular cambium between the secondary xylem and secondary phloem. Look for a zone ca. 1-4 cell layers wide of small diameter, undifferentiated cells.

3) *How can you tell visually that this cambium is meristematic? Answer: the cambial cells are smaller than surrounding cells and have thin cell walls because they had recently divided or were in the process of dividing when the tissue was sectioned. Moreover, the cambial cells give rise to radial files of cells due to their repeated periclinal divisions.*

Phloem – There is one ring of secondary phloem which has formed here. In this species you'll find sieve tube members (large-diameter, living yet anucleate cells) with their companion cells (smaller-diameter, nucleate cells), many fibers (thick-walled, dead and anucleate, small-diameter, narrow-lumened cells) and phloem parenchyma (thin-walled, nucleate cells). The cell walls of sieve tube members, companion cells, and parenchyma should be thin because they are not secondarily thickened and their walls should be blue-staining because they lack lignin. The walls of mature fibers should be very thick and stain red due to lignification.

4) Name the primary function of secondary phloem?

5) *Do you think that the phloem fibers aid the xylem in mechanical support of the stem?*

Rays – Note the radially oriented series of nucleated parenchyma cells extending through the secondary xylem and into the phloem. These are rays.

6) What is the function of rays:
a. *radial transport* ***b.*** *axial transport?*

Transport of what?

Cortex – The ground tissue (usually comprised of parenchyma) between the phloem and epidermis.

Epidermis – The epidermis is surely still intact in a stem of this age, which is still in the very early stages of secondary growth.

b. Prepared slide: *Tilia* 3-yr old stem x-section. Compare this 3-yr old stem to the 1-yr old stem.

1) Can you see the 3 annual rings (i.e., the 3 rings of secondary xylem)?

2) Can you also see three rings of secondary phloem too? Why or why not?

3) Although you may see a few years of phloem accumulation, why are you not likely to see many (e.g., 100) years of phloem accumulation in an old tree?

4) Note how the rays in the xylem are narrow, whereas they are dilated (expanded) in the older (outer) layers of phloem. Indeed, the parenchyma cells of the phloem rays are able to divide anticlinally as the secondary xylem and vascular cambium grow outward, resulting in the widening of the phloem-portion of the rays as they age. *Do you think that the ability of the phloem rays to expand extends the functional life of secondary phloem?* Explain. (Hint: what would happen to the phloem and phloem ray tissue if they could not produce new cells anticlinally?)

5) Are vessel elements and tracheids from the springwood (earlywood) larger or smaller in diameter than such cells from the summerwood (latewood)?

Answer this right and you'll now be able to explain why we can discern annual rings in wood.

3. Hardwoods vs. Softwoods.

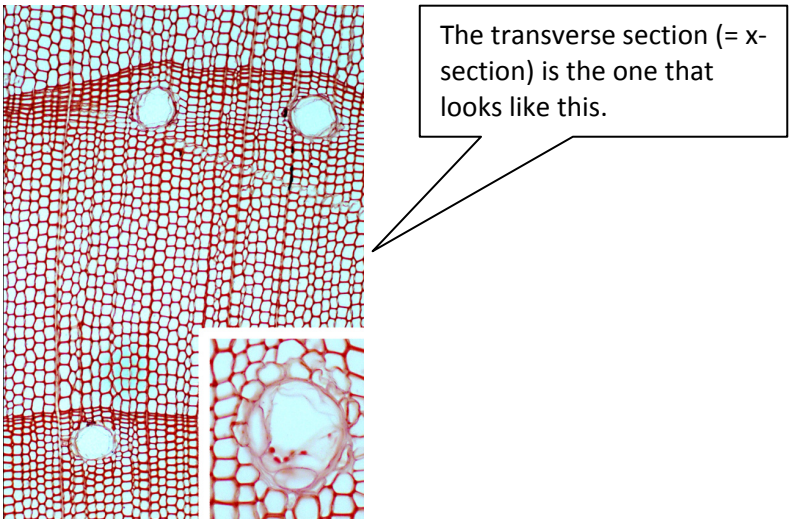
The flooring and timber industries classify woods as hardwoods and softwoods. Botanically, the distinction between hardwoods and softwoods is a taxonomic one: hardwoods are from angiosperms and softwoods are from gymnosperms (Table 1 below). Gymnosperm woods tend to lie towards the bottom of the hardness scale and hardwoods towards the top, but this is not always the case (Appendix 1). For example, wood from the South American balsa tree (an angiosperm) is softer than most gymnosperm woods, yet balsa is technically a "hardwood" because it is an angiosperm.

Table 1. Hardwoods are from angiosperms, whereas softwoods are from gymnosperms. Many, but not all, angiosperm woods are harder than gymnosperm woods due to the presence of fibers in the former. Because angiosperm wood can rely on larger vessels for water transport, the tracheids of angiosperms may also be narrower – thereby working with the fibers to increase the density and, therefore, hardness of the wood relative to gymnosperms.

Taxonomic Group	**Common Name for their Wood**	**Cell types in Wood and their abundance**
Angiosperms (only dicots produce wood)	Hardwood	Vessel Elements (many) Tracheids (many) Fibers (few to many) Parenchyma (variable – mostly in rays)
Gymnosperms (mostly conifers are harvested for their wood)	Softwood	Tracheids (many) Parenchyma (variable – mostly in rays)

a. Prepared Slide of a typical Softwood (*Pinus strobus*, white pine), with transverse, radial, and tangential sections on it.

Examine only the cross (transverse) section (see figure below) and find the axially oriented tracheids and the radially oriented ray parenchyma. Also note the occurrence of larger "holes" which are the resin canals that distinguish conifers. As viewed in transverse/cross section, the resin canal is actually a lacuna (hole or intercellular space) surrounded by parenchyma cells which manufacture and secrete the aromatic resin into the canals.



b. Prepared Slide of a typical hardwood (*Quercus*, oak), with the 3 types of sections on it.

Look at the transverse section and compare it to that of pine.

Draw a portion of a growth ring, large enough to easily diagram and label the following: vessel element, tracheid, fiber, and ray parenchyma cell. In addition to the added complexity (more cell types) of angiosperm wood, note the absence of resin canals.

1) Does the oak appear more or less dense than the pine (a higher density would result from a higher cell wall-to-lumen ratio in the wood)?

2) Density is the single most important predictor of hardness in wood. Thus, do you think that the oak is harder or softer than the pine based on your density estimate?

Look at Appendix 1 to quantify the difference in hardness.

3) How would you rank the fiber abundance in oak: rare or abundant? Do you think that fibers would contribute to the widely recognized hardness of oak wood?

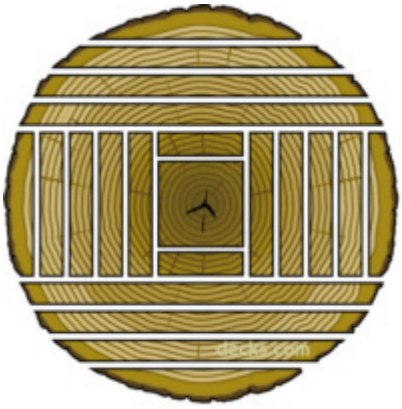
4. Interpreting Grain Patterns in Lumber.

Lumber for boards in construction is not cut randomly, but in an orderly manner with maximizing the number the usable "board feet" per tree a main goal. The way in which each board is sawed determines its strength and appearance (the "grain" of the wood) in particular orientations, and will thus determine its use.

a. Learn to recognize planes of wood cutting, and plane-sawed vs. quarter-sawed boards.

Lumber is never cut transversely from trees because of two reasons: 1) the lumber would break easily and 2) it would be difficult to get long boards from all but the oldest, largest trees (think about it). Sawing a tree longitudinally into boards is typically done one of two ways: plane-sawing and quarter-sawing (as explained below). After reading the description of plane- and quarter sawing techniques below, visit the wood boards and blocks in the room and use their grain patterns to accurately reconstruct their pre-cut orientation in the tree trunk (identifying the direction of the center and outside of the stem) and, if they are boards rather than blocks, whether or not they were plane-sawed or quarter-sawed.

Plane-sawed boards are from a trunk cut tangentially, leaving the end grain appearance of loops and growth "swirls" on the broad, flat surface. This is fastest and most common sawing process.



Plane-sawed

Quarter-sawed

Quarter-sawed boards are from a trunk cut into quarters, then each quarter is rotated 90 degrees back and forth sawing off one board at a time. This process is more labor-intensive but results in stronger boards for use as beams (when load is applied to the narrow side); thus, such boards are generally more expensive than plane-sawn boards. On the broad, flat surface, the grain consists of long, parallel lines. The finest (strongest) quarter-sawed boards are those in which the annual rings are oriented between 80-90 degrees relative to the wide plane of the board.

b. Which makes for a better diving board or plank for walking on: a plane-sawed or quarter-sawed board?

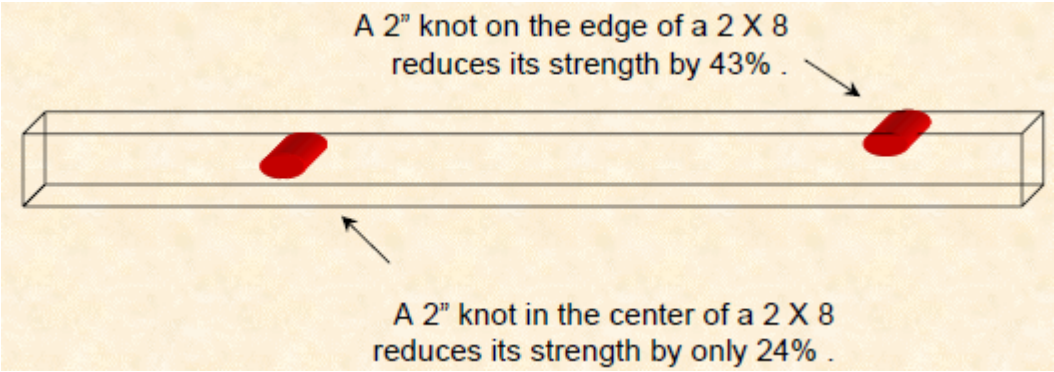
Back at your lab bench, each group of 4 students has two pieces of boards, one of which was plane-sawed and the other of which was quarter-sawed. First determine which is which, and then dry to break them. Which one is easier to break and in which direction was the breaking force applied?

1) After the break test, which sawing method is preferred in the manufacture of planks for diving boards or use in construction scaffolding? Explain.

2) After the break test, which sawing method is preferred in the manufacture of beams for supporting floors in a multi-story house? Explain.

5. You do Not want Knots in your Lumber.

Knots are areas in your wood where there are abrupt, localized changes in the pattern of growth ring deposition (i.e., abrupt changes in the grain). Knots can compromise the mechanical strength substantially because the knot disturbs the direction and continuity of the grain, both of which are important for strength as you learned above.



Picture © Terry Brown

a. What are knots really? *Examine the specimens of knots in the room and tell me "What are knots, botanically speaking?" That is, why and how to they form?*

b. Which would have more knots in it: *lumber from a tree grown in full sun or one grown with other trees in a forest? Explain.*

B. Cork

Like wood, cork is a secondary plant tissue and produced by a lateral meristem. Cork, however, is produced by the cork cambium whereas wood is produced by the vascular cambium. How is cork produced and what biological purpose does it serve? What are its properties that make it so useful to humans? Through the following exercises, you will answer these questions.

1. Cork Cambium.

The epidermis of the primary stem (and root) will be destroyed by the expansive force of the vascular cambium. The cork cambium arises in response to this destructive force, in order to produce a new protective "skin" around the outside of the stem and root. The cork cambium produces most of its cells towards the outside, and these mature as cork. Relatively few cells are produced towards the inside, and these mature as unspecialized parenchyma cells. At maturity, cork cells are dead and the walls are heavily suberized (impregnated with the waxy substance suberin), making the tissue elastic, water proof, and relatively gas proof. A cork cambium plus the tissues it produces are collectively referred to as periderm, which functionally replaces the epidermis.

a. Prepared slide: *Pelargonium* (geranium) stem x-section.

- *Note the well-formed vascular cambium.*
- *Look for newly formed cork cambium to the outside of the vascular cambium, as evidenced by a zone of many newly formed periclinal walls and radial files of small cells.*
- *Did the cork cambium form in the epidermal layer or in the cortex just beneath the epidermis?*

b. Facts and Related Questions.

1) As secondary growth continues, old cork will be sloughed off. Why is this?

2) Inspect the pieces of tree trunks and their bark in the room or outside. Do you see evidence of this tearing & sloughing in the outer bark?

3) Inspect transverse sections of tree trunks in the room for what looks like layers in the outer bark. Layering in the outer bark is caused by two things:

a) Cork is produced annually, and so annual rings of cork that are analogous to those of secondary xylem in wood may be visible.

b) Parts of the cork cambium may be ruptured by the growth of the vascular tissue. This brings the need to form new cork cambia beneath the older, defunct one(s). These new cork cambia form in any remaining cortex or secondary phloem tissue beneath the previous cork cambium, thereby trapping some cortex or phloem tissue amongst cork tissue. Thus, multiple periderms form and this results in a complex appearance and layering to the outer bark.

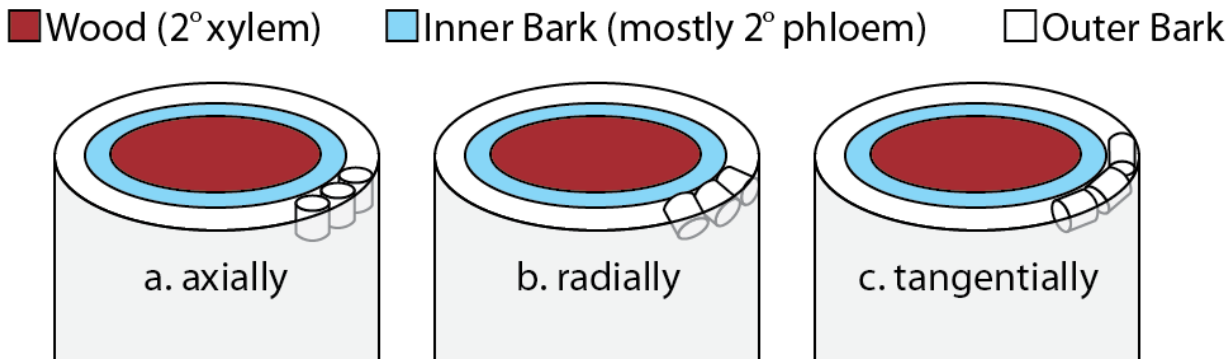
2. The cork of commerce.

"Corks" for bottling and for corkboards are cut from the outer bark of the cork oak, *Quercus suber* of the Mediterranean. They are called "corks" because they consist mostly of actual cork, though there can be small amounts of what was formally phloem or cortex parenchyma that were incorporated during the formation of multiple cork cambia and periderms. While all trees and shrubs produce cork, only the cork oak (*Quercus suber*) of the Mediterranean is used for commerce.

a) Inspect the samples of wine bottle corks, cork flooring, cork stoppers, etc. for the properties of cork. Which samples are made from a single whole, intact piece of outer bark and which are aggregates of many small pieces glued together?

b) In a wine cork or cork stopper that was made from a single, intact piece of outer bark, look for the layering or rings that we expect to see in outer bark. Now, answer the following:

Was the cork bored from the outer bark a) axially, b) radially, or c) tangentially? (See below.) Use the layering visible in the cork, as well as your sense of which bore direction would maximize the strength (think about the stress on the cork when you remove and reinsert corks into a bottle) as well as maximizing the number of long corks cut from a piece of Quercus suber outer bark.



c) Inspect pieces of tree trunks (with their corky outer barks) from other species in the room (e.g., the corky bark of the hackberry, Celtis occidentalis, the Amur cork tree, Phellodendron, or of the winged euonymus, Euonymus alatus). Would these species be a good supply of cork in commerce? Why or why not?

3. Lenticels.

Do you see the soft, raised bumps on a woody twig at your table? These are lenticels, which functionally replace stomata once the epidermis is destroyed and the periderm forms. Lenticels are patches of loose, unsuberized tissue amongst the suberized cork and are conduits for gas exchange between the living cells beneath the cork and the atmosphere.

- *What metabolic process occurs in living, non-photosynthetic cells and what gas(s) must be exchanged?*

- *Why can't gas diffuse through the normal cork cells (that is, why are lenticels even necessary)?*

Prepared slide of a ***Sambucus* (elderberry) lenticel** x-section. Here a single lenticel has been sectioned (as in the figure below).

- For orientation, try to find vascular cambium, the outer bit of secondary xylem, the secondary phloem and, if possible, any remaining cortex and primary phloem bundle tissue.
- Next, find the young cork cambium and the lenticels which looks like a rupture in the cork.
- *How is gas exchange facilitated by the lenticels? (circle all that apply)*
  a. *suberin* *b. no suberin* *c. lenticel tissue is loose (w/ many intercellular spaces).*

C. Gross Morphology of Tree Trunks

Inspect the various trunk cuts in the room and identify the extent and location of the 1) pith, 2) heartwood, 3) sapwood, 4) vascular cambium, 5) inner bark (primarily secondary phloem) and 6) outerbark (this is mostly cork). Use the definitions below to help.

1. Heartwood vs. Sapwood. Heartwood is the older, innermost portion of a plant's wood. It has accumulated gums, resins, oils, tannins and plant metabolic waste over the years and this 1) clogs the conducting cells, preventing them from conducting, 2) often discolors the heartwood, and 3) may even make the heartwood aromatic. Sapwood is the outermost portion of the wood that is still involved in water conduction; often lighter than heartwood in color.

2. Spring (Early) Wood vs. Summer (Late) Wood. Springwood is the first-formed wood of an annual ring. Cell diameters are larger here and wood here is therefore less dense, softer and more "porous". Summerwood is the latter-formed wood in an annual ring; consists of smaller-diameter cells and is therefore denser than springwood. The alternation of spring and summer woods in a woody stem is responsible for the annual rings seen in x-section.

3. Bark. "Bark" is an old but still technical term for everything to the outside of the vascular cambium, including the phloem; it is what is typically stripped off of a woody plant rather easily. Some botanists distinguish between outer bark (from the cork cambium out - consisting mostly of cork) and inner bark (consisting of secondary phloem and any other remnant tissue between the vascular cambium and the cork cambium).

D. Bamboozled by Bamboo

Is bamboo made of wood? If you said yes, you have been tricked by its impressive strength. In the tropics, the many species of bamboo compete with and even surpass wood in importance as construction materials: it is remarkably strong and flexible for its weight. Yet despite its strength and apparent woodiness, bamboo is not wood and it is more closely related to lawn grass and corn than to oaks, for example.

1. Examine the large stems of bamboo in the room and answer the following:

   a) Is the stem completely hollow? Explain.

   b) What are the prominent rings around the stem?

2. Use **dissecting scope** to examine a short specimen of bamboo stem.

   a) A*side from the hollow pith, how is bamboo anatomy apparently different from the woody dicot and gymnosperm stem? Bamboo stem anatomy seems to consist of…*

   a. Growth rings of secondary xylem b. densely packed primary vascular bundles

3. Use **compound scope** to examine a prepared slide of a maize (*Zea mays*) stem x-section. We don't have any microscope slides of bamboo, but maize is in the same family as bamboo AND, like bamboo, is larger than a typical grass and has therefore developed similar adaptations to support its tall stature. Maize pith, of course, is not hollow as in bamboo, but many similarities remain. Note that all the cells staining red in this section are lignified.

   a) What does the anatomy of the maize stem look like (circle all that apply)?

   a. Growth rings of secondary xylem and phloem b. closely spaced primary vascular bundles
   c. a clear vascular cambium is present

   b) If there are vascular bundles visible, how are they reinforced (what type of cells immediately surround them)?

   a. Parenchyma b. tracheids c. vessel elements d. lignified cells, probably fibers e. sieve tube members

   c) What type of evidence indicates that these surrounding cells have secondary walls and are lignified?

   d) Do any cells further from the bundles, possibly in the cortex/ground tissue, look to be reinforced with lignin?

4. As you know, bamboo stems are cylindrical and never get as wide as the trunks of conifers and oaks that boards and lumber come from, yet bamboo is now widely used in flooring and in the manufacture of cutting boards. ***Visit the station with pieces of oak and bamboo flooring.*** *Then answer these questions.*

a) How can flooring and cutting boards made from bamboo be so broad and flat when the bamboo stems they come from are not? Examine samples of bamboo flooring and, if available, cutting boards in the room and learn how these are made.

b) How is a piece of bamboo flooring differently constructed than a piece of wood flooring (e.g., oak)?

5. *Is bamboo flooring softer or harder than white oak flooring (see Table 2)?*
Whether softer or harder, calculate by how much bamboo is softer/harder than white oak. Show your work.

Table 2. Janka Hardness Ratings

Average Janka Hardness Rating* of various woods used for flooring, from highest to lowest and with hardwood/softwood classification indicated (bold are species commonly used for flooring in the United States of America).**

2350 - Brazilian Cherry	Hardwood
2345 - Mesquite	Hardwood
2200 - Santos Mahogany	Hardwood
1820 - Hickory	Hardwood
1820 - Pecan	Hardwood
1450 - Hard Maple	Hardwood
1410 - Natural bamboo (can approach 1700 in some products; varies substantially with manufacturer)	Not a wood
1360 - White Oak	Hardwood
1320 - Ash	Hardwood
1300 - American Beech	Hardwood
1290 - Red Oak(Northern)	Hardwood
1260 - Yellow Birch	Hardwood
1010 - Black Walnut	Hardwood
1000 - Teak	Hardwood
950 - Black Cherry	Hardwood
870 - Southern Yellow Pine (long leaf)	Softwood
690 - Southern Yellow Pine (short leaf)	Softwood
660 - Douglas Fir	Softwood
540 - Chestnut	Hardwood
420 - White Birch	Hardwood
410 - Basswood	Hardwood
380 - White Pine	Softwood

*The relative hardness of wood species is measured using the Janka Hardness test. This test measures the force needed to embed a steel ball (11.28 mm or 0.444 inch diameter) into wood to half the ball's diameter, with the rating measured in pounds of force per square inch in the United States. For example, it takes 1360 psi to do this in white oak, but only 380 psi in white pine; therefore, oak is harder than pine. Wood hardness is an important consideration in the flooring industry and other industries that use wood (baseball bats, axe handles, etc.). It is most commonly discussed in terms of flooring since there are so many woods to choose from here and the customer must weigh the appeal of a given wood's grain or color against his/her desire for hardness and therefore resistance to scratches and dents.

**References: www.ifloor.com and www.hardwoodinstaller.com, retrieved 25 Aug 2010.

E. Glossary of some terms in this lab

Anticlinal = perpendicular to the organ surface.

Bark = an old but still technical term for everything to the outside of the vascular cambium, including the phloem; it is what is typically stripped off of a woody plant rather easily.

Cork Cambium = the lateral meristem responsible for the production of periderm (including cork).

Heartwood = the older, innermost portion of a plant's wood. It has accumulated gums, resins, oils, tannins and plant metabolic waste over the years and this 1) clogs the conducting cells, preventing them from conducting, 2) often discolors the heartwood, and 3) may even make the heartwood aromatic.

Inner Bark = the tissue in a woody stem or root between the vascular cambium and the cork cabmium. Mostly secondary phloem.

Lateral Meristems = meristems (i.e., the vascular cambium and cork cambium) whose action result in secondary growth.

Lumen = The space bounded by the plant cell wall.

Outer Bark = the tissue outside of the inner most cork cambium of a woody stem or root. Most cork tissue. Our undergraduate botany textbook (Bidlack and Jansky 2011) lists this as synonymous with "periderm" but, in actuality, the outer bark can actually be made of multiple periderms with included phloem and other parenchyma tissue.

Periclinal = parallel to the organ surface.

Periderm = the tissue (mostly cork) produced by the cork cambium to the outside of a woody plant, which replaces the epidermis in function after the latter is sloughed off.

Ray = the radially oriented series of cells extending through the secondary xylem and secondary phloem.

Sapwood = the outermost portion of the wood that is still involved in water conduction; often lighter than heartwood in color.

Secondary Growth = the lateral (girth) growth of a stem or root after primary growth. Produced by lateral meristems.

Springwood = the first-formed wood of an annual ring. Cell diameters are larger here and wood here is therefore less dense, softer and more "porous". Synonymous with "earlywood." Contrast with "summerwood."

Summerwood = the latter-formed wood in an annual ring; consists of smaller-diameter cells and is therefore denser than springwood. Synonymous with "latewood."

Vascular Cambium = the lateral meristem responsible for the production of secondary vascular tissues.

Wood = secondary xylem.

F. Credits

This lab was developed by Christopher Hardy. You may cite it as....

Hardy CR. 2016. Wood, cork and bamboo. Pp. 85-102 in CR Hardy, RL Wagner (eds) *Guide to Exercises in Concepts of Botany, 4th Edition*. Millersville, Pennsylvania, USA.

Plant Modifications & Marketplace Vegetables

You have fastidiously examined the structure and growth of the organs of angiosperms in the previous labs. With this background, we ask you to first examine and interpret selected examples of naturally occurring modifications of these organs and then to interpret vegetables structures taken from the supermarket. This should provide lots of fodder for dinnertime or grocery store conversations. All can be interpreted using the terms you have been learning, regardless of how bizarre they may look to you.

Objective for this lab:

1) Apply rules of plant architecture to interpret seemingly bizarre modifications of plant organs occurring in nature or in the supermarket produce isle.

Table of Contents for this lab:

Figure above. Turk's cap cactus (*Melocactus communis*) growing in a native plant garden in Aruba.

A. Plant Modifications Tutorial and Terms

Any of the three types of plant organs (root, leaf or stem-including hypocotyl) or combinations thereof can be modified in peculiar or not so peculiar ways. The previous labs have been concerned with typical basic angiosperm structure, but today we challenge you a bit.

1. Leaves.

Leaves are commonly "modified" from their usual function in capturing light rays for photosynthesis. There are many possibilities, some of which you may see today. Some common modified leaf homologs include:

BRACT = a leaf, may be reduced in size and then not as green or photosynthetic as its full-on vegetative counterpart, or it may be large and with a showy color. Often subtending flowers and inflorescences, or found on horizontal stems called stolons.

INSECT TRAPPING = modifications that attract and/or capture insects. Mechanisms and morphology of insect trapping leaves are variable - e.g. Venus flytraps, bladderworts, pitcher plants, sundews, butterworts.

NEEDLE = a needle-shaped leaf. Common in conifers.

PROPAGATIVE LEAVES = leaves that produce meristematic regions on margin that produce plantlets asexually (vegetative clones)

SCALE = very small, thin, and non-photosynthetic leaf homolog; often on subterranean stems such as rhizomes, or the "bud scales" of winter buds on trees and shrubs.

SHEATH = a leaf that does not have a petiole or blade, and simply ensheathes the stem (e.g., those on a young bamboo shoot).

SPINE = a sharp, pointy leaf homolog.

SUCCULENT LEAF = a leaf swollen with water-storage tissue.

TENDRILS = slender, coiling structure, used for support, modified leaf or leaflet.

VARIEGATION = the appearance of differently colored zones on a leaf. Typically where a green leaf blade is interrupted by white patches or stripes.

2. Stems.

Stems are, in certain species, modified during development and evolution for functions other than support (of the leaves) and conduction of water and sugars between leaf and root. Some of these modifications include:

CLADOPHYLL = flattened stem that resembles a leaf

RHIZOME = an underground, horizontal stem; often a means of colonization of neighboring soils or vegetative (asexual) reproduction as portions of rhizome become fragmented.

SUCCULENT STEM = a stem swollen with water-storage tissue.

STOLON or RUNNER = an above ground, horizontal (or hanging) stem; similar to rhizome in function and morphology.

THORN = a pointy tip of a stem or pointy lateral (branch) stem that does not form leaves.

TUBER = swollen, fleshy, underground storage (e.g., starch, sugar) stem, does not necessarily have a defined orientation. The leaves on the tuber are typically reduced to small scales and may be very difficult to see.

3. Roots.

Roots may be variously modified for storage, solely anchorage, and even photosynthesis!

AERIAL ROOT = English-ivy, poison-ivy, or the vanilla orchid are good examples of stems that produce roots along their stem, above the ground. Many vines produce aerial roots for anchorage to some substrate such as a tree. The aerial roots of many EPIPHYTIC orchids may also be photosynthetic as they are exposed to the light.

PROP ROOT = some plants such as corn, some palms, and mangrove trees have roots which serve to prop and support the stem like flying buttresses do on some buildings.

PROPAGATIVE ROOT = some roots are able to produces plantlets, which is a means of asexual reproduction. Peonies and breadfruit are examples of plants with such roots.

PNEUMATOPHORE = some roots of plants that grow in swamps or mangles, which are able to grow up, above the water. These are generally thought to promote gas exchange.

TUBEROUS ROOT = technically, tubers are stems, and so swollen roots or parts of roots specialized for storage (e.g., starch) are called tuberous roots.

4. Shoots (stems plus leaves).

Examples of whole shoots that are modified include FLOWERS, BULBS, and CORMS.

BULB = usually a subterranean shoot with a very short stem and tightly packed succulent leaves.

CORM = a subterranean shoot consisting of a short, but swollen stem and dry and papery or membranous leaves.

FLOWER = a determinate shoot with modified leaves, some of which bear sporangia.

TANK of a **TANK EPIPHYTE** = a collection of closely overlapping leaves on a stem with very short internodes; found as a strategy in certain epiphytes such as bromeliads to collect water and detritus for nutrition in the tree canopy.

5. Armature.

Some plants are armed and dangerous.

PRICKLE = epidermal outgrowths that project from the epidermis of some organ, rather than being a modification of the *entire* organ.

SPINE = a leaf modified into a hard, prickly structure.

STIPULAR SPINES = where the two paired stipules at the base of a leaf or on the stem at the point of leaf attachment are modified into a hard, prickly structure.

THORN = a stem (or multiple stems through branching) modified into a hard, prickly structure.

B. Plant Modifications Stations

Visit the plant stations placed around the room. Record the name and identify the modification(s) present. There may be multiple modifications present for each plant. Answer the question associated with the specific plant, if applicable:

Number	Name	Modification(s)	Answer to Question:
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			
16			

Number	Name	Modification(s)	Answer to Question:
17			
18			
19			
20			
21			
22			
23			
24			

C. The Market Place

Exactly what part of the plant do you eat when you eat any of the produce in the grocery? Can you apply any of the modification terms from the exercise above (e.g., tuber, rhizome) to the produce? Edible parts include:

leaves
blades
petioles
axillary buds
flower buds
hairs
stems
roots
flowers plus stem
seedlings (which include the radicle, hypocotyl, cotyledons, epicotyls and plumule leaves)

Referenced as "vegetables" in the vernacular.

seeds
(seeds also contain an embryo that has all of the parts of the seedling above).
ovaries
flower usually called
ovaries plus stem

Referenced as "fruits" or "nuts" in the vernacular.

Complete the following exercises with reference to the specified produce available in the classroom.

1. So-called "Tuber" or "Root" Crops.

Which organ makes up the bulk of the underground storage produce we find in various grocery items? Read the following considerations and then answer the questions associated with each produce item that follow.

If stem, then the appropriate medication term is "**tuber**" and you might expect to see modified leaves and even axillary buds in the axils of those modified leaves. Naturally, with the presence of axillary buds, you might also see evidence for branching. Whereas some tubers are determinate in growth, others can keep growing in length (albeit slowly), spread horizontally and therefore also function *also* as a **rhizome**.

If root, then the fat part of the vegetable would be called a "**tuberous root.**" Whereas most tuberous roots are rounded, sometimes tap roots may maintain their overall elongate tap root appearance while at the same time be swollen for starch or sugar storage. Let us classify such roots today as "**tuberous tap roots.**"

As roots, you would not expect to see leaves (or modified leaves) or axillary buds in a regular phyllotactic pattern along their surface, although you might see a rosette of leaves proximally (apically) attached to an extremely short and hardly recognizable stem. As a root, you may see lateral non-tuberous roots or the scars left by lateral roots if these have not been rubbed off prior to and during shipping.

<u>If leaf or leaves</u>, then you may have a "**bulb"** or "**bulblet**" (a small bulb in a cluster of other such bulblets). In a bulb, you will find an entire shoot system of succulent, sugary leaves (or leaf) attached to a very small central stem, and you would expect to see axillary buds or even branching in the axils of the leaves. Often bulbs are borne singly, whereas sometimes they are small (a **bulblet**) and clustered as axillary branches from a central stem system. Since there is stem tissue and these were underground organs, you may see roots coming from the base of the stem if these have not been rubbed off prior to or during shipping.

A final alternative would be **<u>hypocotyl</u>**, and this inference would be based on positional cues. If you recall from your seedling morphology, the hypotocyl is the seedling stem below the cotyledons that transitions to the root. There are no nodes or leaves borne on a hypocotyl.

<u>a. Potato (*Solanum tuberosum*)</u>

The potato is one of the staple foods in US culture. Commonly referred to as a "vegetable," it is consumed for the energy-rich starch it contains.

Is the bulk of the potato a modified leaf(s), stem, or root? What are the "eyes" of the potato? Considering the points above in the introduction to this section and justify your answer with a labeled drawing and use the appropriate modification terms.

<u>b. Sweet potato (*Ipomoea batatas*)</u>

The potato is one of the staple foods in Austrolasian culture. Commonly referred to as a "vegetable," it is consumed for the energy-rich starch it contains.

Is the bulk of the sweet potato a modified leaf(s), stem, or root? Are there any surface features on the sweet potato? If so, do they structurally resemble the eyes of the regular potato or are they different? Considering the points above in the introduction to this section and justify your answer with a labeled drawing and use the appropriate modification terms.

c. Ginger (*Zingiber officinale*)

The ginger is from tropical south Asia and is generally consumed for its aromatic oils as spice although it is also rich in sugar and so can provide abundant energy.

Is the bulk of the ginger a modified leaf(s), stem, or root? Are there any surface features on the ginger? If so, do you think they are modified leaves or parts of leaves? Is there any evidence for branching? Considering the points above in the introduction to this section and justify your answer with a labeled drawing and use the appropriate modification terms.

d. Onion (*Allium cepa*)

The onion is from the Mediterranean region and is generally consumed for its pungent oils as flavor and it is also rich in sugars for energy.

Is the bulk of the onion a modified leaf(s), stem, or root? Considering the points above in the introduction to this section and justify your answer with a labeled drawing and use the appropriate modification terms.

e. Garlic (*Allium sativum*)

Garlic is from the Mediterranean region and is generally consumed for its pungent oils as flavor and it is also rich in sugars for energy.

Is the bulk of a single clove of garlic a modified leaf/leaves, stem, or root? Is this clove borne singly or in clusters? Considering the points above in the introduction to this section and justify your answer with a labeled drawing and use the appropriate modification terms.

f. Radish (*Brassica rapa*)

Revisit your radish plant that you had planted in the first lab. Is there a tuberous structure forming? Is forming in the leaf(s), stem, root or hypocotyl? How can you tell? Return to the Introductory Botany lab to perform the Week 5 exercise.

g. Carrot (*Daucus carota*)

Carrots were originally domesticated in Europe and SW Asia and are generally consumed for their starch and sugar (energy) and carotene pigments that promote healthy corneas. Is the bulk of the carrot a modified leaf(s), stem, or root? Are there any surface features on the carrot? Are there any leaves attached to the carrot and, if so, where are they positioned on the carrot? Considering the points above in the introduction to this section and justify your answer with a labeled drawing and use the appropriate modification terms.

h. Beet (*Beta vulgaris*)

Beets were originally domesticated in the ancient Mediterranean region and is generally consumed for its starch, sugar, and color.

Is the bulk of the beet a modified leaf(s), stem, root or hypocotyl? Are there any surface features on the beet? Are there any leaves attached to the beet and, if so, where are they positioned on the beet? Considering the points above in the introduction to this section and justify your answer with a labeled drawing and use the appropriate modification terms.

2. “Leafy Vegetables” and “Stem Vegetables.”

Vegetables from the above-ground parts of plants are generally leaves, stems, or combinations thereof. If leaves, sometimes we only eat part of the leaves such as the petioles or just the blades. Other times we eat the leaves plus the stem together.

As these vegetables are the products of human intervention in the evolutionary process (artificial selection, aka domestication), we often find the leaves or stems grossly modified in proportion (e.g., very short or small stem and very disproportionately large leaves).

Some vegetables or herbs from the store consist of the leaves alone. Other vegetables come with large leaves attached to a short stem. There are no technical modification terms for such vegetables but the popular vernacular term for these is “leafy vegetables.”

a. Compare and Contrast Brussel Sprouts, Heads of Cabbage, and a Head of Lettuce

Brussels sprouts are borne laterally along the elongate, upright stem of the brussel sprout plant (*Brassica oleracea* var. *gemmifera*). What are these? How do they differ and how are they similar to a head of cabbage (*Brassica oleracea* var. *capitata*). Diagram a longitudinal section through each, label them with the name of the organs involved, and compare and contrast them.

Lettuce (*Lactuca sativa*) is not that closely related taxonomically to cabbage or brussel sprouts, however, it is similar structurally to them. Diagram a head of lettuce, label, and compare and contrast the three.

b. Basil (*Ocimum basilicum*) Observations
Basil is a member of the mint family. *What olfactory similarities to mint can you recognize?*

Basil plants and all members of the mint family are mostly "normal" morphologically, except for their stems. How are their stems unusual? Make a drawing of a contiguous section of shoot that includes two nodes and the attached leaves. Be sure to label all parts including any axillary buds and lateral branches if present.

c. Celery (*Apium graveolens*) Observations
Celery is a member of the parsley family. What part of the celery plant do we eat? Be precise.

How is the bunch of celery from the store similar to a head of cabbage or lettuce? How is it different? Explain and provide a detailed labeled drawing of a celery bunch (a longitudinal section through the bunch would be useful).

d. Asparagus (*Asparagus officinalis*) Observations

Asparagus is a member of the asparagus family, closely related to the lily family. What part of the asparagus plant do we eat? Are there any features of the vegetable to which we could apply some modification terms? Can you determine the phyllotaxy of the asparagus plant based on the asparagus you have before you?

D. Credits

This lab was developed by Christopher Hardy. You may cite it as....

Hardy CR. 2016. Plant modifications and marketplace vegetables. Pp. 103-114 in CR Hardy, RL Wagner (eds) *Guide to Exercises in Concepts of Botany, 4th Edition*. Millersville, Pennsylvania, USA.

Water Relations

Water, in all organisms, is the solvent in which life-supporting chemical reactions occur and provides polar environment integral to everything from membrane and protein structure and function to the intra- and intercellular transport of important soluble molecules and ions. However, these are not the only important roles of water in plants.

In plants, water also is the solvent that carries mineral nutrients from the soil into the plant via the roots. In chloroplasts, it is the source of electrons that drive photosynthesis in the presence of light. Much like the flow of water into a water ballon, it is the uptake of water by young cells that drive their expansion and, thus, the very process of plant growth. Lastly, it is water that fuels the the turgor-based hydrostatic skeleton supporting the primary bodies of stems, leaves and flowers.

In Biology 101, you learned about the process of osmosis (local diffusion of water across semipermeable membranes) and that water diffuses from areas of high water concentration (low **solute** concentration; **hypotonic** solutions) to areas of low water concentration (high solute concentration; **hypertonic** solutions) until equilibrium is reached. However, osmosis is not sufficient alone to explain all aspects of water movement in plants. The concept of **Water potential** (Ψ_w) provides a more comprehensive understanding of water movement. The simple rule is that water flows from an area of high Ψ_w to low Ψ_w (that is, down the Ψ_w gradient). For our purposes, it is sufficient to consider Ψ_w as consisting of two components as follows:

$$\Psi_w = \Psi_s + \Psi_p$$

Ψ_s refers to **solute potential** (also known as osmotic potential). Pure water has a solute potential of zero. Adding solute lowers Ψ_s into negative values. Ψ_p refers to **pressure potential**. Pure water at atmospheric pressure has both a solute and pressure potential of zero. Applying positive or negative pressure to a system will raise or lower Ψ_p. Figure 1 illustrates how these two components work.

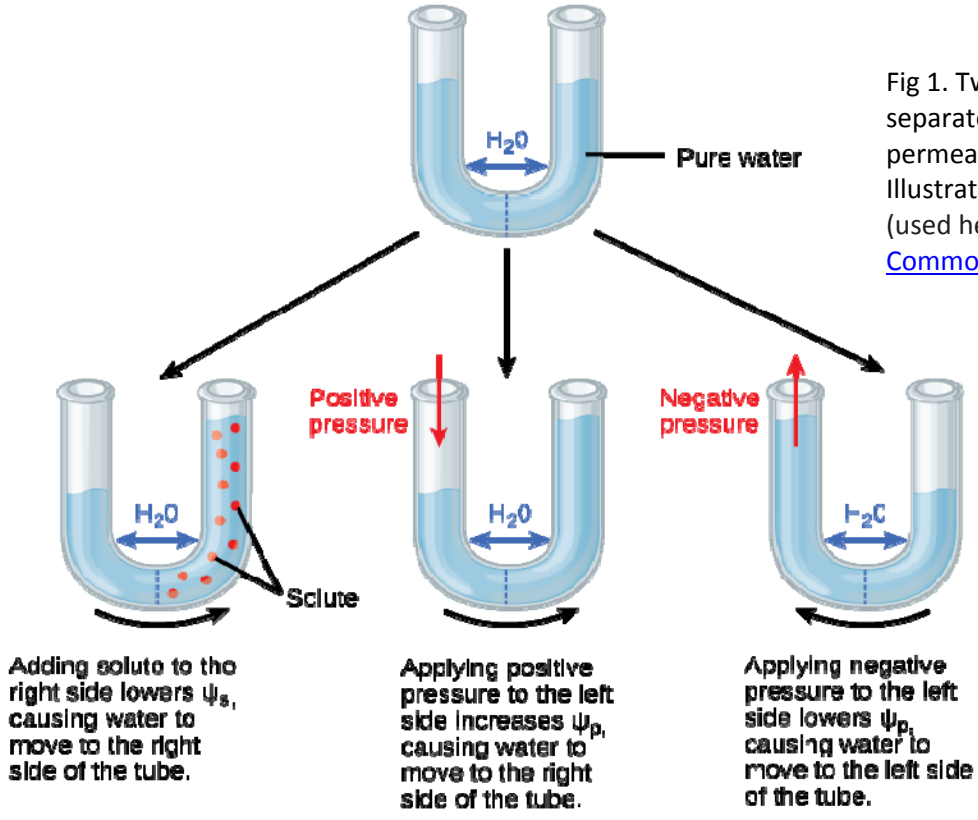


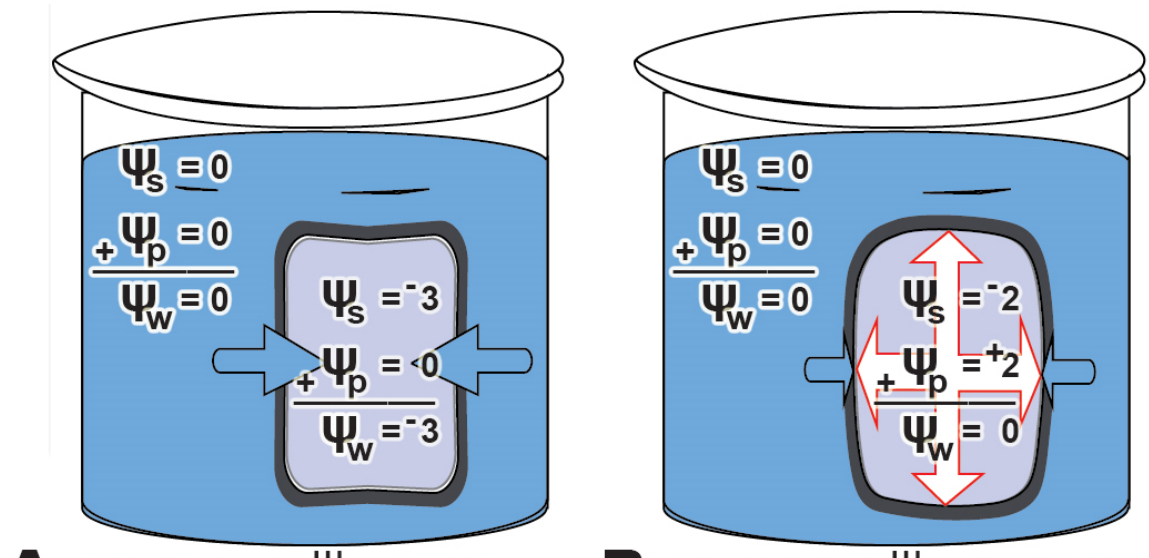
Fig 1. Two aqueous systems separated by a semi-permeable membrane. Illustration by OpenStax (used here under Creative Commons license 4.0).

I. The Role of Water in Turgor Pressure & Mechanical Support

You may have noticed that the leaves and other herbaceous parts of a plant's primary body wilt when the plant does not get enough water. In contrast, the woody parts of plants never noticeably wilt because they are supported by an abundance of fibers, vessels and/or tracheids whose secondary cell walls are heavily lignified. Thus, the observation of wilting in herbaceous plant parts leads to the hypothesis that water is somehow involved in mechanical support in herbaceous plant parts. But before we test this hypothesis, it is important to gather background information from the literature on precisely how water is thought to work in this capacity.

Evert and Eichhorn (2013, page 81), for example, describe how the protoplast of a plant cell in a solution of relatively high Ψ_w will expand due to water influx, thereby placing positive a pressure on the surrounding cell wall, which itself can stretch and bulge outward to a limited extent yet is strong enough not to rupture (Fig 2). This positive hydrostatic pressure is called turgor (aka turgor pressure) and cells that experience it are said to be turgid rather than flaccid. Thus, herbaceous parts of plants are stiff when their cells are turgid, and herbaceous parts wilt when their cells become flaccid.

Today we will test this hypothesis about the role of water in turgor-based mechanical stiffness (turgidity). The following two experiments will manipulate cellular water content in plant tissue by manipulating the Ψ_s component of Ψ_w in the plants' environment.



A. Flaccid cell with $\Psi_W = -3$ just after being placed in pure water.

B. Turgid cell with $\Psi_W = 0$ some time later in pure water.

Fig 2. A cell before (A) and after (B) an equilibrium in water potential between it and its environment has been reached. Note that there is no net flow of water into the cell in B, even though its environment is still hypotonic.

A. Experiment 1: Can a plant wilt even when it gets plenty of water?

For this experiment, you will take two turgid, 1-week old radish seedlings intact in the tubes in which they are rooted. To the rhizosphere of one you will add 150 ml of Solution A and to the rhizosphere of the other you will add 150 ml of Solution B and then you will wait and see which if any solution has an affect on the turgidity of the seedling. One of these solutions is pure (distilled) water and the other is a 7% saline solution; however, you will not be told which is which and, thus, you will have to figure that

out based on the affect on the seedling that you see. Below in Table 1, make your qualitiative predictions about what to expect given the two treatments.

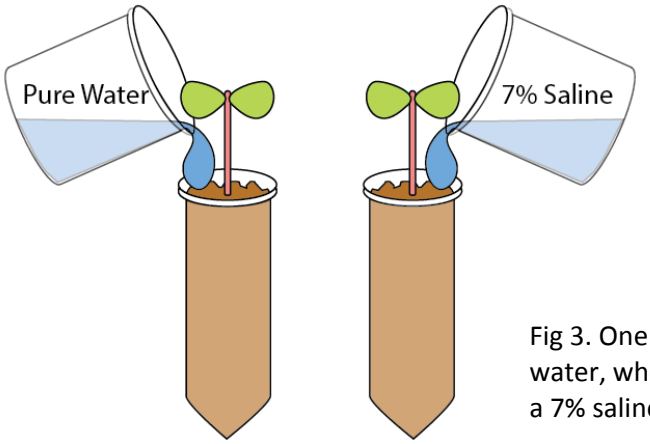


Fig 3. One seedling is to receive pure water, whereas the other will receive a 7% saline solution.

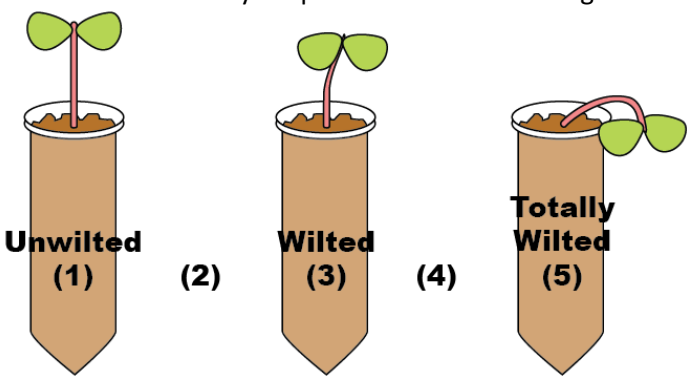
Table 1. Record your hypotheses below about the affect that each type of solution will have on the various components of water potential in the soil immediately surrounding the roots (the rhizosphere), as well as the hydration, turgidity and wilt status of the seedling.

	Treatment Group	
Predicted Affect on…	0.0% Saline Solution	7.0% Saline
Ψ_S of the rhizosphere (+, -, or none)		
Ψ_P of the rhizosphere (+, -, or none)		
Ψ_W of the rhizosphere (+, -, or none)		
hydration of seedling tissue (+, -, or none)		
turgidity of seedling tissue (+, -, or none)		
wilt status (unwlted or wilted)		

1. Procedure

a. Grab two erect seedlings intact in their tubes of vermiculite. If there are more than one seedling per tube, use scissors to remove extras such that each tube has one seedling approximately the same size as the other one.

b. Record initial observations of seedling posture and a semi-quantitative wilt status using the following scale. Note that your plants should be starting at or near stage 1 (unwilted).



Wilt Status of seedling to receive Solution A?

_____

Wilt Status of seedling to receive Solution B?

_____

Fig 4. A semi-quantitative scale with which to record wilt status of your seedlings before and after their treatments.

c. Apply 150 ml of Solution A to one seedling (labeled appropriately) and the same volume of Solution B to the other.

d. After 2 hours, record final wilt status of each using the same semi-quantitative scale (Fig 4).

Wilt Status after one hour of seedling receiving Solution A?

_____

Wilt Status after one hour of seedling receiving Solution B?

_____

2. Analysis

a. Calculate a mean final wilt status for Solution A and B treatments based on the data from each lab group below.

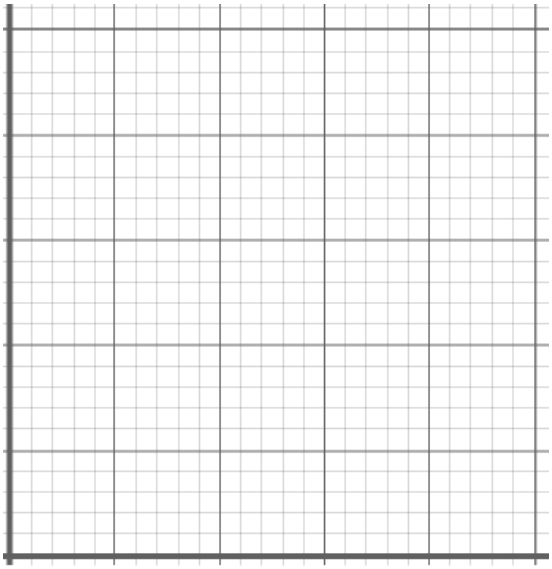
Table 2. Final wilt status after treatment with Solution A and Solution B for 2 hours.

	Final Wilt Status	
Group	**Solution A**	**Solution B**
Group 1		
Group 2		
Group 3		
Group 4		
Group 5		
Group 6		
Mean		

b. Generate a graph depicting the effect of Solutions A and B on final wilt status using the available graph space below.

Before graphing,

- Use the Graphing Appendix if needed to decide whether a bar graph or scatterplot is most appropriate;
- Which of the two variables, Mean Final Wilt Status or Treatment should go on the x-axis and which on the y-axis?



3. Dicussion & Conclusions

a. Which Solution, A or B, do you think was the 7% saline? Explain your answer with reference to real numbers and your figure.

b. Is is possible for a plant to wilt even in the presence an abundance of water in its environment? Explain.

B. Experiment 2: Quantitative assessment of the role of water in turgitity in plant tissue.

1. Procedure

a. Establish 6 sucrose solutions. Set up and label six beakers each with 60 ml of one of the following sucrose solutions: 0.0, 0.2, 0.4, 0.6, 0.8, and 1.0 M.

b. Unfold 6 wet paper towels on your bench in front of each sucrose solution.

c. Prepare 30 1 x 1 x 1 cm cubes from a single fresh potato (*Solanum tuberosum*) tuber. Avoid skin and rotten parts. Rinse with tap water using a strainer and then place 5 cubes into each of the wet paper towels in front of the beakers and cover them with the wet paper towel to prevent desiccation. Gently blot the cubes once covered to remove any excess tap water from their surface.

d. Record initial weights of each set of 5 potato cubes. Quickly weigh each set of 5 cubes together. Record into Table 1 and return cubes to their respective wet paper towels to prevent their desiccation.

Table 1. The weights of each set of 5 potato cubes before and after their exposure to varying sucrose solutions.

	Initial Weight of 5 cubes (g) 0 min	Final Weight of 5 cubes (g) 25 min	Water Flux (% Weight Change)
0.0 M Sucrose			
0.2 M Sucrose			
0.4 M Sucrose			
0.6 M Sucrose			
0.8 M Sucrose			
1.0 M Sucrose			

e. Soak the cubes for 25 min. Soak each set of 5 cubes in their respective solution for 25 minutes.

f. Leave the wet paper towels on the bench and keep moist.

g. After their 25 min soak, remove the cubes. Over the sink, dump each set of 5 cubes into the strainer, rinse with tap water and then return them to the waiting wet paper towels where you can cover them to prevent desiccation. Gently blot if necessary to remove excess tap water.

h. Record final weights of each set of 5 potato cubes. Quickly weigh each set of 5 cubes together. Record into Table 1 and return cubes to their respective wet paper towels to prevent their desiccation.

i. Record mechanical sag for each set of 5 potato cubes. Use a spudometer (Fig 2) to quickly record the mechanical sag of each cube. Record into Table 2.

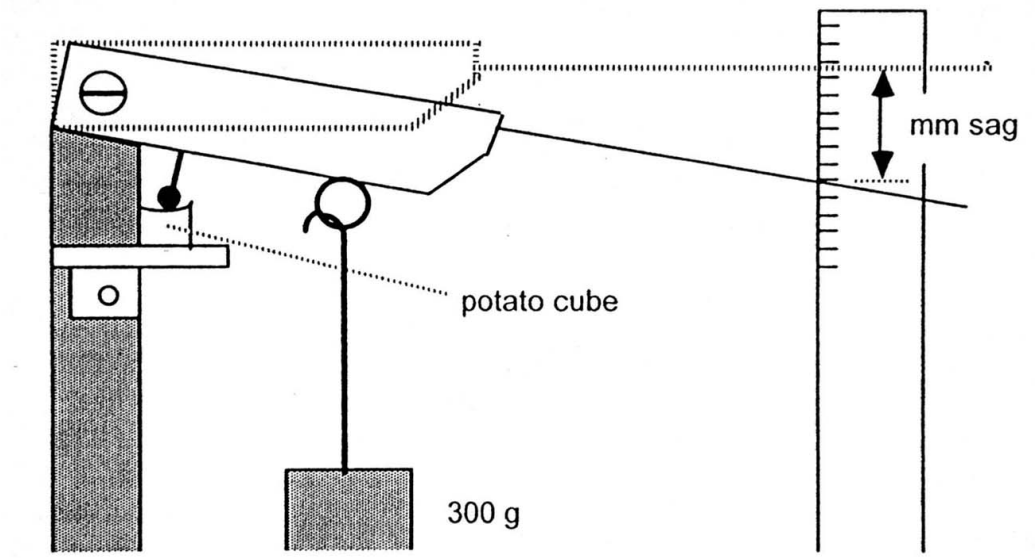


Fig 2. Measuring 'Mechanical Sag' of Potato Cubes Using the Spudometer. The dotted lines show the position of the arm **before** the 300 g weight is added. The solid lines show the position of the arm **after** adding the 300 g weight. Illustration and Spudometer construction by Larry Reinking, Professor Emeritus, Millersville University.

Table 2. The effect of varying sucrose solutions on the mechanical sag of white potato cubes.

Treatment	**Mechanical Sag (mm)**						**Mean Turgidity (mm^{-1})** (1/mean sag or mm^{-1})
	Cube 1	Cube 2	Cube 3	Cube 4	Cube 5	Mean	
0.0 M Sucrose							
0.2 M Sucrose							
0.4 M Sucrose							
0.6 M Sucrose							
0.8 M Sucrose							
1.0 M Sucrose							

2. Analysis

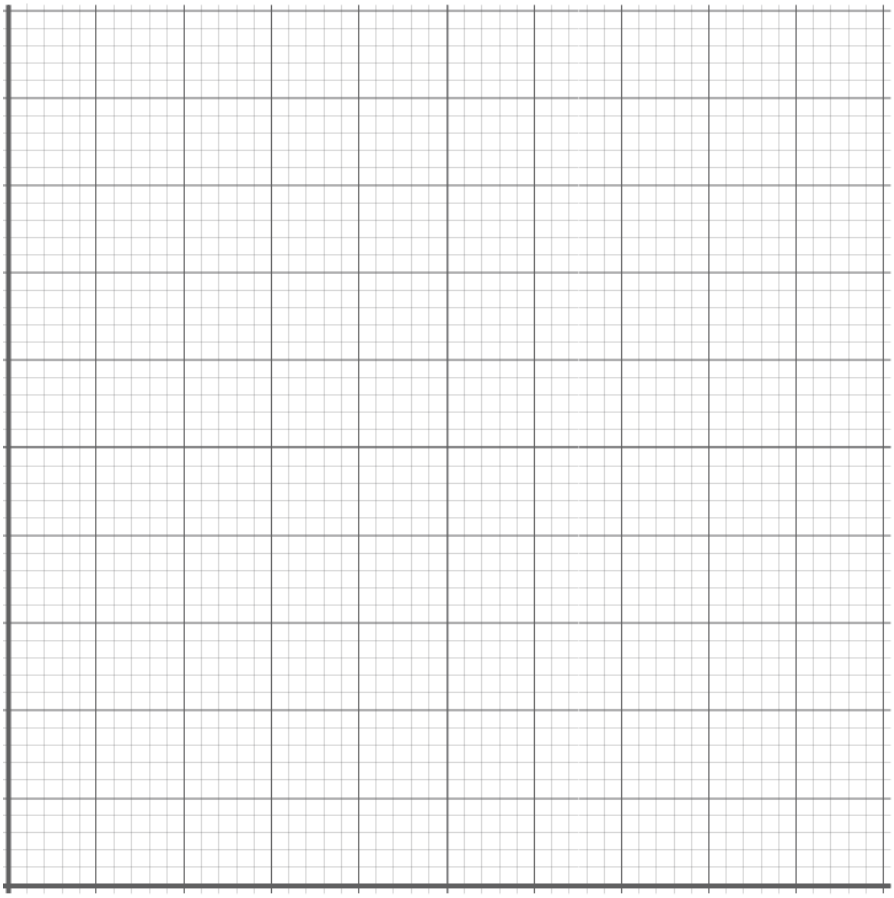
a. Complete Table 3 and generate a graph to assess the correlation between water flux and turgidity in potato tissue. Transfer Water Flux from Table 1 and Turgidity from Table 2 to Table 3. Then use the graph space that follows to create a graph from the data in Table 3 in order to assess the relationship between changes in turgidity and water flux.

Table 3. Water flux and turgidity in potato cubes as a function of sucrose concentration.

	Water Flux (% weight change)	Turgidity (mm^{-1})
0.0 M Sucrose		
0.2 M Sucrose		
0.4 M Sucrose		
0.6 M Sucrose		
0.8 M Sucrose		
1.0 M Sucrose		

Before graphing,

- Use the Graphing Appendix to decide whether a bar graph or scatterplot is most appropriate;
- Which of the two measured variables, water flux or turgidity would you hypothesize to be dependent on the other? Put the dependent variable on the y-axis;
- After plotting the data, decide whether a linear or some curvilinear relationship between the two variables exist and draw a best-fit line accordingly.
- Use your Simple Regression Appendix to perform a regression analysis and to calculate an R^2 value for your line.



3. Dicussion & Conclusions

a. How did the concentration of the sucrose solutions affect water content in the potato tissue?

b. Which concentrations of sucrose were hypotonic and which were hypertonic to the potato tissue?

c. How did hypotonic solutions affect turgidity?

d. How did hypertonic solutions affect turgidity?

e. Is the relationship between cellular water content and turgidity negative or positive? Linear or non-linear?

f. Use the R2 value to assess the strength of the relationship between cellular water content and turgiity.

g. Does this experiment support your about the role of water in generating turgidity? Explain.

h. Use your graph to estimate the turgidity of a raw cube of potato before exposure to any sucrose solution. Explain the principle behind your ability to use the graph in this way.

i. What are the theoretical upper and lower limits of percent weight change in potato tissue?

II. Transpiration and the Environment

Water moves through the plant continuously due to severe differences in water potential between the soil, the plant and the atmosphere. The xylem vascular tissue utilizes the physical properties of water (**surface tension, cohesion, adhesion**) in order to move water from the roots to the shoot. No energy is expended in this process but an important sacrifice is made: that is, water must be lost through **transpiration** (transpiration is the loss of water vapor through stomata). Water availability is a major limiting factor for growth of a plant, and if the **rate of transpiration** exceeds the uptake of water from the soil, then wilting can occur. In the wilted state, the plant cells do not have positive pressure in them and thus cannot grow. So the need for regulation of transpiration is present.

Guard cells regulate the flow of water out of primarily the leaves (where most stomata are) but, due to the nature of photosynthesis, guard cells need to be open for CO_2 to be available. This interaction results in a complex regulation of **stomatal aperture** in order to maximize photosynthesis while minimizing water loss. Like it or not, water is going to be lost (and a large amount of it at that) but the rate will depend on various environmental factors in addition to the anatomy of the plant.

A. Experiment

"Potomeer" Measurements under Environmental Conditions

1. Remove the plunger and needle (if present) from a 1ml syringe.

2. Seal the plastic "needle end" with parafilm.

3. Add 0.8ml of water to the sealed syringe using the second larger syringe with needle. **Record your precise starting volume** in the table below.

4. Place the syringe in a glass test tube → this is your transpirometer!

5. Cut a leaf from the supplied plant material and **IMMEDIATELY** place it into the prepared syringe. Do this very quickly. If you take too long, air will enter the xylem = problems with water uptake. *Optional: gently wrap the top of the syringe with parafilm to seal around the petiole.*

6. Label the tube so you can recognize it (don't cover the number scale on the syringe).

7. Prepare a total of four transpirometers as described above.

8. Place one plant/transpirometer set-up in each of the conditions below for 60-75 minutes:

   a. High Light (back bench light apparatus)
   b. Medium Light (on your lab bench)
   b. Darkness (place in cabinets under lab benches)
   c. Medium Light + Wind (fan station)

9. Record the amount of water lost (beginning volume minus final volume).

10. Weigh the leaf tissue for each experiment (do this last) and calculate area as cm^2.

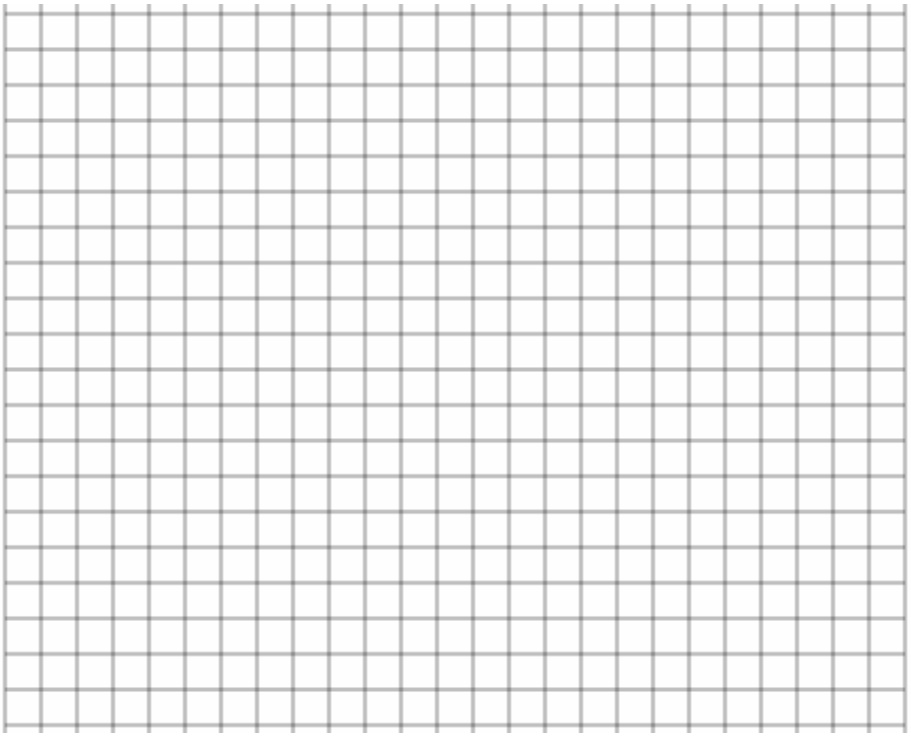
Hint: Determine total leaf area by first calculating the weight/cm^2 of the bean leaf by cutting a square leaf section 3 cm X 3 cm and weighing this leaf section. Divide the leaf section weight by 9 cm^2 to find the weight of 1cm^2 section of leaf. By dividing the total mass of the leaves by the mass of 1cm^2, you will determine the surface area in cm^2 of the leaves on your bean plant.

11. Calculate the rate of transpiration as ml/min/cm^2.

Transpiration Rate Data:

	Leaf Treatments			
	High Light	Med Light	Dark	Wind
Starting H_2O Volume				
Final H_2O Volume				
H_2O Lost				
Time				
Total Leaf Weight				
Weight/cm^2				
Total Leaf Area				
Transpiration Rate (ml/min/cm^2)				

12. NOW, plot the transpiration rates for each treatment on the graph below. Determine what graph format would be the best for comparison. Label the graph appropriately.



a. Which environment caused the most water loss? Why?

b. How & why does light environment affect transpiration?

c. How & why does a windy environment affect transpiration?

d. Did transpiration occur in the dark? Explain this using biological relevant information.

e. Why and how does total surface area need to be considered for all treatments?

f. Name and describe 2 or more reasons that plants transpire at all?

III. Further Exercises: Plasmolysis of onion epidermal cells

Based on our understanding of water potential gradients, we can make a prediction regarding the direction of water movement into or out of a cell if we have some additional information. Two extreme environments that plant cells can be exposed to include a) pure water and b) water with a high salt concentration.

Predict what will happen to red onion epidermal cells if they are exposed to the two environments indicated:

Pure Water:

High Salt (NaCl) Concentration:

Prepare two wet mount slides of red onion epidermal peels (use the red, outer epidermis). Prepare both mounts in pure water initially.

Make observations of the status of the epidermal cells (pay attention to the extent of color distribution which is associated with the vacuole).

Now to one of the wet mounts, add a volume of provided salt solution to one end of the cover slip and draw the liquid under the cover slip by using a paper towel to wick from the opposite side of the cover slip. This will draw the salt solution under the cover slip and into contact with the red onion epidermis.

Make observations and drawings of the status of the epidermal cells. Explain your observations in terms of water potential.

Indicate in the drawing if there is evidence of plasmodesmata.

IV. Credits

This lab was developed from contributions by Christopher Hardy and Ryan Wagner. You may cite it as.... Hardy CR, RL Wagner. 2016. Water relations. Pp. 115-128 in CR Hardy, RL Wagner (eds) *Guide to Exercises in Concepts of Botany, 4th Edition*. Millersville, Pennsylvania, USA.

Photosynthesis

Plants use the energy from light to make sugars (their food) from CO_2 and H_2O through the process of photosynthesis. Thus, plants are **autotrophs**. This is in contrast to **heterotrophs** such as animals and fungi. Today we will be studying photosynthesis from multiple perspectives.

Table of Contents for this lab:

A. Pigment Analysis via Paper Chromatography

It is known that the light absorbed by plants for photosynthesis falls somewhere within the **visible light** portion of the **electromagnetic (em) spectrum** of radiation that comes from the sun (Fig. 1). This visible light portion includes em radiation with **wavelengths (λ)** ranging from just under 400 nm to just over 750 nm. Molecules called **pigments** are, by definition, responsible for the absorption of these wavelengths. We all know that the green pigment chlorophyll is necessary for photosynthesis to occur, but today we wish to determine if other pigments might be involved in driving photosynthesis.

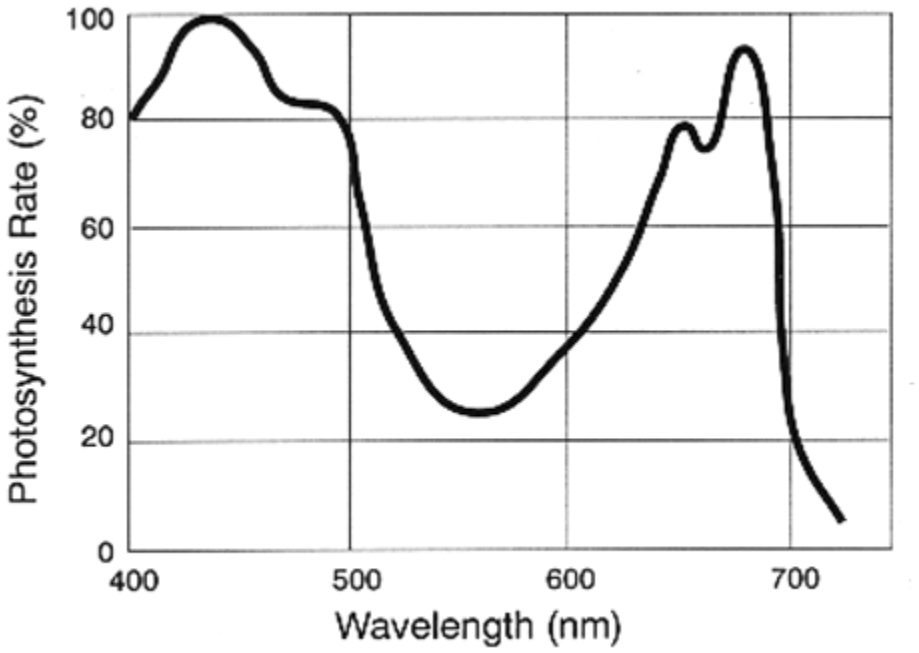


Fig. 1. Action spectrum for photosynthesis in the visible light portion of the electromagnetic spectrum rangeing from violet (400 nm) to red lights (750 nm).

One only has to look to the changing foliage color of deciduous trees in autumn in the temperate latitudes to know that leaves are capable of producing multiple types of pigments. Before the leaves drop, one can see green fading to yellow, reds, orange, or even purple. Over the years, plant biochemists have identified a variety of different pigments such as **chlorophyll a**, **chlorophyll b**, and various carotenoids such as **β-carotene** and **xanthophylls** (Fig. 2). All of these differ in color from one another and so they have different **absorption spectra** that distinguish them (Fig. 3).

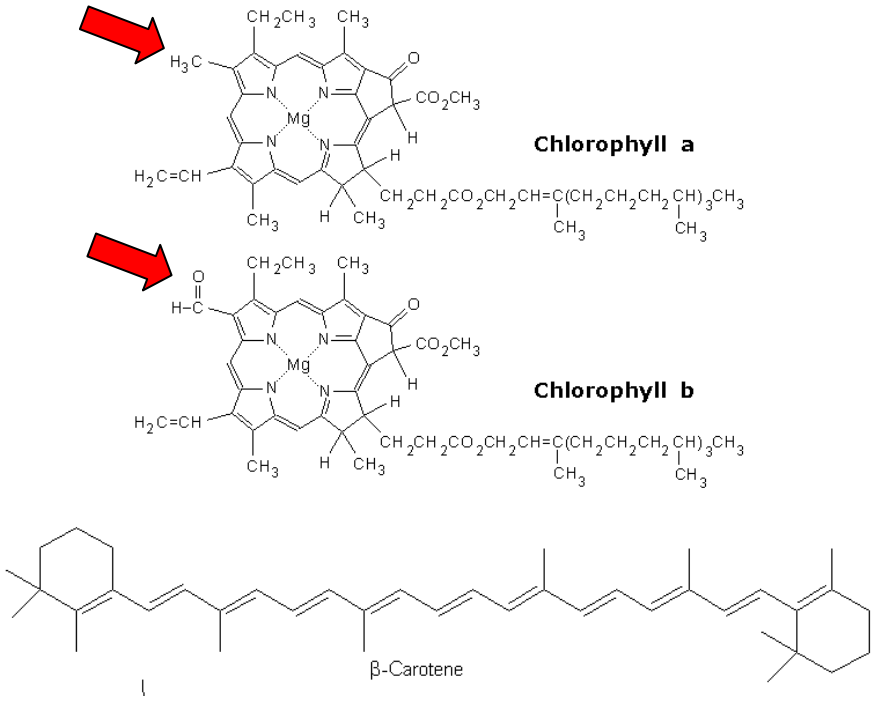


Fig. 2. Molecular structures of chlorophyll a, chlorophyll b and a carotenoid, β– carotene. The arrows indicate the atomic differences between chlorophyll a and b.

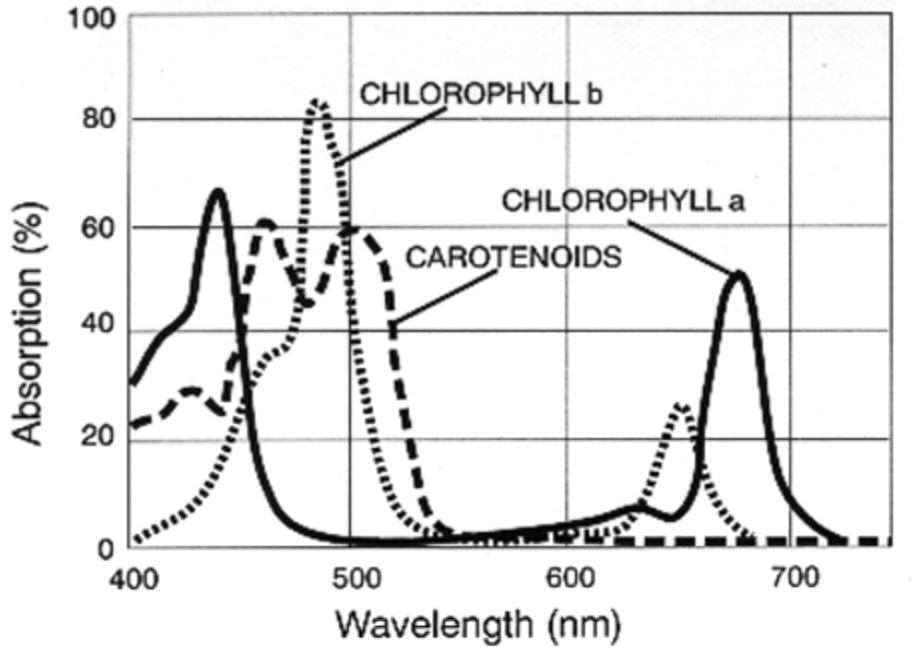


Fig. 3. Absorption spectra for chlorophyll a, chlorophyll b and various carotenoids collectively.

Today we will extract pigment(s) from dark green, living spinach leaves to determine the number and identity of pigments with absorption spectra falling within the action spectrum for photosynthesis. We will use a technique called **paper chromatography**. Paper chromatography will separate multiple pigments if they are present.

Your **INSTRUCTOR** will prepare a crude extract of spinach leaves for you to analyze by extracting the pigments from spinach leaves. In a Waring Blender, 20g of spinach and 100ml of **ACETONE** are combined and macerated for 3 minutes. This will be followed by vacuum filtration to separate the cellular material from the soluble extract. The soluble extract will be collected in a 200-400ml beaker and this will be **YOUR SOURCE** for the paper chromatography.

1. Separate individual lipid-soluble pigments via paper chromatography. Working in groups of 2-4.

a. Obtain a small amount of the stock extract in a 50 ml beaker or flask. Take it back to you bench.

b. Mark lightly a pencil line 2 cm from the bottom of the sheet of chromatography paper.

c. Apply a thin, dark band of the extract to the pencil line using a paintbrush or glass capillary tube. to apply the extract on a pencil line 2 cm. This will need to be repeated several times: allow drying between applications; be sure not to tear or etch the paper if using a capillary tube. Label the top of the chromatography paper in pencil, with your name.

d. Place 100 ml 9:1 v/v petroleum-ether/acetone solvent into a chromatography tank. The height of this liquid in the tank should be 1.5-2.5 cm. (This may have been done previously by the instructor.)

e. Fold the top of the chromatography paper approximately 1 cm from the top of the sheet. Hang the folded top edge of the chromatography paper over a string spanning the top of the tank. Make sure the bottom of the chromatography paper is submerged in the chromatography solvent mixture by 1 cm, but below the line of leaf extract that was applied.
    a. **MAKE SURE YOUR PAPER DOES NOT TOUCH THE TANK WALLS OR OTHER PAPERS.**

f. After sufficient time to separate any pigments, but before the solvent front reaches the top of the chromatography paper, remove and dry the chromatograms.

g. If multiple pigments are present, then they will have separated according to their different polarities.

2. Preliminary Questions about chromatography results.

a. How many pigments can you visualize?

b. What color(s) do you detect?

c. Can you see the chlorophylls and/or carotenoids? Which?

d. *The chromatography solvent mixture was strongly nonpolar (hydrophobic). With that in mind, if you do see multiple pigments, are the pigments at the top of the chromatogram highly polar or highly nonpolar?*

   Explain.

   Why did we use a strongly hydrophobic chromatography solvent mixture to extract and separate photosynthetic pigments?

3. Diagram of chromatography results.

Below, list the color bands identified (in order from top to bottom) and PREDICT which pigment they represent.

(top of chromatogram)

1.

2.

3.

4.

5.

(bottom of chromatogram)

4. Analysis and identification of pigments.

Identify the unknown pigment molecules separated by paper chromatography by determining the absorption spectrum of each unknown and comparing it to the absorption spectrum of known pigments.

a. Turn on your spectrophotometer so it warms up (~15 minutes). Refer to **Appendix A** for the operating procedure of the SpectroMaster 415.

b. Isolate an individual pigment by cutting out a single colored band and then cutting the chromatography paper into strips approximately 2 cm long. Be careful not to combine different colored pigments on your cut paper. Then place the pieces into a labeled test tube (use separate test tubes for each pigment being purified).

c. Add 5 ml of acetone and elute the pigments into solution. USE THE MINIMUM SOLUTION NECESSARY to cover the paper! Elute the pigments for 5 minutes or until all color is removed from the paper.

d. Do this for all of your pigment bands. Keep each band separate so that if you have a minimum of four bands, you should have four test tubes in the next step.

e. Transfer the acetone pigment extract from C (minus the paper) to a clean glass cuvette. Label the very top of the cuvette with the pigment ID number: **Be sure that your label does not cover the area of the tube that will fit into the spectrophotometer slot**. *Do not mix up your samples.*

f. Adjust the spectrophotometer wavelength to **400nm** and insert a cuvette BLANK containing 5 ml of acetone with **NO PIGMENT**. This tube is your **BLANK**. Push the "Set Ref" button to calibrate the absorbance to zero. Remove the BLANK.

g. Then insert each tube unknown pigment cuvettes successively and record absorbance for all pigments before moving to the next wavelength.

****IMPORTANT: DO ALL OF YOUR SAMPLES BEFORE MOVING****
TO NEXT WAVELENGTH!

h. Remove your last sample, increase the wavelength by **20nm** and re-insert the BLANK. Push to the calibrate button and then record the absorbance for each of your pigments.

i. Repeat steps until you have finished with your measurements at **700nm**. Record all values in the provided table.

Table 1. Pigment absorbance in relation to wavelength.

Pigment #:		Pigment #:		Pigment #:		Pigment #:	
λ (nm)	Absorbance	**λ (nm)**	Absorbance	**λ (nm)**	Absorbance	**λ (nm)**	Absorbance
400		400		400		400	
420		420		420		420	
440		440		440		440	
460		460		460		460	
480		480		480		480	
500		500		500		500	
520		520		520		520	
540		540		540		540	
560		560		560		560	
580		580		580		580	
600		600		600		600	
620		620		620		620	
640		640		640		640	
660		660		660		660	
680		680		680		680	
700		700		700		700	

j. Plot the absorbance (*NOTE: absorbance does not have a unit*) as a function of wavelength for EACH UNKNOWN PIGMENT. Graph paper is attached to the back of this manual. If using one graph, use different colors or data symbols or line patterns to clearly distinguish one line from the next. If using one graph per pigment, then different colors and symbols, etc. is not necessary. Use the absorption spectra on the first page of today's lab manual to IDENTIFY the pigments. Clearly label each pigment band as the number it was on the paper AND as the type of pigment it represents.

5. Conclusion Questions.

a. *How many types of pigments could you identify from your green spinach leaves and what are their identities?*

b. *Are the autumn colors you see in leaves the result of pigments that were present all summer or of a succession of pigments produced in the autumn only?*

c. *Do the pigments absorb all wavelengths equally or do they exhibit differential absorption?*

d. *Do any of the bands being analyzed seem to have similar absorption patterns?*

e. *Relate the pigment identified to the color of the leaf and explain why it was or was not originally seen in the leaf.*

B. The effect of light wavelength or color on photosynthesis

Photosynthesis is a complex set of reactions but it can be represented by the following summary equation.

$$6CO_2 + 12H_2O \rightarrow C_6H_{12}O_6 + 6O_2 + 6H_2O$$

From this equation, it can be seen that one can determine the rate of photosynthesis under an experimental set of conditions by measuring the rate at which reactants are consumed or products are produced. Today we will use the rate at which CO2 is consumed as a measure the rate of photosynthesis under different light regimes.

1. Procedure. Each group of 4 should perform one group experiment as follows:

1. Be sure your Vernier CO_2 probe and data collection box and cables are plugged into your computer, a power source, and is turned on.

2. Set probe for low CO_2 levels (0-10,000 ppm) rather than high.
   Note that outdoor atmospheric CO_2 levels are about 380 parts per million (ppm), whereas indoor CO_2 levels will typically vary between 700 and 2000 ppm, depending on who is talking and how much.

3. OPEN the Logger Pro software on your computer and confirm CO_2 readings.

   Once the software is open, you should start to see live CO_2 readings in the lower left of the program window. Be sure these are in ppm. It will take about 1-2 min before the readings are accurate. In order to check on the responsiveness of your probe, you may blow or speak into, resulting in a temporary increase in the CO2 concentration reported.

   If the units are not ppm, change them as follows:
   Experiment
   Change Units
   Carbon Dioxide Gas
   Select “ppm”

4. Close the large digital graphing window since we will be collecting data and graphing by hand, not automatically. Be sure that your close only the graphing window and not the entire program.

5. EXPERIMENTAL CONDITIONS:
   a) Place a water-filled heat shield between the light and the plant specimen chamber/probe set up. Light should be at least a 120 or 150 watt bulb.

   b) Use four to six leaves of hibiscus, holly, or some other plant your instructor has made available. Place the leaves in the plastic chamber so that the adaxial (upper, dark green) sides are all on one side of the chamber that will be exposed to the light. You might need to curl the leaves so that the adaxial (dark/top side) of the leaf faces outward.

      Insert the CO_2 probe into the chamber. Try not to let the leaves touch the probe.

For all of the light treatments below: The leaf chamber should be immediately against (behind) the heat shield and not more than 15 cm from the light source.

c) **Dark treatment for 10 min:** Wrap the chamber in foil and, after 5 minutes, start recording data into Table 1 every minute for 10 minutes.

d) **Red Light treatment for 10 min**: Remove the foil, air out the chamber by removing the probe, then reinsert the probe, expose to red light using red filter and, after 5 min wait, start recording data every minute for 10 min.

e) **Green Light treatment for 10 min**: Remove the foil, air out the chamber by removing the probe, then reinsert the probe, expose to green light using filter and, after 5 min wait, start recording data every minute for 10 min.

f) **Blue Light treatment for 10 min**: Remove the foil, air out the chamber by removing the probe, then reinsert the probe, expose to blue light using filter and, after 5 min wait, start recording data every minute for 10 min.

Table 1. Atmospheric CO_2 over time in a leaf chamber exposed to various light and distance regimes.

Time (min)	Dark (ppm)	Red light (ppm)	Green light (ppm)	Blue light (ppm)
0				
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				

2. Results and Analysis.

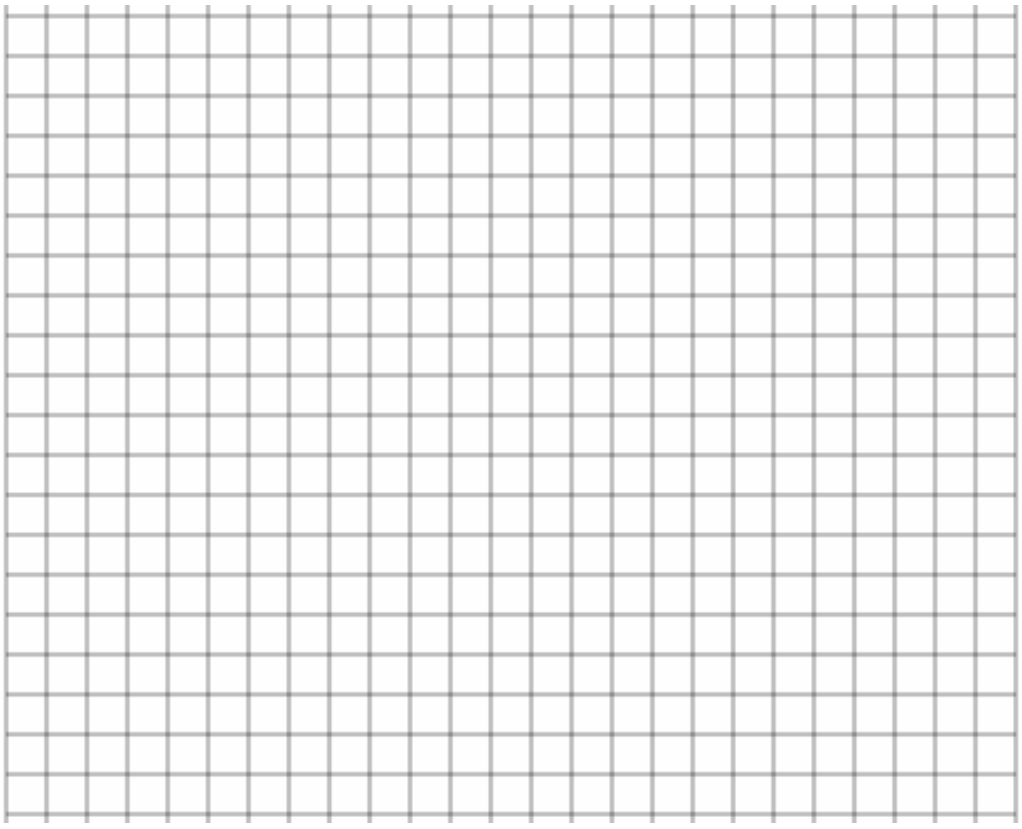
a. Determine the rate of respiration

1) Graph CO_2 over time in the dark. Calculate the slope of a best fit line through the plotted data, which should be a positive number with units ppm/min. This is your rate of respiration and it is assumed to be the same for all treatments. Explain why that is so, using the equation for respiration in the introduction to this lab.

   Remember that the general formula for slope $= (Y_2-Y_1)/(X_2-X_1)$. This is translated into reaction rates as follows:

   If you started in the dark at time 1 (1.0 min) with 600 ppm and ended at time 2 (8.0 min) with 800 ppm, then the rate of respiration calculation is as follows:

   Rate of Respiration (RR) $= (Y_2-Y_1)/(X_2-X_1)$ = (Final CO_2 – Initial CO_2)/(Final time – Initial time)
   = (800 ppm – 600 ppm)/(8.0 min – 1.0 min)
   = 200 ppm/7.0 min
   =**29 ppm/min**



b. Determine the rate of photosynthesis in each light

1) Rate of photosynthesis in red light

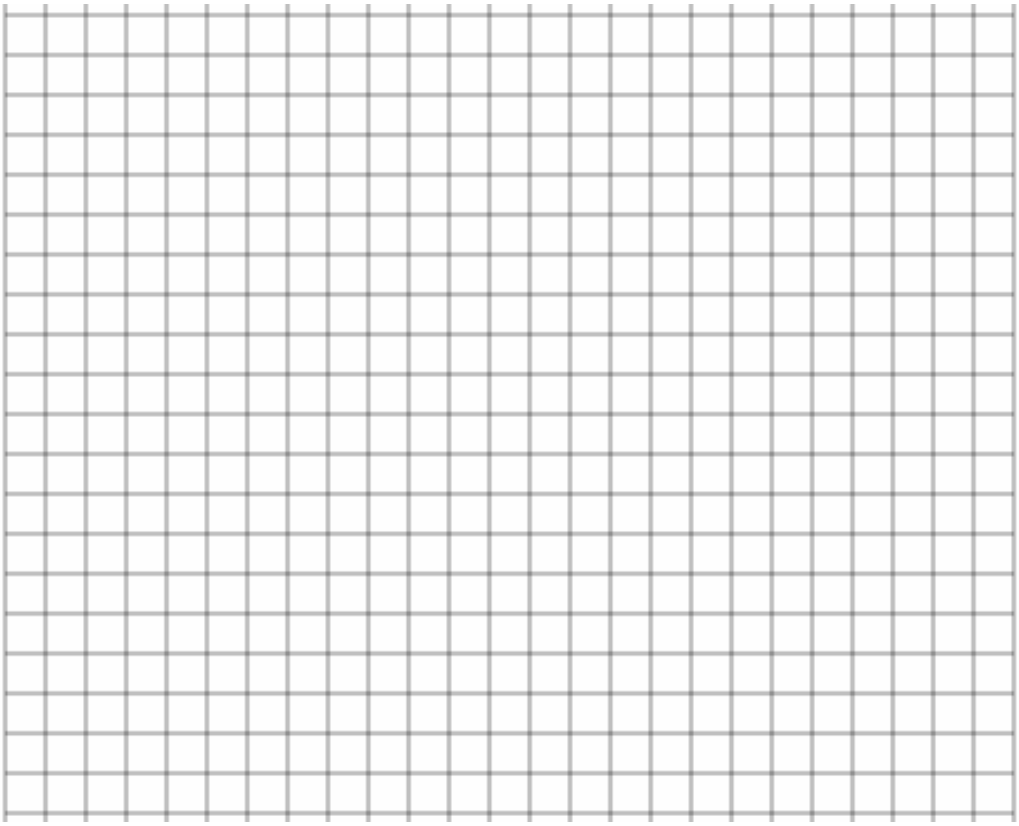
a) Determine the net rate of photosynthesis in red light: Graph CO_2 over time, then calculate the slope of a best fit line through the plotted data, with units ppm/min. Then times this by -1 to yield your net rate of photosynthesis. It can be a negative or positive value. If positive, it means that there was more CO_2 removed from the atmosphere through photosynthesis than was being produced via the simultaneous process of respiration.

$$NRP_{red} = slope_{red} \times -1$$

b) Determine the gross rate of photosynthesis in red light: Add the rate of respiration (calculated during the dark treatment) to the net rate of photosynthesis in red light as follows;

$$GRP_{red} = NRP_{red} + RR$$

2) Rate of photosynthesis in green light



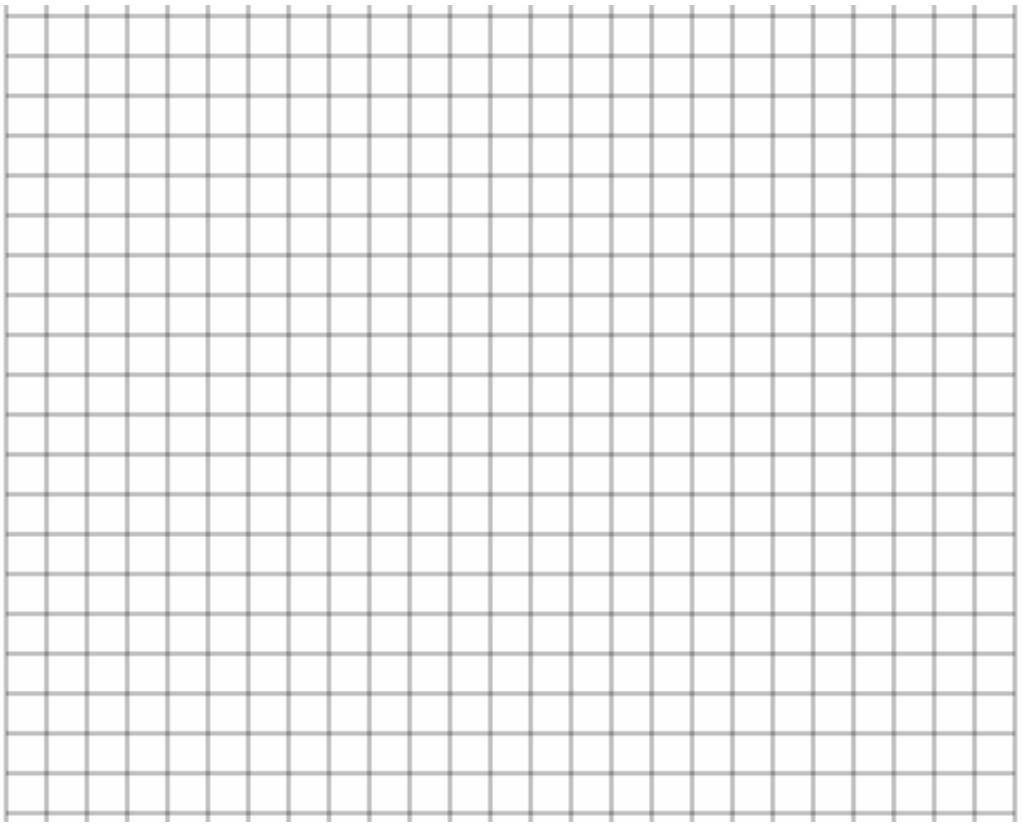
a) Determine the net rate of photosynthesis in green light in a manner similar to that for the red light but using data collected during the green light period.

$$NRP_{green} = slope_{green} \times -1$$

b) Determine the gross rate of photosynthesis in green light: Add the rate of respiration to the net rate of photosynthesis as follows;

$$GRP_{green} = NRP_{green} + RR$$

3) Rate of photosynthesis blue light



a) Determine the net rate of photosynthesis in blue light by using data collected during the blue light period.

$$NRP_{blue} = slope_{blue} \times -1$$

b) Determine the gross rate of photosynthesis in blue light: Add the rate of respiration to the net rate of photosynthesis as follows;

$$GRP_{blue} = NRP_{blue} + RR$$

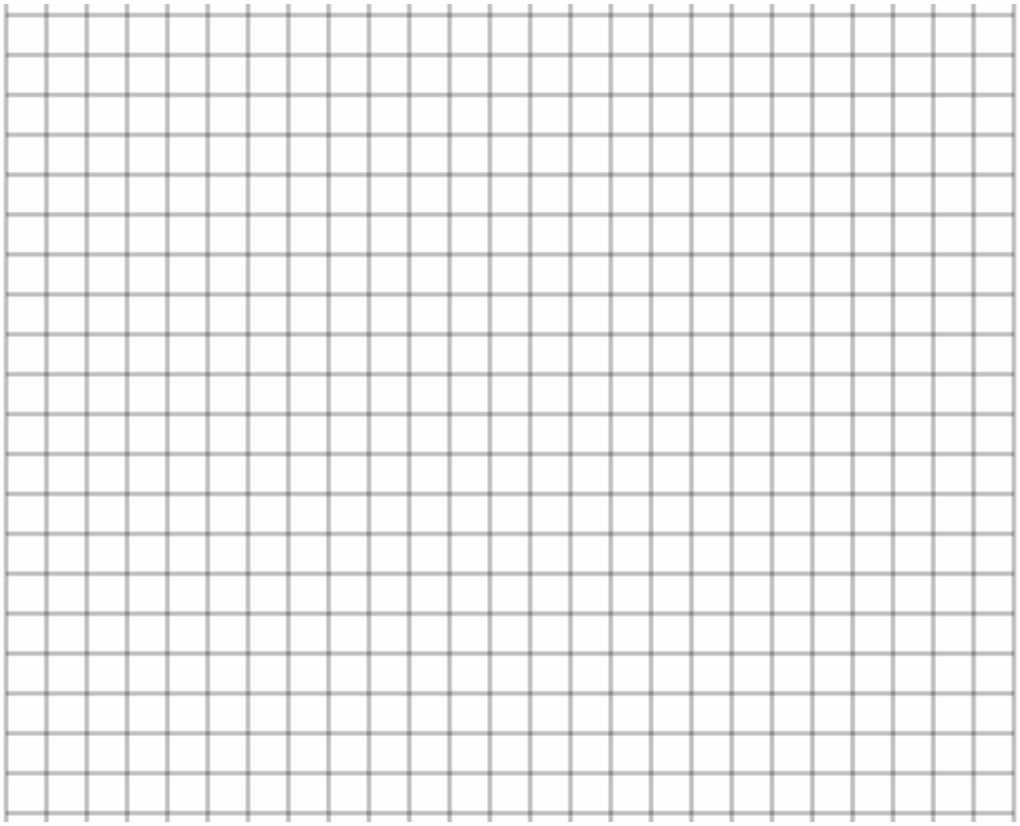
3. Discussion and Conclusions.

a. *How did CO_2 concentration change during the dark treatment?*

   What process occurs in dark that influences gas use/production

   Explain your answer:

b. *How do different light regimes affect the rate of photosynthesis?*
   Explain your answer using specific rate values and by creating a new graph below showing gross rate of photosynthesis (GRP) as a function of light color (do not include dark data of course). This is your all-important conclusions-figure in which you communicate to your audience the results of your experiment. Decide whether or not a line or bar graph is appropriate here.



Which light yielded the strongest photosynthetic response? Which the weakest? Does this make since? Explain

Compare these results to your absorbance spectral analysis. Are they consistent?

C. Credits

This lab was developed by Ryan Wagner and Christopher Hardy. You may cite it as....
Wagner RL, CR Hardy. 2016. Photosynthesis. Pp. 129-148 in CR Hardy, RL Wagner (eds) *Guide to Exercises in Concepts of Botany, 4th Edition*. Millersville University, Millersville, Pennsylvania.

Appendix A. Operation of the SpectroMaster Model 415

Basic Operation:

1. Turn on and allow it to warm up for 10-15 minutes.

2. Switch the display to absorbance (abs) rather than transmittance.

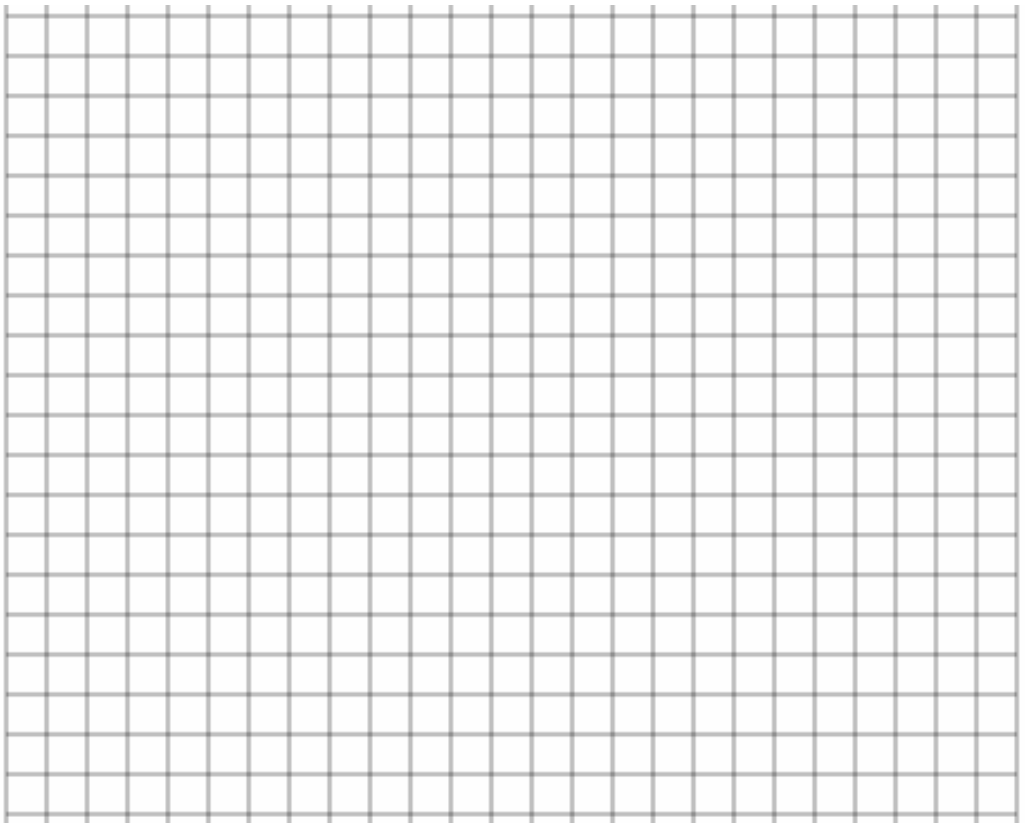
3. Adjust with dial on right to the desired wavelength.

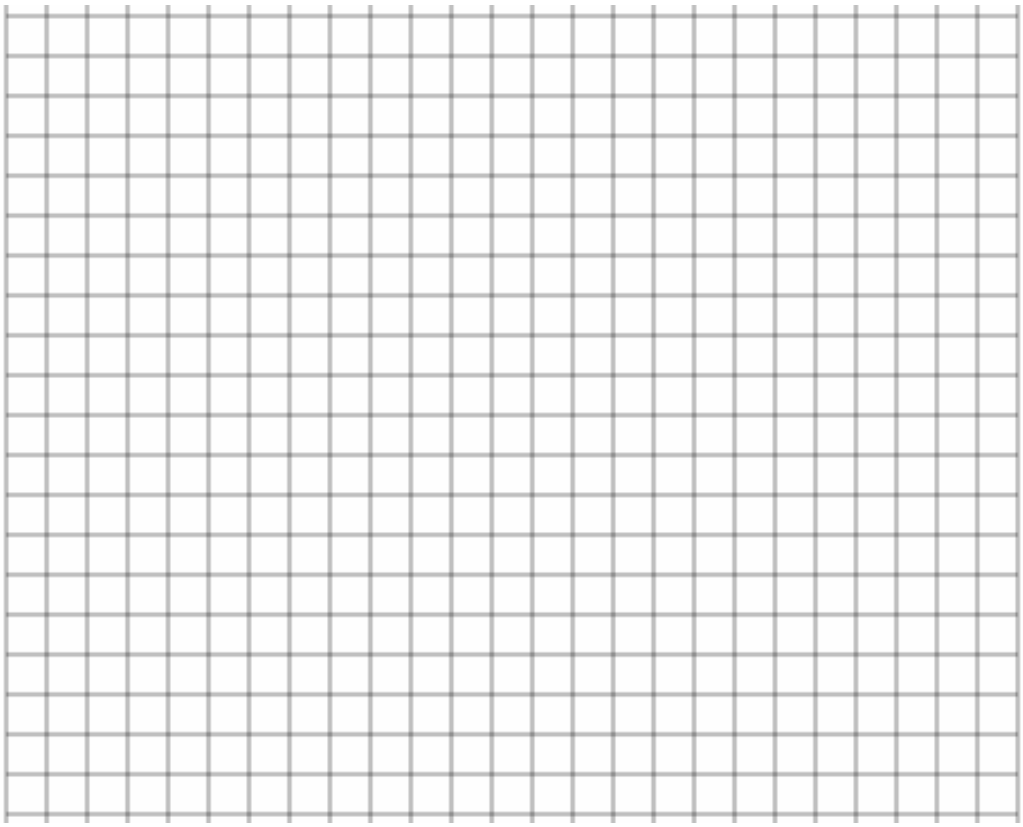
4. Open chamber lid and adjust black filter knob to the correct filter number that is appropriate for the wavelength.

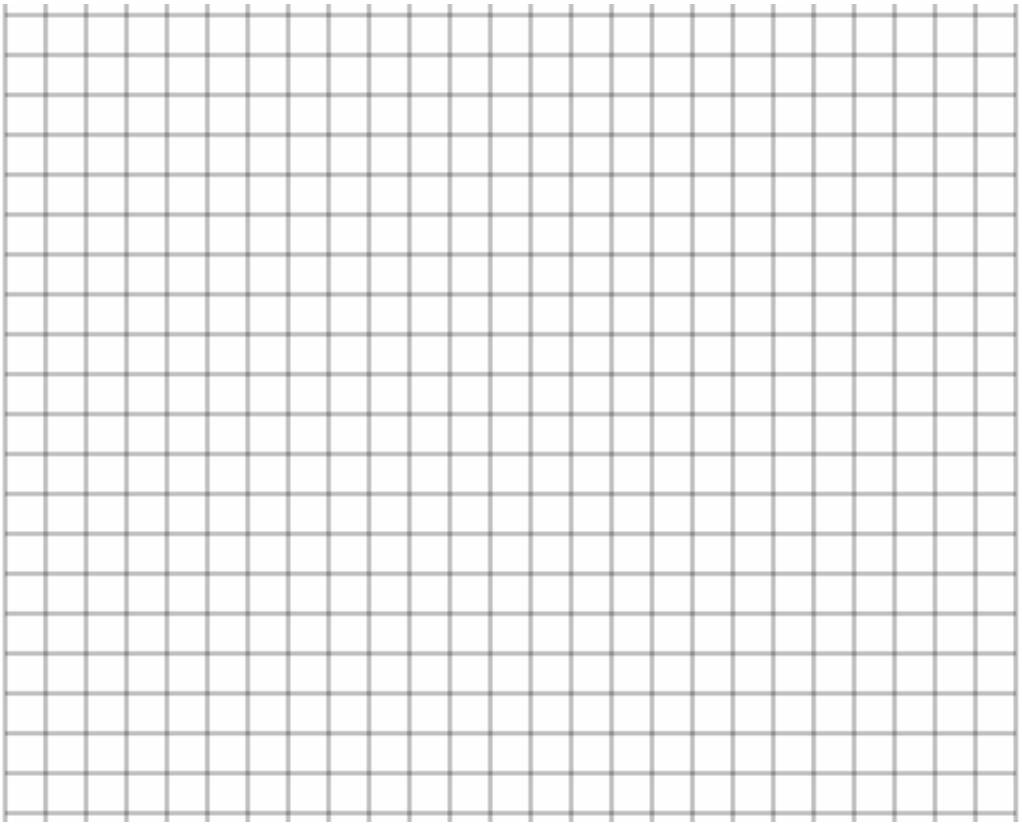
5. Place a reagent blank in the chamber and push “set ref” to calibrate the machine with zero absorbance for a blank.

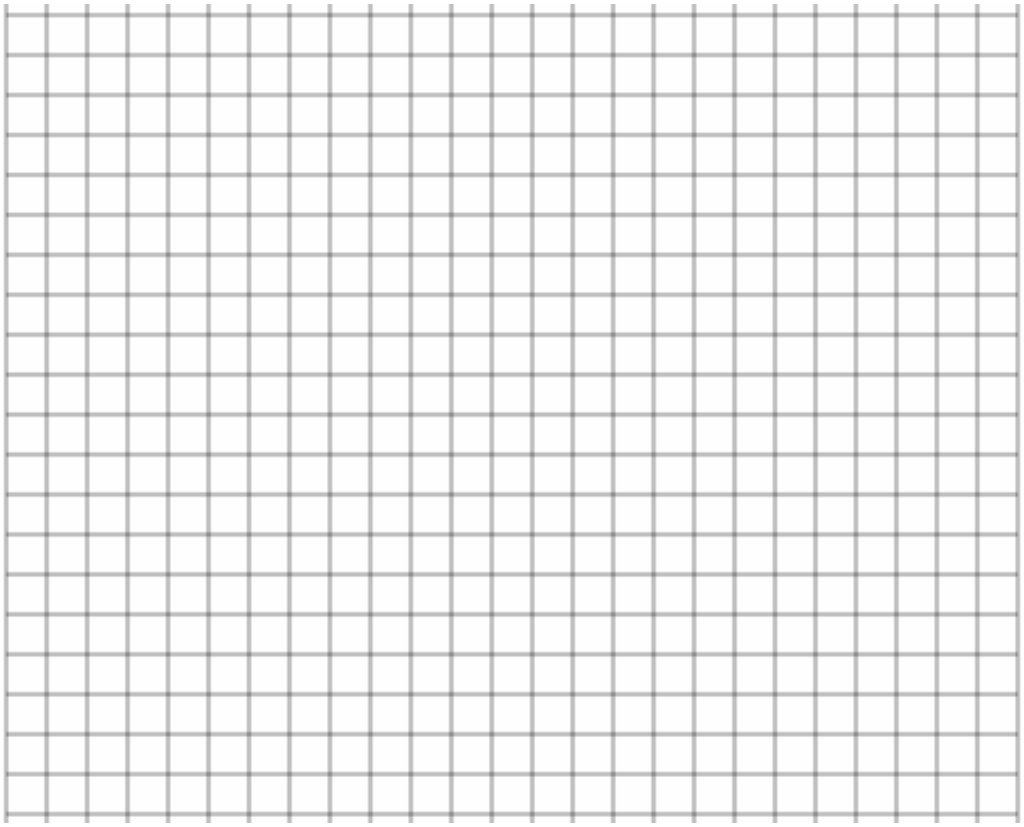
6. Place sample(s) to be read into chamber and record absorbance.

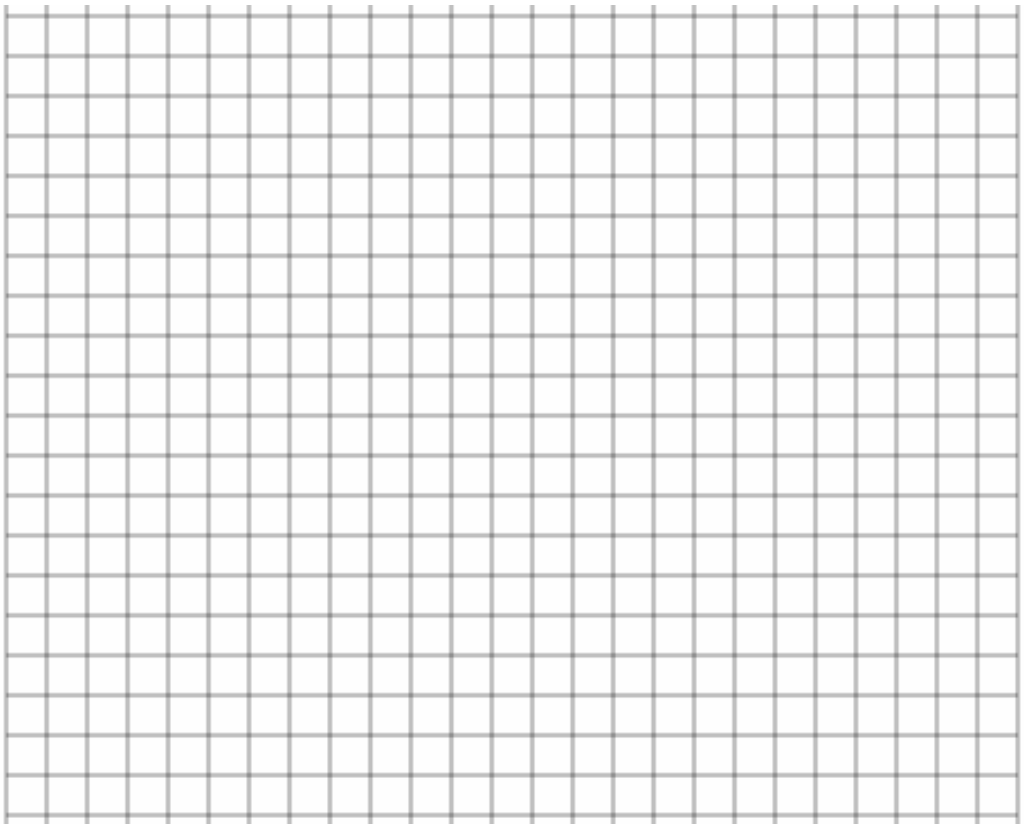
7. To read at a different wavelength, repeat steps 2-6.

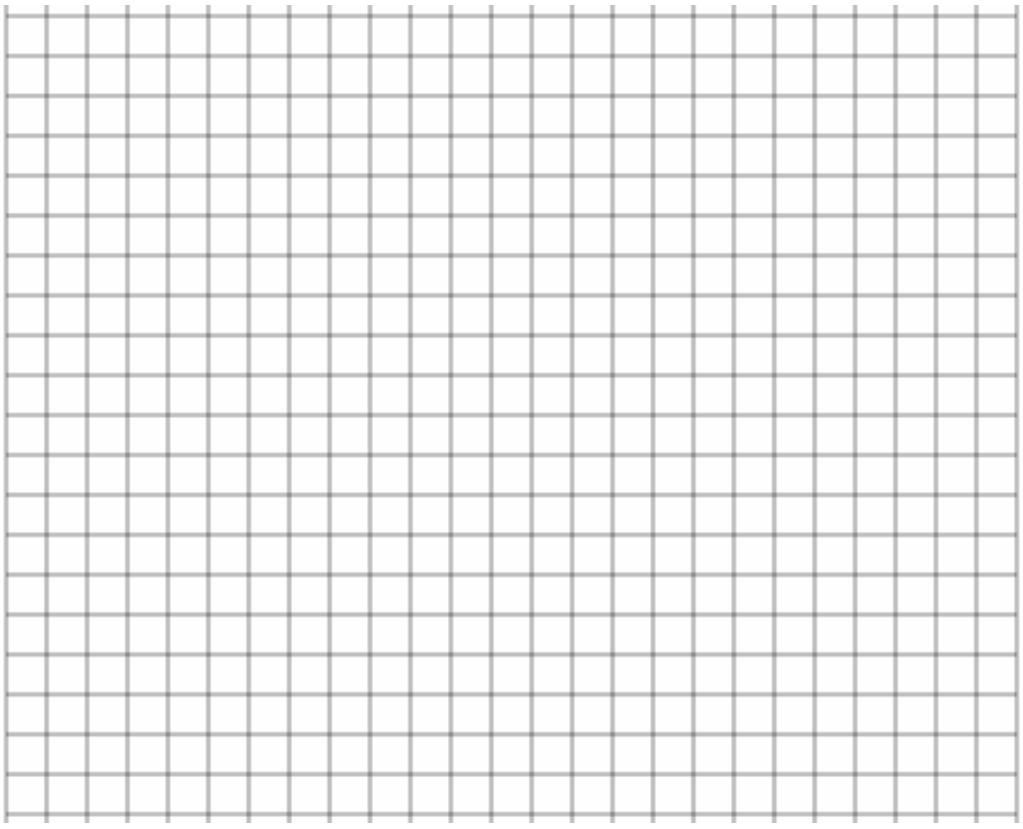












Hormones & Tropisms

Growth and responsive behaviors in plants are the result of **phytohormones** that are synthesized and distributed in response to environmental and internal signals. Some of the most basic aspects of plant development are inseparable from hormones, including cell division, cell elongation and cell differentiation. Currently six hormone classes have been identified in plants: **Auxins**, **Cytokinins**, **Ethylene**, **Gibberellins**, **Abscisic Acid**, and the **Brassinosteroids**. All of these phytochemicals have specific functions critical to the completion of the plant life cycle. These phytohormones all display the characteristics of hormones in general: they are extremely potent such that they can act in very low concentrations (on the order of parts per million or **ppm**), and they may be transported in the plant over great distances to act in tissues far removed from their site of synthesis. While the biochemical response to a stimulus and the distribution of hormones can be quite rapid, often the physical manifestation of the hormonal signals can take a while to see/observe. Therefore we will examine several aspects of hormonal regulation in today's lab over the course of hours or days, depending upon the experiment.

Table of Contents for today's lab:

I. Tropisms

A tropism is a directional and irreversible growth response to some stimulus such as light (**phototropism**), gravity (**gravitropism**), or touch (**thigmotropism**). Growth towards the stimulus is a positive tropic response (e.g., positive phototropism is growth of the organ towards light), whereas growth away from the stimulus is a negative tropic response (e.g., negative gravitropism is growth away from gravity). Auxin is the primary hormone class that regulates the tropisms. The tropisms should not be confused with other plant movements that are driven by reversible and localized changes in turgor (so-called turgor movements). The closing of the Venus flytrap leaf in response to touch, for example, is not thigmotropism, because it is driven by turgor changes in special leaf cells: it is called instead a thigmonastic movement.

A. Phototropism in the Shoot

We will attempt to experimentally dissect the visible light spectrum to determine which wavelengths (colors) of light are responsible for signaling positive phototropic responses in the shoot (primarily stem).

1. Procedure.

a. Perform this setup at the beginning of the lab period. Set up 5 "**LIGHT CHAMBERS**" by using a film canister that has a single hole punched in the side.

b. Set up 5 light treatments by placing the appropriate cover/cellophane filter over the hole of each as follows: 1) DARK (the one chamber with no hole in side), 2) white (clear cellophane over the hole), and 3) Red, 4) Blue, and 5) Green.

c. Fit a layer of moist paper to the inside of the lids.

d. Prepare **one radish or Fast Plant seedling (*ask instructor which is available*) for each chamber** by de-rooting it and placing the cotyledons on the moist filter paper. Complete quickly to avoid desiccation.

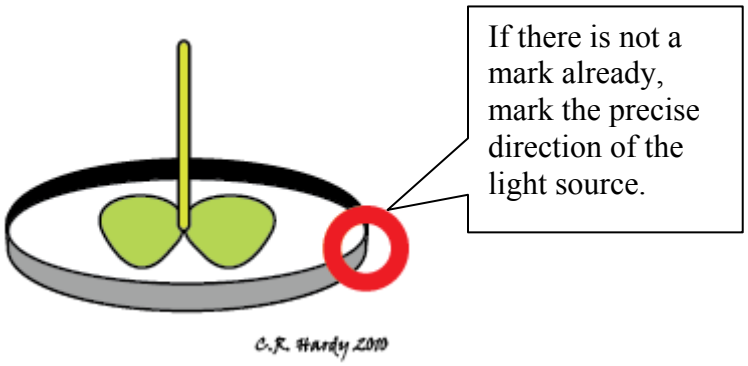


Fig 1. De-rooted radish seedling adhering to wet paper on inside of upside-down film canister lid.

e. On the cap, mark the side of the hole (light source) so that we can know if the hypocotyl grew towards or away from the light source.

f. In Figure 2 (top row), make a drawing of the upside-down seedling and its starting orientation as if the light source is to the right side of your paper. Pay careful attention of the angle of the hypocotyls with respect to the table and the direction in which the light lays (It should be approximately 90° - if it's not, then that's okay, but you will rely on your drawing to see how much it subsequently curves (if any) towards or away from the light).

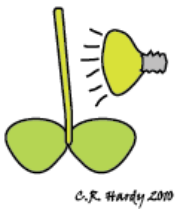
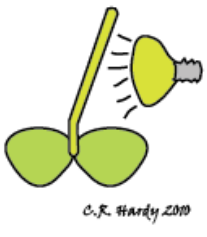
 Sample Initial	Dark Initial	White Initial	Red Initial	Blue Initial	Green Initial
 Sample Final	Dark Final	White Final	Red Initial	Blue Final	Green Initial

Fig 2. Appearance and curvature of radish or Fast Plant hypocotyls before and after exposure to various light regimes. Light source presumed to be <u>to the right</u> in figure.

g. Place the canister over the plant (inverted, see figure below) and put in front of the light source for 90 min. THE PLANT CANISTER SHOULD BE 10cm FROM THE LIGHT SOURCE WITH THE WATER HEAT SHIELD BETWEEN THE LIGHT AND THE CANISTERS.

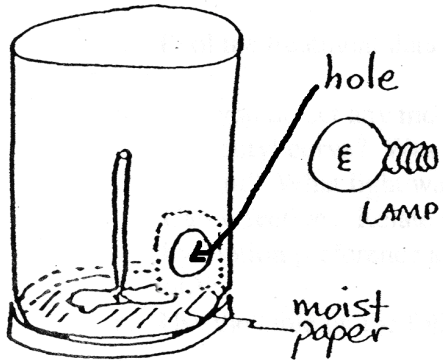


Fig 3. Complete set up.

h. After exactly 90 min, redraw your seedling in the bottom row of Table 1 with respect to the light source and determine how much curvature there was.

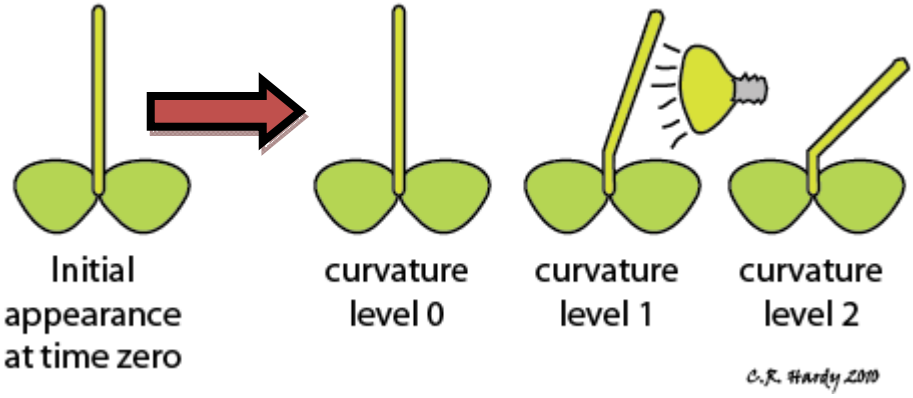


Fig 4. How to quantify the level of curvature. If curvature was towards the light, source, then these numbers (0, 1, or 2) are positive. If away from light, then numbers are negative.

Table 1. Degree and direction of curvature to or from a light source of radish hypocotyls after 90 minutes subjected to that light source. Key as follows: -2, strong curvature away from opening (usually the light source except in the dark treatment), -1 = weak curvature away, 0 = no curvature, 1 = weak curvature towards light source, 2 = strong curvature towards light source.

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Class Sum Total
Dark							
White Light							
Red Light							
Blue Light							
Green Light							

i. Sum the totals for each treatment from all groups to quantify which light treatment caused the greatest and least amount of phototropic response.

2. Conclusions.

a. *Which light treatment(s) caused the greatest positive phototropic responses?*

b. *Which light treatment(s) caused the least positive phototropic responses?*

c. *Can any wavelength stimulate phototropic movements?*

d. *Which way and where did the hypocotyl curve?*

e. *What color pigment(s) might be involved?*

f. *Does it make evolutionary sense that plants do not respond to all wavelengths of light for phototropism to occur?*

g. *Provide scenarios in nature in which phototropic movements benefit the plant?*

B. A Classic Battle: Gravitropism vs. Phototropism

We will attempt to experimentally determine if gravitropism occurs in the stem and then, if so, which is a stronger response, phototropism or gravitropism.

1. Procedure.

a. Radish or Fast Plant seeds have been previously planted in centrifuge tubes containing vermiculite and grown under light for several days. Select **three** of the tubes and label them appropriately for identification by your group.

b. Each group should place a labeled germinated plant/tube in each of the following environments as early in the lab as possible:

1) Dark/Horizontal
2) Top Light/Horizontal
3) Bottom Light/Horizontal

c. Be sure that the plants are oriented parallel to the surface they are placed on (the stem should be horizontal). Make drawings of the plants at the start as follows (you'll need this to compare with the final orientation):

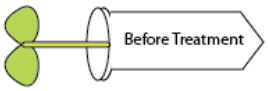
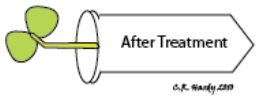
 Sample Initial	Dark/Horizontal Initial	Top Light/Horizontal Initial	Bottom Light/Horizontal Initial
 Sample Final	Dark/Horizontal Final	Top Light/Horizontal Final	Bottom Light/Horizontal Final

Fig 5. Appearance and curvature of radish or Fast Plant hypocotyls before and after exposure to various light / gravity regimes.

Predict how the plant in the DARK/HORIZONTAL environment will respond:

Predict how the plant in the TOP LIGHT/HORIZONTAL environment will respond:

Predict how the plant in the BOTTOM LIGHT/HORIZONTAL environment will respond:

d. Observe the phenotype of the specimens **at the end of the lab period**. Notice any curvature, severity of curvature, or other developmental changes present. Make a drawing of each below and score the degree of curvature as above phototropism experiment.

2. Conclusions.

a) *How did the DARK/HORIZONTAL plant respond to the treatment?*

b) *How did the TOP LIGHT/HORIZONTAL plant respond to the treatment?*

c) *How did the BOTTOM LIGHT/HORIZONTAL plant respond to the treatment?*

d) *Indicate for each scenario, whether the tropic response was positive or negative.*

e) *Which tropism was dominant?*

f) *Provide scenarios in nature in which these behaviors would benefit the plant?*

3. If instructor asks...

*Observe the phenotype of the specimens **after 12-24 hours**. Notice any curvature, severity of curvature, or other developmental changes present. Answer the following questions.*

How did the DARK/HORIZONTAL plant respond to the treatment?

How did the TOP LIGHT/HORIZONTAL plant respond to the treatment?

How did the BOTTOM LIGHT/HORIZONTAL plant respond to the treatment?

Indicate for each scenario, whether the tropic response was positive or negative.

Which tropism was dominant?

How did the results change (if at all) after a longer exposure time?

II. Hormonal Regulation of Non-Tropic Growth Processes

A. The Role of Gibberellins in Internode Elongation

Gibberellins (aka **gibberellic acids**) are known to be the primarily hormone stimulating stem (internode) elongation in plants. Today you will set up an experiment that will test a hypothesis about the reason a dwarf cultivar of pea, *Pisum sativum* 'Little Marvel', is dwarf by apply GA to the plants and letting them grow for a week. We would expect normal plants to increase their rate of stem elongation in response to added GA. But what about the mutant?

After following the procedure below (i.e., getting your pots labeled and plants measured), return to this section and speculate here in writing on possible causes of the dwarf phenotype and then develop a hypothesis regarding the outcome of your experiment on the dwarf cultivar.

1. Procedure.

a. Each group of 4 students has two pots of ***Pisum sativum*** '**Little Marvel'** pea cultivars and will be responsible for the measurement of 10 plants in each pot.

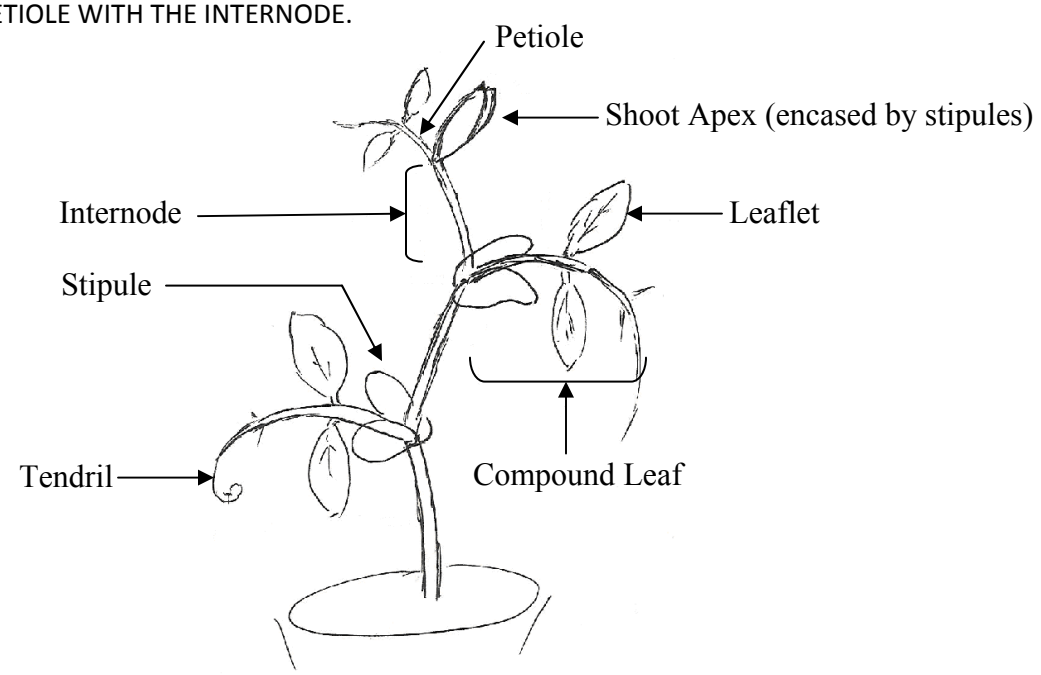
b. If there are more plants than 10 per pot, remove the extras without disturbing the remaining plants.

c. Designate each pot as a different treatment group as follows. Use tape and/or labels to label each pot with your group name and treatment:

Control (water plus tween-20)
10 **ppm GA** plus tween-20

d. Also write your group's name on the labels.

e. Number each plant in each pot 1-10 with a marker. On each plant, also identify and mark the first and second internodes with a marker. These are the youngest internodes closest to the growing tip. REMEMBER, PEAS HAVE COMPOUND LEAVES…BE CAREFUL TO NOT CONFUSE THE PETIOLE WITH THE INTERNODE.



f. As a group of 4, record the following measurements for each plant in the following tables:

- Length in mm of the first (distalmost) internode extending from the apex
- Length of the second (second distalmost) internode from the apex.

g. Determine the average lengths for both the first and second internodes in the following tables.

i. **<u>As a class and with your instructor:</u>** Take the Control and GA treatment pots to the hall or outside and apply the foliar spray treatment.

> Be sure to completely cover the leaves of all pea plants in the pots. Be careful not to cross-contaminate with drift from the other groups that may be spraying.

J. Return the pots to the greenhouse for light and watering (by staff) until the next lab period.

k. After 1 week, measure the lengths of the same internodes again. KEEP IN MIND THAT THESE INTERNODES WILL NO LONGER BE THE FIRST AND SECOND INTERNODES.

Table 2. 'Little Marvel' cultivar results with CONTROL treatment.

	Internode 1 (distal) length (mm)			Internode 2 (proximal) length (mm)		
	day 0	day 7	% change	day 0	day 7	% change
plant 1						
plant 2						
plant 3						
plant 4						
plant 5						
plant 6						
plant 7						
plant 8						
plant 9						
plant 10						
Average						

Table 3. 'Little Marvel' cultivar results with GA 10 ppm treatment.

	Internode 1 (distal) length (mm)			Internode 2 (proximal) length (mm)		
	day 0	day 7	% change	day 0	day 7	% change
plant 1						
plant 2						
plant 3						
plant 4						
plant 5						
plant 6						
plant 7						
plant 8						
plant 9						
plant 10						
Average						

2. Conclusions.

a. *What were your starting hypotheses regarding the effect of each treatment?*

b. *Did your results support your hypotheses? Explain:*

c. *How did the plant growth change in response to gibberellin application compared to the control?*

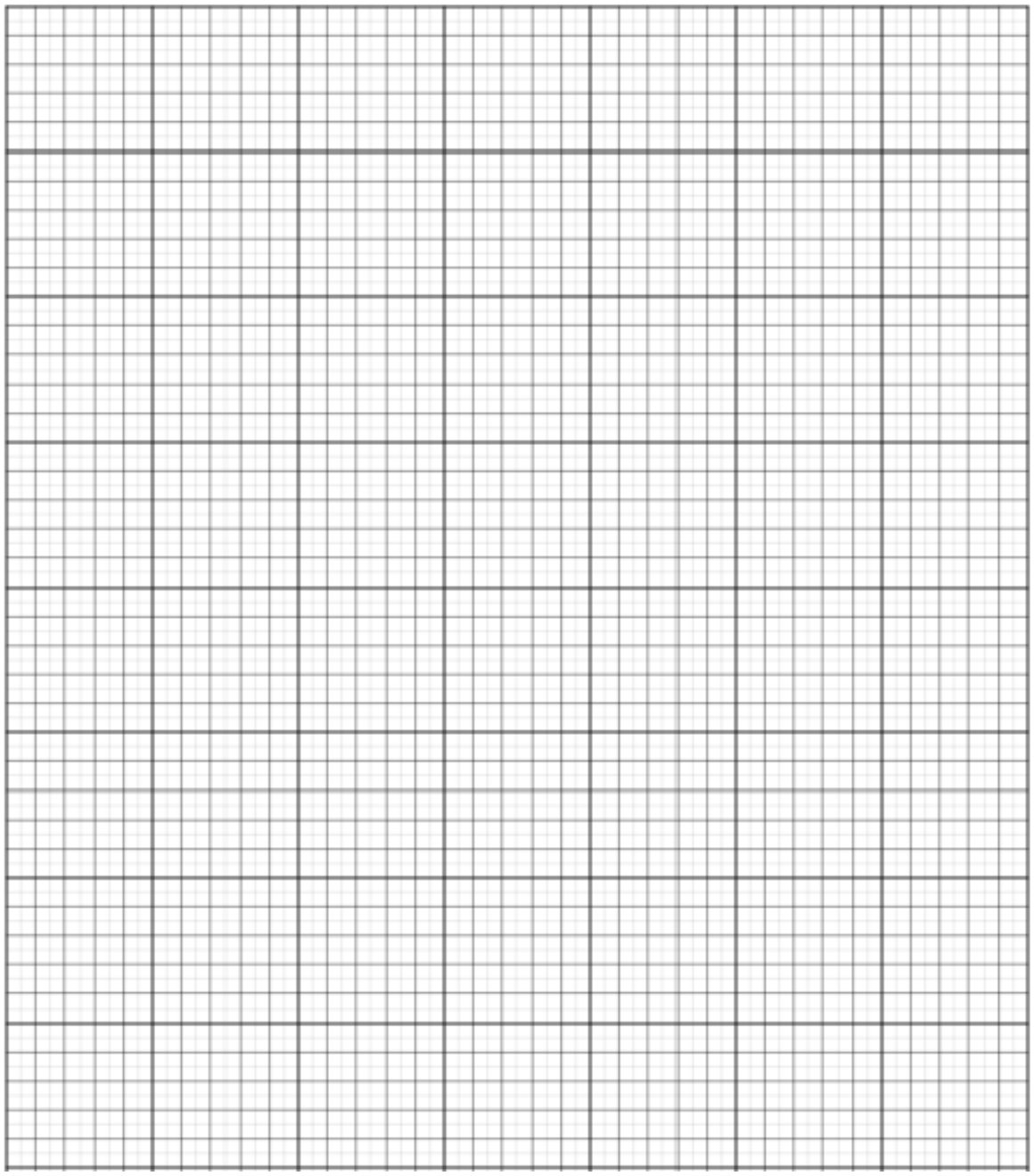
d. *Is there a difference between the two internodes measured for any of the treatments?*

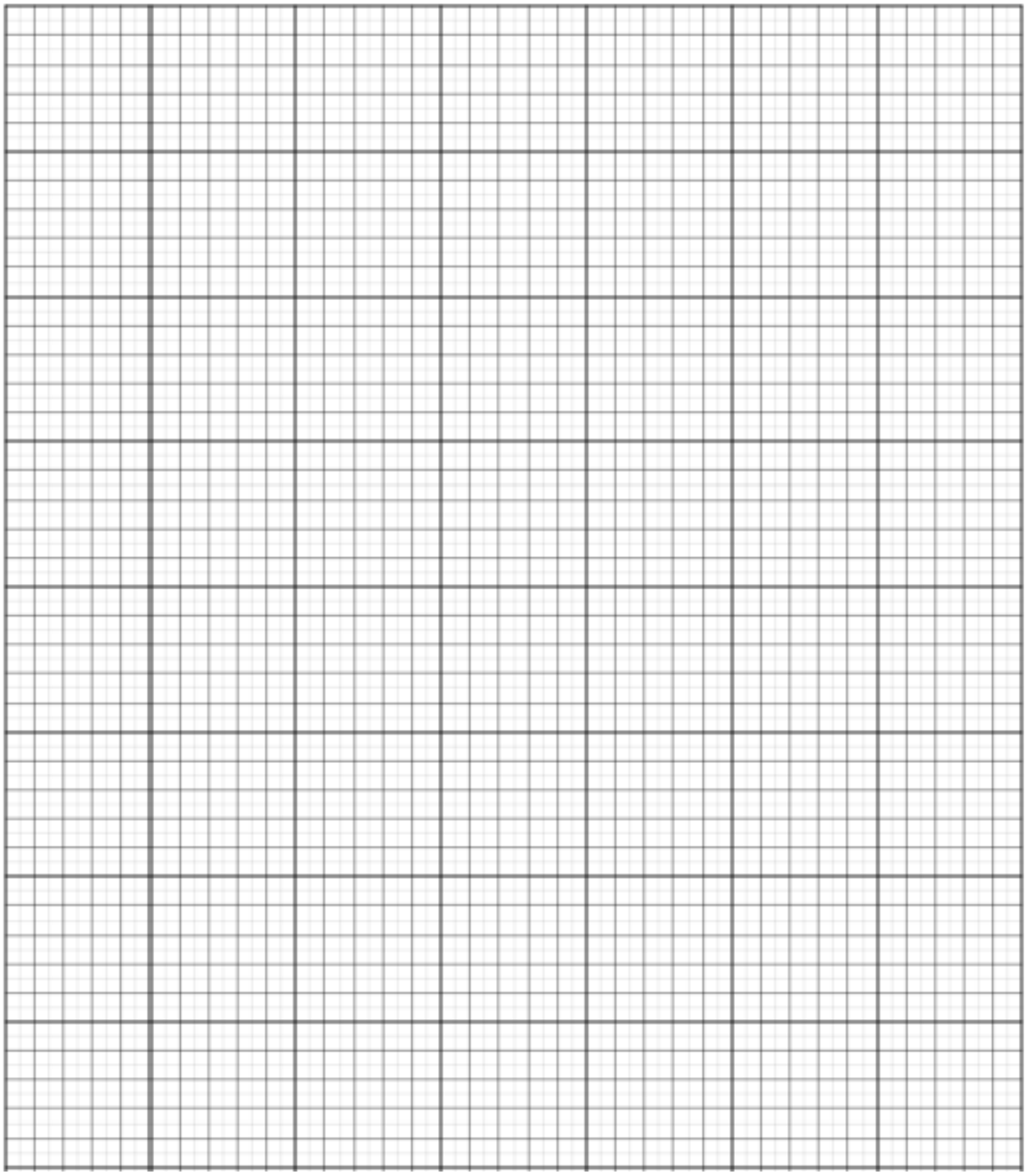
e. *Explain why any differences in the internodes might have occurred.*

f. *Formulate a theory to explain your results?*

III. Credits

This lab was developed by Christopher Hardy and Ryan Wagner. You may cite it as....
Hardy CR, RL Wagner. 2016. Hormones and tropisms. Pp. 149-164 in CR Hardy, RL Wagner (eds) *Guide to Exercises in Concepts of Botany, 4th Edition*. Millersville, Pennsylvania, USA.





The Ethnobotany of Plant Secondary Metabolism

Phytochemicals not involved directly in plant growth and development are called plant secondary metabolites (those that are involved directly, such as growth hormones and sugars, are called primary metabolites). Examples of the former include chemicals used to attract pollinators or seed dispersers, and those that deter or repel herbivores, pathogens, or competitors. Today we will focus on the ethnobotany of some of these chemicals.

HEALTH NOTE: The exercises on coffee, tea, and chocolate today call for tasting. All of these contain caffeine. If you have allergies or are opposed to caffeine consumption, then by all means do not partake. Furthermore, the chocolate was made in a facility that also processes peanuts. Thus, if you have peanut allergies, you will want to avoid the chocolate. However, if you are not involved in tasting, please participate in all other aspects of this lab with your group.

Objectives for this lab:

1) Learn the basics of dyeing with natural plant dyes.
2) Acquire basic systematic and morphological knowledge of plants and their parts used to make coffee, tea, and chocolate.
3) Conduct a systematic comparison of the properties of the beverages or confections made from such plants.
4) Understand the plant biology and ecology that underlies the use of such plants and their chemicals by humans.

Table of Contents for this lab:

Before starting this lab:

It will be interesting to compare your preferences against those of the class, and to see if today's lab has any influence on your preferences.

Table i. Class preferences for the three major tea types before and after conducting today's lab.

	Green	**Oolong**	**Black**	**No preference**
Number of Students preferring before lab				
Number of Students preferring after lab				
Percent Change				

Table ii. Class preferences for the three major chocolate types before and after conducting today's lab.

	Dark	**Milk**	**White**	**No preference**
Number of Students preferring before lab				
Number of Students preferring after lab				
Percent Change				

I. Dye Plants

Dye plants have been used for millennia to color textiles, hair, or even skin. Biochemically, the dyes extracted from plants are typically secondary metabolites. Depending on the plant species, dyes are obtained from the root, leaves, bark, seeds, or flowers. Two popular sources of natural dyes are walnut husks (e.g., the outer covering of the fruit of *Juglans nigra*, the black walnut) and turmeric (from the rhizomes of *Curcuma longa*, a relative of ginger). We will use one or both of these species in lab, depending upon their availability.

The active dying agents in walnut husks are juglone (which oxidizes brown-black), plumbagin (a yellow pigment) and tannins, which oxidize dark brown and also act as a mordant. The juglone is known to be allelopathic and antiherbivorous. Tannins, found in many different plants, have antiherbivory properties since they bind with protein in an herbivore's stomach and thereby decrease the nutritional value of the food to the consumer.

The dying agent in turmeric is curcumin, which has an earthy, slightly bitter and slight chili-like taste. Turmeric has antimicrobial activity and is used by South Asians not only for spice, color, and as a food preservative, but also to self-medicate against bacterial infections (Ronita et al. 2009) and many other ailments. Ecologically, the antimicrobial activity is thought to defend the rhizome against microbial attack in the soil.

Working in groups of 4.

A. Inspect unprocessed walnut husks, turmeric powder

Today you will be dyeing fabric with one or more these dye plants, as instructed by your instructor .

Which color to you think each will dye your fabric?

After the dyeing and rinsing process, which color did your fabric come out to be? Was it the same as your prediction?

B. Dye your fabric

Your instructor may have prepared the dye solution ahead of time in order to allow for time for the other exercises today. If that's the case, you only need to follow the procedure below. Otherwise, you will need to break off the husks of the walnuts if you will be dyeing with walnuts. Do not use a knife for this because you might accidently hurt yourself; rather, remove the husk by crushing with a piece of wood (e.g., a small piece of two-by-four). The recipe for both is as follows:

Walnut Dyeing

1. Add as much broken or ground walnut husk to a pot of water as you like (the more the darker your dye).
2. Bring to gentle boil and then reduce to simmer.
3. Mark your fabric (or shirt tag) with marker to indicate it is yours.
4. Wet and wring-out your fabric with water
5. Add shirt to liquid for 2 hours up to several days.
6. Afterwards, remove, rinse, and wash in cold water separately from other clothing the first time.

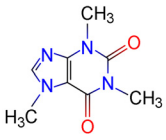
Turmeric Dyeing

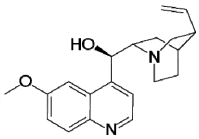
Do the same as above, only add the turmeric powder and stir to mix before bringing to boil.

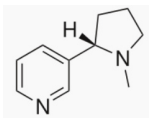
II. Stimulatory Plants

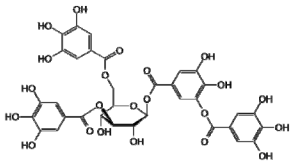
Coffee, tea, and chocolate are three different species from three different families, yet they all have the alkaloid caffeine in common. Alkaloids are a class of N-rich, psychoactive secondary metabolites affecting the central nervous systems (CNS) of the animals that ingest them. Alkaloids generally are bitter to the taste.

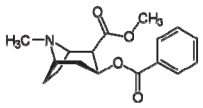
Five of the six secondary metabolites below are classified as alkaloids: *inspect all six and 1) determine which of them are alkaloids and 2) deduce one more thing (about their names) that alkaloids tend to have in common.*

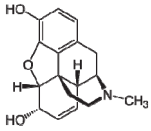

Caffeine


Quinine


Nicotine


Tannic Acid


Cocaine


Morphine

Caffeine itself is found in at least 60 other plant species, and its ecological role suggested by experiments that have shown it to deter herbivory as well as the growth of nearby plants (Dearing et al. 2001; Kim et al. 2006). In humans, caffeine is a CNS stimulant and is mildly addictive. These latter properties of caffeine surely contribute in making these three species the most economically and socially important plants behind the major food crops.

Working in groups of 4, complete the following exercises.

A. Coffee.

Coffee comes from the seeds of plants in the tropical genus *Coffea*. The seeds are packaged in a red berry (called coffee "cherries" by those in the industry) and, once extracted, are allowed to dry and then they are roasted for varying amounts of time at varying temperatures, depending upon the "roast"(e.g., Italian or French roasts are among the darkest, whereas Colombian roast is a medium roast). Generally speaking: the darker the roast, the stronger the flavor.

1. Systematics. Use books in the classroom or the internet to answer.

What is the scientific name for the coffee species?

What is the scientific name of the family it is in?

What malaria medicine comes from this family? What is the genus that this medicine comes from? What type of water (popular to mix drinks with) is flavored with this medicine?

To which country(s) are coffee plants native?

Above: Coffee plant from Köhler (1887). Image processed by Thomas Schoepke at http://www.plant-pictures.de.

2. Morphology. Examine the coffee plants in the room. We only recently received them and so they are still small seedlings. Then use the books in the room, your text, or the internet to answer these questions.

a. *Looking at the internet:*
Is the fruit a berry or a capsule?

What color is the ripe (mature) fruit?

b. *Looking at the coffee plant:*
Habit (herb, shrub or tree):
Phyllotaxy (alternate, opposite or whorled):
Leaf stalking (petiolate or sessile):
Leaf venation (pinnate, palmate or parallel):
Blade margin (entire, toothed or lobed):

c. *Looking at the coffee beans (seeds):*

A coffee fruit typically has two seeds in it, which are appressed tightly against one another and the central fruit septum.

Use this knowledge to explain why the seeds are typically flat on one side.

Find the longitudinal groove on a seed. This is the hilum. Look up hilum and define it below.

Why isn't the hilum on the round side of the seed?

Peaberries. Peaberry is a term used by coffee makers for a coffee seed that is rounded on all sides rather than being flat on the one side. They form naturally at a certain frequency. Sometimes they are selected out and sold separately; however, the coffee at your table has a mix of normal seeds and peaberries (i.e., the naturally occurring peaberries on the trees at the plantation were not sorted out for special processing).

Find a peaberry, compare it to a normal flat-sided seed, and hypothesize why the peaberry is rounded on all sides rather than flat on one.

Do you think peaberries taste any different than the flat-sided seeds?

Now determine the frequency with which peaberries form naturally by determining their frequency in the coffee you have. Each person should count 50 randomly drawn seeds and score them in a table you make below as "peaberry" or "flat-sided". Then, as a class we can tally all results for an accurate estimate of the frequency with which they form.

Table 1. Number and percentage of peaberries relative to regular (flat-sided) coffee beans.

	Your Count	**Your Group's Count**	**Class Count**
Peaberry beans			
Flat-sided beans			
% Peaberries			

3. Roasting. Ripe, red fruits are harvested and the seeds removed. Once cleaned, the clean seeds are placed in a plastic bag and allowed to ferment overnight. After slight fermentation, the seeds are spread across a surface and dried in the sun or at low temperature in oven or with lamp. The dried seeds ("green" coffee beans) can be stored in a jar or in zip-locked bag until roasting. Coffee beans are roasted for 10-15 min at 200 ^{O}C (for light roasts) to 230 ^{O}C (for dark roasts).

How does the appearance and smell compare between the green and roasted coffee beans at your table?

Which smell nicer to you?

As a group of 4, place a fifth of a small Dixie cup's worth of green beans into a ca. 8x8 cm foil boat and place in a toaster oven in the greenhouse that is set to 230 ^{O}C (**NOT THE CUP, ONLY THE SEEDS ON THE FOIL!)**. Since the toaster oven uses the Fahrenheit scale, manually convert 230 ^{O}C into ^{O}F in order to set the oven properly.

The conversion formula for ^{O}C to ^{O}F is: _____

The conversion is: 230 ^{O}C = _____ ^{O}F

Roast for 10-15 minutes and then carefully remove the beans with a spoon onto a napkin or small plate.

Do these beans now smell better than when they were green?

What type of roast does this look like? (light, medium, or dark roast)

4. Brewing. Combine your roasted coffee with others in the class and produce the *Class Special Blend*^{TM} Grind this lot in the coffee grinder in the side or back of the room and use the coffee maker to brew your own coffee. Taste.

Do we have the knack to start our own coffee roasters? Could our coffee fetch $14-16 per pound, retail, as do fine coffees?

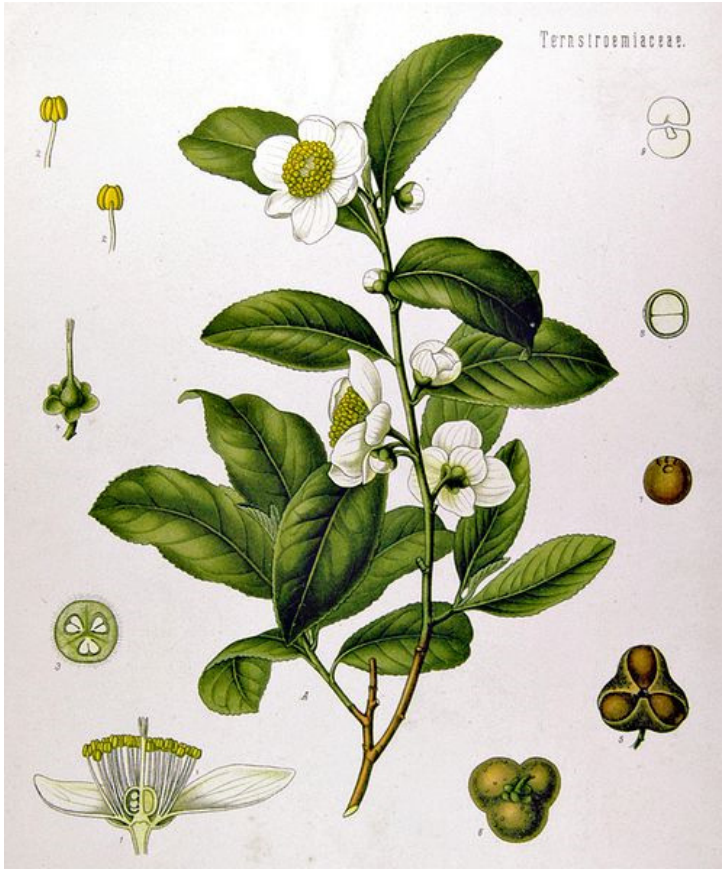
B. Tea.

Strictly speaking, tea comes from the leaves of just one species – a species of the genus *Camellia*. There are three major classes of tea: black (fermented), green (unfermented), and oolong (semifermented) (Ukers 1935: 453). The term "ferment" used in the tea industry is a misnomer because the process is actually oxidation. Black, green and oolong teas all come from the same species, but they are prepared differently following harvesting of the leaves.

Black tea processing: After harvest, the fresh tea leaves are allowed to wilt to about 2/3 their original water content then, while still somewhat moist, they are typically crushed (bruised) or torn and rolled in some way and then allowed to "fully" oxidize. When the tissue is damaged, catechins in the exposed tissues oxidize to form thearubigins and theaflavins. The theaflavins are yellow and lend tea its brisk and bright taste and smell, whereas the thearubigins are orange-brown and provide tea with its depth and body of taste and smell. Chlorophylls are also converted to the dark pigment pheophytin, helping to make the tea leaves dark. After so-called "full" oxidation, the leaves are dried and packed for sale.

Green tea processing: After harvest, the leaves are not allowed to wilt or oxidize (i.e., no "fermentation"). Rather, they are quickly killed by heating with steam or by baking, which prevents oxidation. The leaves are then dried and packaged for sale.

Oolong tea processing: After harvesting, the leaves are allowed to wilt to about 2/3 moisture then they are tumbled (e.g., in a basket) to promote bruising and oxidation. Oxidation is halted earlier than for black tea (e.g., between 30-70% of that for black tea) by steaming or baking of the leaves. After oxidation, the leaves are dried and packed for sale.



Left: Tea plant from Köhler (1887). Image processed by Thomas Schoepke at http://www.plant-pictures.de.

1. Systematics. Use the Introduction, books in the classroom, or the internet to answer the following questions about the systematics of this species.

What is the scientific name for the tea species?

What is the scientific name of the family it is in?

Name one other member of this family that is used in ornamental horticulture.

To which country(s) is the tea plant native?

2. Morphology. Determine the following.

<u>Use the internet books or the illustration above to determine the habit of the tea plant</u>:

Habit (herb, shrub or tree):

<u>Looking at actual tea leaves</u>:

Take a few leaves of whole-leaf tea and rehydrate it using hot water in the two-cup aluminum sauce pan on the hotplate. Once rehydrated, spread the leaf out and determine the following.

Leaf stalking (petiolate or sessile):

Leaf venation (pinnate, palmate or parallel):

Blade margin (entire, toothed or lobed):

<u>Looking at the picture of the tea plant above</u>:

Phyllotaxy (alternate, opposite or whorled):

3. Tea Beverage Study & Comparison. For this you will work in groups of 4 and need the following materials:

- 2 bags of black tea
- 2 bags of oolong tea
- 2 bags of green tea
- 1 two-cup aluminum sauce-pan
- 3 clean (food-grade) 200 ml glass beaker
- Popcorn or peanuts
- Small Dixie or other cups/small (food-grade) beakers for each person to taste tea from.
- 1 hotplate

Directions: Boil one cup or 500 ml of water in the sauce pan. While you are waiting, put a fresh bag of black, oolong, and green teas separately into three separate, clean, small glass containers (200 ml beakers) and label so as not to mix up tea types. Upon boiling:

<u>Black tea</u> – pour 1/3 cup (or 150 ml) into the black tea beaker and steep for 2 minutes.

<u>Oolong tea</u> – wait until water has cooled for 1 minute (to 80-85 °C), then pour 1/3 cup (or 150 ml) into the oolong tea beaker and steep for 2 minutes.

Green tea – after pouring the oolong water, do the same for the green tea and steep for 2 minutes.

After 2 minutes of steeping time for each tea, remove and discard the bags. Pour into your small drinking cups, taste by slurping a little onto your tongue and record observations into Table 2. To cleanse your palate between teas, eat some popcorn or peanuts provided, or take a drink of water.

Table 2. Comparison of beverages made from tea leaves in lab. Use the descriptors provided in the left-most column. Note that you can use the same descriptor for multiple teas.

	Green	**Oolong**	**Black**
Darkness (light; darker; darkest)			
Color			
Aroma (grassy; sweet; smoky)			
Aroma Strength (1-5, mild to strong)			
Rank their tastes as to your liking (0 = least; 1 = more; 2 = most)			

After you are done tasting teas, feel free to make yet another tea but this time add milk and/or sugar. Does this improve the taste for you?

4. Dry Tea Study & Comparison. As a group of 4, take one tea bag each of the black, green, and oolong teas.

How are the tea bags constructed? Draw one below and answer the questions.

Are they simply one simple bag with a single central compartment in which all of the tea sits? If not, then how are they different?

How does this design facilitate a more rapid infusion during the tea-making process than a more simple bag design?

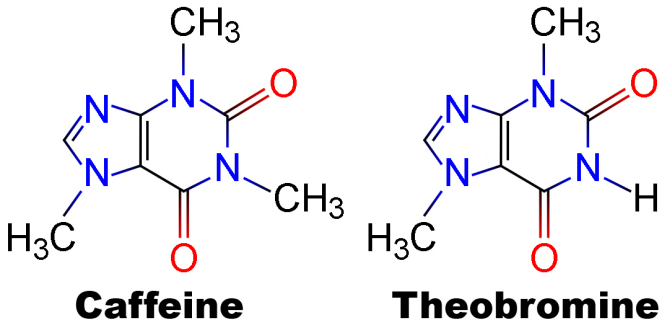
Open each bag and study the tea inside. Then record your observations in Table 3.

Table 3. Comparison of dried leaves available in lab. Use the descriptors provided in the left-most column. Note that you can use the same descriptor for multiple teas.

	Green	**Oolong**	**Black**
Leaf Color (green, dk. brown w/ green tinge, or dk. brown to black w/ reddish tinge)			
Aroma (e.g., grassy, sweet, or smoky)			
Aroma Strength (1-5, mild to strong)			
Rank their aroma as to your liking (0 = least; 1 = more; 2 = most)			

C. Chocolate.

The Aztec and Maya were the first peoples to use the seeds of the cacao tree. They used them to make a drink that is distinctly different than the confection "chocolate" we know today. Like coffee and tea, chocolate has caffeine in it, as well as a similar alkaloid, theobromine, with a similar, but lesser, stimulatory effect on humans.



Above: The structures of caffeine and theobromine. As is typical for structural formulas, the structure is abbreviated by not writing the carbon atoms that are a part of rings, as well as not indicating any hydrogen atoms that are bonded directly to the ring carbons.

There are three major classes of chocolate confection: dark, milk, and white. Unsweetened Baking Chocolate (essentially "raw" chocolate) is not considered a "confection" since it has no sugar added. These all come from the same species and the same part (the seeds), but they are prepared differently (Simpson & Orgorzaly 1995: 458).

Bitter, Baking & Unsweetened Chocolate are all names for the solid form of chocolate liquor or raw chocolate.* It has no sugar or milk added and so can be considered "raw" chocolate. To produce it, the cacao seeds (called "cocoa beans" by English speakers in the

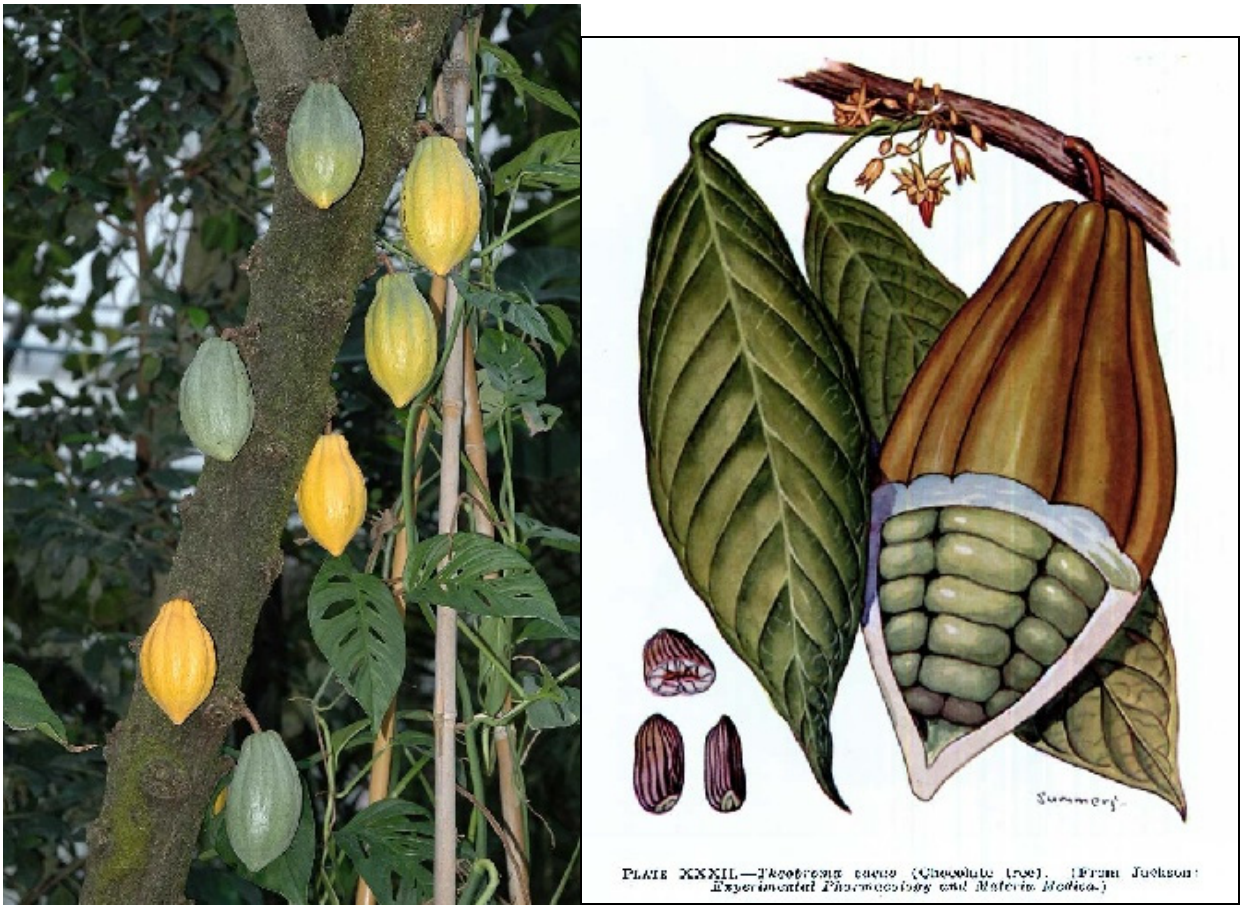
chocolate industry) are fermented, dried, roasted and separated from their seed coats (called "shells" by those in the industry). The seeds are then ground into cocoa paste which is then melted to become chocolate liquor. The liquor is then cooled and molded into blocks of unsweetened chocolate.

*Note that some baking chocolate has had some of the cocoa butter (fat) removed to increase the shelf life since fats such as cocoa butter will spoil before the cocoa solids. Raw chocolate from which all of the cocoa butter has been removed is dry and, when powdered, is called cocoa powder.

Dark Chocolate is produced by adding sugar and typically some additional cocoa butter to chocolate liquor. It either completely lacks or has only a very small amount of milk products in it. There is no official definition of "dark chocolate," but in practice it is a confection with relatively high chocolate liquor content (e.g., 50-85%) and little to no milk.

Milk Chocolate is produced by adding sugar, additional cocoa butter, and milk powder, milk and/or condensed milk to liquor. Because of the high sugar and milk products content, milk chocolate necessarily has much lower percentage of chocolate liquor in it than dark chocolate.

White chocolate is a white or ivory-colored confection of cocoa butter (the chocolate liquor minus the cocoa solids), sugar, and milk. Note that cheap "white chocolates" substitute some or much of the cocoa butter with solid vegetable oils. Without the cocoa solids, white chocolate contains only trace amounts of theobromine and caffeine. Much on the market has vanilla flavoring added to it.



Chocolate plant from www.wikipedia.org.

1. Systematics. Use books in the classroom, or the internet to answer the following questions about the systematics of this species.

What is the scientific name for the chocolate species?

What is the scientific name of the family it is in?

Name one other member of this family (by genus) that is used to make a drink that is rich in caffeine.

To which country(s) is the chocolate tree native?

2. Morphology & Taste of the Seeds. Inspect and taste the raw chocolate seeds available in the room.

What is the shape of the seed?

What is the color of the exterior and interior of the seed?

Can you find a small embryo inside the seed?

Remove the seed coat and taste a seed. Describe it as sweet, semisweet, or bitter.

3. Chocolate Comparative Study. As a group of 4, taste the various forms of chocolate at your table (take one small piece of each chocolate type per person). Then fill in Table 4.

Table 4. Comparison of chocolates available in lab. Use the descriptors provided in the left-most column. Note that you can use the same descriptor for multiple types.

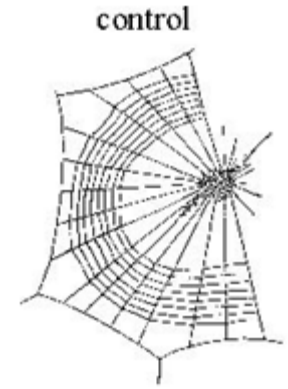
	Raw Seeds	**Dark Choc.**	**Milk Choc.**	**White Choc.**
Color (black, dk.-brown, brown, or white)				
Sweetness (not sweet; semi-sweet; very sweet)				
Bitterness (weak to none; somewhat bitter; very bitter)				
Rank them as to your liking (0 = least; 1 = more; 2 = even more; 3 = most)				

Use information in the Introduction to rank the three chocolate types above as to relative content of the stimulants caffeine and theobromine.

Does this ranking correlate with bitterness? Use information from the Introduction to explain this.

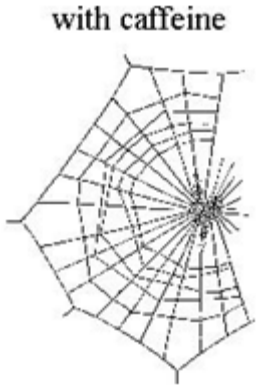
III. Further Analysis

1. It is generally hypothesized that certain plants evolved the ability to make alkaloids such as caffeine as adaptations to deter herbivory. In the experiment below, scientists fed spiders insects, some of which were laced with exogenously applied caffeine. The scientists then looked at the effect of the caffeine on the spider's ability to make their webs.

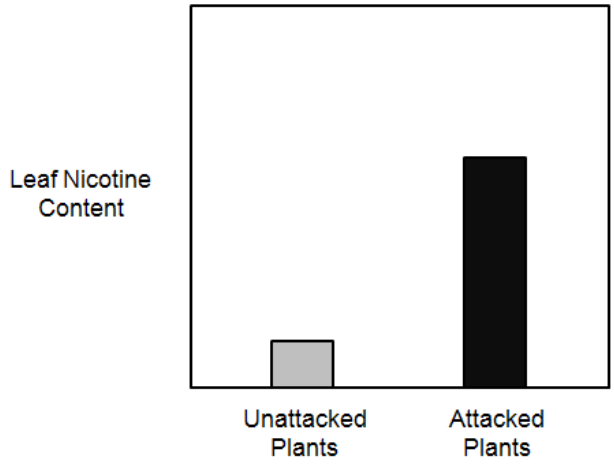


a. What aspect of the results figure below provides some relevance to the testing of this hypothesis?

b. What about this experiment weakens it as a test of this hypothesis?



2. Below is a figure from Baldwin (2001) that looked at plant-herbivore interactions in tobacco, *Nicotiana tabacum*, which produces the notorious alkaloid nicotine.



a. Based on this figure alone, and without reading the article or any other information, come up with a reasonable and testable hypothesis that Baldwin might have tested.

b. Design a simple experiment that could test this hypothesis.

c. Based on your hypothesis and this figure, what all can you conclude about the nicotine content of tobacco leaves?

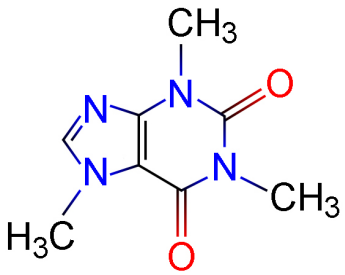
3. Catechins are the antioxidants in tea that are largely responsible for tea's healthfulness. Based on the introductory reading on tea, explain why green tea is said to have more antioxidant power than black tea.

4. Go around the room and collect everyone's scorings regarding the aroma of the tea leaves from Table 3 and average. Which tea type has a fuller-bodied and more pleasant aroma on average? Does the introductory reading on tea provide an explanation for these findings?

From Table 3. Class scores and average regarding the aroma of tea leaves.

	Green	**Oolong**	**Black**
Aroma Strength (1-5, mild to strong)			
Rank their aroma as to your liking (0 = least; 1 = more; 2 = most)			

5. Pictured below is caffeine. It is common knowledge that caffeine acts as a CNS stimulant and can make you less sleepy. The technical explanation for this is that caffeine acts as an antagonist to adenosine, an important regulator of sleep. During waking hours, extracellular adenosine accumulates in the brain, attaches to adenosine receptors on neurons, and this progressively induces sleepiness.



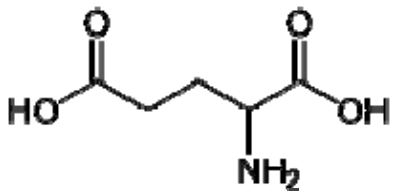
Caffeine

a. Which of the following is the definition of antagonist?

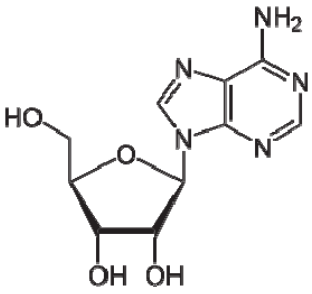
A. A chemical that binds to a cellular receptor adapted for some other chemical and induces the same response as that chemical.
B. A villian in a story.
C. A hero in a story.
D. A chemical that binds to a cellular receptor adapted for some other chemical and does not induce (i.e., prevents) the same response as that chemical.
E. A chemical that destroys the cellular receptor adapted for some other chemical.

b. Based on your understanding of "antagonist" and that caffeine is an antagonist of adenosine, which of the following is most likely adenosine?

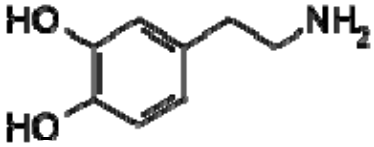
A.



B.



C.



6. Using information from question 5 above, explain in your own words how caffeine works to make you less sleepy?

7. Using Tables 5 and 6 below, and any relevant instrument in the room:

Table 5. Caffeine in parts of species used to make stimulating products (Simpson and Orzogoly 1995). The amount of caffeine that makes it into the actual beverage or product depends on how the product is made.

Plant, part	**Caffeine** (% by weight)
Coffee, unroasted, dried seeds	1-1.5
tea, dried lvs.	2.5-4.5
Cacao, dried or fresh seeds	0.6-0.8
Kola, fresh seeds	2.0
Guarana, dried fruit	3.0-4.5

Table 6. Average caffeine content in products (most amounts from the Center for Science in the Public Interest, 2007; chocolate amounts from Simpson and Orzogoly 1995).

Product	**Caffeine (to the nearest mg)**
Coffee (Starbucks)	
12 oz drip coffee	240
1 oz espresso	75
12 oz drip decaf coffee	19
Tea (various)	
12 oz brewed tea	80 (60-180)
12 oz Nestea	26
12 oz Snapple	14-32
Cocoa and chocolate (various)	
12 oz, from powder	14 (4.5-20)
1 oz baking choc	35
1 oz dark choc	20
1 oz milk choc	6
Soda (various)	
8.3 oz Red Bull	80
12 oz Jolt Cola	72
12 oz Mountain Dew	54
12 oz Dr. Pepper	42
12 oz Pepsi	38
12 oz Coca-Cola Classic	35

a. Prepare a flow chart that describes how you would calculate the number of coffee beans that you would have to eat to match the caffeine intake of one espresso.

b. Now follow your flow chart to derive an answer to this problem.

c. Do the same as above, but with chocolate-covered coffee beans. Assume that dark chocolate is used and that for such candies there is a 1-to-1 ratio of chocolate to coffee by weight

IV. Literature Cited

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V. Credits

This lab was developed by Christopher Hardy. You may cite it as....

Hardy CR. 2016. Ethnobotany of plant secondary metabolism. Pp. 165-184 in CR Hardy, RL Wagner (eds) *Guide to Exercises in Concepts of Botany, 4th Edition*. Millersville, Pennsylvania, USA.

VI. Glossary

- **Allelopathy** - the process whereby one plant's chemicals inhibit the growth of neighboring plants.
- **Antiherbivory** – deterrence of herbivory.
- **Antimicrobial** – a substance that kills or retards the growth of microbes.
- **Berry** – A fruit that does not open at maturity, and whose wall is entirely fleshy.
- **Capsule** – A fruit composed of multiple segments (carpels) that is dry and opens at maturity to release or expose the seeds.
- **Chocolate liquor** - unsweetened baking chocolate in its melted state.
- **Ethnobotany** – derived from ethnology (the study of human culture) and botany (the study of plants): the study of plants in human culture.
- **Herbivory** – the eating of plant parts by an herbivore.
- **Hilum** – the scar on a seed that is left from its attachment during development to the inner wall or septum of the fruit. Analogous to the belly button in humans.

- **Mordant** – a substance (chemical) that facilitates the binding of a dye to fiber.
- **Peaberry** – a coffee seed that, instead of being flat on the side with the hilum, is rounded on all sides.
- **Phytochemical** – a chemical made by a plant.
- **Secondary metabolism** – biochemistry that is not directly involved in growth and development of the organism.
- **Primary metabolism** – biochemistry that is directly involved in the growth and development of the organism.

Algae

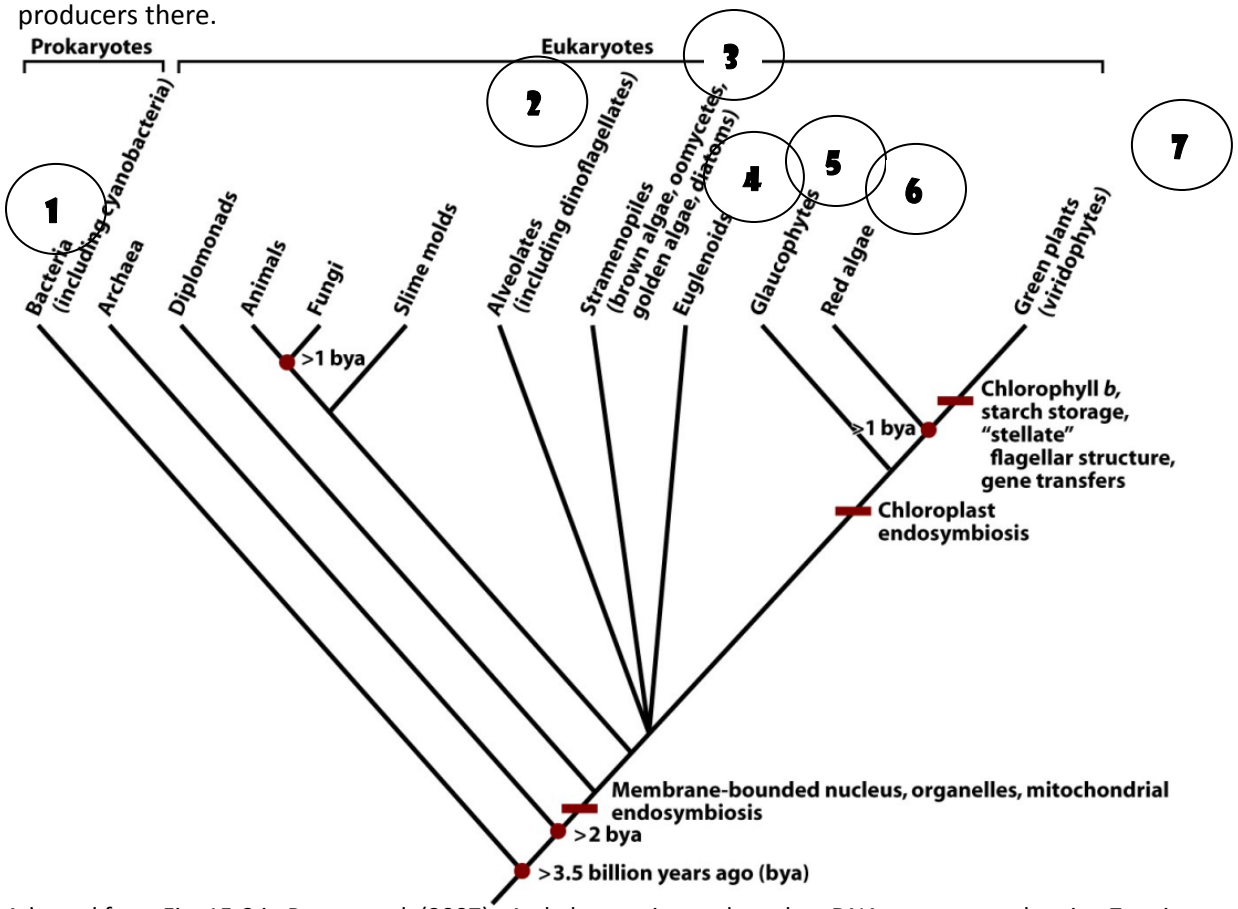
"Algae" (or singular "alga") is a term historically applied to an unnatural assemblage of photosynthetic, mostly aquatic organisms that lack the characteristics of members of the plant kingdom. Algae have been classified as members of the traditionally recognized eukaryotic Kingdom Protista (where all simple eukaryotic organisms went under the 5-kingdom system) or, in the case of the blue-green algae, the Domain Bacteria. Some authors prefer to restrict the term "algae" to photosynthetic, eukaryotic protists, and prefer to label the photosynthetic bacteria formerly called "blue-green algae" as "blue-green bacteria" or "cyanobacteria."

Among the eukaryotic algae, the various groups have been classified into taxonomic groups based mainly on distinctive chloroplast accessory pigments, and secondarily based upon other characteristics such as cell wall components, reproductive structures, and distinctive food storage molecules. Because of their distinctive set of pigments, many groups of algae have been known by their color: hence red, green, and brown algae, etc.

The phylogenetic tree below provides an overview of the major lineages of life and how they are thought to be related to one another: the 7 groups numbered below are the 7 major lineages of photosynthetic organisms and they're obviously not all that closely related to one another.

Structurally, some algae are unicellular, but others are larger and multicellular organisms that are called "seaweeds" when growing in saltwater bodies. Within the algae you can see the major trend in the evolutionary development of multicellularity reflected in the diversity of organismal forms: i.e., unicellular >> filamentous (exhibiting 1D multicellular growth) >> planar/thalloid (exhibiting 2D multicellular growth) >> 3D forms that begin to resemble plants in their complexity. This is important because it is this evolutionary trend that gave rise to the Kingdom Plantae from amongst the green algae in particular. Some, such as the green alga *Volvox*, and some bluegreens (cyanobacteria), are colonial.

Ecologically, algae are very important in aquatic environments since they are the primary producers there.



Adapted from Fig. 15-3 in Raven et al. (2007). A phylogenetic tree based on DNA sequences, showing 7 major groups of photosynthetic organisms.

Table of Contents for today's lab:

A. Dichotomous Keying of Freshwater Algae

A key is an identification tool. Use the dichotomous key to freshwater algal genera (Section F) to identify the 10 or so unknown monocultures of algae to their respective genera.

The algal samples in the numbered vials are largely monocultures, but the presence of other species as contaminants is possible. Often there are small protozoa (microscopic animals) feeding on the algae in these vials, but do not be distracted by them. Focus your keying effort on the "majority" alga in each vial.

See instructor for the answers at the end of class. Important details are viewed at 200X and 400X magnifications.

Procedure

0. Working in pairs, make wet mounts (with coverslips) of two of the numbered algae tubes at a time from the back/side. Be sure to remember which number each slide is of – perhaps by numbering the slides with tape and pen.

a. Do this by placing a drop onto slide (be sure to get some algae in it – you may have to flush the tube once with the pipette).

b. Cover with coverslip (be careful laying the cover slip down so as not to destroy large colonial forms such as *Volvox*).

c. Do not let these wet mounts dry out until you are done looking at them.

1. Inspect the alga for color since this is a characteristic you will often need to identify them. Do this one (or both) of two ways:

a. hold the tube up to the light or against white paper.

b. then be sure to confirm this color again once you've got it under the scope since some of the tubes have soil contaminants in them and will make algae in the tube look brown when the cells are actually green, for example.

2. Work your way through the key to identify the genus. You may visit section C in the lab manual with information about each algal group to confirm your tentative identification with pictures or descriptions.

3. Make drawings on the following pages and label with genus name, the magnification used for the drawing, and other structures such as **CHLOROPLAST(S), FLAGELLUM(FLAGELLA), PYRENOID(S), EYESPOT, NUCLEUS,** etc. Also record the color of your alga, as seen with the naked eye.

4. After making your identification, go to section C in the lab manual on the group and answer the questions.

1.	2.
3.	4.
5.	6.

7.	8.
9.	10.

B. Practical Algal Taxonomy

Taxonomy is the scientific discipline that deals with the formal naming, classification, and identification of organisms. A taxonomic key (which you used above) is a very useful tool that biologists and people who monitor water quality use to help identify which algae are found growing in the bodies of water they are interested in. Use the skills from Part A above to answer some of these practical questions.

1. What types of algae grow in the Roddy Pond?

Additionally, do different species grow at different depths? It is conceivable that different algal species have different preferences in light intensity and temperature and we will test this hypothesis.

Sample from Roddy Pond the following (note that in winter months [November through March] most of the algae are low in the water column or on the bottom near the shore since they are not very photosynthetically active).

1. Surface (the "pond scum")
2. Sample deeper, such as around 50-100 cm depth, but do not scrape the pond bottom. You may need to sample from the pier for this deeper sample.
3. Place into glass jars/vials with lids and label.

4. Use the same dichotomous key to algae to help identify samples of photosynthetic organisms taken from the pond.

2. Is Roddy Pond safe for cattle or cows to drink from?

Farmers sometimes have major problems with cattle drinking from ponds with high densities of cyanobacteria (see the snippet of the 1997 article by Mez et al. below). Some cyanobacterial species can make potent toxins (neurotoxins, hepatotoxins, or dermatotoxins) that can kill cattle. You can use your dichotomous key to algae to identify samples of photosynthetic organisms taken from the pond. Pretend you are a farmer, and 1) if you find cyanobacteria that are 2) in great abundance, then you may have cause for concern.

Eur. J. Phycol. (1997), **32**: 111–117. Printed in the United Kingdom

Identification of a microcystin in benthic cyanobacteria linked to cattle deaths on alpine pastures in Switzerland

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(Received 1 October 1996; accepted 28 January 1997)

During the last two decades, more than 100 cattle deaths have been reported from 11 alpine sites in south-eastern Switzerland. Pathological findings and the histological examination of their organs strongly indicated acute hepatotoxicosis. Clinical symptoms suggestive of neurotoxicity were also observed in some cases. To elucidate the etiology of these poisonings, different water bodies in one of the affected alpine pastures were investigated for cyanobacterial toxins. The waters were highly oligotrophic, cold and turbid, and the ice-free period was limited to 3–4 months. The algal community in these waters consisted mostly of benthic cyanobacteria forming dense mats on the surface of sediments and on submerged rocks. *Oscillatoria limosa* and *Phormidium konstantinosum* (= *Oscillatoria tenuis*) dominated these populations, but occasionally other species of *Oscillatoria*, *Phormidium*, *Tychonema* and *Pseudanabaena* also occurred in the mats. Samples from the cyanobacterial mats yielded positive results in a protein phosphatase inhibition assay, reacted with antibodies against microcystins in an enzyme-linked immunosorbent assay and were hepatotoxic in a mouse bioassay. The same cyanobacterial material also included neurological effects in mice. High-performance liquid chromatography was used to identify a microcystin, in these cyanobacterial samples as well as in the corresponding lake water. To our knowledge, this is the first documented example of hepatotoxicity associated with benthic cyanobacteria, and the first report of toxic cyanobacteria from the remote, oligotrophic alpine environment.

C. Groups of Algae

Regardless of whether or not you've seen living examples in lab today, read each section and answer the questions.

C1. Group 1: Cyanobacteria (blue-green algae)

Cyanobacteria are photosynthetic bacteria sometimes called "blue-green algae". Unlike other algae, however, cyanobacteria are prokaryotes. Whereas plants and eukaryotic algae have chloroplasts in their cells to carry out photosynthesis, the whole cell of a cyanobacterium is homologous to a chloroplast. Like other prokaryotic cells, the cells of cyanobacteria are much smaller than eukaryotic cells. Although cyanobacteria have chlorophyll, their different mix of accessory pigments (namely the presence of phycobilins) make them appear bluish-green. In addition to having carbohydrate storage reserve, they have an additional storage, cyanophycin, which is nitrogenous and thereby unique amongst the algae. Many are unicellular, others are filamentous, while others are colonial.

1. Question: *If you were to have a filamentous (or unicellular) cyanobacteriumand a filamentous (or unicellular) eukaryotic alga, how might you tell them apart? List at least two ways, based on your reading of the above paragraph.*

2. One of the unknown samples from section A was *Oscillatoria*, which has filaments which can move or oscillate, causing the mass of filaments to move!

Did you see evidence for that?

What color were the masses of filament in the stock culture tubes or under the microscope?

3. *Which other genus or genera of cyanobacteria did you find in the mystery monocultures in part A?*

Cross reference these answers to your drawings in part A above.

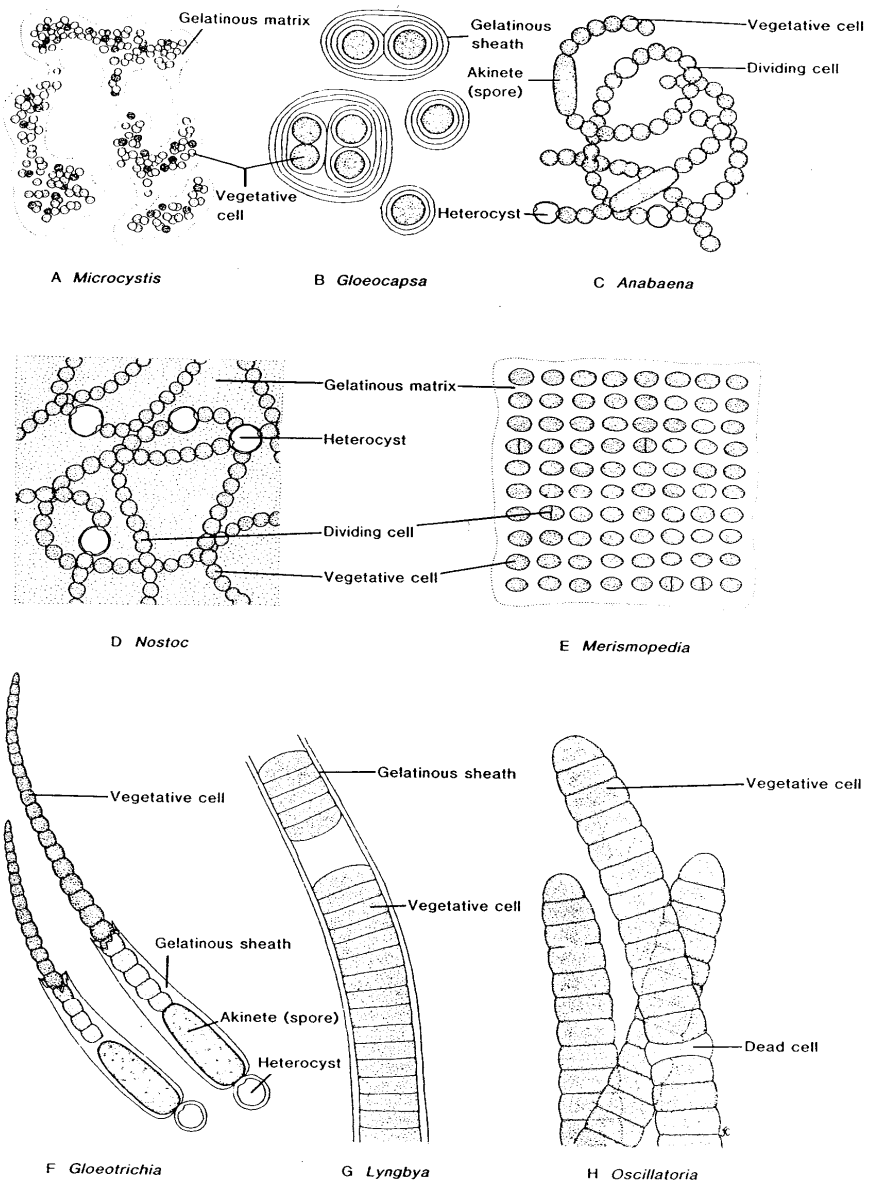

Figure at left: some cyanobacteria. A-B and D-E are more or less colonial, since the cells and/or filaments stick together in a gelatinous matrix.

<u>Above</u>: Cyanobacterial genera. Drawing copyright of Carolina Biological Supply.

C2. Group 2: Dinoflagellates

Dinoflagellates are not bacteria but unicellular protists.

Thus, are dinoflagellates prokaryotes or eukaryotes?

Other characteristics of dinoflagellates are their storage reserve being starch, elaborate shapes, "armor" of cellulosic plates, their olive-brown color due in part to the accessory pigments peridinin or fucoxanthin, and their motility due to the action of two flagella oriented perpendicular to one another.

We do not have dinoflagellates in any of the monocultures in lab today, but Roddy Pond sometimes has dinoflagellates in it (note: your particular lab may be too early in the season to see any). Interesting phenomena in nature include bioluminescence and red tides caused by some marine species.

How many cells is each dinoflagellate?

Inspect your samples and the pictures below: How many flagella do these have and how is each arranged to help them move?

Read in your textbook about the "armor" or "plates". Are the plates inside or outside the cell(s)? What are they made of? How is this the same or different than a plant cell wall?

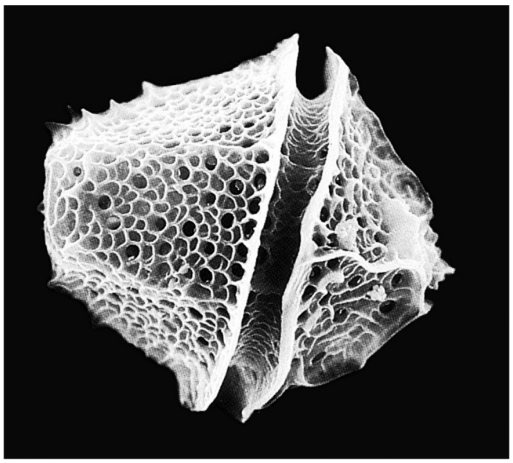

Figure 15-5c
Biology of Plants, Seventh Edition
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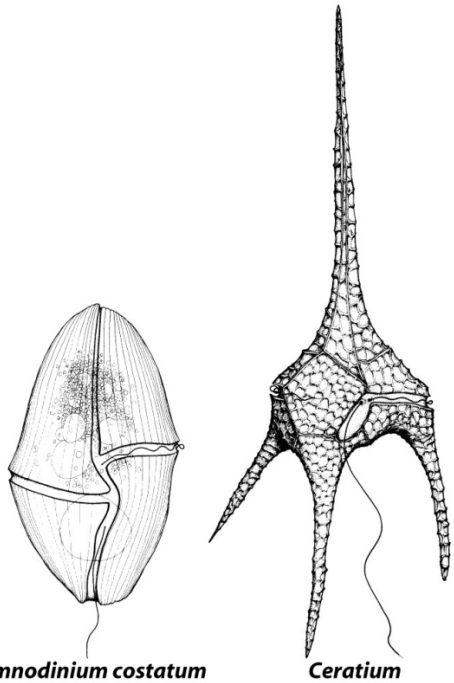

Gymnodinium costatum ***Ceratium***

Figure 15-6
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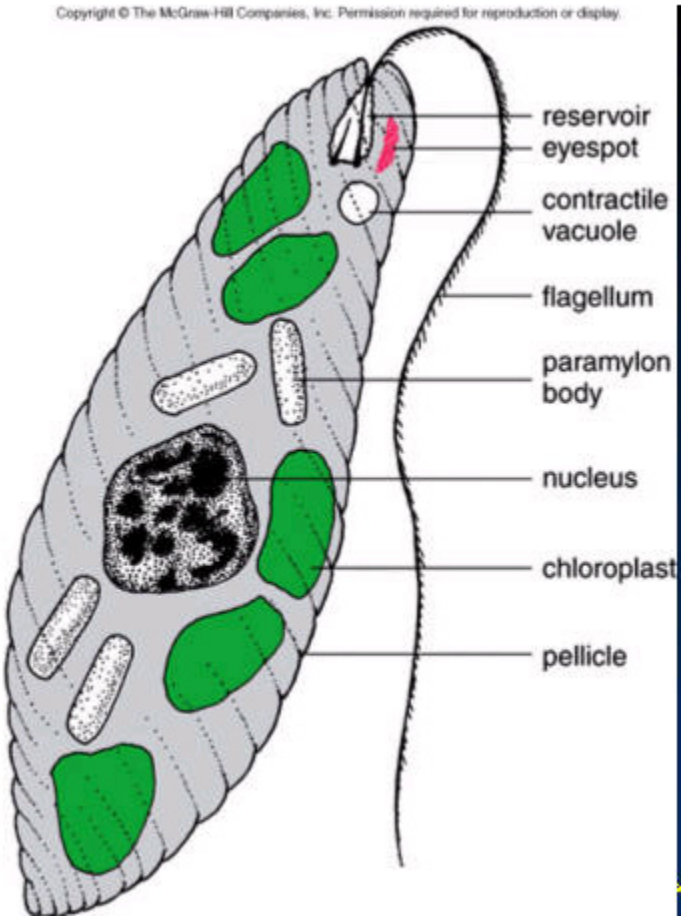
Figure above: Various forms of dinoflagellates.

C3. Group 3: Euglenoids

EUGLENA: PLANT OR ANIMAL? Remove a drop or two of the freshwater sample containing *Euglena gracilis* *Euglena gracilis* is just one of many (ca. 800-900) unicellular species of the genus *Euglena*. They do not have common names like birds or trees, so we have to refer to them by their scientific name *Euglena*.

Euglena are interesting because they combine superficial characteristics of both plants and animals. They can make their own food like a plant, through photosynthesis, but they can also eat other things, "like" an animal (although they actually eat more like an amoeba – through phagocytosis-- than an animal). They can also swim and move thanks to their single functional flagellum. Scientists argued for years about which Kingdom to put them in: Animalia or Plantae? Right now they are in neither: according to some classifications they are in the Kingdom Protista with other simple (often microscopic) eukaryotes, such as amoeba and paramecium. Protists are eukaryotes, typically small or microscopic, which lack sufficient characteristics to otherwise justify their inclusion in the animal, plant, or fungal kingdoms.

A euglena's body is unicellular. The outermost envelope is the plasma (cell) membrane, beneath which lies the pellicle (a matrix of protein bands that spiral down the length of the cell). The pellicle helps the euglena maintain its distinctive shape, although it is quite flexible and can be flexed by the cell in an inch-worm type fashion. Euglena typically store their excess carbohydrates as paramylon (a polymer of glucose related to starch), distributed throughout the cytoplasm as "paramylon bodies." Their chloroplast pigment profile is similar to that of green algae and plants: chlorophyll a, b, and carotenoids. At one end of a cell is a contractile vacuole, which helps expel excess water absorbed by the cell from its freshwater environment.



Sometimes, since they live in water, if there are millions of euglena together, they form a mat on the surface of a pond or marsh that you can see. It looks slimy. Some people say it looks like “pea soup”. Euglena get into swimming pools too, if they are not cleaned regularly.

1. Are euglena unicellular or multicellular?

2. Are euglena animals, plants, protists, or bacteria? Prokaryotic or eukaryotic?

3. What organelle carries out photosynthesis?

4. Are euglena autotrophic or heterotrophic?

5. How many flagella did the euglena from the unknown algal cultures above have?

6. Does the flagellum or flagella work by “pulling” or “pushing” the cell through the water?

7. What is the eyespot used for?

8. What is the function of the contractile vacuole? What would happen if the cell did not have this organelle.

9. Did the shape of Euglena cells change while you observed them? Or were they rigid?

C4. Group 4: Brown, Golden-brown, and Yellow-brown Algae

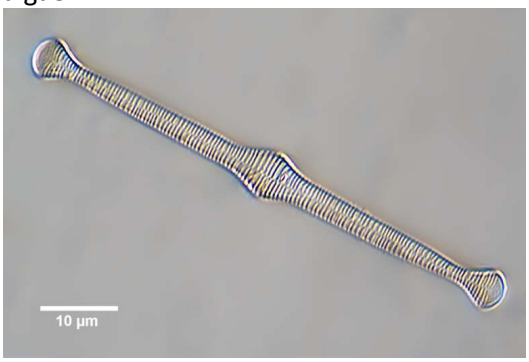
A diverse group united by the accessory pigment fucoxanthin and carbohydrate reserves laminarin or the very similar chrysolaminarin.

C4.1. Diatoms

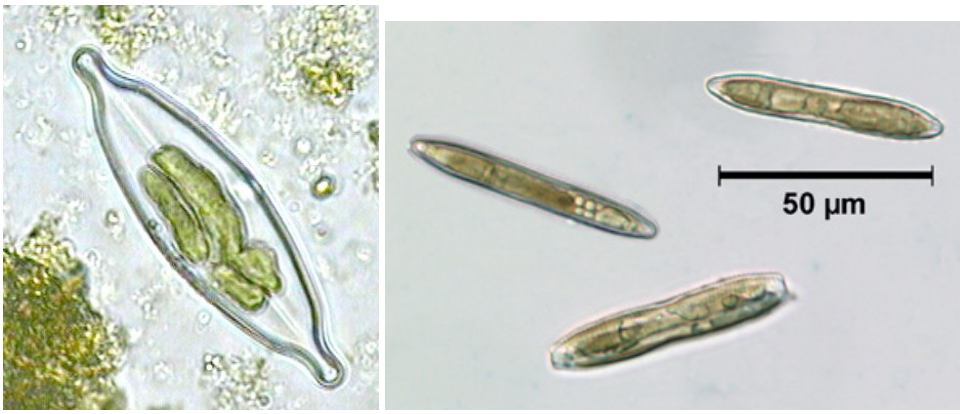
Diatoms include at least 5000 species of algae that are mostly unicellular and golden-brown in color due to their brown accessory pigment, fucoxanthin, and possess a silica-based (glass-like) cell wall. Some diatoms co-occur as colonies of cells. Their carbohydrate reserves are in the form of laminarin, a starch relative. Although some diatom species are terrestrial (e.g., in soils), the majority are aquatic (fresh- or saltwater) and grow on submerged surfaces, including those of other aquatic plants, in the intertidal sands of beaches, or as plankton suspended in the water column.

The cell walls of diatoms (called frustrules) come in two halves that fit together like the two halves of a petri-dish. The frustules themselves have an overall symmetry when viewed on their broad face (e.g., radial, bilateral, or disymmetric) and are highly ornamented with species-specific patterns of pores and grooves that can be discerned under compound light or scanning electron microscopy. While many diatoms are non-motile and generally lack flagella, some are motile through their ability to glide or "walk" over the surface of a substrate by the extension and contraction of fibrils through frustrule pores or grooves, through which cytoplasm protrudes, leaving a mucilaginous trail in their wake. Biotechnologists have begun to look at manipulating diatoms (through artificial selection of their frustrules) to produce nanotech valves for nanoscale drug delivery, etc.

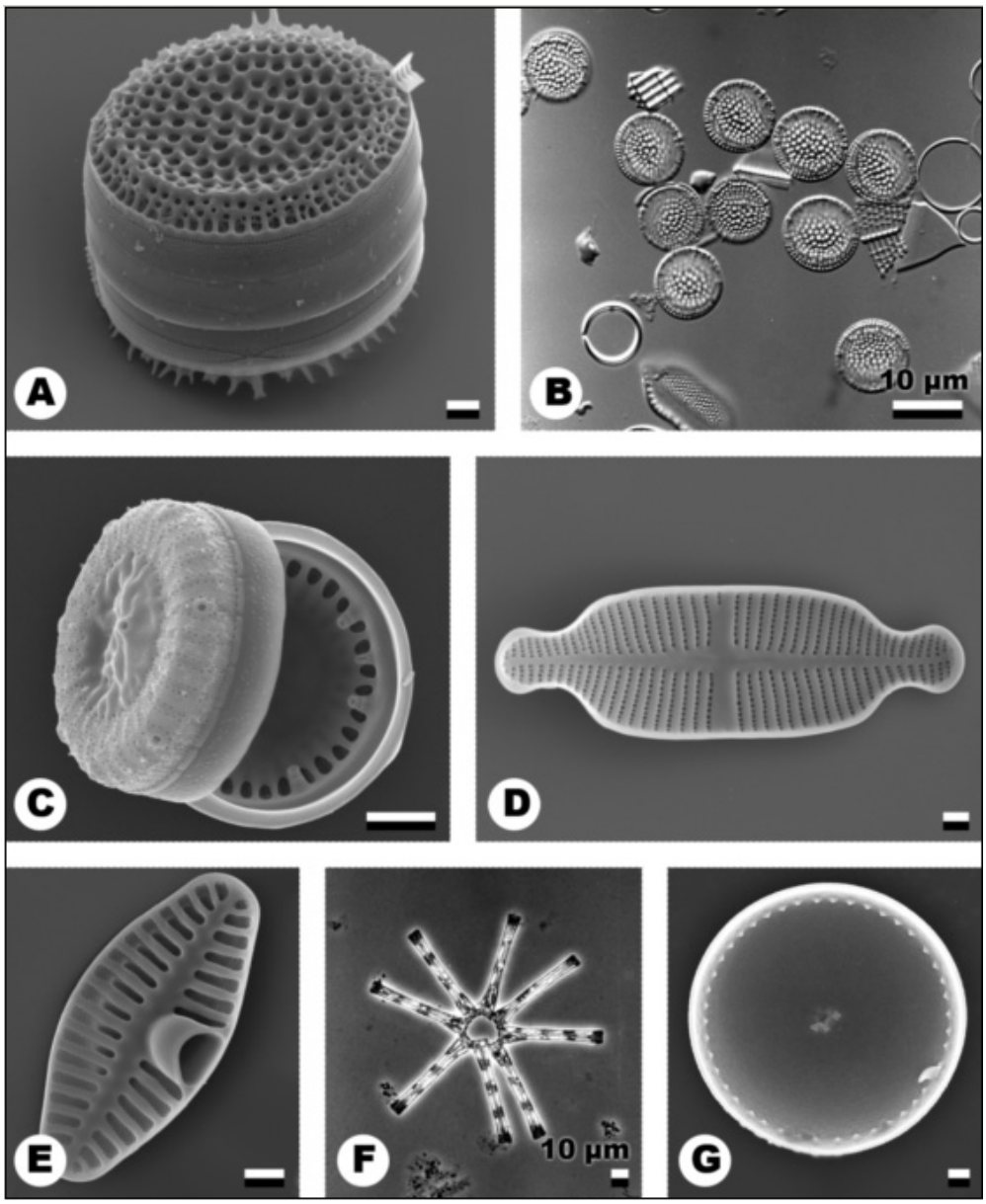
Fossil evidence indicates that they evolved during or before the Jurassic. Fossil beds of diatoms are often called "diatomaceous earth," which is often used by humans as a water filter matrix (e.g., pool water filters are often diatomaceous earth), or as a polishing agent. Our oil deposits in world are mostly the transformed organic remains of diatoms and other marine algae.



Above, *Tabellaria* from a freshwater pond.



Above: *Navicula* (left) and *Synedra* (right). (*http://biology.missouristate.edu/phycology*)



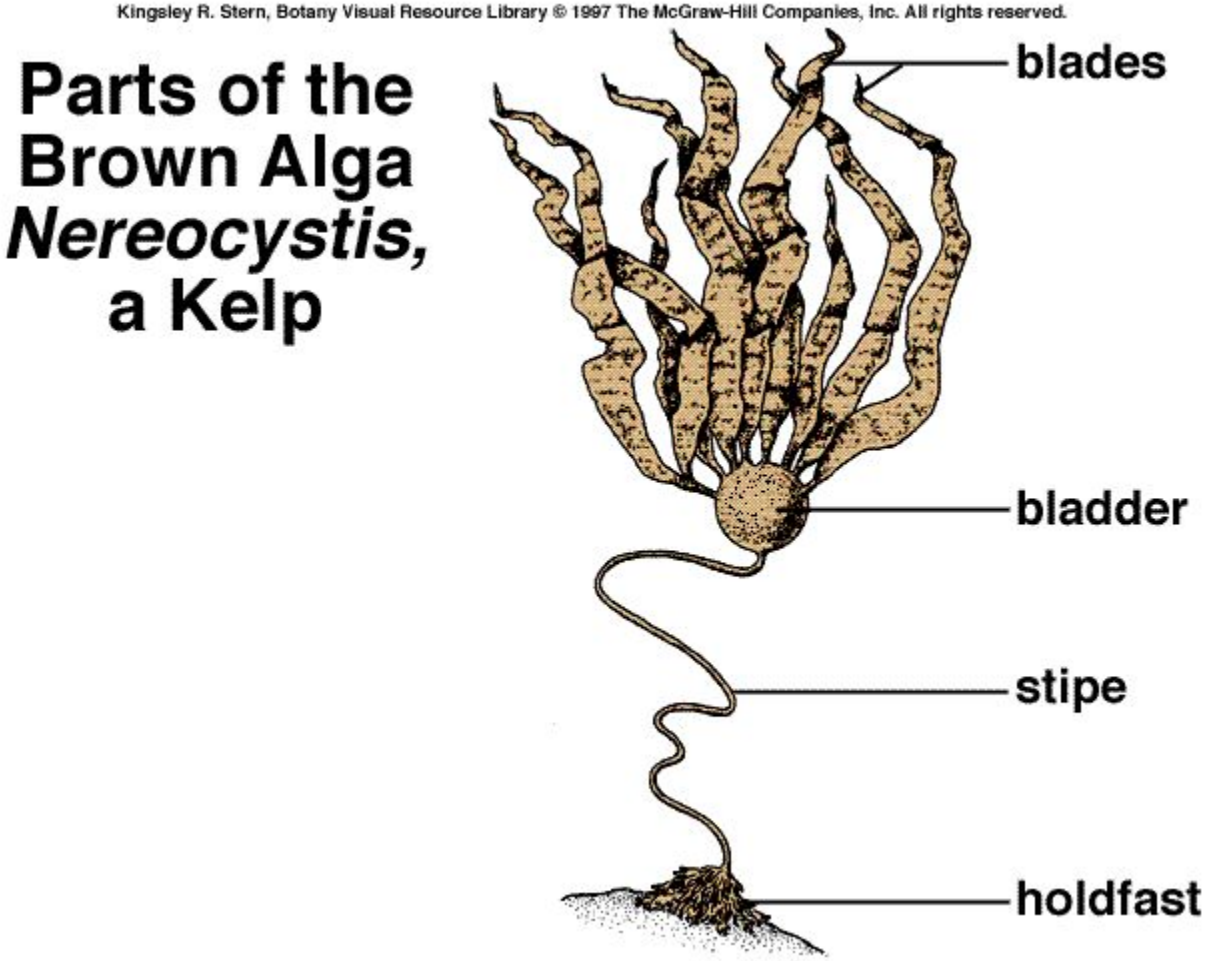
Above: Diatoms come in a variety of forms and are found in a wide diversity of habitats. Scale bar = 1 µm, except where otherwise indicated. A–C: Some marine diatoms from coastal waters of south-eastern North America (A and B, *Thalassiosira cedarkeyensis*; C, *Cyclotella choctawhatcheeana*). D–G: Some freshwater diatoms from south-eastern North America (D, *Acanthidium exiguum*; E, *Planothidium lanceolatum*; F, The colonial *Asterionella formosa*; G, *Conticribra weissflogii*). A, C, D, E, and G are scanning electron micrographs. B and F are light micrographs using differential interference contrast and phase contrast, respectively. Photos courtesy of Akshinthala K. S. K. Prasad, Department of Biological Science, Florida State University.

C4.2. Brown Algae

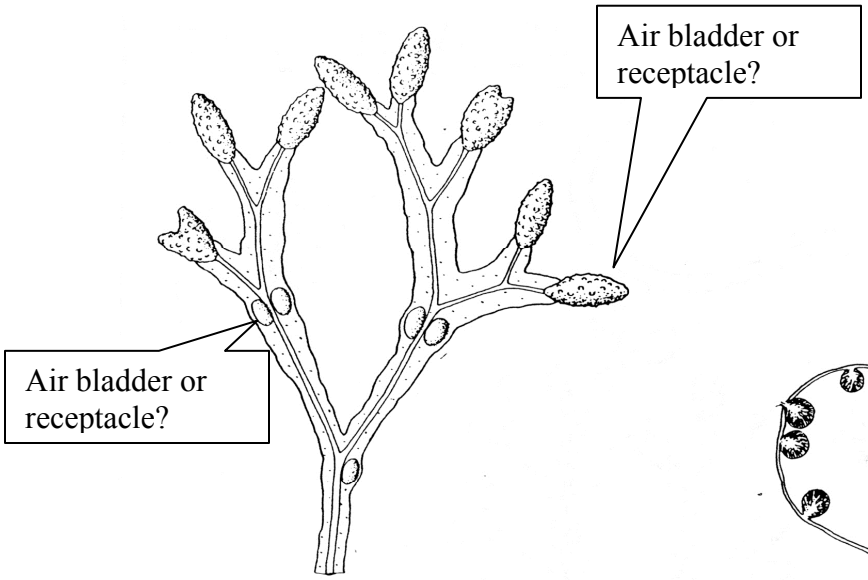
When marine algae get big (macroscopic), they are called seaweeds. Most browns are relatively big, and all are multicellular. Only 6 of 265 genera occur in freshwater. Although they have chlorophyll, their special carotenoid fucoxanthin (which they share with diatoms and many dinoflagellates) impart a brown color to them. These algae store excess carbohydrates in the form of laminarin, a relative of starch. Brown algae have cellulosic cell walls.

Well known members include *Fucus* (rockweed), *Laminaria* and *Macrocystis* (the kelps).

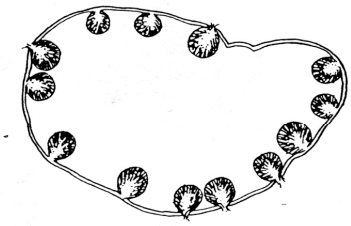
Common structures will include a HOLDFAST, a STIPE, A BLADDER (for buoyancy; aka pneumatocyst), and BLADES to function like "leaves".



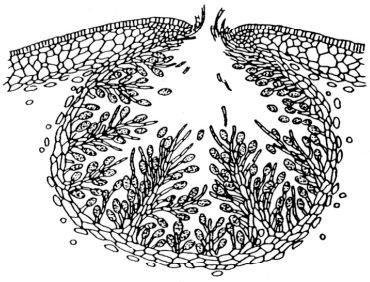
Holdfast Logic Question: *In what way(s) do holdfasts resemble roots, and in which way(s) do they not? HINT: think about function(s).*



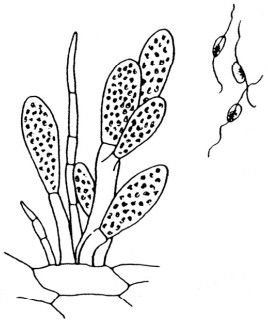
A. *Fucus*, plant with receptacles and air bladders.



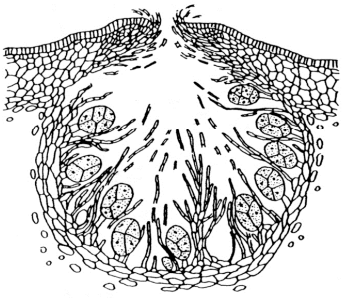
B. x-section of receptacle and smaller conceptacles.



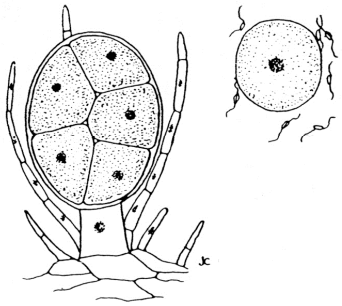
C. Male conceptacle with antheridia.



D. Antheridia with sperm.



E. Female conceptacle with oogonia.



F. Oogonium with eggs.

Above: *Fucus*, illustrations of the plant, as well as the reproductive parts.

C5. Group 5: Glaucophytes

Extremely small group of algae closely related to red and green algae. Will not be covered in lab.

C6. Group 6: Red Algae

The red algae are largely marine and multicellular "seaweeds". A class of accessory pigments (phycobilins) makes them reddish. Red algae store carbohydrates as floridean starch, a starch relative. Red algae have cellulosic cell walls.

We typically do not have any living red algae in the lab, but there will at least be herbarium specimens for your observations.

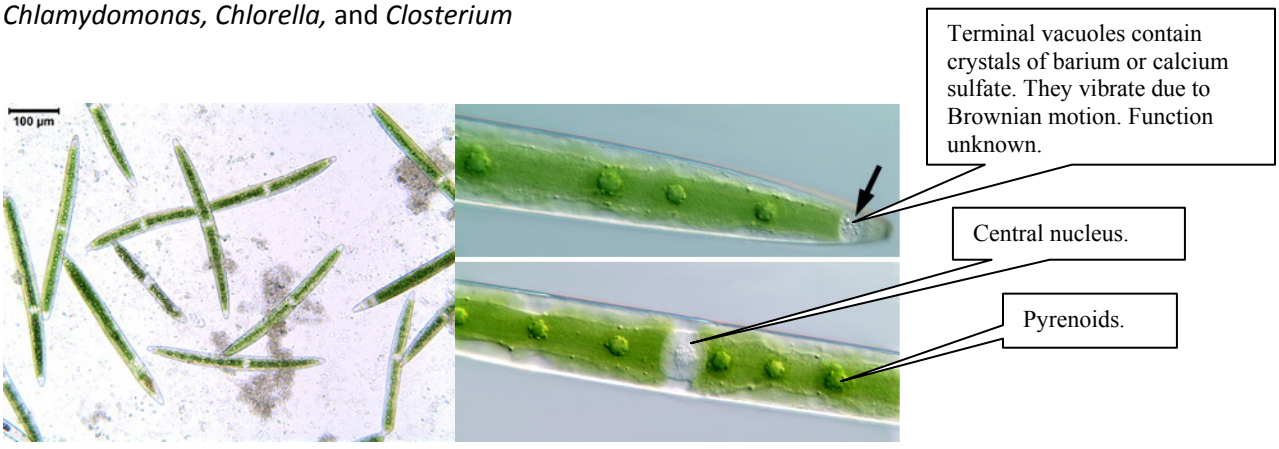
C7. Group 7: Green Algae

The green algae are a very large and diverse group. They range from unicellular organisms such as *Chlamydomonas* or *Chlorella* to colonial organisms such as *Volvox*, to mulicellular organisms such as *Ulva* (sea-lettuce) and *Chara* (stonewort). Green algae share with plants the same chloroplast pigment profile (chlorophyll a, b, and the carotenoids beta-carotene and xanthophylls). When a cell wall is present, it is cellulosic. Their carbohydrate storage reserve is starch.

Many of the unlabeled monocultures of algae in the lab were green algae. Presumably, you have now identified them all.

C7A. Unicellular forms: all three of the algae below were represented in the unknown algal cultures. Which numbers were which?

Chlamydomonas, Chlorella, and *Closterium*



Above: Closterium. This unicell is bilaterally symmetric, with two equal halves and a single chloroplast in each half; such unicellular algae with symmetrical halves are called "desmids." Like many desmids, *Closterium* can move in a somersaulting manner by secreting mucilage from alternating ends of the cell through special pores in the cell wall.
(http://silicasecchidisk.conncoll.edu/LucidKeys/Carolina_Key/html/Closterium_Main.html)

Which of the algae above is an indicator of polluted fresh or salt waters? See the poster in the back or side of the room.

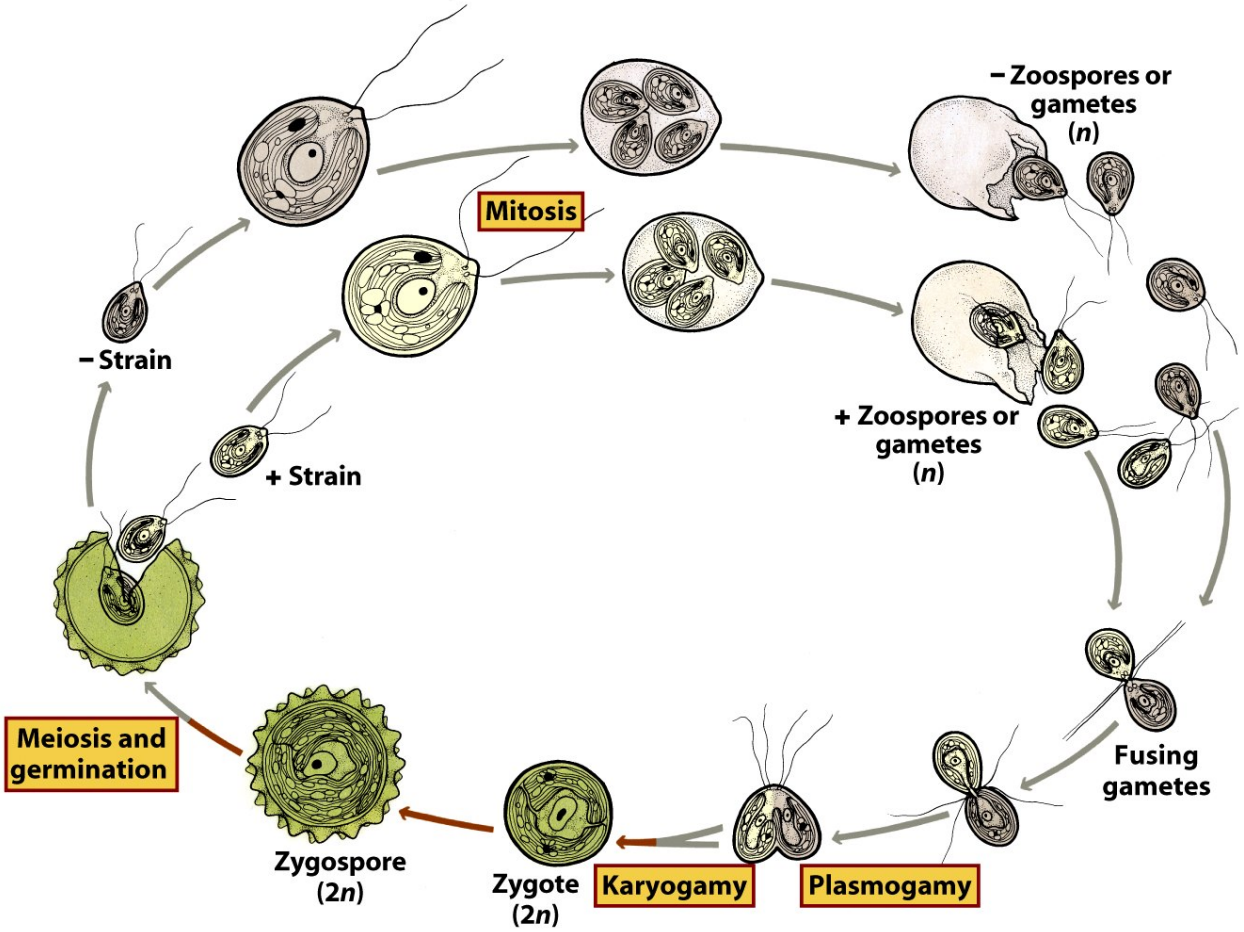


Figure 15-41
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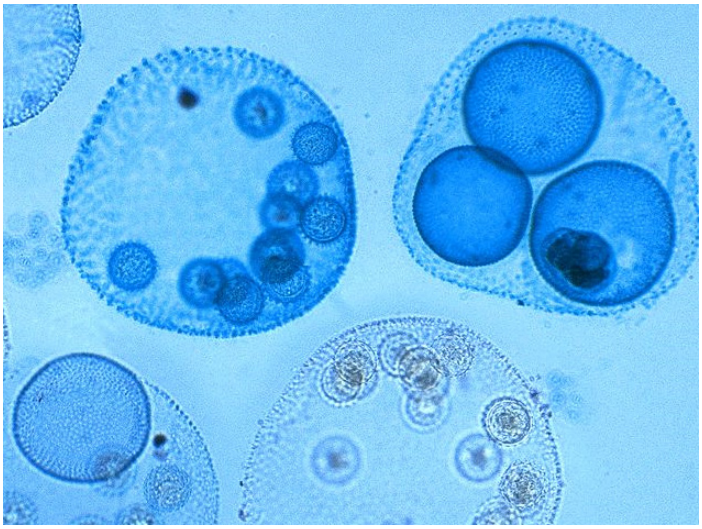
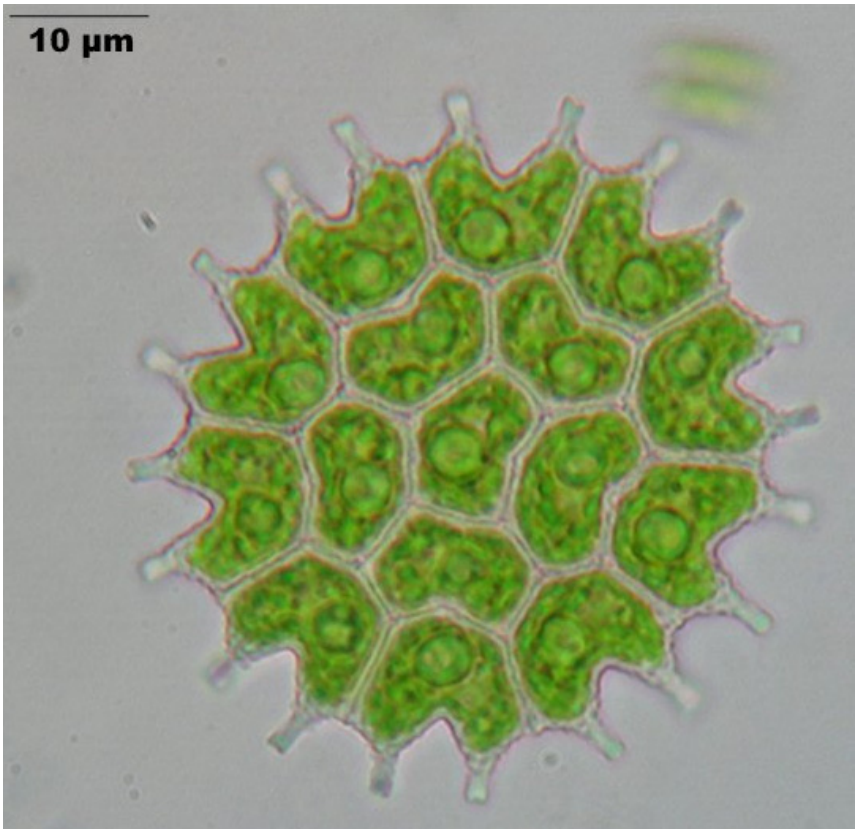
Above: Life cycle of *Chlamydomonas*, a unicellular green alga.

C7B. Colonial forms: e.g., *Volvox* & *Pediastrum* (sometimes found in the pond). Can you recognize *Volvox* on-sight? (See figure below.)



Above: *Volvox* with daughter colonies inside larger parent colonies.



Above: *Pediastrum*, a plate-like colonial freshwater (pond) alga.

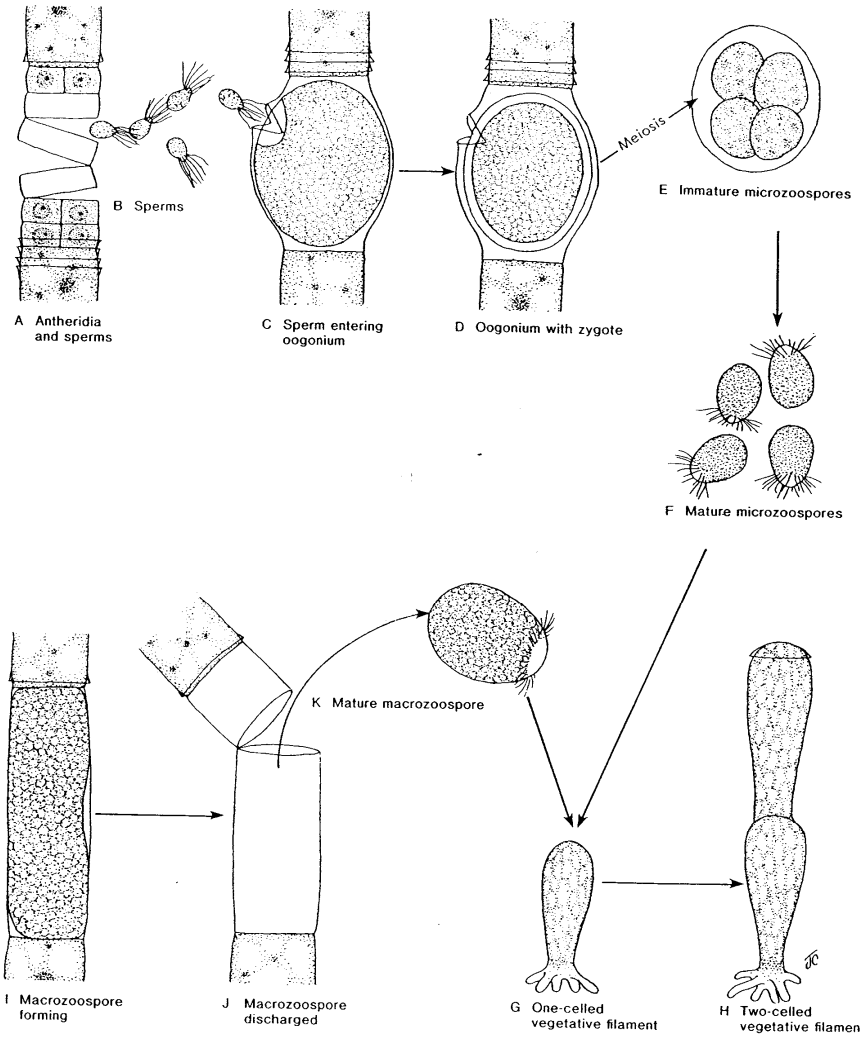
C7C. Filamentous forms: *Spirogyra* was represented in the algal cultures and possibly the pond water. How about *Oedogonium*?

Why is Spirogyra named as such?

Can you spot pyrenoids (protein-rich sites of carbon fixation and starch synthesis) in Spirogyra chloroplasts?

How do you distinguish these two genera (look at the key or the figure below)?

Figure 23.2 *Oedogonium*



Above, life-cycle of the green alga *Oedogonium* from Carolina Biological Supply Co.

C8. Macroscopic (seaweed) forms:

See pictures, posters, herbarium specimens or living specimens (when available) at stations around the periphery of the room.

D. Credits

This lab was developed by Christopher Hardy. You may cite it as....
Hardy CR. 2016. Algae. Pp. 185-206 in CR Hardy, RL Wagner (eds) *Guide to Exercises in Concepts of Botany, 4th Edition*. Millersville, Pennsylvania, USA.

E. Some Useful Terms for Algae in this lab

Bladder – see *pneumatocyst*.
Blade – the flattened analog of a leaf in many seaweeds.
Coenocytic – an organism whose cell(s) contain multiple nuclei (e.g., the alga *Vaucheria* exists as a long, sometimes branched filament that is just one or few giant cells).
Colonial – an "organism" actually made up of multiple organisms. In algae, these individual organisms are typically unicellular and they stick to one another by a gelatinous matrix they secrete.
Eyespot – a patch of pigment on a membrane inside a cell that can detect light. These are typically red and found only in motile algae since the cell, once it detects light, can them swim towards it (or away from it if it is too much).
Filamentous – an organism that is long and thin (sometimes branching), and typically made up of a string of cells.
Holdfast – the branching structure at the base of many seaweeds that holds them to a substate.
Motile – to have directed mobility. Cells able to propel themselves by some means are said to be "motile." Usually this is achieved by way of a flagellum or two (e.g., *Chlamydomonas*). However, the filamentous cyanobacterium *Oscillatoria* can move by way of the entire filament and mass of filaments oscillating or twirling, and desmids such as *Closterium* can move in a somersaulting motion by secreting jets of mucilage through special pores in the cell wall alternatingly from different ends of the cell.
Pneumatocyst – an air-filled bladder-like organ on many seaweeds that functions for buoyancy to keep the blades afloat in a position to intercept sunlight.
Protist – a eukaryotic organism classified traditionally in the kingdom Protista: lacking the characteristics of members of plant, animal or fungal kingdoms.
Pyrenoids - centers of carbon dioxide fixation or starch synthesis within the chloroplasts of algae and hornworts, visible because of the density of enzymes in that region. Pyrenoids are not membrane-bound organelles, but specialized areas of the chloroplast that contain high levels enzymes such as ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO). A pyrenoid typically has several starch grains near it.
Seaweed – a macroscopic marine alga. This term has no real technical definition and no taxonomic significance since some "seaweeds" are more closely related to unicellular or microscopic algae than they are to other "seaweeds".
Stipe – the stalk between the holdfast and the blades of many seaweeds.

F. Dichotomous Key to Some Freshwater Algal Genera

1. Alga unicellular and cells round, ovoid, or elliptic (cells may be clumped together but do not confuse this with a colony- which should be regular and ordered- or a filament)....2
1. Alga colonial or filamentous (if colonial, then cells regularly and orderly grouped into spherical clusters or clusters of 2-4 cells).....7

2. Cells with light golden-brown chloroplasts, with highly transparent glass-like cell walls, shape elliptic (football-shaped) or long, thin and bone-shaped or rectangular (Diatoms)..... 3
2. Cells bright-green; round, ovoid, or slightly crescent-shaped..... 4

3. Cells elliptic (football-shaped) or oblong (ends tapering).....Synedra (Diatom)
3. Cells rectangular or bone-shaped (ends not tapering, often bulbous)..... Tabellaria (Diatom)

4. Cells narrow (longer than wide, although sometimes changing shape).. 5
4. Cells rounder or more ovoid..... 6

5. Cell shape unchanging; appearing non-motile or motile; very thin, with pointy ends, slightly crescent-shaped, with two equal halves – the nucleus in the middle and each half containing one chloroplast.....Closterium (Desmid Green Alga)
5. Cell shape changing frequently; definitely motile; not so thin and with rounded ends; patch of red pigment near flagellum insertion..... Euglena (Euglenoid)

6. Cells ovoid, usually rather motile, with a small groove on the narrower end where two flagella insert; red eye-spot typically visible..... Chlamydomonas (Green Alga)
6. Cells round, not apparently motile (not flagellate), no red eye-spot..... Chlorella (Green Alga)

7. Alga colonial and cells grouped into spheres or clusters 2-4 cells or globular clusters, & held together by gelatinous matrix..... 8
7. Alga a filament, branched or not..... 9

8. Colonies of 2-4 cells, cells blue-green..... Eucapsis (Cyanobacterium)
8. Colonies round, some cells green or golden-green.....Volvox (Green Alga)

9. Cells blue-green (Cyanobacteria)..... 10
9. Cells or chloroplasts green or yellow-green..... 12

10. Filaments unbranched.....11
10. Filaments branched frequently..... Tolypothrix (Cyanobacterium)

11. Filaments with constrictions between the cells..... Anabaena (Cyanobacterium)
11. Filaments without constrictions between the cells; sometimes moving Oscillatoria (Cyanobacterium)

12. Filament branched and apparently coenocytic (all one cell or very few cells – that is, with few or no internal partitions)..... Vaucheria (Yellow-Green Alga)
12. Filament obviously multicellular.....13

13. Chloroplast(s) in each cell not round, instead somewhat coiled..Spirogyra (Green Alga)
13. Chloroplast(s) round..... Oedogonium (Green Alga)

Bryophytes & Pteridophytes

In this lab we cover members of the Kingdom Plantae that are sometimes collectively referred to as the "free-sporing plants" or "spore-bearing plants". These terms refer to the fact that the spores are dispersed free of the sporophytes that make them, and the gametophytes that develop from those spores are therefore free-living. These plants also do not produce seeds. Many like to conveniently distinguish between two groups of free-sporing plants: the bryophytes and pteridophytes. Today you will learn to recognize these various groups and subgroups, and learn about their similarities and differences. Today's lab is organized as follows:

I. Bryophytes

Bryophytes is a general term for the non-vascular, free-sporing plants we know as mosses, liverworts, and hornworts. Their characteristics are as follows:

- No xylem or phloem (i.e., non-vascular);
- No seeds or pollen (free-sporing: spores are released from sporophyte and must find moist spot to grow);
- Cuticle poorly developed;
- Gametophyte large (relative to other plants): it is the green, persistent, dominant phase of the life-cycle;
- Require external water for fertilization (i.e., sperm from male gametophyte must swim to egg of female gametophyte);
- Gametophytes have simple dichotomous branching if at all; sporophytes smaller than gametophytes & unbranched;
- No true leaves or roots.

See Section V for a glossary of some relevant terms.

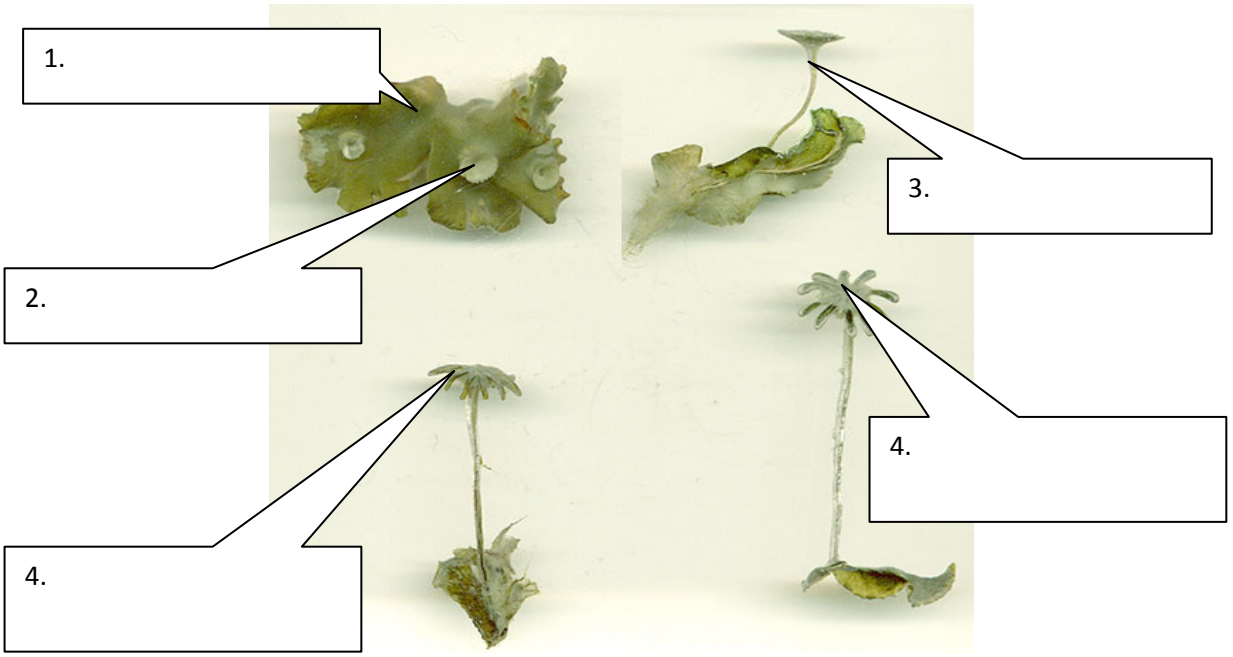
A. Liverworts (Raven pp. 369-377)

We will focus our study on the so-called thalloid/thallose liverworts, which have very simple vegetative structure to the gametophytes – in that their body consists of a thallus (flattened body) that is *not* differentiated into stem, leaf and root. The thallus has unicellular projections called rhizoids that help anchor the plants to the substrate and may also absorb water.

1. Gametophytes.

a) Labeling of Picture with aid of Book Figures.

Based on figures in your text book, label the pictures below with the appropriate term.
Thallus- the flattened green body of a thalloid liverwort.
Gemmae Cup- a cup like structure that produces little clonal discs that, when ejected from the cup by rain drops, can grow into a new liverwort plant.
Antheridiophore- a stalk holding antheridia.
Archegoniophore- a stalk holding archegnoia.



Which parts above are from a female gametophyte? *3 or 4?*

Which are from a male gametophyte? *3 or 4?*

b) Living or Pickled Material, Dissecting Scope, & BOOK.

From back/side of room, grab a portion of thallus and take it back to your bench to examine. It will be from a species of *Marchantia* &/or *Conacephalum*.

Study **Figure 16-4 from Raven**. Then, with a dissecting scope, look at the top surface and observe the polygonal outlines of the air chambers that were diagrammed.

Go up to 40 x magnification and draw your field of view, labeling the features as they relate to **Fig. 16-4**.

We will not call the pore in **Fig. 16-4** a stoma since it cannot be opened & closed (i.e., it is always open) b/c there are no guard cells surrounding it as in angiosperms or gymnosperms (higher plants).

c) Prepared Slides & Compound Scope.

Obtain prepared slides of liverwort antheridium and archegonium from the side/back of room. Observe and draw the following structures at high mag & use Fig. 16-6 in Raven to help you.

Antheridium

Archegonium

2. Sporophytes.

The sporophyte is the diploid, spore-producing organism in the plant lifecycle. In liverworts it is very small (microscopic), simple, short-lived, and nutritionally dependent upon the female gametophyte.

a) Prepared slide: *Marchantia* sporophyte & Cmpd Scope.

From the side/back of the room. Liverwort sporophytes are small (< 2 mm long) and so really only visible with the compound scope. Make observations and drawings of the sporophyte in this slide, use **Raven Fig. 16-7** to aid in labeling your drawing.

B. Mosses (Raven pp. 378-387)

The gametophytes and sporophytes of mosses are a bit more advanced than thalloid liverworts in their structure. Examine living material of various species available.

1. Gametophytes and Sporophytes.

a. Living Material of Various Species.

Be sure you can recognize and interpret all you see as follows:

1) Gametophytes. The conspicuous green and leafy plants are the gametophytes.

a) This is the multicellular, haploid phase of the life cycle.

b) The "leaves" on this gametophyte are really not true leaves and are rather called phyllids (they are not vascularized, are not organized into tissues –being only 1 cell thick except at the midrib).

2) Sporophytes. On some of the female gametophytes (those which carried the egg and the archegonium) there are long stalks (either light green or brown) terminating in a capsule. These are the sporophytes and they are multicellular and diploid. They are rooted by a foot in the archegonium base of the female gametophyte, then they have a long, unbranched stalk (called a seta) terminated by a sporangium (called the capsule). The young capsules wear a calyptra (capsule cover, actually the vestige of the archegonium that was torn off as the sporophyte started to grow out of it). This calyptra will come off when the capsule is ready to release its spores.

See the moss life cycle in Raven (Fig. 16-28) to relate all of these terms.

b. Microscopic Observations with Cmpd Scope.

Make observations of the vegetative and reproductive parts of various moss species, using prepared slides in the back/side of the room.

1) Prepared Slide & Cmpd Scope: Female Gametophyte w/ Archegonium.

Make drawing of an archegonium atop a female gametophyte.

2) Prepared Slide & Cmpd Scope: Male Gametophyte w/ Antheridium.

Make drawing of antheridium atop a male gametophyte.

II. Pteridophytes

Pteridophytes is a general term for the vascular, free-sporing plants we know as ferns, lycopods (club-mosses, horsetails and scouring-rushes. Their characteristics are as follows:

- Xylem & phloem (for conduction & support);
- No seeds or pollen (free-sporing: spores must find moist spot to grow);
- Gametophyte very small, inconspicuous, short-lived, at or just below the soil surface;
- Sporophyte dominant, large, photosynthetic, persistent phase of the life-cycle;
- Require external water for fertilization (sperm must swim through water on surface of gametophyte to fertilize egg in archegonium);
- Most species (except *Psilotum*) have leaves & roots.

See Section VI for a glossary of some relevant terms.

A. Whisk-ferns (Raven pp. 415-416)

The representative we will study in this lab is the genus *Psilotum* (pronounced with a silent "P"). Characteristics of sporophytes (and, where specified, the gametophytes) are as follows:

- No roots;
- No leaves (only enations, which are minute leaf-like appendages lacking vasculature);
- Sporophyte with dichotomous branching;
- Groups of 3 sporangia fused to form synangium;
- Gametophyte unbranched, less than 2 cm long, underground and not photosynthetic.

1. Living *Psilotum* Plant & Naked Eye & Cmpd Scope

Potted plants in side/back of room or there is one pot near the sink of each 8-student bench.

a. Vegetative Characteristics.

This funny looking plant resembles the earliest vascular plants we see in the fossil record.

What organs (leaf, stem, and/or root) is missing on these sporophytes?

Contrast the branching pattern in this plant with that of a pine tree. Why does this look so odd?

b. Sporangia.

The sporangia on this species appear 3-lobed b/c they actually consist of 3 fused sporangia which, together, the structure is called a synangium. At maturity they are yellow/brown.

Is there an enation subtending each synangium? Draw this.

c. Spores.

Extract some spores from mature (yellow to brown) synangia. Make a wet mount, cover with coverslip, observe at high mag and draw them.

B. Lycopods: aka club-mosses, ground-pines, spike-mosses (Raven pp. 403-407)

Characteristics of sporophytes (and, where specified, the gametophytes) are as follows:

- Roots;
- Spirally arranged microphyll leaves (small, simple, and with just one vein per leaf);
- Sporophyte with dichotomous branching;
- Sporangia semi-lunar or kidney-bean shaped, born in the axil of sporopylls, typically aggregated into strobili;
- Gametophyte short-lived, variable in form, but small (<8 mm), amorphous and colorless (when underground) or green (when on soil surface).

1. Living & Herbarium Material of *Lycopodium*.

Side/back of room. There various specimens of *Lycopodium* & close relatives such as *Diphasiastrum* & *Huperzia*.

a. Vegetative Characteristics.

Study the vegetative parts of these plants.

Although they are not mosses, why do you think laypersons gave them the name "club-mosses" or "spike-moss". Hint: how do they resemble mosses?

Name one anatomical and one ploidal (referring to haploid vs. diploid) difference between the green plants of true mosses and these lycopods.

b. Reproductive Characteristics.

Study the sporangia and (if present) strobili of these plants.

2. Prepared Slides of Stroblius with Cmpd Scope

From side/back of room. Draw a strobilus that has been sectioned longitudinally. Be sure to label sporophylls, sporangia, spores (or spore mother cells).

C. Horsetails & Scouring-rushes (Raven pp. 419-427)

Characteristics of sporophytes (and, where specified, the gametophytes) are as follows:

- Roots;
- Stems (internodes) hollow, ribbed, many species' cell walls impregnated with glass-like silica;
- Whorled microphylls fused in collar at node;
- Branching (if present) whorled (not dichotomous);
- Sporangia in strobili at ends of stems;
- Gametophyte short-lived, small (<8 mm), lobed and "lettuce-like", above ground, green.

Branching forms are generally called horsetails, nonbranching forms are called scouring-rushes. One extant genus, *Equisetum*, with 15 species.

1. Living & Herbarium Material of *Equisetum*.

a. Study the various specimens for the characteristics above. Make drawings where necessary.

b. Test the scouring potential of *Equisetum hyemale* (a local species of scouring-rush) by take the stems and rubbing them against the palm of your hand or against another stem.

Can you hear and feel the abrasion?

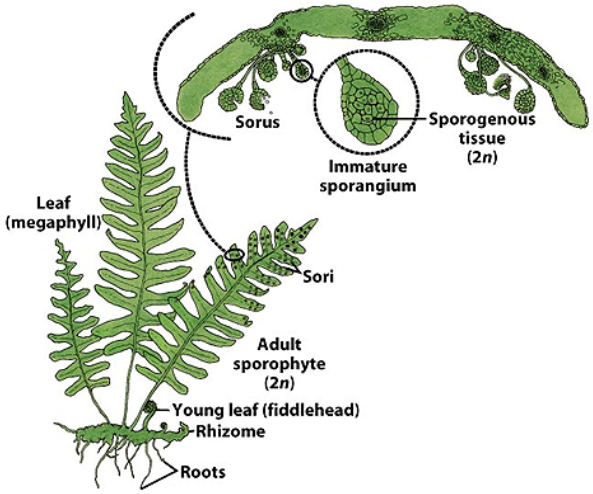
What in the stems (specifically the cell walls) causes this?

D. Ferns (Raven pp. 417-423)

Characteristics of sporophytes (and, where specified, the gametophytes) are as follows:

- Roots;
- Leaves macrophylls and alternate, often pinnate, with young leaves resembling fiddleheads;
- For some reason or another, fern leaves are traditionally called fronds;
- Stem a rhizome, leaves therefore appearing to come from ground;
- Branching various and not diagnostic of the group;
- Sporangia clusters sori,(singular sorus) on undersides of leaves;
- Sori "naked" or covered by flap of tissue called indusium;
- Leptosporangia;
- Gametophyte short-lived, small (<8 mm), hear-shaped, above ground, green.

1. Sporophyte.



a. Herbarium Specimens.

At side/back of room. Compare 3 different fern species for leaf form, as well as sorus shape, abundance, distribution, and indusia. Refer to poster of fern in room.

Wood Fern Christmas Fern Hay-scented Fern

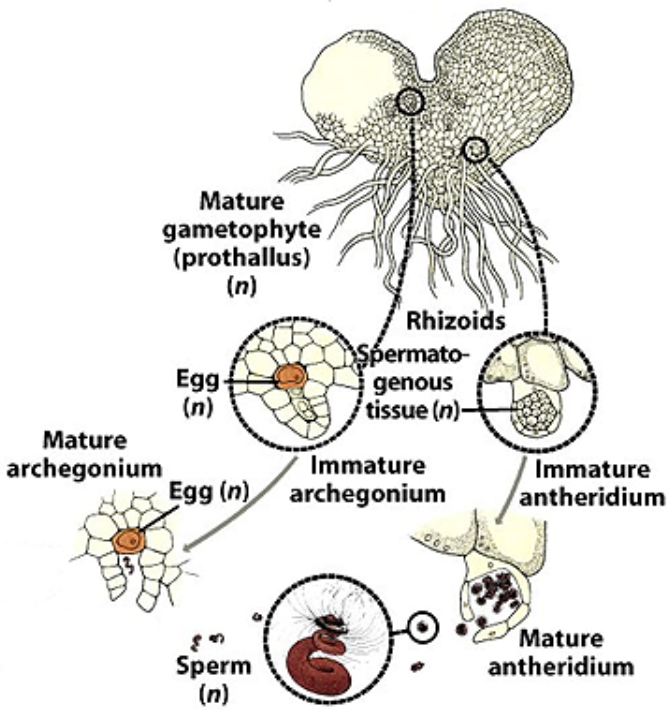
b. Living Specimens.

At side/back of room. These living plants may or may not have sori on them. Make observations of these living plants and revel in their beautiful foliage. NASA has investigated one of these plants, the Boston fern, quite extensively and found that it can remove many airborne indoor air pollutants (such as formaldehyde, a carcinogen used in iron-free plants and as part of the fire-retardants used in carpets, blankets, toys). Thus, it is a healthy plant to keep around the house.

Fern1

Fern 2

2. Gametophyte.



a. If available: Living Fern Gametophytes & Microscopes.

Study first with dissecting scope to see shape and rhizoids on bottom, then make wet mount and look for cellular detail and antheridia / archegonia with compound scope.

Do you see any gametophytes with young sporophytes growing from them?

III. Ethnobotanical references if you are inclined to learn more about the uses of bryophytes and ferns

Nwosu, MO. 2002. Ethnobotanical studies on some pteridophytes of southern Nigeria. *Economic Botany* 56: 255-259.

Thieret, JW. 1956. Bryophytes as economic plants. *Economic Botany* 10: 75-91.

IV. Credits

This lab was developed by Christopher Hardy. You may cite it as....

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V. Some useful terms relevant to bryophyte biology

Antheridiophore – a stalked structure on the gametophytes of some bryophytes that bears on it antheridia.

Anteridium (plural anteridia) – A capsule-like structure, sometimes borne on an antheridiophore, in which mitosis occurs to produce sperm.

Archegoniophore - a stalked structure on the gametophytes of some bryophytes that bears on it archegonia.

Archegonium (plural archegonia) – A flask-shaped structure that contains one egg cell inside at the base of a long neck and canal.

Capsule – usually the swollen distal portion of a bryophyte sporophyte in which meiosis occurs to produce spores. Synonymous with "sporangium" in bryophytes.

Foot – the basal portion of a bryophyte sporophyte that is rooted into the gametophyte.

Free-sporing – spores are released from sporophyte and must find moist spot to grow. Spores are not retained and, thus, gametophytes do not develop within sporophytic tissue.

Gametophyte – the multicellular haploid plant in the life cycle that produces gametes in plants.

Gametangia (singular gametangium) – a structure on a gametophyte that produeces sperm through mitosis. The two types are antheridia and archegonia.

Gemma Cup- a cup like structure on the gametophyte of a liverwort where small clones of itself (gemmae, singular gemma) are produced.

Phyllid – the leaf-like organs on a moss gametophyte. Except sometimes at the midrib, phyllids are just one cell thick and therefore not considered true leaves.

Protonema – the filamentous, but branching juvenile phase of the gametophyte of mosses.

Rhizoid – unicellular or filamentous projections from the epidermis of bryophytes that are analogous to roots.

Seta – the stalk of sporophyte in bryophytes.

Sporangia (singular sporangium) – the spore-producing structure of a sporophyte.

Sporophyte – the multicellular diploid plant in the life cycle that produces spores in plants.

Thallus – a flattened gametophyte plant of some liverworts that is not differentiated into root, stem or leaf.

VI. Some useful terms relevant to pteridophyte biology

Circinate Vernation – the way that a young fern leaf is rolled in bud. Results in the fiddlehead-like appearance to a young fern leaf.

Enation – a minute leaf-like appendage lacking vasculature; found in whisk-ferns.

False Indusium --- a cover of a sorus that is merely the folded margin of the leaf, rather than a distinctly specialized structure for this purpose. Contrast with *indusium*.

Frond – term used traditionally by fern experts as synonymous with the leaf of a fern.

Indusium – a special structure on fern leaf that covers a sorus.

Pinna – the leaflet of a fern compound leaf. Plural is *pinnae*.

Sorus – a distinct cluster of sporangia on the underside of a fern leaflet. Plural is *sori*.

Sporophyll – a pteridophhyte leaf modified to bear sporangia on them or in their axils. Typically similar in shape and form to a normal leaf, but lacking chlorophyll.

Strobilus – a distinct, terminal portion of the shoot of some pteridophyte sporophytes in which there is a cone-like aggregation of sporophylls or sporophyll-like structures bearing sporangia.

Gymnosperms

Gymnosperms are a group of seed plants united and distinguished from the angiosperms by their naked seeds (*gymnos* is Greek for naked; *sperma* is Greek for seed). Otherwise, the three or four major lineages of gymnosperms do not have much else in common. There are some new terms to be introduced here, and you can find the important ones in the glossary provided by Section E.

Objectives for this lab:

1) become familiar with gymnosperms in general,
2) learn how to distinguish them from other non-gymnospermous taxa,
3) learn how to distinguish the major gymnosperm lineages from one another, and
4) learn to use a dichotomous key to identify conifer genera.

Table of Contents for this lab:

Sequoiadendron giganteum (giant redwood)

A. Conifers

These are the largest extant (not extinct) group, as well as the most economically important group of gymnosperms. The group is defined by their ovulate cones (conifer means "cone-bearing") and resin ducts (aka resin canals) that run through all parts. A more comprehensive list of characteristics is as follows:

- Trees or shrubs with a branched stem.
- Resin ducts (resin canals) throughout.
- Leaves typically evergreen (some deciduous) and needle-shaped.
- Sexual system monoecious or dioecious.
- Microsporangia (male) borne in pendulous or erect strobili; strobili often in clusters.
- Megasporangia (female) borne in ovules borne on scales in cones (each scale subtended by a bract).
- Seed coat dry, papery, often winged for wind dispersal.
- **Other notes:** source of "pine tar" and resin (turpentine + rosin); major lumber products (e.g., pine from *Pinus*); major source of pulpwood for paper (e.g., *Tsuga* –hemlocks, *Abies* –firs, *Picea* –spruces); popular as ornamentals; other notable genera include *Sequoia* (coastal redwood), *Sequoiadendron* (giant redwood), *Pseudotsuga* (Douglas-fir), and *Taxus* (yew).

A1. Conifer economic botany.

Most of the value of conifers come from their aesthetic qualities as ornamental plants, or their wood that is used for lumber or paper making. However, there are other important products. At some point today and when the crowd is not too large there, visit the Conifer Economic Botany station to see, smell, and in the case of pine "nuts" taste, various products of conifers.

a. Turpentine.

1) What is turpentine used for?

2) From what of the conifer is it made from?

b. Pine "nuts."

1) Technically speaking, a "nut" is a type of fruit produced by certain angiosperms such as oaks. Thus, pine "nuts" are not truly nuts. What are they and for what purpose are they used?

2) With your group, take 1 pine nut per person and try roasting them on a foil boat in the toaster oven. Be careful not to burn them. Then compare the taste of a raw pine nut with a roasted one.

c. Gin.

1) What does gin smell like?

2) How is gin made and, particularly, with what is gin flavored?

3) Relative to homologous structures of other conifers, what is unusual about the structures that are used to flavor gin?

A2. Compare & contrast a strobilus with a cone.
All gymnosperms produce pollen in a simple male strobilus and many non-conifer gymnosperms also produce seeds in a simple female strobilus. Although many textbooks used the term "cone" loosely to refer to, for example, the "pollen cones" or "seed cones" of conifers, we will restrict our application of the term cone to the female (ovuliferous) structure unique to conifers.

In conifers, the male strobilus is a simple (unbranched) modified stem axis with modified leaves called sporophylls on which sporangia produce male spores which then mature into several-celled pollen grains (microgametophytes). The cone of conifers is different in that is a compound (branching) shoot system where ovules (then seeds) are borne on woody lateral appendages called "seed scales" (aka "cone scales") that form as branches from axils of small bracts attached directly to the main cone axis.

Strobili, Cones & Dissecting Scope

Use your naked-eye and dissecting scope to contrast the male strobilus and female cone of a conifer in terms of size and texture.

On the male strobilus, locate and draw a sporophyll with sporangia.

On the female cone, locate and draw a cone scale with seeds: then locate the small bract subtending the scale (note that in Douglas-fir the subtending bract is 3-pronged and easily visible, whereas in other genera such as pines the subtending bract is much smaller than the scale and so not easily visible).

How does the presence of the bract subtending the cone scale reveal that the cone scale is actually a lateral branch rather than a sporophyll?

Prepared Slide & Compound Scope

Prepared slide of “Young Male & Female Cone” in Pine. Compare and contrast a male strobilus with a developing female cone in pine. Find the sporophylls and sporangia in the male strobilus, and a cone scale and ovules in the cone. Also in the developing cone, look for a cone scale and its subtending bract.

A3. Conifers have resin canals.

Prepared Slides & Compound Scope

Locate the resin canals in a transverse section of pine wood and needle (leaf) obtained from side/back of room. Resin canals are lined with parenchyma cells that produce and secrete the aromatic resin we are all familiar with.

A4. Conifer leaves are needle-like or scale-like.

Live Specimens & a Key to Conifers

Conifer genera and species can be distinguished based on the form and arrangement of their leaves. Use the Key to Conifer Genera (Section F, which includes an illustrated glossary of important terms) to identify the genus of up to 20 conifer specimens in the room or outside, and record your answers below. In the process, you will familiarize yourself with the various forms that conifer leaves can take.

1.

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B. Cycads

Cycads comprise an ancient lineage of tropical trees and shrubs: they existed before and with the dinosaurs, and there are even fossils of these tropical plants on Antarctica. Familiarize yourself with cycad morphology and be sure you can properly distinguish cycads from the other gymnosperms in the room.

- Shrubs or trees with a single, typically unbranched stem.
- Leaves typically evergreen; in a terminal rosette; pinnate and leathery; leaflets typically with dichotomous leaf venation; circinate vernation common.
- Sexual system dioecious.
- Microsporangia (male) borne on sporophylls and grouped into a strobilus.
- Megasporangia (female) borne in ovules which are borne on loosely clustered megasporophylls (*Cycas*) or into a strobilus (*Zamia*).
- Seed coat may become fleshy and colored to attract animal dispersers.
- **Other notes:** produce neurotoxins – harmful only if ingested (eaten) in large quantities over period of years; thus, otherwise a harmless, ancient, and wonderfully beautiful lineage that is extremely popular in ornamental horticulture.

B1. Carefully examine a male cycad strobilus.

Male Strobilus & Naked Eye.

In the back/side of the room is male strobilus from a cycad. All male strobili in cycads are very similar to one another.

a. Unlike some other taxa, cycad male sporophylls have more than one sporangium on a sporophyll. About how many sporangia do you count on these large sporophylls?

b. Are the sporangia borne on top or beneath the sporophylls?

B2. Compare & contrast form & arrangement of female sporophylls in *Cycas* vs. *Zamia*.

Live Plants/Photos/Cuttings of *Cycas* & *Zamia*.

In the back/side of room are some specimens and photographs showing the female sporophylls in the genus *Cycas* and *Zamia* (which are quite different from one another).

a. *Which genus has the sporophylls arranged in a tight strobilus? Zamia or Cycas?*

b. *Which genus/genera has/have pubescent sporophylls?*

c. *Draw a sporophyll of Zamia: be mindful of its shape and the number of seeds on each sporophyll.*

d. *Draw a sporophyll of Cycas: be mindful of its shape and the number of seeds on each sporophyll.*

C. Ginkgo

Although there were dozens of species living millions of years ago throughout North America and Northern Asia, today there is just one extant (surviving) member of this interesting lineage, *Ginkgo biloba* (the maidenhair tree).

- Trees with a branched stem.
- Leaves deciduous; typically clustered on lateral short shoots; simple, fan-shaped, with dichotomous venation.
- Sexual system dioecious.
- Male sporangia borne in pendulous strobili clustered on short shoots.
- Ovules in clusters of 2 or 3 on pendulous peduncle from short shoots.
- Seed coat becomes fleshy at maturity and stinks.
- **Other notes:** produce various terpenoids thought to promote circulation and thereby brain function; popular as ornamentals, ancient lineage; a "living fossil."

C1. Ginkgo leaves are very distinctive.

Live or Herbarium Specimens of *Ginkgo* Leaves.

Specimens are in back or side of room. Draw a leaf of a ginkgo below, showing petiole, blade, and venation.

a. How is the shape of the blade distinctive?

b. Is the venation type pinnate, palmate, parallel or something quite different? Explain.

C2. Ginkgo reproductive structures are very distinctive.

Fertile Live/Herbarium/Photo Specimens of *Ginkgo*.

Specimens are in back or side of room.

a. How are the male and female structures of ginkgo different from one another and different from other gymnosperms in room?

D. Credits

This lab was developed by Christopher Hardy. You may cite it as....
Hardy CR. 2016. Gymnosperms. Pp 223-234 in CR Hardy, RL Wagner (eds) *Guide to Exercises in Concepts of Botany, 4th Edition*. Millersville, Pennsylvania, USA.

E. Glossary of terms relevant to gymnosperms

Cone – the female structure of conifers, referred to a "compound strobilus" by some: a compound (branching) shoot system where ovules (then seeds) are borne on woody lateral appendages called "scales" that form as branches from axils of small bracts attached directly to the main cone axis.

Dichotomous venation – major veins of leaf branch dichotomously (forking). Occurs in the leaf blades of ginkgo and many cycads.

Dioecious – when the male and female structures in a species are borne on separate plants: plants are thus unisexual and either male or female. Contrast with monoecious and synoecious.

Hetersporous – Producing large (female) megaspores and small (male) microspores.

Integument – the diploid outer layers of an ovule; will ripen into the seed coat.

Megagametophyte – same as female gametophyte; a haploid, egg-producing plant. In seed plants, the megametophyte consists of anywhere from seven (angiosperms) to several hundred or thousand (gymnosperms) cells, is termed "embryo sac" in the vernacular, and is contained within the ovule.

Megasporangium – a sporangium producing megaspores (female spores). In seed plants, it is fleshy and also called the "nucellus," and there is one megasporangium contained in each ovule.

Megaspore – a spore that is large relative to a microspore and will develop into a megagametophyte.

Megasporophyll – a sporophyll that bears one or more megasporangia.

Microgametophyte – same as male gametophyte; a haploid, sperm-producing plant. In seed plants, the mature microgametophyte is made up of three (angiosperms) to several (gymnosperms) cells (including the two sperm it produces) and is termed "pollen grain" in the vernacular.

Microsporangium – a sporangium producing microspores (male spores). In seed plants, microspores will develop into pollen grains.

Microspore – a spore that is small relative to a megaspore and will develop through mitotic activity into a microgrametophyte.

Microsporophyll – a sporophyll that bears one or more microsporangia.

Monoecious – when the male and female structures in a species are borne on the same plant but in separate structures (e.g., separate strobili): plants are thus hermaphroditic. Contrast with dioecious and synoecious.

Nucellus – the fleshy megasporangium of seed plants.

Ovule – the structure in a female strobilus or cone that will ripen into the seed; contains the female gametophyte (haploid tissue) surrounded by diploid sporophytic tissue of 1-2 integuments.

Peduncle – the stalk of a strobilus, cone, or other reproductive structure.

Pollen – the male (micro) gametophyte in seed plants that consists of 2 or 3 cells when mature, and 3 or 4 cells when one of those cells divides to become two sperm.

Seed – the structure that ripens from an ovule, consisting of an embryo (from the fertilized egg of the ovule), some nutritive tissue (megagametophytic tissue in gymnosperms; typically endosperm or the cotyledons in angiosperms), and a seed coat (the integument(s) of the ovule).

Short shoots – found in ginkgo and some conifers: short side branches of long main stems, the short shoots grow very slowly (due to very short internodes) and typically bear a cluster of leaves during the growing season.

Synoecious – when the male and female structures of a plant are borne in a single structure (e.g., a bisexual flower or bisexual strobilus): plants are thus hermaphroditic. Contrast with monoecious and dioecious.

Strobilus (plural, strobili) – a cone-like aggregation of sporangia (each subtended by sporophylls) at the distal end of stem.

F. Key to Conifer Genera ©MU Botany 2013 onwards

A. Plants not evergreen

B. Branchlets (secondary branches) short and stubby, persistent, alternate..... ***Larix*, Larch**

BB. Branchlets elongate, deciduous, opposite..... ***Metasequoia,* Dawn Redwood**

AA. Plants evergreen

C. Leaves needle-shaped or otherwise elongate and well-diverging from the stem

D. Leaves in fascicles of 2, 3, or 5..... ***Pinus*, Pine**

DD. Leaves borne singly or tufted on stubby side-branches (but not fascicled)

E. Leaves tufted on stubby side-branches..... ..... ***Cedrus*, Cedar**

EE. Leaves borne singly

F. Leaf ending at stem, so stem is woody in texture and color.

G. Leaves sharp-pointed and square in cross-section, with small woody peg-like leaf-stalk..... .... ***Picea*, Spruce**

GG. Leaves rounded or round-pointed, and flattened, woody peg-like leaf-stalk absent or if present not very prominent

H. Leaves <1.25 cm long, with distinct leaf-stalk; cones 1-2 cm long ....***Tsuga*, Hemlock**

HH. Leaves >1.5 cm long, with no distinct leaf-stalk (although perhaps with a gradually narrowed leaf-base); cones >2.5 cm long

I. Needles flat, rounded and blunt at tip, with swollen round base, base not persistent and leaving a round leaf scar on twig; cones erect with scales deciduous at maturity ..... ***Abies*, Fir**

II. Needles flat, pointy (but not sharp) at tip; cones pendulous, scales persistent, with very long 3-lobed bracts that look like the rear-end of a mouse; needle-base not swollen, leaf-scar either not round or not very big..... ***Pseudotsuga*, Douglas Fir**

FF. Leaf-base decurrent along (i.e., the base runs along) the stem for some distance, such that twig stem to which leaves are attached appears green.

J. Leaves flattened in cross-section; cones with just a single seed which is partially enclosed in fleshy red covering (aril); often bushes or shrubs .....***Taxus*, Yew**

JJ. Leaves angular (not flattened) in cross-section and very sharp-pointed; cones with multiple woody scales present, no fleshy red covering (aril) around seed(s); trees or shrubs.

K. Typically bushes to small trees; cone small (< 1 cm diam), soft and berry-like, blue-gray; needle-like leaves (juvenile) and scale leaves (adult) often on same plant..... .....***Juniperus*, Juniper**

KK. Larger trees; cone large and round (> 1 cm diameter) ....***Cryptomeria*, Japanese Cedar**

CC. Leaves scale-like (or at least not especially elongate)

L. Branchlets (secondary branches) forming flattened fan-like sprays

M. Twiglets (smallest twigs) much flattened; cones elongate; cone-scales flattened, 8-12 . ..... ***Thuja*, Arbor Vitae**

MM. Twiglets rounder; cones round; cone-scales shield-shaped, 4-8 ....***Chamaecyparis,* Cypress**

LL. Branchlets forming 3-D clusters not at all fan-like

N. Cones soft and berry-like, needle leaves (juvenile) and scale leaves (adult) often mixed..... .....***Juniperus*, Juniper**

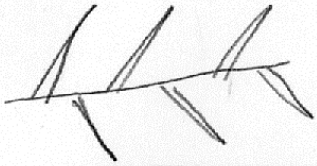
NN. Cones woody

O. Cones < 0.5 cm; leaves uniformly scale-like (<2 mm long)....***Chamaecyparis,* Cypress**

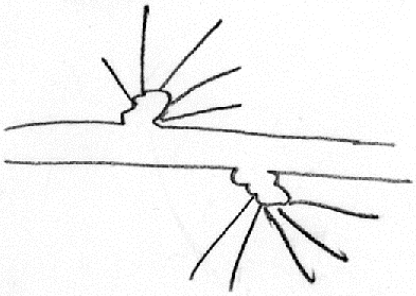
OO. Cones >0.5 cm; leaves longer (>2 mm long). ..***Cryptomeria*, Japanese Cedar**

Leaves alternate vs. opposite

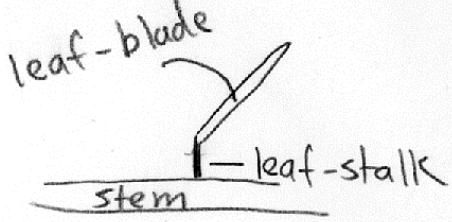
Leaves needle-like vs. scale-like



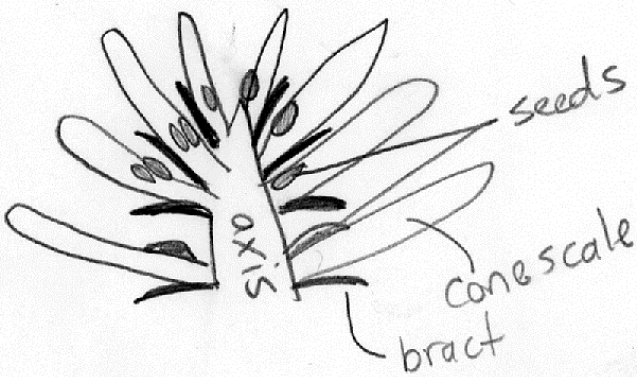
Leaves fascicled vs. tufted on short branches



Leaf Morphology



Cone Morphology



Angiosperms

The angiosperms are seed plants which, unlike the gymnosperms, have flowers and fruits. The flower itself is a type of strobilus. It evolved from a gymnosperm strobilus (Fig 1A) ca. 150 million years ago through a conduplication and marginal fusion of the megasporophyll to form what is called the carpel of angiosperms (Fig 1B & 1D). Microsporophylls became the stamens and lower, sterile sporophylls or modified leaves became the perianth (Fig 1B & 1E). Subsequent shortening of the strobilus axis and reduction in floral organ number produced a whorled phyllotaxy to each organ class and the perianth differentiated more sharply into petals and sepals (Fig 1C).

The key structure that distinguishes the angiosperm flower from the gymnosperm strobilus is the carpel that encloses the ovule(s). The term angiosperm itself is derived from *Angeion* (Greek for "vessel") and *sperma* (Greek for "seed"): it is the carpel(s) that develops into the fruit wall (i.e., the vessel), and the ovule(s) that develops into the seed(s) inside.

Objectives for this lab:

1) Become familiar with the angiosperms in general
2) Learn to dissect and interpret the parts of a flower
3) Learn to interpret and name the types of inflorescences
4) Learn to name the different types of fruits
5) Relate fruit structure to that of the flower from which it came

Table of Contents for this lab:

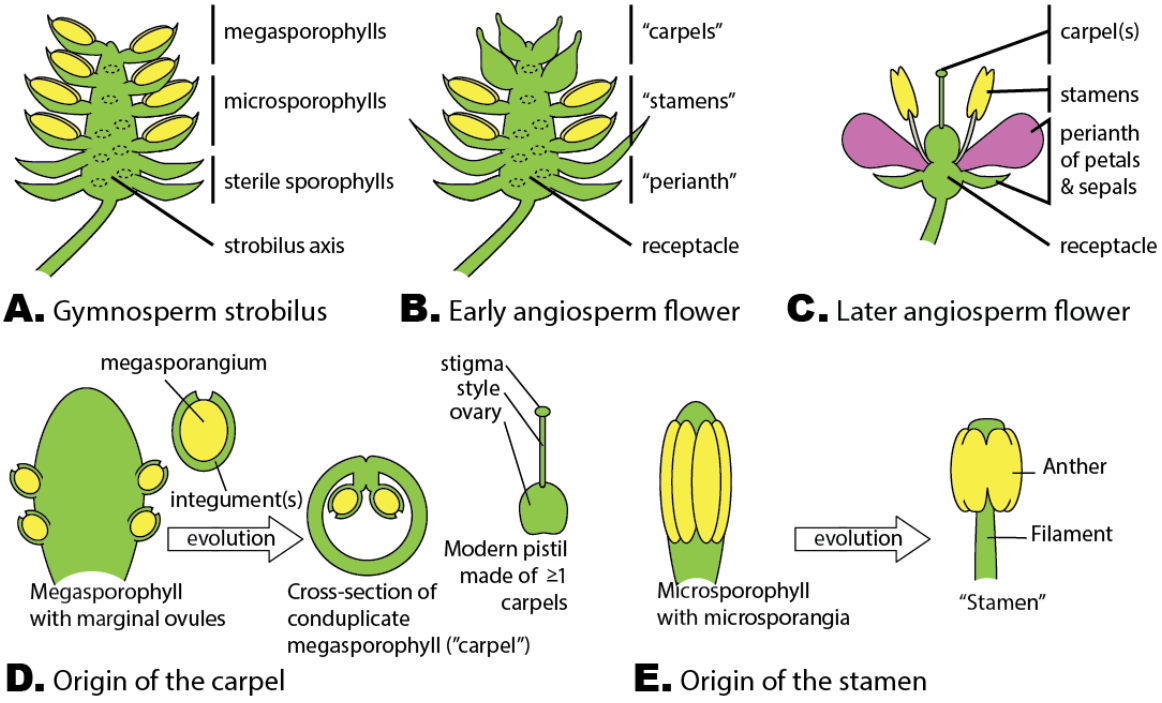


Fig 1. An evolutionary sequence for the flower having originated from a bisexual gymnosperm strobilus. Under this scenario, unisexual flowers are then derived from bisexual flowers. Alternatively, evolution of unisexual flowers from unisexual gymnosperm strobili also is possible, but under this scenario bisexual flowers were then derived from unisexual flowers.

I. Flowers & Inflorescences

A. Floral Morphology

1. Basic floral structure.

Flowers are compact shoot systems consisting of organs derived ultimately from the evolutionary modification of leaves. A single flower consists of up to 4 organ classes inserted onto a receptacle (the floral axis). From the outside-in, these organ classes, sometimes called organ "whorls" since they usually exhibit whorled phyllotaxy, are the calyx (consisting of sepals that serve to protect the flower in bud), corolla (consisting of petals which serve generally to attract pollinators), the androecium (consisting of stamens which produce pollen), and the gynoecium (consisting of 1 or more carpels which bear ovules). The calyx and corolla are sterile (i.e., do not have sporangia) and are collectively referred to as the perianth. When the sepals and petals are not well differentiated from one another and look alike, they are called tepals. Tepals are usually petaloid (petal-like in color) and this condition is common in monocots such as lilies and tulips.

If a flower is stalked, then the stalk is called a peduncle if the flower is solitary, or a pedicel if the flower is part of a larger inflorescence. A flower with a pedicel is said to be pedicellate. If the flower is unstalked, it is said to be sessile.

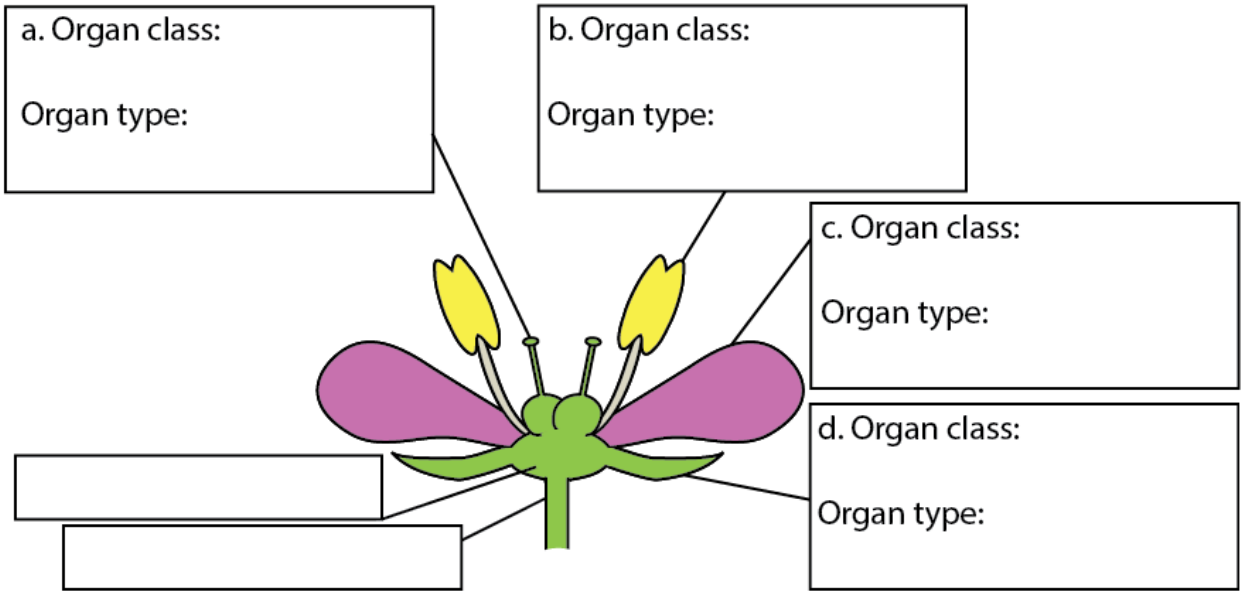


Fig 2. A longitudinal section through a flower, showing 2 sepals, 2 petals, 2 stamens and 2 carpels.

2. Androecial structure.

The classic stamen is differentiated into a filament (the stalk) and an anther (see Fig 1E). The anther produces the pollen. Laminar stamens lack a narrow filament and thereby more closely resemble the gymnospermous microsporophylls from which they evolved. Laminar stamens are not that common, but are present in some angiosperms with primitive-looking flowers.

3. Gynoecial structure.

The classic gynoecium consists of one or more carpels that comprise one or more pistils. The textbook pistil is differentiated into ovary (containing the ovules), a style, and a stigma (which receives the pollen during pollination) (Fig 1D). Whenever there are multiple pistils per flower (as in Fig 2), each pistil consists of just one carpel. However, the pistil of a unipistillate flower (e.g., Fig 3) may consist of one or more carpels. There are several ways to determine the number of carpels that make up a pistil. A cross-section of the ovary may reveal any number of locules (chambers) and the number of locules often

equals the number of carpels. Other times there may be multiple styles, stigmas or stigma lobes and these will typically equal the number of carpels. The ovary may also be lobed, and the number of lobes may reveal the number of carpels.

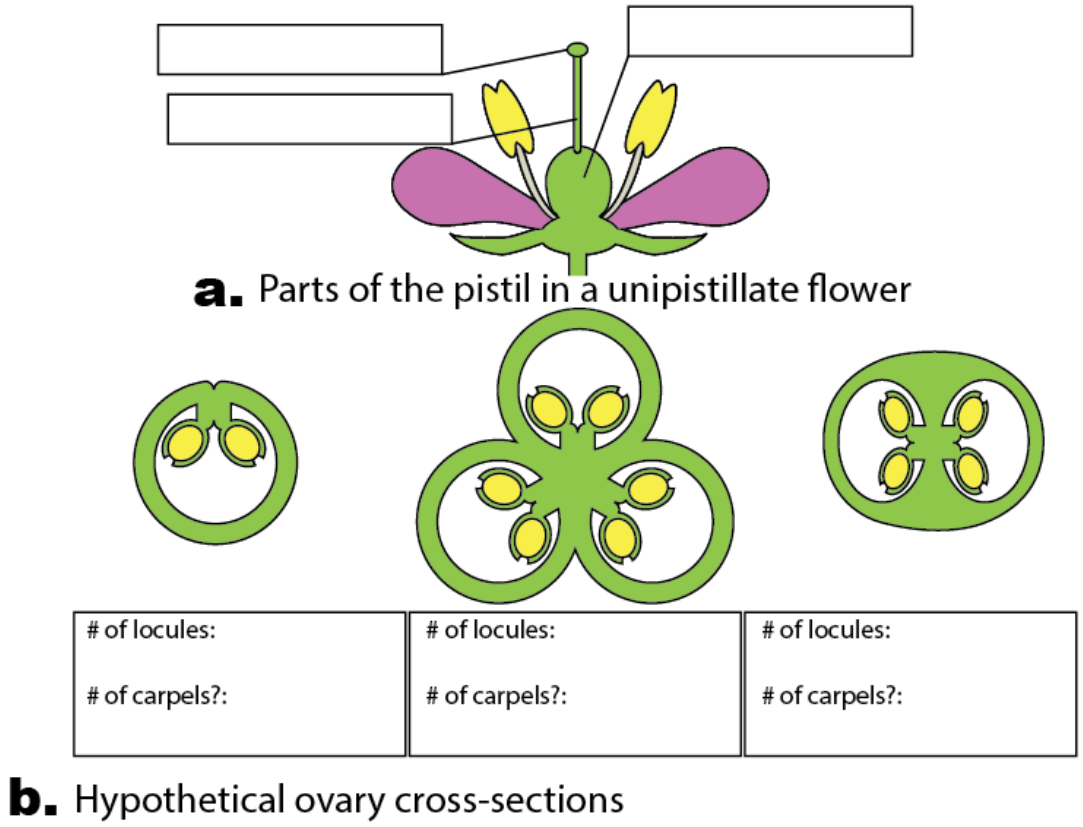


Fig. 3.

4. Floral Completeness & Perfection.

Complete flowers are those possessing all 4 organ classes. An incomplete flower is one lacking one or more of these organ classes. Perfect flowers are those possessing both functional stamens and carpels (i.e., a bisexual flower), whether or not sepals or petals are present. Imperfect flowers are those with only one functional type of sexual organ (i.e., unisexual flowers). Staminate flowers are imperfect flowers possessing functional stamens rather than carpels. Pistillate (aka carpellate) flowers are imperfect flowers possessing functional carpels rather than stamens.

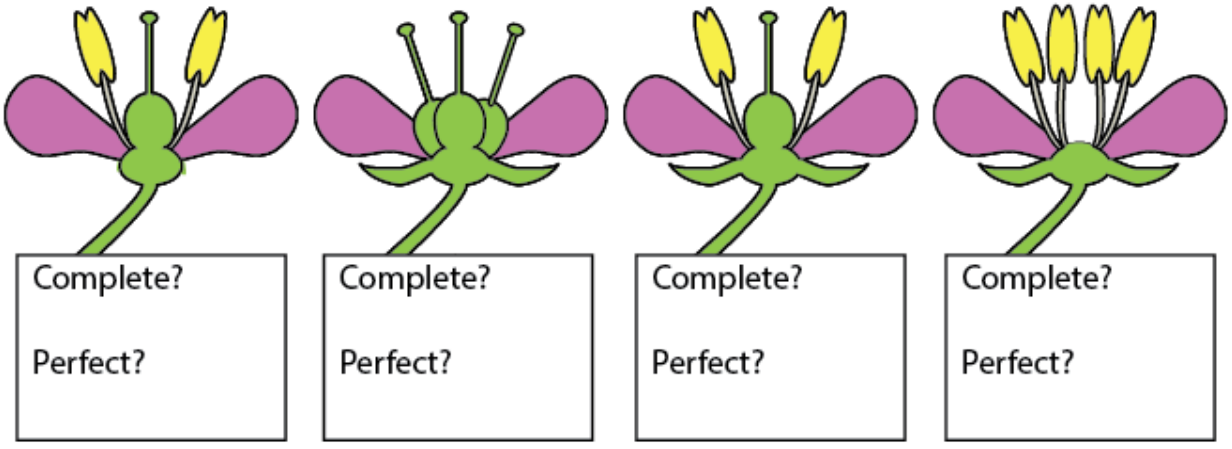
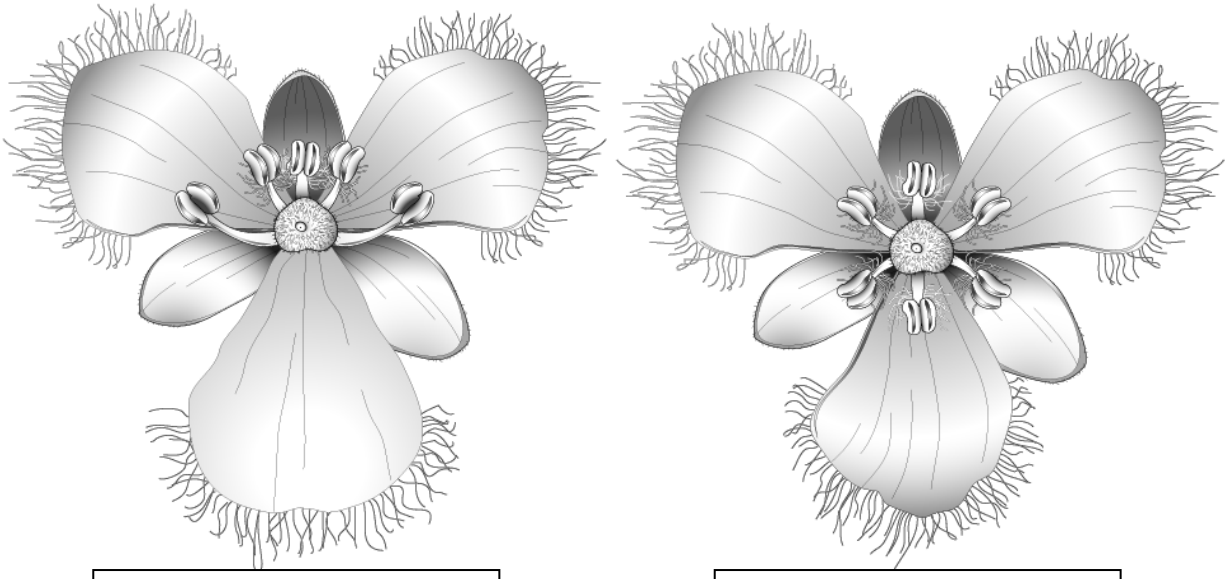


Fig. 4.

5. Symmetry.

Parts of the flower or the entire flower itself may exhibit various forms of symmetry, depending upon the species. Polysymmetric (sometimes AKA regular, radial, actinomorphic) flowers possess more than 2 planes of symmetry. Monosymmetric (sometimes AKA irregular, bilateral, zygomorphic) flowers possess just one plane of symmetry. Other symmetries such as dissymmetric (two planes of symmetry) and asymmetric (no plane of symmetry) are rare.



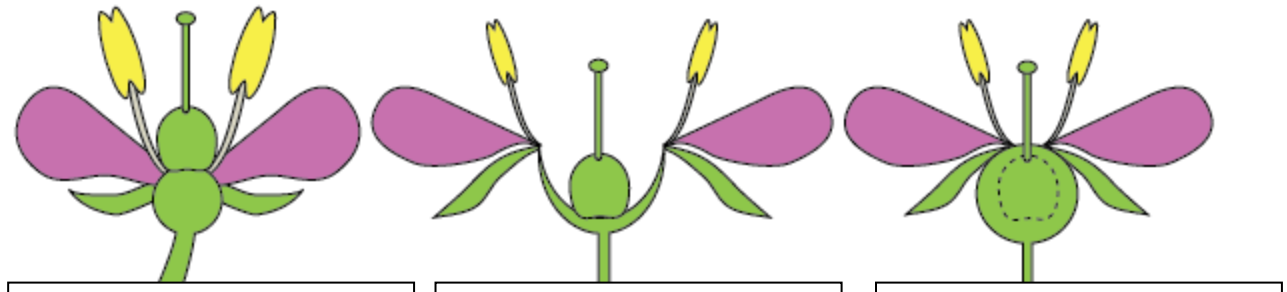
Symmetry?

Symmetry?

Fig. 5. Floral symmetry varies with the species.

6. Ovary position.

The ovary(s) of a flower may be either superior (where the ovary sits on top of the receptacle, above the insertion of all other organs) or inferior (where the ovary is embedded in the receptacle or sits below the insertion of all other organs). Beware of flowers with a hypanthium (floral cup) in which an ovary sits and from the rim of which depart the other floral organs: ovaries inserted on the bottom of this hypanthium are still considered to be superior.



Ovary position:

Ovary position:

Ovary position:

Fig. 6. Ovary position can vary.

Which flower above has a hypanthium?

7. Fusion: Connation and Adnation.

Parts of the same organ classes that are fused are said to be connate. Parts of different organ classes that are fused are said to be adnate.

Where exactly in either of the two diagrams below is adnation or connation discernible?

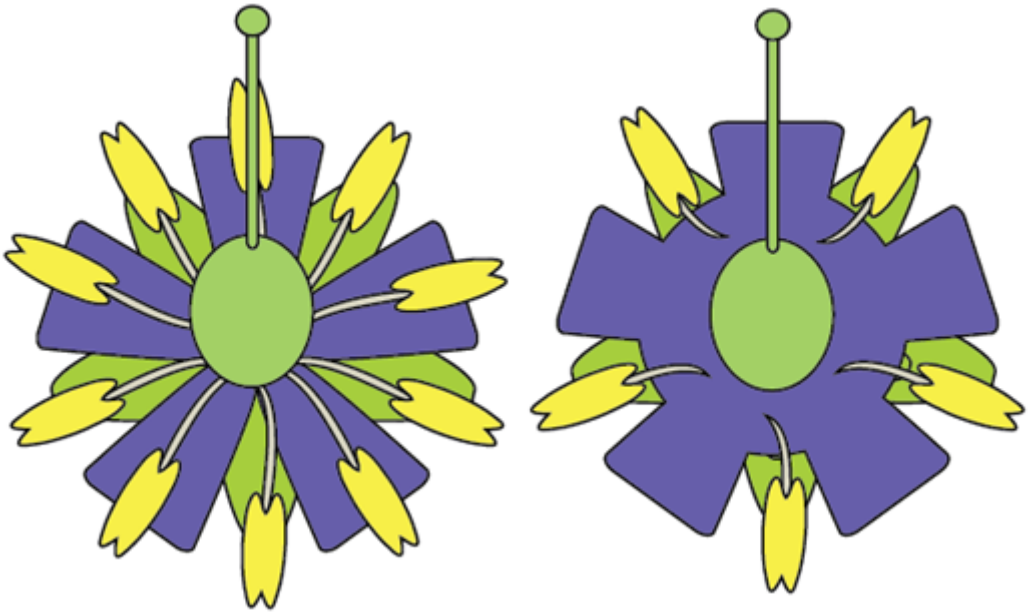


Fig 7.

8. Merosity.

The number of organs in each whorl follows an underlying numerical pattern or merosity in most species. Monocots and some primitive dicot angiosperms, for example, have parts in 3's and are thus said to be trimerous: for example, you find 3 sepals, 3 petals, and 3, 6, or 9 stamens (in multiples of 3). Eudicots tend to have flower parts in 4's and 5's and are thus said to be either tetramerous or pentamerous, respectively. A pentamerous flower, for example, will typically have 5 sepals, 5 petals, and 5 or 10 stamens. Exceptions to this rule occur in the androecium where there may be one less that the merosity (e.g. 4 stamens in an otherwise pentamerous flower) or many more. Exceptions also occur in the gynoecium where the number of pistils and carpels is less than the number of sepals and petals.

What is the merosity of each of the two flowers illustrated in Figure 7 above?

What is the merosity of each of the flowers illustrated below in Figure 8?

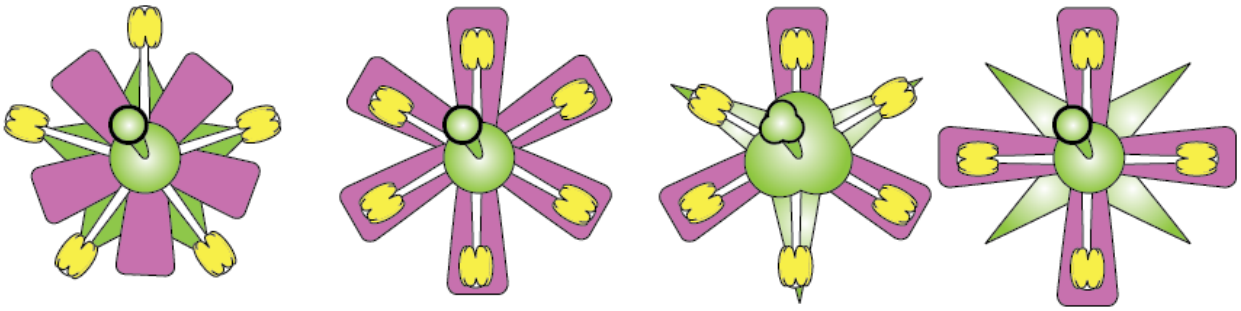
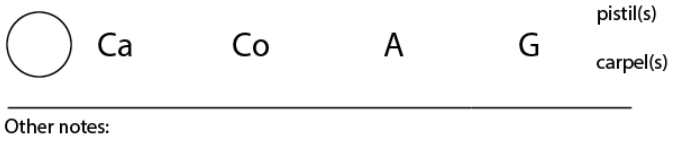


Fig 8.

B. Floral Formulas & Flower Dissections

Now you will apply your knowledge to actual flowers, using a device called a floral formula. A floral formula helps you organize your observations of flowers as you learn floral structure. A floral formula is a succinct, one-line description of the flower. A second line may be used to note other diagnostic features not easily accommodated in the floral formula. For each flower you observe today, you will start with a blank floral formula template onto which you will insert data from your observations (Fig 9).

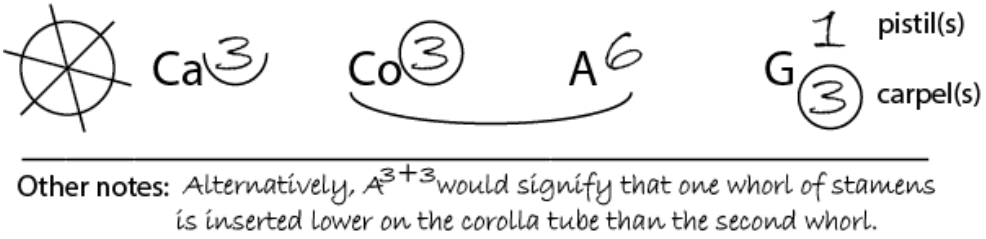


Where:

- ○ = circle for floral symmetry onto which the # of lines drawn equals the number of planes of floral symmetry (1 for monosymmetry, 3 for polysymmetry).
- Ca = the number of sepals, degree of fusion and other info for the calyx.
- Co = the number of petals, degree of fusion and other info for the corolla.
- A = the number of stamens, degree of fusion and other info for the androecium.
- G = the number of pistils, carpels, degree of fusion, and ovary position in the gynoecium.

Fig 9. Floral formula template onto which you will record data from your observations of a flower.

As an example, the completed floral formula (below) for the flower in Figure 10 indicates an polysymmetric flower, 3 basally connate sepals, 3 mostly connate petals, 6 stamens adnate basally to the corolla, and 1 pistil of 3 connate carpels and a superior ovary.



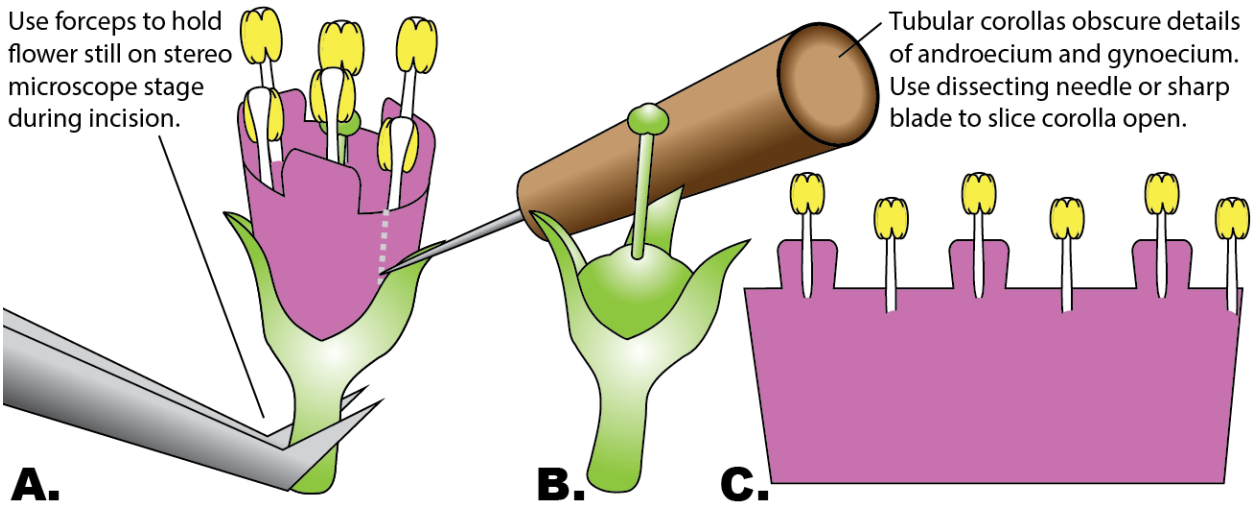


Fig 10. Flowers with tubular corollas (A) can obscure details of stamens and pistil(s) to the inside. Surgically removing the corolla is a way to study the gynoecium (B) and precise number and attachment of the stamens (C).

As a second example, the completed floral formula (below) based on the flower in Figure 11 indicates that the flower is monosymmetric with 4 free sepals, 1 large and 3 smaller free petals, 3 free stamens and 1 pistil of a single carpel and an inferior ovary.

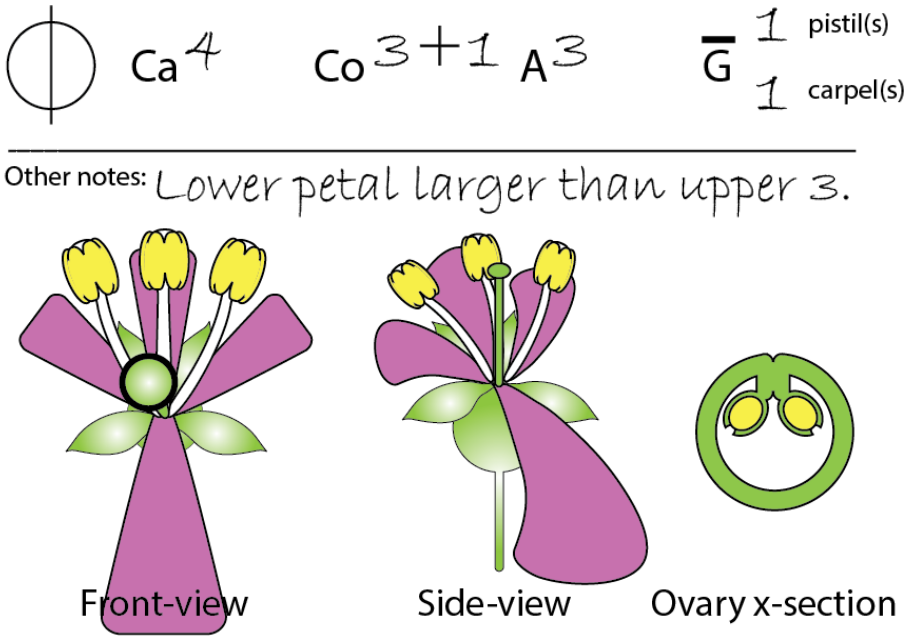


Fig 11. Flower from above (left), the side (middle) and its ovary in cross-section (right).

As a third example, the completed floral formula (below) based on the flower in Figure 12 indicates that the flower is polysymmetric with 6 free tepals, 6 free stamens with pubescent filaments, and 1 pistil of 3 connate carpels and a pubescent, superior ovary.

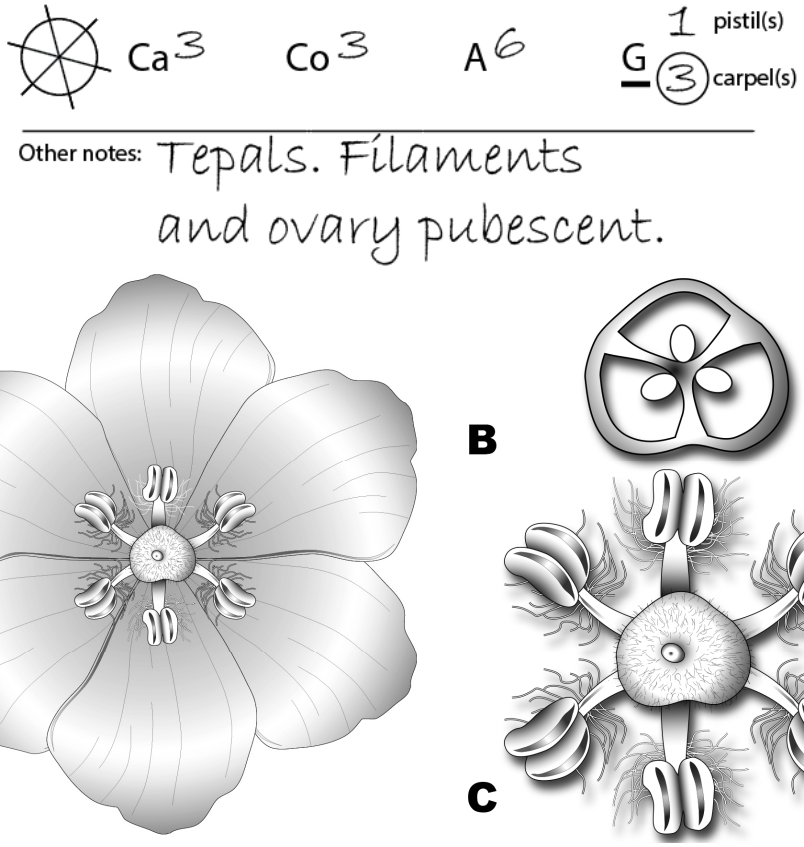
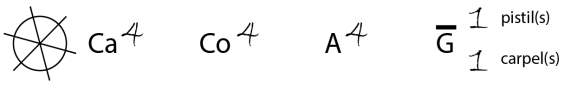


Fig 12. A flower. Front-view (A), ovary x-section (B), androecium and gynoecium close-up (C).

1. Test yourself

Below, match (with connecting lines) the floral formulas on the left with the correct illustrations on the right.



Other notes: None.

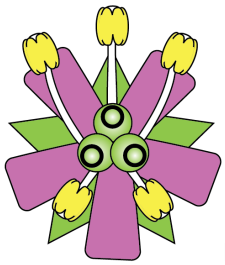
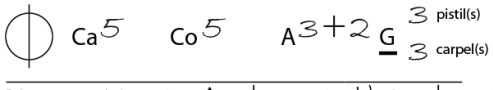


Fig 13



Other notes: Upper A3 longer than lower A2.

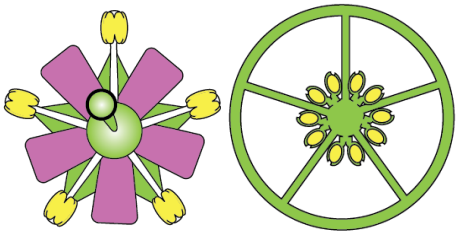
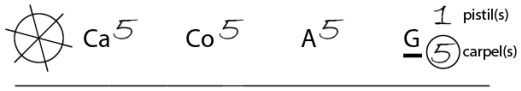


Fig 14



Other notes: None.

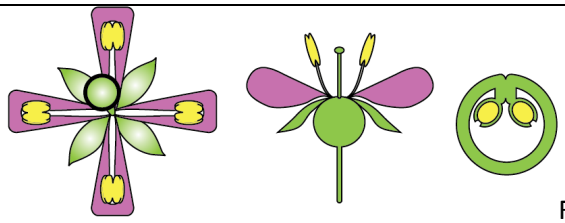


Fig 15

2. Apply to real flowers

Visit the flower stations and perform flower dissections or study dissections presented in posters. Complete the floral formulas below for each flower. If the calyx and corolla are comprised of

a. Plant Name: _____

Other notes:

b. Plant Name: _____

Other notes:

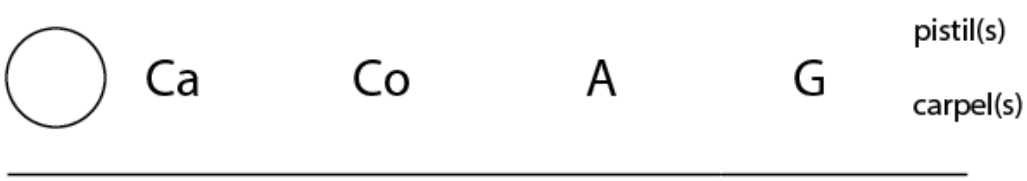
c. Plant Name: _____

Other notes:

d. Plant Name: _____

Other notes:

e. Plant Name: _____



Other notes:

C. Inflorescence Morphology

1. Simple Inflorescences.

Flowers may be solitary or they may be borne in clusters called inflorescences. Simple inflorescences consist of a main axis from which single flowers emerge from the axils of bracts. A raceme is an inflo with an elongate axis on which are borne stalked lateral flowers. A spike is similar to a raceme only that the flowers are not stalked. An umbel is a flat-topped or round inflo in which stalked flowers all arise from a common attachment point. A head is an inflo in which sessile flowers all arise from a common attachment point or a common, expanded receptacle. A corymb is a flat- or round-topped inflo formed by lateral, stalked flowers with progressively shorter stalks. A catkin is a pendulous inflo of unisexual flowers. A helicoid cyme is a curved inflo comprised of terminal flowers that grow as successive branches from the previous flower's axis. These are only some of the more common inflorescence types.

The basal stalk of an inflorescence or of a solitary flower is called a peduncle. After the first flower, the rest of the inflorescence axis, when present, is called the rachis.

Apply these terms where appropriate below. Terms may be used more than once.

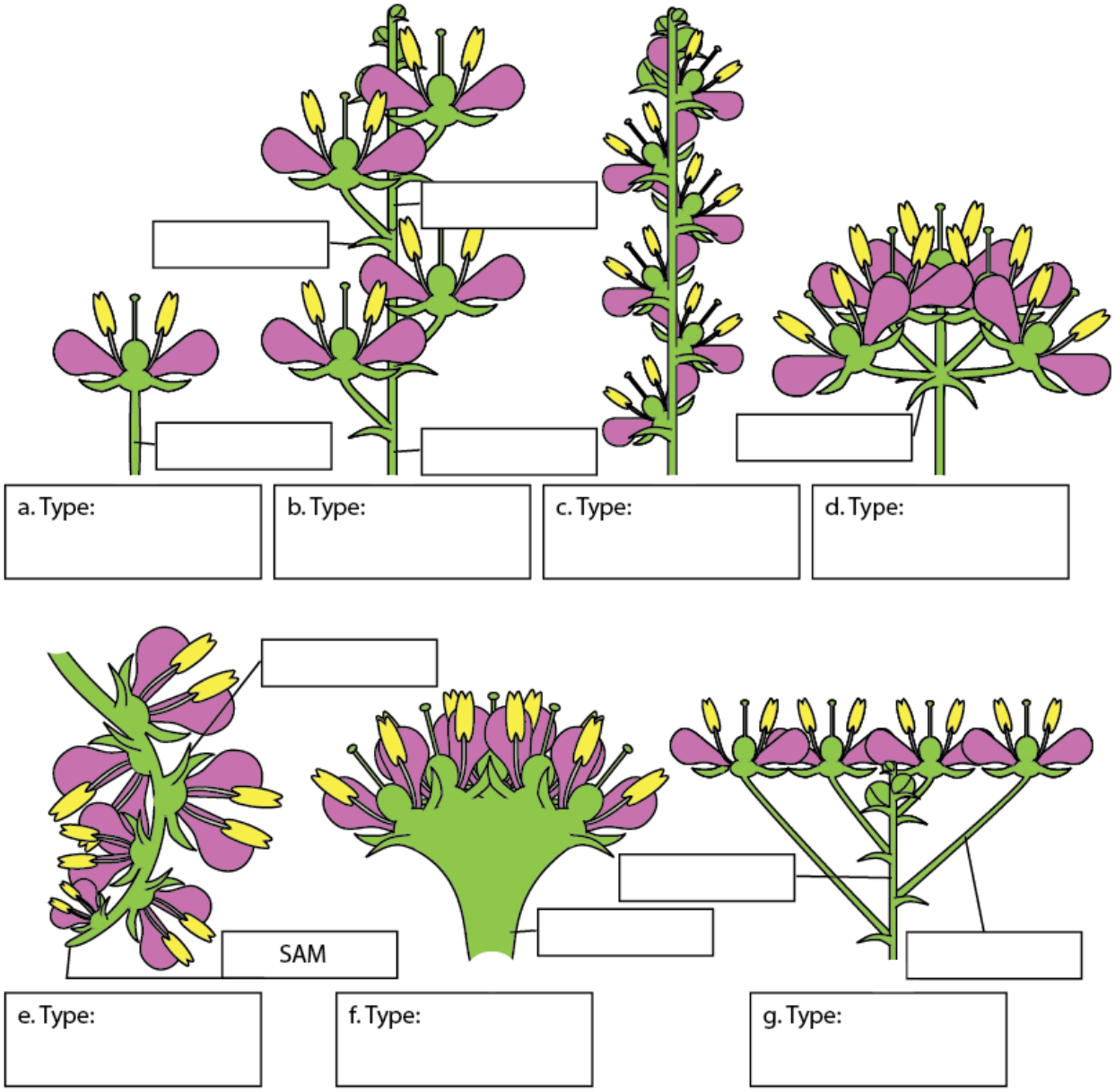


Fig 16. Some types of simple inflorescences.

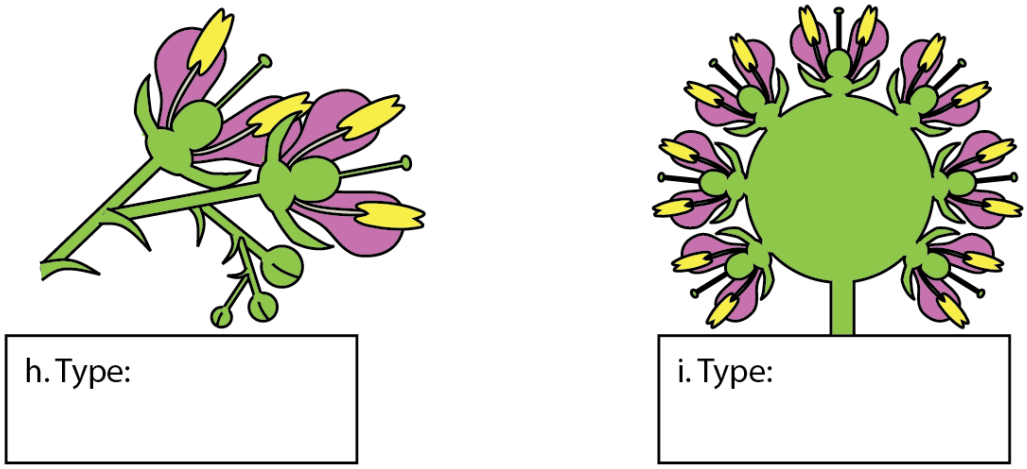


Fig 16 continued. Some additional types of simple inflorescences.

2. Compound Inflorescences.

Compound inflorescences are inflorescences in which there are multiple simple units arising as branches in a greater, larger inflorescence. Common ones include a compound umbel (consisting of multiple umbels arranged in a greater umbel), a panicle (consisting of multiple lateral racemes, spikes, or heads), and a thyrse (consisting of multiple lateral cymes).

Apply these terms as appropriate below. The terms peduncle and rachis still apply to the main inflorescence axis, but additional terms include rachilla (the rachis of secondary and higher-order inflo axes), and bracteole (a bract subtending a flower on secondary and higher-order inflo axes). Label these terms below.

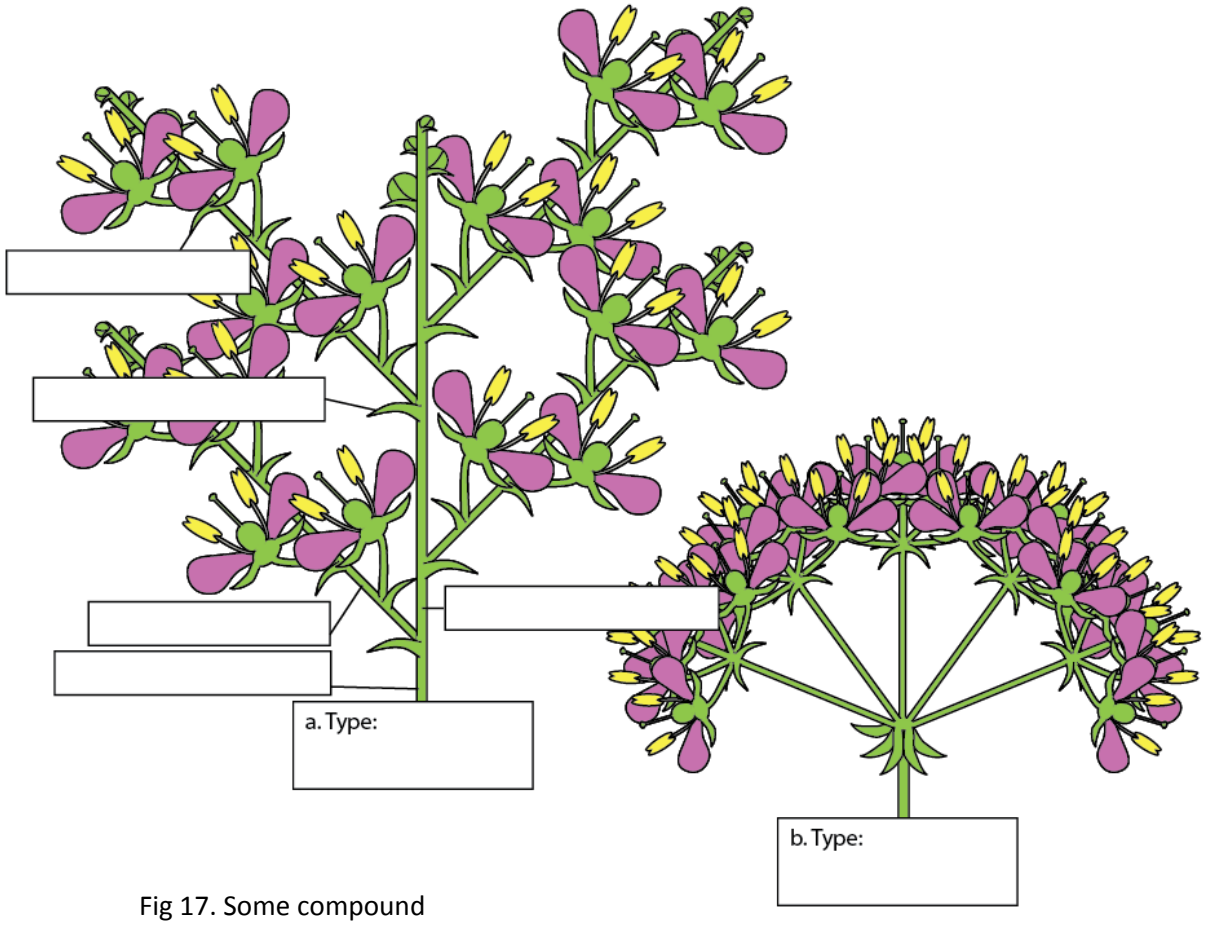


Fig 17. Some compound inflorescences.

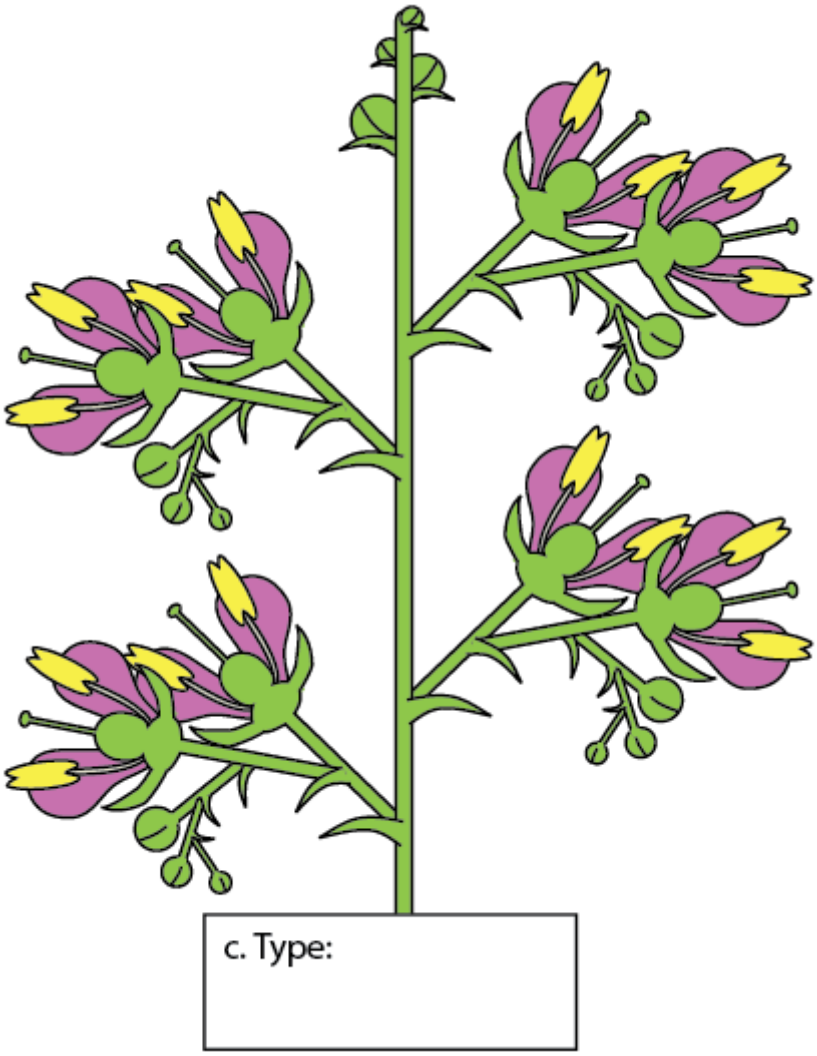


Fig 17 continued. Yet another compound inflorescences.

D. Inflorescence Survey Using Real Plants

Review the inflorescences diagrams and names above, then apply them as appropriate to the sample inflorescences at the inflorescence station in the room. Some of these may be in fruit but, since fruits come from flowers, you can still determine the inflorescence type.

Plant or Family	Inflorescence Type
1.	
2.	
3.	
4.	
5.	
6.	
7.	
8.	
9.	

E. Pseudanthia

A pseudanthium (from the Greek *pseudo*, false, and *anthus*, flower) is a structure that looks like a single flower but is actually (usually) made up of multiple (several to thousands of) small flowers and often some closely associated bracts that have evolved to resemble sepals and / or petals through convergent evolution, a form of natural selection (Fig 18). The individual flowers are often called florets.

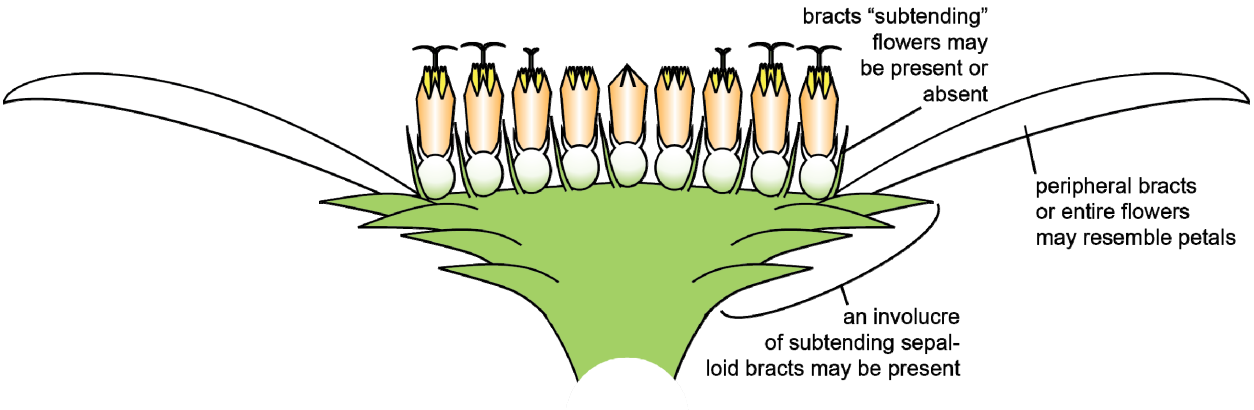


Fig 18. Pseudanthia may resemble individual flowers, but they are much more. The hypothetical pseudanthium pictured here is based on a head of flowers.

1. The Dogwood pseudanthium.

The blossom of dogwoods (species of *Cornus*) consists of many small, inconspicuous flowers subtended by showy bracts. Below, make a drawing of the dogwood pseudanthium, being sure to label any bracts, whether or not they are sepaloid or petaloid, and provide an estimate of the number of actual flowers in a dogwood pseudanthium. Also draw an individual flower, being sure to indicate the number of and position of sepals, petals, stamens and pistils in the floral formula template below.

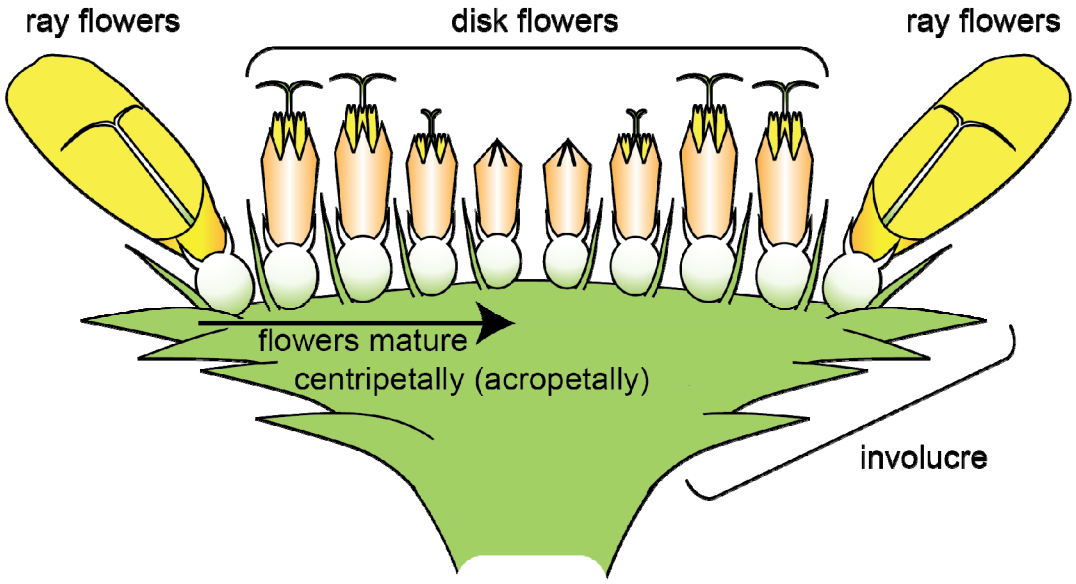
a. The dogwood pseudanthium **b. A dogwood flower**

Other notes:

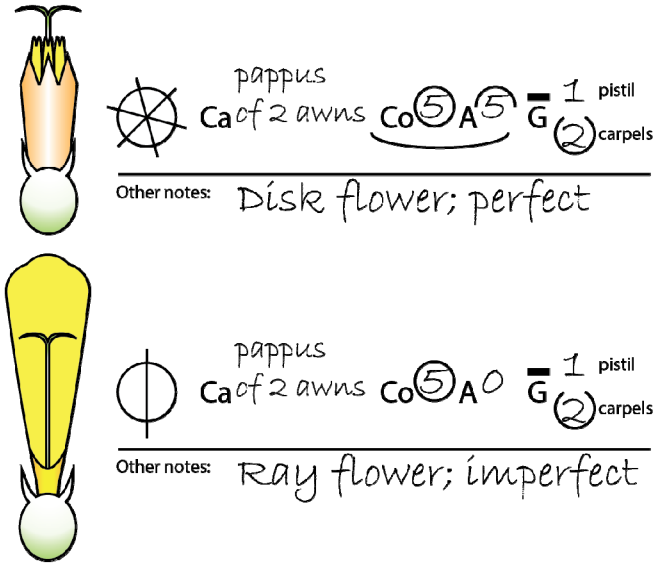
c. Complete the floral formula for a single flower

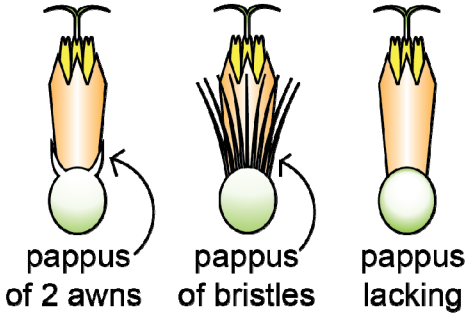
2. The Composite pseudanthium.

The apparent "flower" of a sunflower, aster or any of their relatives in the Composite family, Asteraceae, is actually a composite of many small flowers (Figs 19 A and B). The calyx of each flower is highly modified as a "pappus" which varies in form throughout the family (Fig 19C) and generally persists in fruit to aid in seed dispersal. In the sunflower and other similar pseudanthia in many of its relatives, "ray flowers" around the periphery look like petals and serve to attract pollinators, while "disk flowers" in the center serve in pollination and seed production. The involucre and its bracts function like sepals.



A. The head of a sunflower is a contracted rachis subtended by an involucre of bracts. The apparent centripetal pattern of flower maturation follows the acropetal pattern expected along inflorescence rachises generally.



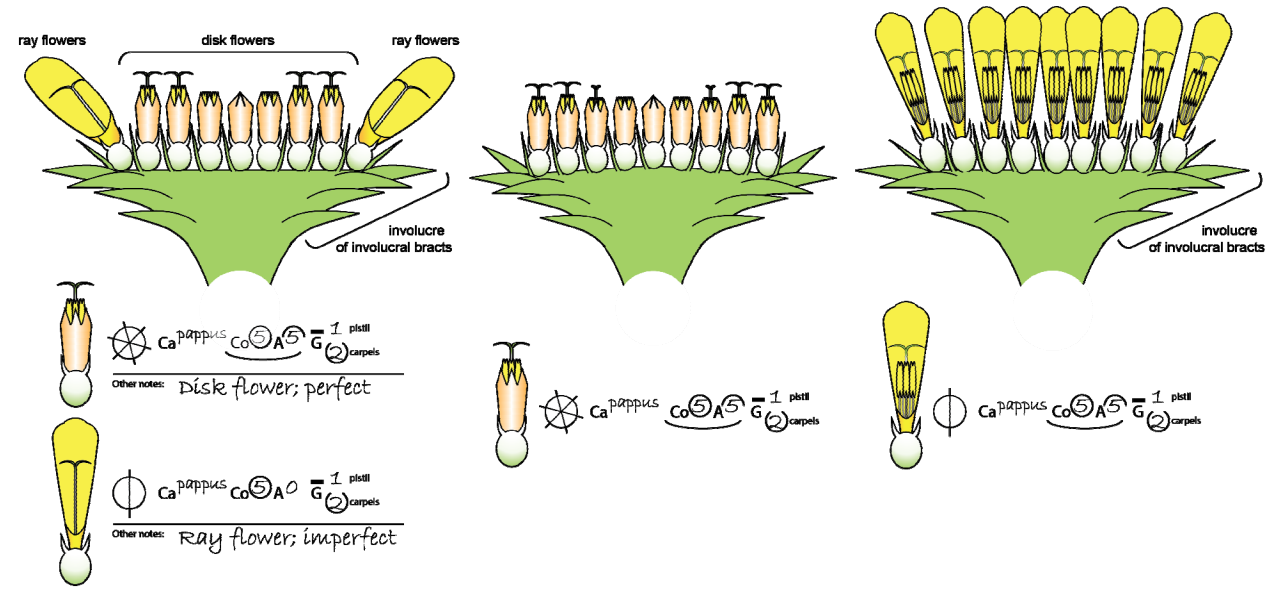


B. The sunflower pseudanthium has two types of flowers.

C. Pappus variation in the Composite family.

Fig 19. The small flowers in the Composite family collectively form showy inflorescences that resemble individual flowers (A, B). The calyx of individual flowers is typically modified to form a "pappus" (C) that serves in many species to aid seed dispersal.

a. 3 head types. Broadly speaking, there are 3 types of heads in the Asteraceae. In a typical radiate head, disk flowers in the center are surrounded by ray flowers around the periphery (see below). Other heads have only disk-like flowers, and these are called discoid or disciform heads. Other heads have only ray-like flowers and these are called ligulate heads.



Head type:	Head type:	Head type:

Fig 20. Three head types in the Composite family, Asteraceae

b. Application to real plants. *Make a study of each of the other sunflower family representatives in lab today. Working in pairs, make a medial longitudinal section through the heads of the plants below and draw them.*

1) Plant Name:

a. Is this a radiate, ligulate, or discoid head?

b. Draw an individual flower. Label the ovary, pappus (if present), corolla, stamens (when present), and pistil.

2) Plant Name:
a. Is this a radiate, ligulate, or discoid head?

b. Draw an individual flower. Label the ovary, pappus (if present), corolla, stamens (when present), and pistil.

3) Plant Name:
a. Is this a radiate, ligulate, or discoid head?

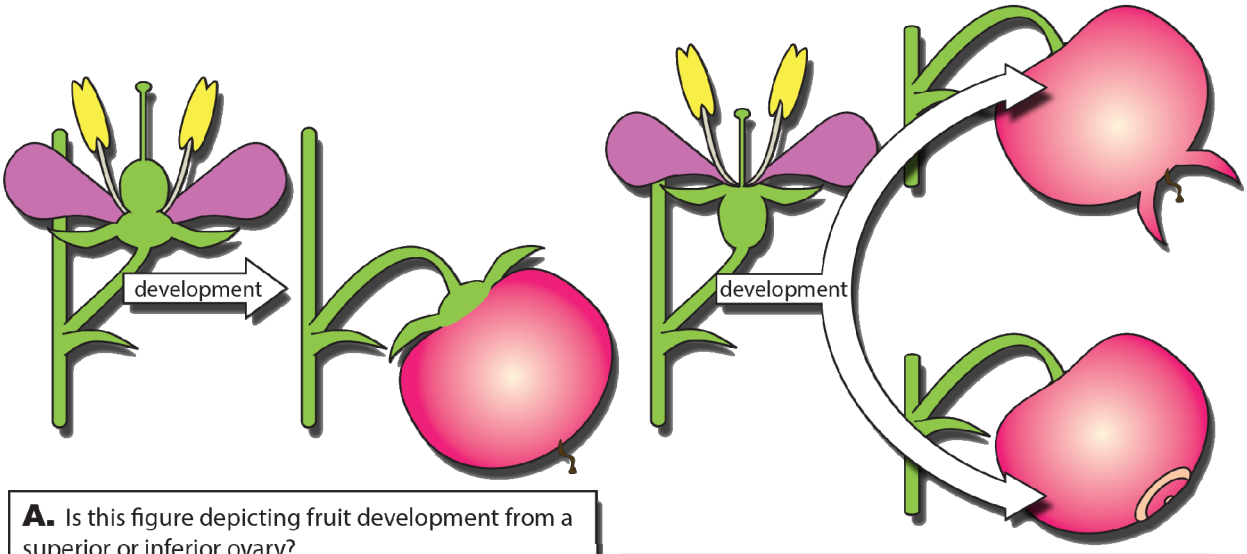
b. Draw an individual flower. Label the ovary, pappus (if present), corolla, stamens (when present), and pistil.

4) Plant Name:
a. Is this a radiate, ligulate, or discoid head?

b. Draw an individual flower. Label the ovary, pappus (if present), corolla, stamens (when present), and pistil.

II. Fruits & Infructescences

As the flowers in an inflorescence ripen into fruits, the inflorescence transitions to an infructescence. Individual fruits, strictly speaking, develop from the ovary of a flower's pistil (Fig 21). However, there are a great variety of forms and complexities that fruits in a more general sense come in. Yet despite these complexities, you should always be able to connect aspects of the fruit back to the flowers and, in some cases, the inflorescences from which they came. **Answer the questions in the boxes in Fig 21 below.**



A. Is this figure depicting fruit development from a superior or inferior ovary?

What features of the fruit are evidence of this?

B. Is this figure depicting two alternate paths of fruit development from a superior or inferior ovary?

What features of the fruits are evidence of this?

Fig 21. Flowers turn into fruits. Answer the questions in the boxes.

A. Fruit Morphology & Anatomy

1. Fleshiness

Some fruits are fleshy at maturity, whereas others are dry at maturity. **Apply these terms to the following sentence:**

Acorns are _____ at maturity,

whereas blueberries are _____ at maturity.

2. Dehiscence

Fruits that open at maturity are dehiscent, whereas those that remain closed are indehiscent. **Apply these terms in labeling below.**

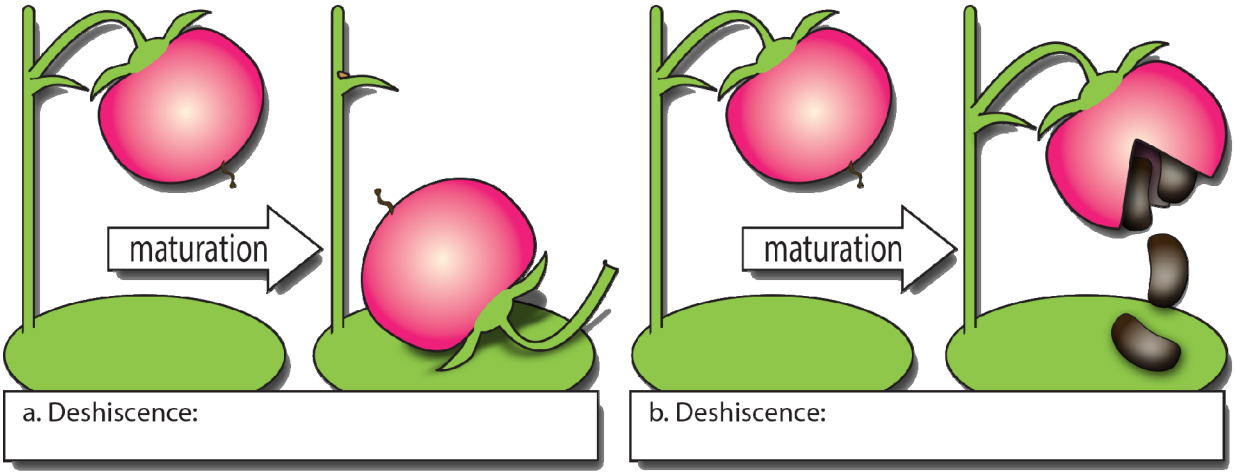


Fig 22. Dehiscent vs. indehiscent fruits.

3. Complexity

Simple fruits are those that develop from single ovaries. Aggregate fruits are composites of multiple ovaries from a single flower. Multiple fruits are composites of multiple ovaries from multiple flowers. **Apply these terms below.**

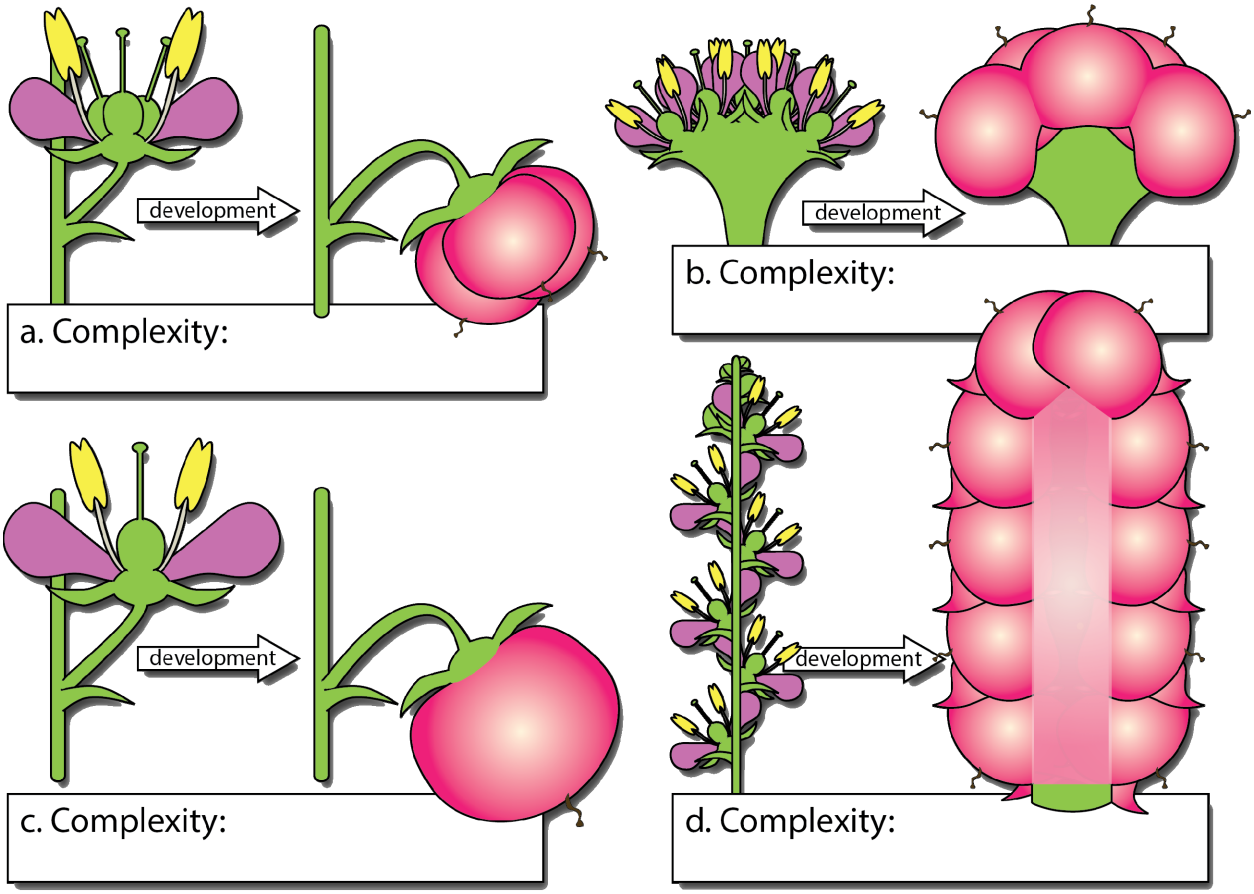


Fig 23. Simple, aggregate and multiple fruits.

4. Anatomy

The ovary wall becomes the pericarp in fruit. The pericarp is more or less (sometimes not discernible to the human eye) differentiated into the exocarp (the outer layer), mesocarp (the middle layer) and endocarp (the innermost layer, sometimes thick, other times thin and membranous). **Apply these terms as appropriate below.**

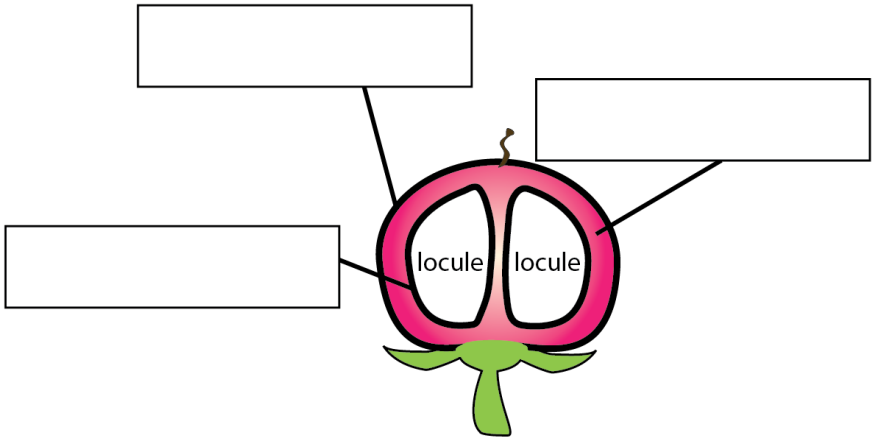
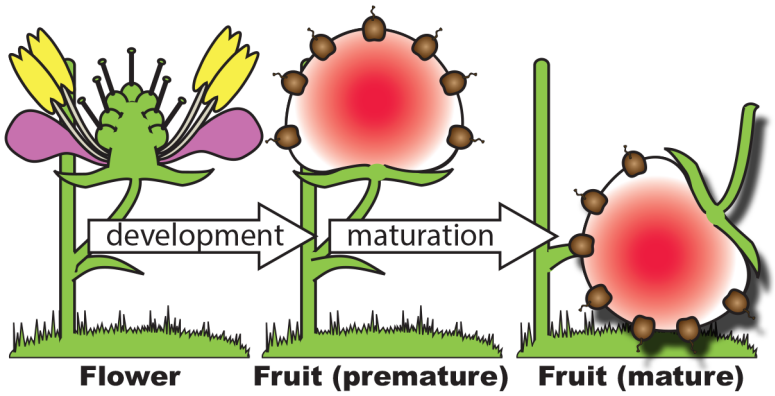


Fig 24. The layers of the pericarp (ovary wall in fruit).

5. Accessory Tissue

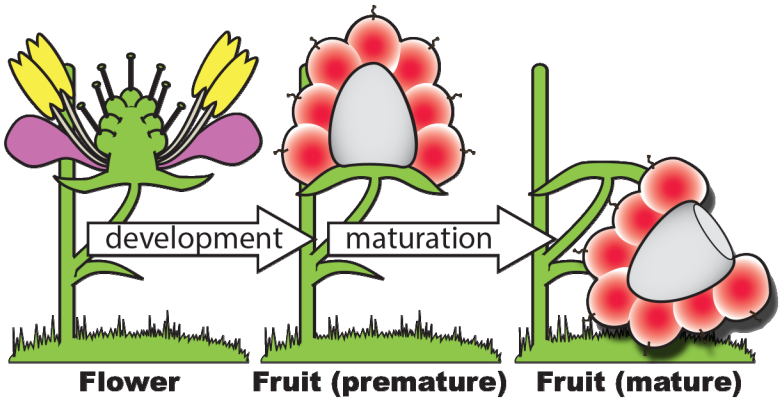
Any type of simple, aggregate or multiple fruits may also be accessory fruits. Accessory fruits are those in which tissue other than that of the carpel(s) contribute to the "fruit" body, beyond the normal persistent sepals or pedicel affixed to the base of the fruit. Any fruit derived from an inferior ovary, for example, necessarily has more or less material from the receptacle or fused perianth and stamen organ bases incorporated into the wall of the fruit. Aggregate fruits, for example, may or may not have receptacle tissue incorporated into the fruit body at maturity (maturity being when the fruit dehisces or, in the case of indehiscent fruits, detaches from the maternal plant). Multiple fruits will typically have at least inflorescence rachis and bracts incorporated into the fruit at maturity. Apply this information to the figures and question boxes that follow.



Is the mature, fallen fruit on the right an accessory fruit?

If so, what accessory tissues are present?

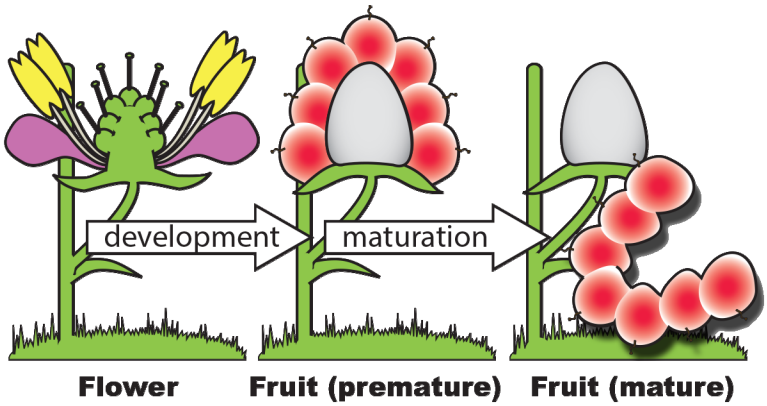
Fig 25.



Is the mature, fallen fruit on the right an accessory fruit?

If so, what accessory tissues are present?

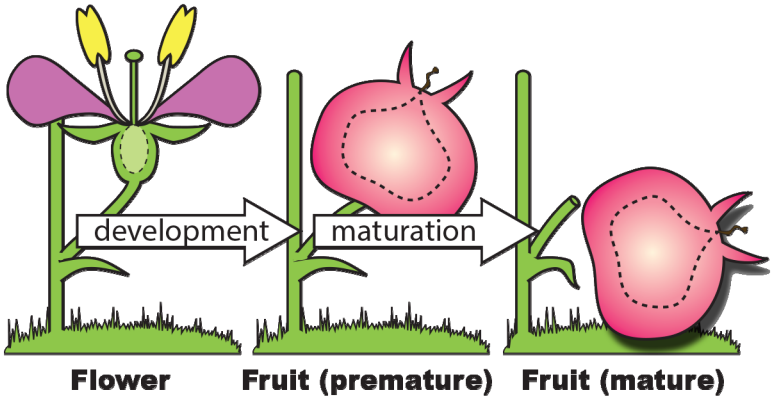
Fig 26.



Is the mature, fallen fruit on the right an accessory fruit?

If so, what accessory tissues are present?

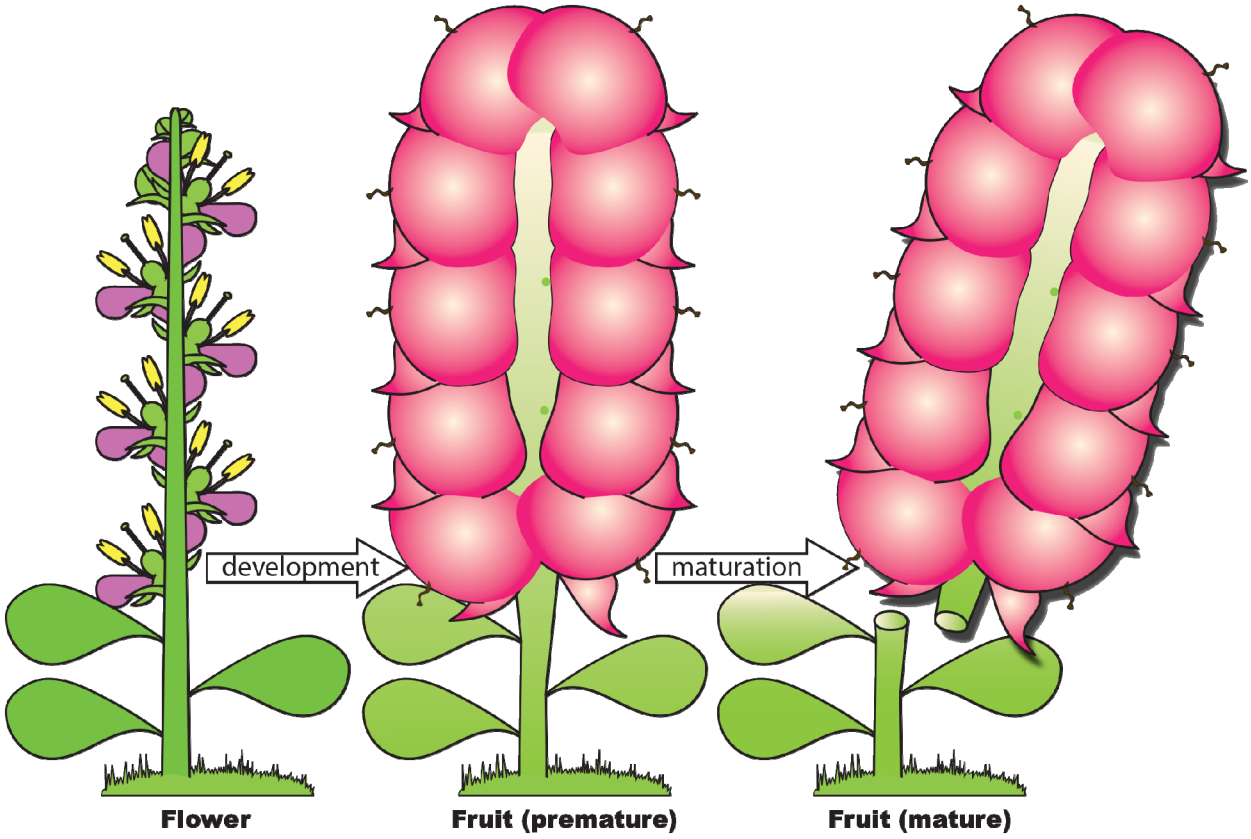
Fig 27.



Is the mature, fallen fruit on the right an accessory fruit?

If so, what accessory tissues are present?

Fig 28.



Is the mature, fallen fruit on the right an accessory fruit?

If so, what accessory tissues are present?

B. Fruit Studies

Apply your knowledge and the classification of specific fruit types in Appendix 1 at the end of this lab to the questions about the fruits situated around the classroom.

1. Tomatoes & Eggplants (*Solanum* spp.)

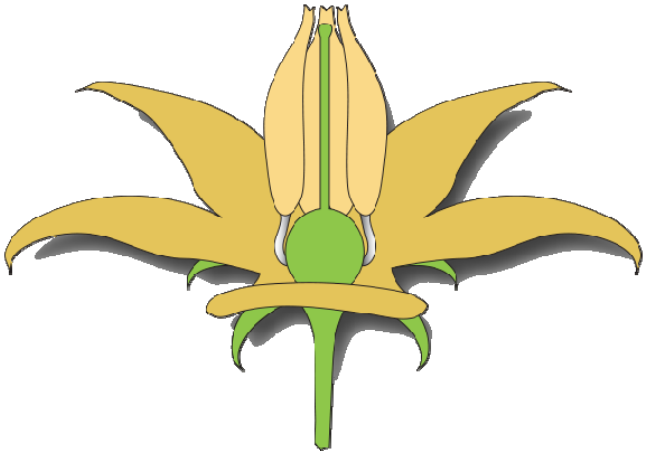
a. Look at but do not cut a large vine-ripened tomato and/or eggplant

1) *Can you see the pedicel and sepals of the flower from which the tomato came?*
2) *How can you tell that it came from a superior ovary?*

b. Take a cherry tomato

1) *Look for any pedicels, sepals, or scars of where these had been attached.*
2) *Cross-section the fruit and count the number of locules. How many carpels do you think make up this fruit?*

c. Next to the flower below, draw one vine-ripened tomato and label any flower parts or their scars that are present in fruit.



d. Fruit type:

1) *Fleshiness (dry or fleshy):*
2) *Dehiscence (dehiscent or indehiscent):*
3) *Complexity (simple, aggregate, or multiple):*
4) *Accessory fruit (if yes, record "accessory"):*
5) *Specific fruit or fruitlet type (e.g., berry, capsule, pome, etc.; use the Fruit Classification on the last page of this lab):*

2. Iris fruits (*Iris* spp.)

a. Inspect the outside:

1) *Did it come from a superior or inferior ovary?*
2) *By how many valves does it open?*
3) *How many carpels made up this fruit?*

b. Next to the flower below, draw the fruit and label the valves of dehiscence:

c. Fruit type:

1) *Fleshiness (dry or fleshy):*
2) *Dehiscence (dehiscent or indehiscent):*
3) *Complexity (simple, aggregate, or multiple):*
4) *Accessory fruit (if yes, record "accessory"):*
5) *Specific fruit or fruitlet type (e.g., berry, capsule, pome, etc.; use the Fruit Classification on the last page of this lab):*

3. Avocadoes (*Persea americana*)

a. Draw and label the probable attachment points of the pedicel and style prior to fruit maturation.

b. Fruit type:

1) *Fleshiness (dry or fleshy):*
2) *Dehiscence (dehiscent or indehiscent):*
3) *Complexity (simple, aggregate, or multiple):*
4) *Accessory fruit (if yes, record "accessory"):*
5) *Specific fruit or fruitlet type (e.g., berry, capsule, pome, etc.; use the Fruit Classification on the last page of this lab):*

4. Raspberries or blackberries (*Rubus* spp.)

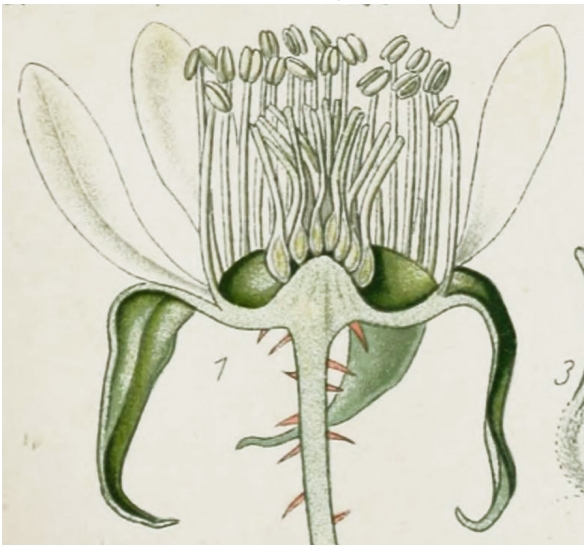
a. Take one raspberry or blackberry (both of which are from the same genus, *Rubus*). This came from a single flower.

1) How can you tell that this fruit came from multiple ovaries?

2) Is this a simple, aggregate or multiple fruit (see fruit classification on last page)?

3) Can you see any remnant styles or stigmas attached to the little fruitlets? Draw this.

b. Next to the flower below, draw a fruit and label any persistent floral parts.



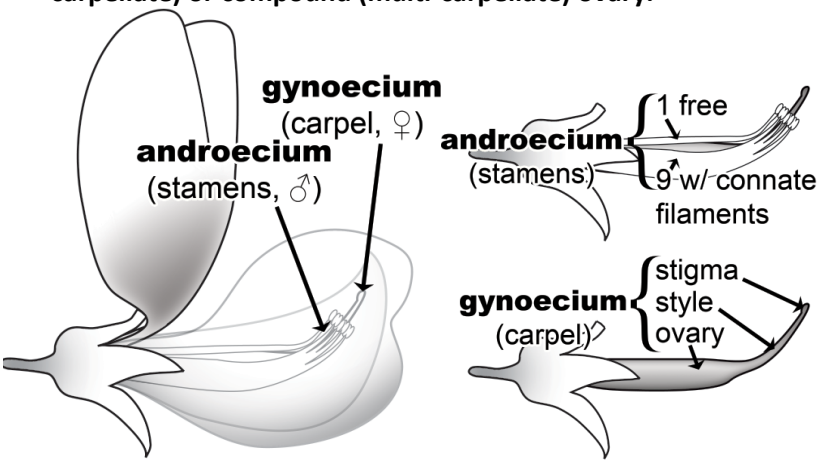
c. Fruit type:

1) *Fleshiness (dry or fleshy):*
2) *Dehiscence (dehiscent or indehiscent):*
3) *Complexity (simple, aggregate, or multiple):*
4) *Accessory fruit (if yes, record "accessory"):*
5) *Specific fruit or fruitlet type (e.g., berry, capsule, pome, etc.; use the Fruit Classification on the last page of this lab):*

5. Pea and bean family (Leguminosae spp.)

a. Compare unripe fruits from the supermarket with ripe ones from various localities.

b. Inspect a dehiscent fruit and determine whether or not it likely came from a simple (1-carpellate) or compound (multi-carpellate) ovary.



c. Snow peas, snap peas and green beans are consumed whole-fruit. Why is it that the immature fruit rather than the mature fruit is consumed?

d. Fruit type:

1) *Fleshiness (dry or fleshy):*
2) *Dehiscence (dehiscent or indehiscent):*
3) *Complexity (simple, aggregate, or multiple):*
4) *Accessory fruit (if yes, record "accessory"):*
5) *Specific fruit or fruitlet type (e.g., berry, capsule, pome, etc.; use the Fruit Classification on the last page of this lab):*

6. Strawberries (*Fragaria* spp.)

a. Fruit type:

1) *Fleshiness (dry or fleshy):*
2) *Dehiscence (dehiscent or indehiscent):*
3) *Complexity (simple, aggregate, or multiple):*
4) *Accessory fruit (if yes, record "accessory"):*
5) *Specific fruit or fruitlet type (e.g., berry, capsule, pome, etc.; use the Fruit Classification on the last page of this lab):*

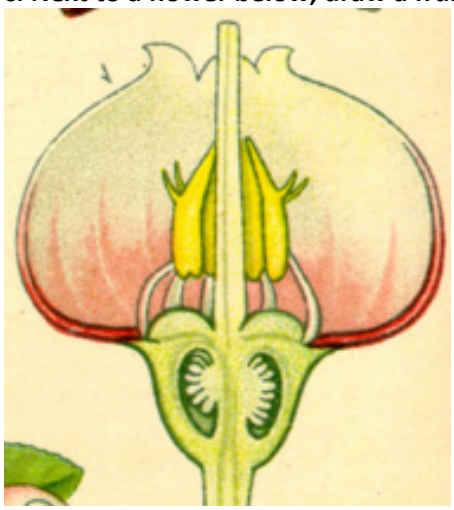
b. Next to the flower below, draw a strawberry and label what you see.

7. Blueberries & Cranberries (*Vaccinium* spp.)

a. Did this come from a superior or inferior ovary?

b. Are there any persistent other floral parts or their scars?

c. Next to a flower below, draw a fruit and label these features named above.



d. Fruit type:

1) *Fleshiness (dry or fleshy):*
2) *Dehiscence (dehiscent or indehiscent):*
3) *Complexity (simple, aggregate, or multiple):*
4) *Accessory fruit (if yes, record "accessory"):*
5) *Specific fruit or fruitlet type (e.g., berry, capsule, pome, etc.; use the Fruit Classification on the last page of this lab):*

8. Bell & chili peppers (*Capsicum* spp. & cultivars)

a. Did this come from a superior or inferior ovary?

b. Are there any persistent other floral parts or their scars?

c. Next to the flower below, draw one and label these features.

d. Fruit type:

1) *Fleshiness (dry or fleshy):*
2) *Dehiscence (dehiscent or indehiscent):*
3) *Complexity (simple, aggregate, or multiple):*
4) *Accessory fruit (if yes, record "accessory"):*
5) *Specific fruit or fruitlet type (e.g., berry, capsule, pome, etc.; use the Fruit Classification on the last page of this lab):*

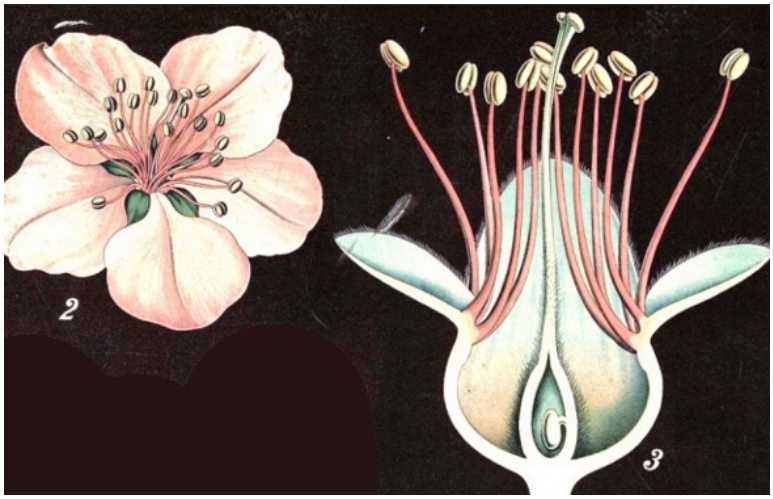
9. Peaches, Nectarines, Apricots, Almonds, Cherries & Plums (*Prunus* spp.)

a. Did this come from a superior or inferior ovary?

b. Are there any persistent other floral parts or their scars?

c. These fruits are known as stone fruits. What does that mean?

d. Next to the *Prunus* flower below, draw a long-sectioned fruit and label the 3 layers of pericarp and seed.



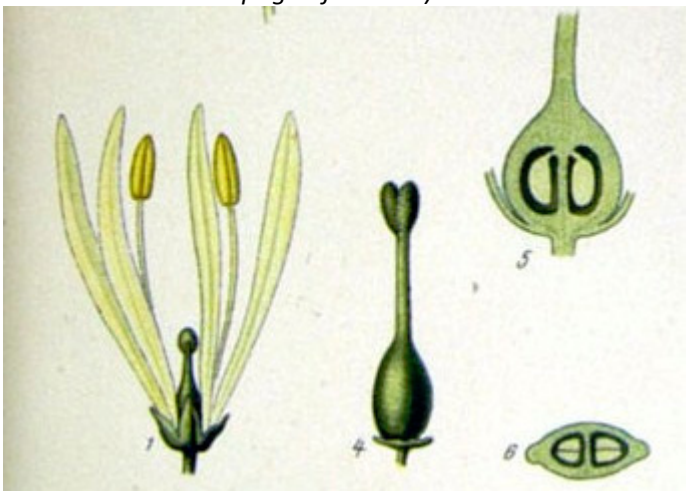
e. Fruit type:

1) *Fleshiness (dry or fleshy):*
2) *Dehiscence (dehiscent or indehiscent):*
3) *Complexity (simple, aggregate, or multiple):*
4) *Accessory fruit (if yes, record "accessory"):*
5) *Specific fruit or fruitlet type (e.g., berry, capsule, pome, etc.; use the Fruit Classification on the last page of this lab):*

10. Ash (*Fraxinus* spp.) or tree-of-heaven (*Ailanthus altissima*) fruits.

a. Fruit type:

1) *Fleshiness (dry or fleshy):*
2) *Dehiscence (dehiscent or indehiscent):*
3) *Complexity (simple, aggregate, or multiple):*
4) *Accessory fruit (if yes, record "accessory"):*
5) *Specific fruit or fruitlet type (e.g., berry, capsule, pome, etc.; use the Fruit Classification on the last page of this lab):*



11. **Maple** (*Acer* spp.)

Maple fruits are simple (derived from a single ovary), dry at maturity, indehiscent (do not open to release the seeds), and take the form of a pair of wings. Inspect the available maple flowers and study the pistil to see if you can see any hint of that pair of wings in the ovary.

a. Below, draw a fruit and draw the pistil of the flower side by side, being sure to label the ovary, style(s), and stigma(s). How many carpels do you think this pistil and fruit is made of?

b. Do the winged segments remain attached or do they separate at maturity?

c. Fruit type:

1) *Fleshiness (dry or fleshy):*
2) *Dehiscence (dehiscent or indehiscent):*
3) *Complexity (simple, aggregate, or multiple):*
4) *Accessory fruit (if yes, record "accessory"):*
5) *Specific fruit or fruitlet type (e.g., berry, capsule, pome, etc.; use the Fruit Classification on the last page of this lab):*

12. **Apples & Pears** (*Malus* & *Pyrus* spp.)

a. Make a drawing of <u>one that</u> <u>has not been cut open</u>.

- Label the pedicel or the position of its insertion if it's no longer present.
- Label the parts that can be seen in or around the "naval". What are they?
- Label whether or not the fruit was derived from a superior or inferior ovary.

b. Make a drawing of <u>one that has been</u> <u>transversely sectioned</u>.

- Count and label the locules in the drawing. Indicate the number of carpels you infer from this.

c. Make a drawing of <u>one that has been longitudinally sectioned</u>.

- The apple/pear is one type of an accessory fruit. In your drawing, inspect the section for the boundary between the true pericarp (ovary wall) and the accessory tissue. Where is the accessory tissue and what is it?
- Locate the endocarp.

d. Fruit type:

1) Fleshiness (dry or fleshy):
2) Dehiscence (dehiscent or indehiscent):
3) Complexity (simple, aggregate, or multiple):
4) Accessory fruit (if yes, record "accessory"):
5) Specific fruit or fruitlet type (e.g., berry, capsule, pome, etc.; use the Fruit Classification on the last page of this lab):

13. Mangoes (*Mangifera* spp.)

a. In the one that has been sectioned open for you, is there a well differentiated layering to the pericarp? Can you find an endocarp? A seed? Next to the flower below, draw and label these features.

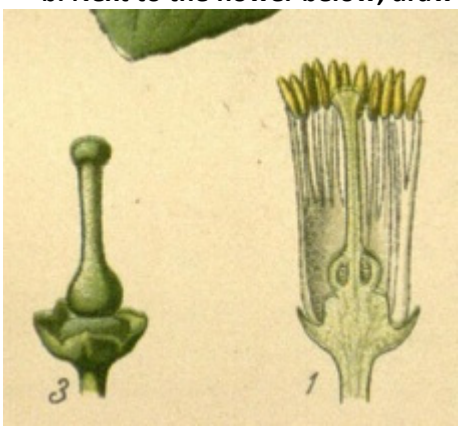
c. Fruit type:

1) *Fleshiness (dry or fleshy):*
2) *Dehiscence (dehiscent or indehiscent):*
3) *Complexity (simple, aggregate, or multiple):*
4) *Accessory fruit (if yes, record "accessory"):*
5) *Specific fruit or fruitlet type (e.g., berry, capsule, pome, etc.; use the Fruit Classification on the last page of this lab):*

14. Oranges, Lemons & Grapefruits (*Citrus* spp.)

a. Inspect the transversely sectioned one. Locate the exocarp, mesocarp and the thin, soft endocarp. What does the endocarp have projecting from it into the locules of the fruit?

b. Next to the flower below, draw a whole fruit and locate any persistent sepals and stylar scars.

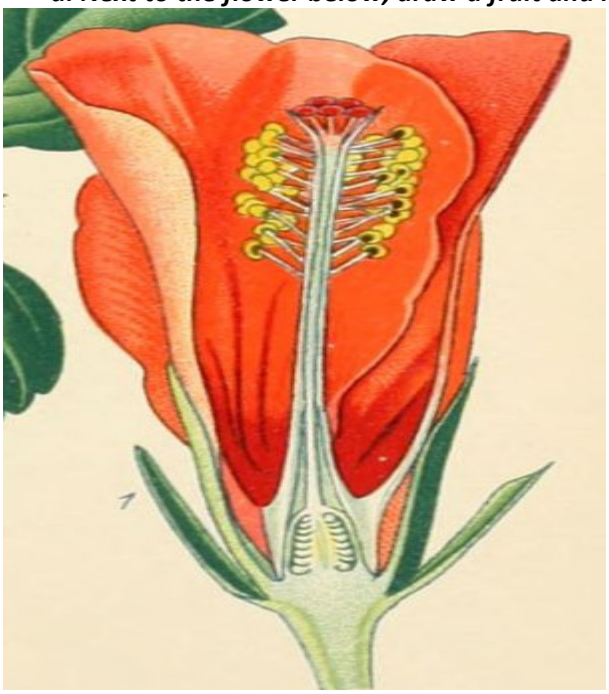


c. Fruit type:

1) *Fleshiness (dry or fleshy):*
2) *Dehiscence (dehiscent or indehiscent):*
3) *Complexity (simple, aggregate, or multiple):*
4) *Accessory fruit (if yes, record "accessory"):*
5) *Specific fruit or fruitlet type (e.g., berry, capsule, pome, etc.; use the Fruit Classification on the last page of this lab):*

15. Hibiscus fruits (*Hibiscus* spp.)

a. Next to the flower below, draw a fruit and label its valves of dehiscence.

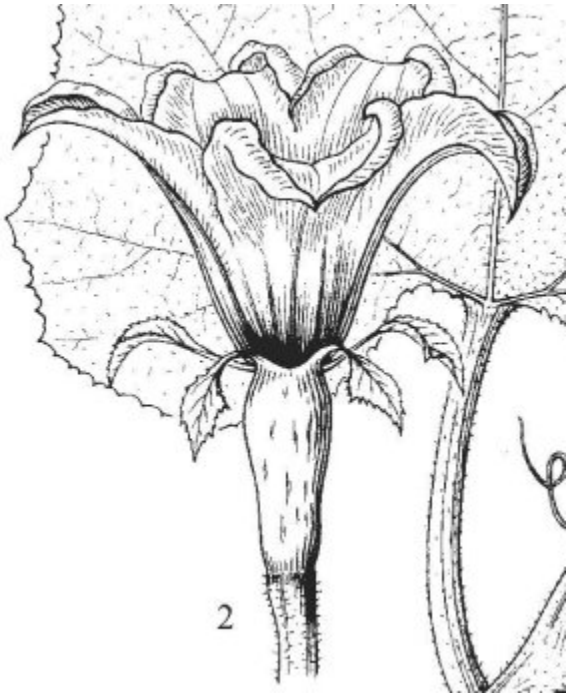


b. Fruit type:

1) *Fleshiness (dry or fleshy):*
2) *Dehiscence (dehiscent or indehiscent):*
3) *Complexity (simple, aggregate, or multiple):*
4) *Accessory fruit (if yes, record "accessory"):*
5) *Specific fruit or fruitlet type (e.g., berry, capsule, pome, etc.; use the Fruit Classification on the last page of this lab):*

16. Pumpkins or acorn squash (*Cucurbita pepo* cultivars)

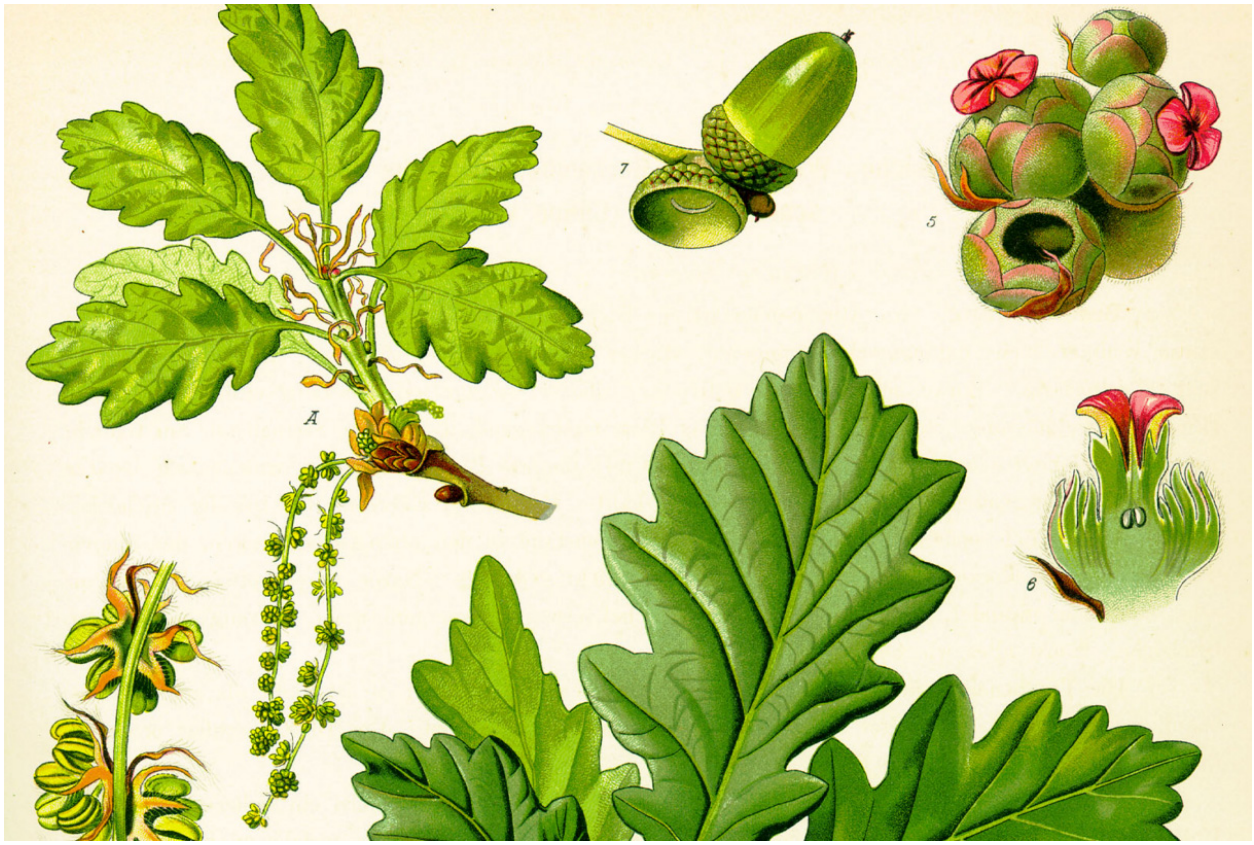
a. Next to the pistillate flower below, draw a squash it from the outside and label the pedicel or its attachment scar. Label also the scar from the attachment of the corolla and style.



b. Fruit type:

1) *Fleshiness (dry or fleshy):*
2) *Dehiscence (dehiscent or indehiscent):*
3) *Complexity (simple, aggregate, or multiple):*
4) *Accessory fruit (if yes, record "accessory"):*
5) *Specific fruit or fruitlet type (e.g., berry, capsule, pome, etc.; use the Fruit Classification on the last page of this lab):*

17. Acorns (*Quercus* spp.)

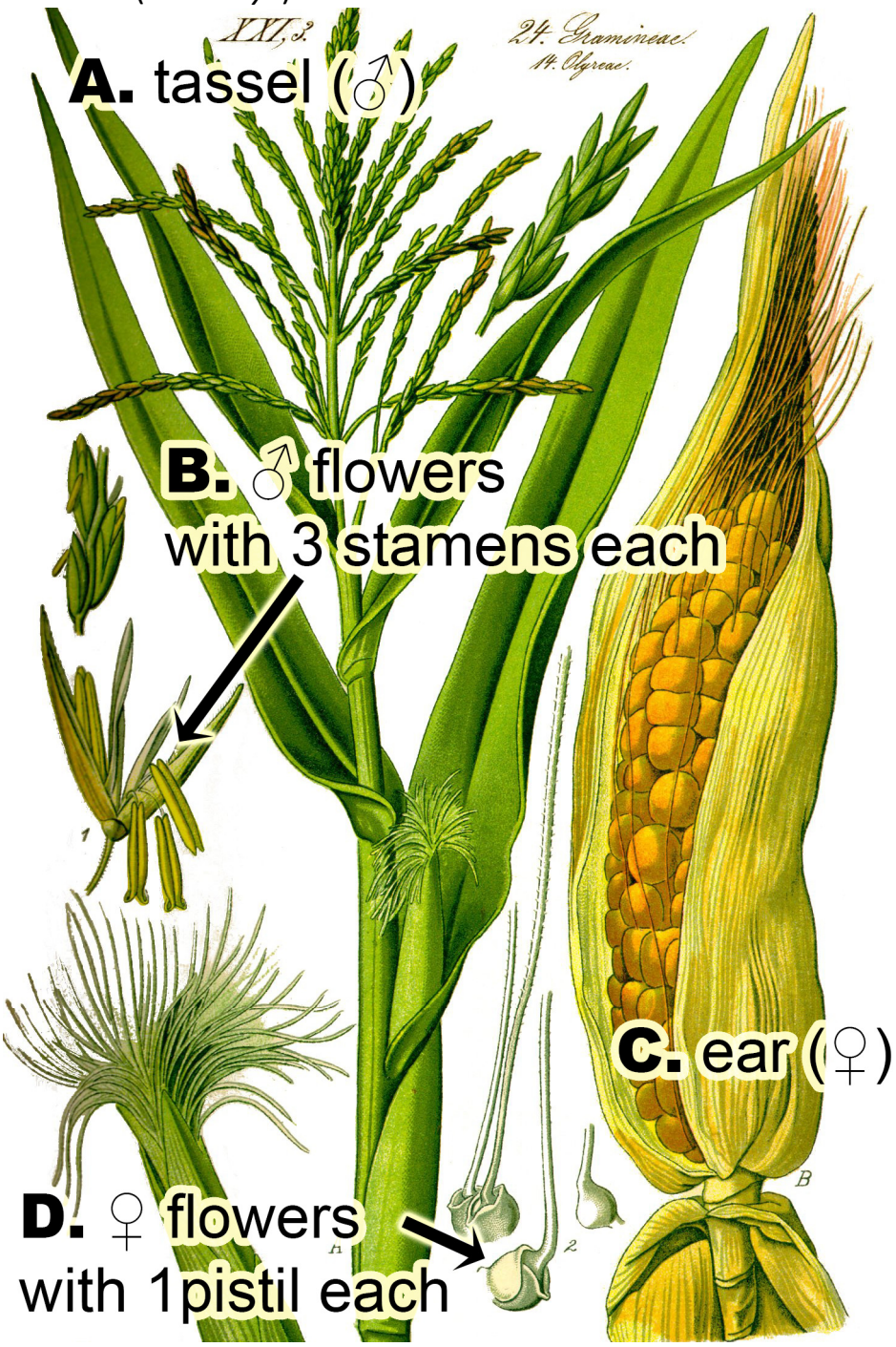


a. Inspect the illustration of staminate and pistillate oak flowers above, then draw a fruit below and label the subtending cupule and any remnant styles and stigmas.

b. Fact, this came from an inferior ovary. Now determine the fruit type:

1) *Fleshiness (dry or fleshy):*
2) *Dehiscence (dehiscent or indehiscent):*
3) *Complexity (simple, aggregate, or multiple):*
4) *Accessory fruit (if yes, record "accessory"):*
5) *Specific fruit or fruitlet type (e.g., berry, capsule, pome, etc.; use the Fruit Classification on the last page of this lab):*

18. Corn (*Zea mays*)



a. Inspect an ear of corn which, most likely, has lost its silks. Each kernel is a small fruitlet from a superior ovary in which the seed coat of the single seed is fused to the thin pericarp. What specific type of fruit is this kernel? (use the fruit classification on last page).

b. Now draw a portion of the ear showing multiple kernels. To each kernel there had been one silk attached. Find and label the scar from the attachment of the silk.

c. What part of the flower is the "silk"?

d. If one looks at the corn cob as a whole, classify its fruit type as follows:

1) *Fleshiness (dry or fleshy):*
2) *Dehiscence (dehiscent or indehiscent):*
3) *Complexity (simple, aggregate, or multiple):*
4) *Accessory fruit (if yes, record "accessory"):*
5) *Specific fruit or fruitlet type (e.g., berry, capsule, pome, etc.; use the Fruit Classification on the last page of this lab):*

19. Coconuts (*Cocos nucifera*)

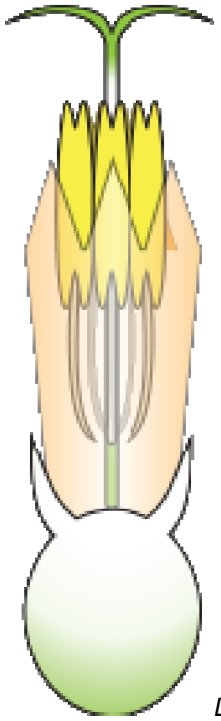
a. Inspect the illustration below and the coconut artifacts at the table, then classify its fruit type as follows:

1) *Fleshiness (dry or fleshy):*
2) *Dehiscence (dehiscent or indehiscent):*
3) *Complexity (simple, aggregate, or multiple):*
4) *Accessory fruit (if yes, record "accessory"):*
5) *Specific fruit or fruitlet type (e.g., berry, capsule, pome, etc.; use the Fruit Classification on the last page of this lab):*

20. Sunflower fruits (*Helianthus annuus*)

Inspect the sunflower fruit, intact. Then open one up and remove the seed. With the flower drawing to help (below), determine the fruit type as follows:

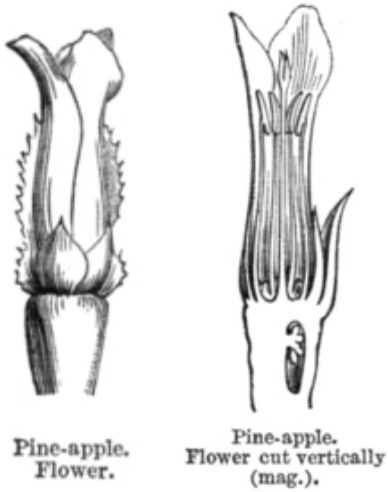
1) *Fleshiness (dry or fleshy):*
2) *Dehiscence (dehiscent or indehiscent):*
3) *Complexity (simple, aggregate, or multiple):*
4) *Accessory fruit (if yes, record "accessory"):*
5) *Specific fruit or fruitlet type (e.g., berry, capsule, pome, etc.; use the Fruit Classification on the last page of this lab):*



Disk flower of sunflower; corolla shown as semi-transparent to facilitate sight of inside.

21 . Pineapple (*Ananas comosus*)

1) *Fleshiness (dry or fleshy):*
2) *Dehiscence (dehiscent or indehiscent):*
3) *Complexity (simple, aggregate, or multiple):*
4) *Accessory fruit (if yes, record "accessory"):*
5) *Specific fruit or fruitlet type (e.g., berry, capsule, pome, etc.; use the Fruit Classification on the last page of this lab):*



Pine-apple. Flower.

Pine-apple. Flower cut vertically (mag.).

22. **Kiwi** (*Actinidia deliciosa* & related spp.)

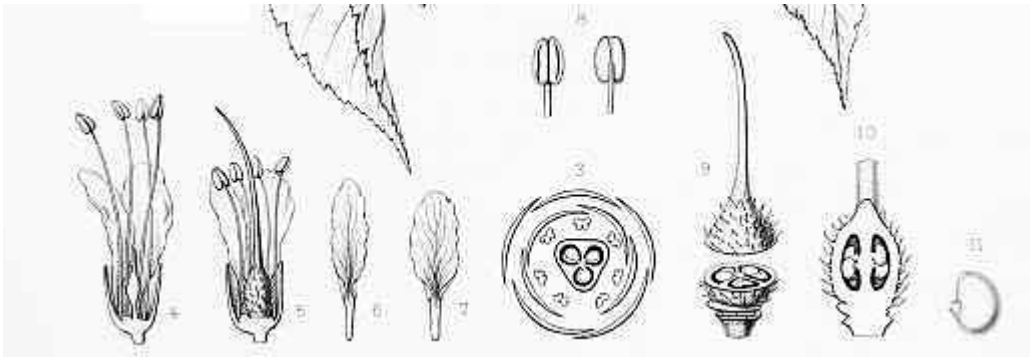
a. Compare the live fruit with the pendent flower in the picture below. Make a drawing of the whole fruit that labels any persistent floral parts.

b. Now determine the fruit type:

1) *Fleshiness (dry or fleshy):*
2) *Dehiscence (dehiscent or indehiscent):*
3) *Complexity (simple, aggregate, or multiple):*
4) *Accessory fruit (if yes, record "accessory"): *
5) *Specific fruit or fruitlet type (e.g., berry, capsule, pome, etc.; use the Fruit Classification on the last page of this lab*

23. **Ohio Buckeye** (*Aesculus glabra*)

a. Compare the live fruit with the flower illustrations in the picture below. Make a drawing of the whole fruit that labels the valves of dehiscence.



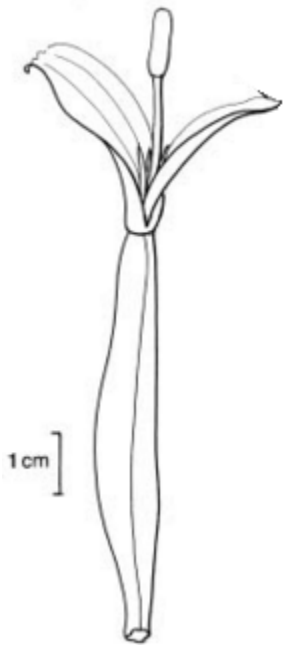
b. Now determine the fruit type:

1) *Fleshiness (dry or fleshy):*
2) *Dehiscence (dehiscent or indehiscent):*
3) *Complexity (simple, aggregate, or multiple):*

4) *Accessory fruit (if yes, record "accessory"):* \
5) *Specific fruit or fruitlet type (e.g., berry, capsule, pome, etc.; use the Fruit Classification on the last page of this lab*

24. Cavendish Bananas (*Musa acuminata* cv. Dwarf Cavendish)

a. Compare the live fruit with the flower illustration below. Make a drawing of a banana next to the flower illustration, trying to orient the fruit in the same manner as the ovary in the flower.



b. Now determine the fruit type:

1) *Fleshiness (dry or fleshy):*
2) *Dehiscence (dehiscent or indehiscent):*
3) *Complexity (simple, aggregate, or multiple):*
4) *Accessory fruit (if yes, record "accessory"):* \
5) *Specific fruit or fruitlet type (e.g., berry, capsule, pome, etc.; use the Fruit Classification on the last page of this lab*

III. Credits

This lab was developed by Christopher Hardy. You may cite it as....
Hardy CR. 2016. Angiosperms. Pp. 235-272 in CR Hardy, RL Wagner (eds) *Guide to Exercises in Concepts of Botany, 4th Edition*. Millersville, Pennsylvania, USA.

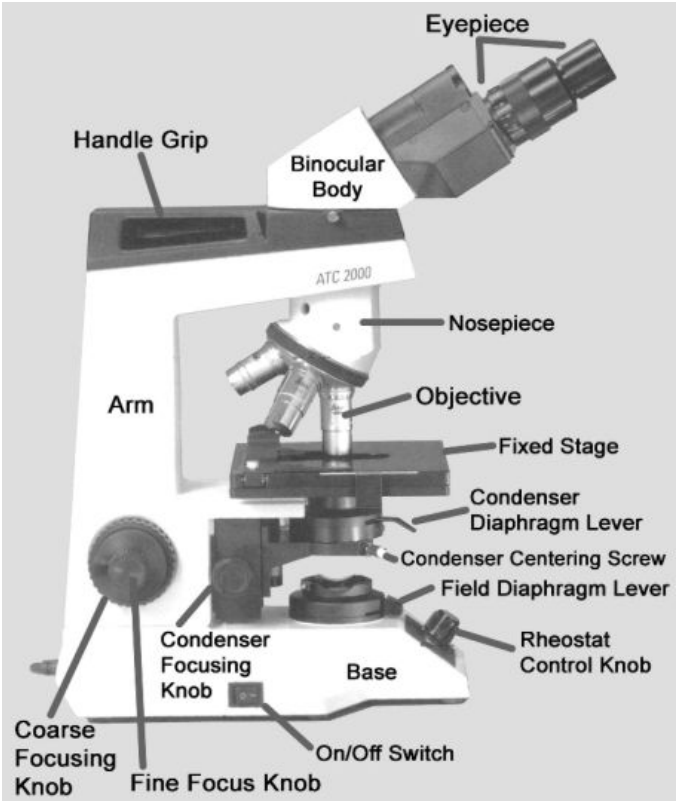
Appendix 1. Classification of Fruit Types

Most of the following classification and typological terms apply to any **simple fruit**. **Aggregate fruits** (derived from multiple ovaries of 1 flower) would be aggregates of such types (e.g., an aggregate of drupes), whereas **multiple fruits** (derived from ovaries of >1 flower) would be multiples of such types (e.g., a multiple of berries). Where accessory (i.e., non-ovarian) tissue is also present beyond the persistent receptacle, sepals or pedicel normally present , then such fruits would also be **accessory fruits**.

A. Fleshy Fruit (outer wall fleshy and living at maturity)
   1. Dehiscent
      a. **Fleshy Capsule** (opens variously)
   2. Indehiscent
      a. **Berry** (entire wall , including endocarp, is fleshy)
         1) normal **Berry** (wall succulent all the way to the seeds)
         2) **Hesperidium** (wall generally leathery, fragrant and rind-like, except endocarp membranous and with succulent trichomes protruding into locules)
         3) **Pepo** (entire wall thick and rind-like)
      b. **Drupe** (so-called "stone fruits" or "pitted" fruits; caused by hard, thin or thick endocarp surrounding seed)
         1) normal **Drupe**
         2) **Pome** (derived from an inferior ovary, where bulk of flesh derived from the hypanthium/receptacle surrounding the ovary to which it is fused)
      c. **Hip** (enlarged hypanthium enclosing but not fused to fruitlets to the inside; probably always consisting of multiple nutlets and thus, a type of aggregate fruit)

B. Dry Fruit (outer wall dry, fibrous and dead at maturity)
   1. Dehiscent (opens when ripe)
      a. **Follicle** (from a simple pistil, dehiscent along one side)
      b. **Legume** (from a simple pistil, dehiscent along two sides)
      c. **Capsule** (from a compound pistil, opens in a variety of ways)
      d. **Silique** (from a simple pistil, opening by lateral wings and leaving behind seeds attached to a persistent free-standing partition)
   2. Indehiscent (does not open when ripe)
      a. **Achene** (thin-walled, 1-seeded fruit with seed coat free of pericarp)
      b. **Nut** (hard-walled, 1-2 seeded fruit with seed coat free of pericarp; usually subtended or wholly or partially enclosed by hypanthium, bracts, or bract-like structures)
      c. **Caryopsis** or **Grain** (1-seeded and nutlike, but seed coat adnate to pericarp)
      d. **Samara** (winged wall)
      e. **Schizocarp** (breaks into 1-carpellate seeded units)
         1) regular **Schizocarp** (units variously shaped but not winged)
         2) **Samaroid Schizocarp** (units are winged)

Appendix A. Care and custom-tuning of your Leica ATC 2000 Compound Light Microscope and Obtaining an Optimal Image



1. Care.

a. Always carry the scope with two hands, one hand on the microscope arm and the other hand under the base.

b. WHEN YOU MOVE YOUR SCOPE ON THE LAB TABLE, DO NOT SLIDE THE SCOPE INTO POSITION. THE VIBRATIONS FROM THIS SLIDING CAN BREAK LOSE THE MIRRORS & INTERNAL LENSES LOCATED IN THE MICROSCOPE.

c. Please put the plastic cover on your scope when you finish the lab exercise. A big cause of microscope problems is dirt in vital places, especially on the eyepiece and objective lenses. If you wear eye make-up, clean the eyepiece lens after each use, with lens paper only.

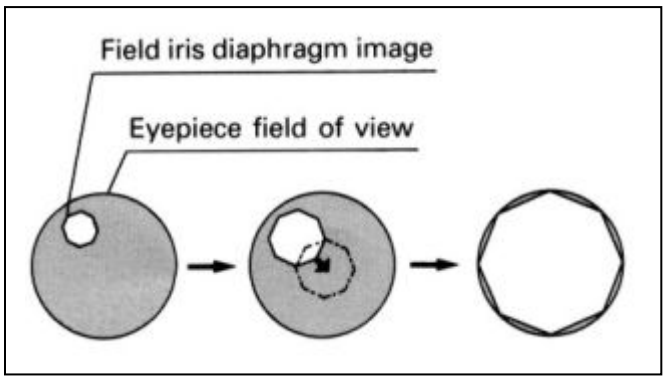
d. **DO NOT CLEAN ANY OF THE LENSES WITH ANYTHING EXCEPT LENS PAPER (NOT PAPER TOWELS, TOILET PAPER, KIMWIPES, MICROWIPES, FACIAL TISSUE, ETC.).** If you cannot get the lens clean with lens paper, notify your professor.

2. Obtaining an optimal image.

Obtain a letter "e" slide and carefully follow the directions below. Most of this procedure (steps 1-11) should be done each time you use the microscope.

1) Turn the lamp on by pushing down the black **on/off toggle switch** on the left side of the **microscope base**, and use the **rheostat control knob** to adjust the light intensity to a level that is comfortable to your eyes.

2) Make sure the low-power **objective** (4X) is pointing straight down (is in its usable position), and use the **condenser focusing knob** to move the **condenser** to its highest possible position; make sure that the **condenser diaphragm** and **field diaphragm** are wide open (maximum amount of light is getting through).
3) Place a microscope slide in the **slide-clip** and position it (with the **mechanical stage traverse knobs**) such that the cover slip on the slide is directly beneath the low-power objective.
4) Use the **coarse** and **fine focusing knobs** to bring the image into sharp focus (use only the right eye for this focus).
5) While using only your right eye to look through the right **ocular**, focus on the slide first using the outer, larger focus ring (**course focus knob**) and then the inner, smaller focus ring (**fine focus knob**).
6) Now close your right eye and as you look through the left **ocular** with your left eye, rotate the base of the ocular around until the image is in focus for your left eye.
7) While looking through the scope with both eyes, adjust the distance between the two oculars to match the distance between your eyes by pulling the oculars apart or pushing them together.

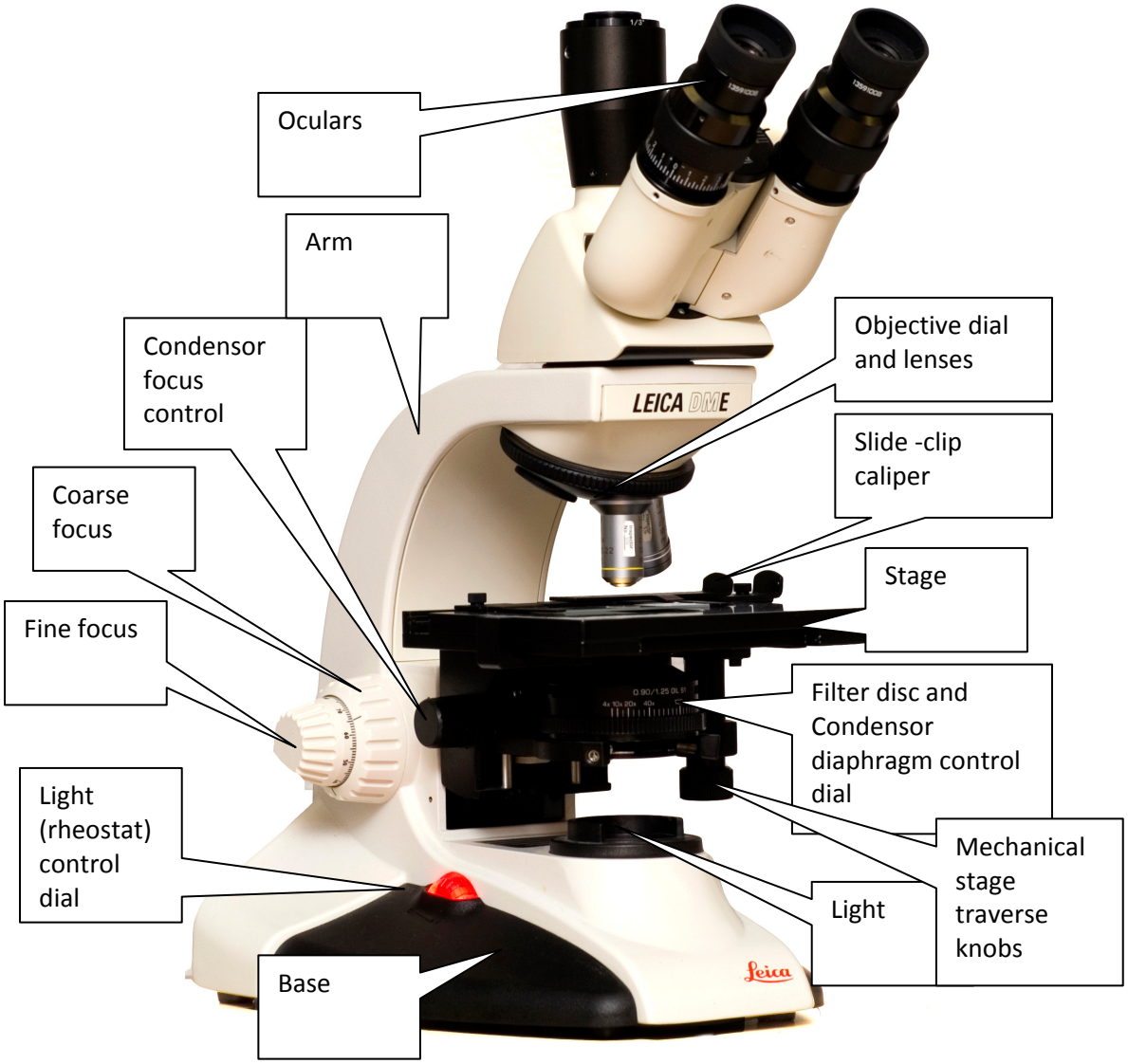


8) While looking through the microscope, slowly start closing the **field diaphragm** lever until the field iris diaphragm image is only a small fraction of the total field of view (forming a smaller circle of light in the field of view).
9) Turn the **condenser focusing knob** until the inner edges of the field iris diaphragm image is in sharp focus (the condenser should be very close to its highest possible position just below the fixed stage).
10) Use the two **condenser centering screws** to center the diaphragm image (circle of light) within the field of view.
11) Open the diaphragm back up slightly until the field iris diaphragm image extends just outside of the eyepiece field of view.

Steps 1—11 should only have to be done once by you each time you use the microscope.

12) When you need to increase magnification to 400X, simply rotate the nose-piece such that the 40X objective is pointing straight down. The image should stay close to focus since this microscope should be *parfocal*.
13) Altering Contrast:
You can alter the contrast via two ways:
a. Adjust the condenser diaphragm level (via the condenser focusing knob) or the field diaphragm aperture.
b. For fresh materials, you can use a stain such as toluidine blue before fixing a cover slip.

Appendix B. Care and custom-tuning your Leica DME Compound Light Microscope and Obtaining an Optimal Image



1. Care.

a. Always carry the scope with two hands, one hand on the microscope arm and the other hand under the base.

b. WHEN YOU MOVE YOUR SCOPE ON THE LAB TABLE, DO NOT SLIDE THE SCOPE INTO POSITION. THE VIBRATIONS FROM THIS SLIDING CAN BREAK LOSE THE MIRRORS & INTERNAL LENSES LOCATED IN THE MICROSCOPE.

c. Please put the plastic cover on your scope when you finish the lab exercise. A big cause of microscope problems is dirt in vital places, especially on the eyepiece and objective lenses. If you wear eye make-up, clean the eyepiece lens after each use, with lens paper only.

d. **DO NOT CLEAN ANY OF THE LENSES WITH ANYTHING EXCEPT LENS PAPER (NOT PAPER TOWELS, TOILET PAPER, KIMWIPES, MICROWIPES, FACIAL TISSUE, ETC.).** If you cannot get the lens clean with lens paper, notify your professor.

2. Obtaining an optimal image.

Obtain a letter "e" slide and carefully follow the directions below. Most of this procedure (steps 1-9) should be done each time you use the microscope.

1) Turn the light by turning the **light rheostat control dial** on the left side of the **microscope base**, and use this knob to adjust the light intensity to a level that is comfortable to your eyes.
2) Just beneath the stage, be sure that the reading on the **filter disc** is "**BF**" for **bright field**.
3) Make sure the lowest-power **objective** (4x or 10x) is pointing straight down (is in its usable position), and use the **condenser focusing knob** to move the **condenser** to its highest possible position but not touching your slide.
4) Place a microscope slide (use the Letter "e" slide) in the **slide-clip** and position it (with the **mechanical stage traverse knobs**) such that the cover slip on the slide is directly beneath the low power objective.
5) Use the **coarse** then **fine focusing knobs** to bring the image into sharp focus.
   Notice how the "e" is in focus only when it is in the focal plane (i.e., at the correct distance) for the given objective lens.
   For the 10 x objective, this distance is about 1 cm. You should start any observations with the 10 x objective first.
6) While using only your right eye to look through the right **ocular**, focus on the slide first using the outer, larger focus ring (**course focus knob**) and then the inner, smaller focus ring (**fine focus knob**). Bring the image in your right eye in sharp focus.
7) Now close your right eye and as you look through the left ocular with your left eye, rotate the base of the eyepiece (ocular) around until the image is in sharp focus for your left eye.
8) While looking through the scope with both eyes, adjust the distance between the two oculars to match the distance between your eyes by pulling the oculars apart or pushing them together.
9) Adjust the light intensity with the red dial to ensure sufficient but not too much light is reaching your eyes.

Steps 1—9 should only have to be done once by you each time you use the microscope.

10) When you need to increase magnification to 400X, first you should center what you wish to look at, then simply rotate the nose-piece incrementally such that the 10x, 20x, then 40x objective lenses are pointing straight down. At each magnification, make sure the object you wish to see is centered and in focus. The image should stay close to focus since your microscope has a parfocal lens system.
11) Altering Contrast:
    You can alter the contrast via two ways:
    a. Adjust the condenser diaphragm level (via the condenser focusing knob) or the field diaphragm aperture.
    b. For fresh materials, you can use a stain such as toluidine blue before fixing a cover slip.

Appendix 01. Descriptive Statistics

Descriptive statistics describe data collected from samples. They come in a variety of forms, including measures of central tendency and measures of dispersion (described below). They are different than inferential statistics, which are used to make inferences or conclusions about what the data "tell you" about a statistical population. Inferential statistics include those commonly employed to test hypotheses, such as the standard error (Appendix 4), chi-square test (Appendix 5) and *t*-test (Appendix 6) described in other appendices.

I. Measures of Central Tendency

These provide a single value that summarizes a data set by identifying the central position within the data set. Such measures include the mean, median, and mode.

A. Mean. The mean (often referred to as "average") is the most often used measure of central tendency. The mean can be that of a statistical population (μ = the population mean; e.g., all red oak trees in a forest) or of a sample from that population ($\bar{x}$ = the sample mean; e.g., a random sample of 10 red oak trees from the larger population in a forest). It is usually not possible or not feasible to measure all members of a population and so μ must be estimated by $\bar{x}$. Individuals of a sample are ideally taken at random and number as many as is feasible. The equation for sample mean is as follows:

$$\bar{x} = \frac{\Sigma x}{n}$$

Where
- Σ = the sum of…
- x = individual sample measures
- n = the number of samples

For example, if we have the 5 sample values of 10, 8, 11, 9, and 22, our mean is calculated as follows:

$$\bar{x} = \frac{\Sigma x}{n} \qquad \bar{x} = \frac{10 + 8 + 11 + 9 + 22}{5} \qquad \bar{x} = 12$$

In Excel: compute the mean by clicking on an empty cell and typing "=average(" and then select the range of cells with numbers for which you want to compute the mean, and then close parentheses with ")" and hit enter.

Important: When reporting a mean, it is important that you also indicate the *n*-value (i.e., the number of independent samples on which your mean is based). Doing so gives the reader a rough idea of the reliability of the mean and how likely it is that your sample mean is close to the true population mean. Below is an example of how to do this:

> "The mean length of adult male yellowfin damselfish (*Microspathodon chrysurus*) was found to be 12.2 cm (*n* = 5)."

Larger n's evoke greater confidence in your conclusions than smaller n's. You would probably be more confident that the true mean length of adult male yellowfin damselfish was close to 12.2 cm had the *n* been 500 instead of 5.

B. Median. The median is the middle value when all measured values are ordered by magnitude. Sometimes the median is used because it is generally less affected by outliers and skewed data than the mean. As an example, if we have the following values in a sample (10, 8, 11, 9, 22), after ordering them by magnitude (8, 9, 10, 11, 22), we would determine "10" to be the median because there are exactly 2 numbers below and 2 numbers above it. Note that this sample yields a median of 10 when the same sample yielded a mean of 12 above. That is because the mean was affected strongly by the outlier "22" in the data set. The mean and median, however, typically yield similar values for large datasets. In cases where there is an even number of sample values, the median is taken as the mean of the two middle values.

> **In Excel:** compute the median by clicking on an empty cell and typing "=median(" and then select the range of cells with data for which you want to compute the median, and then close parentheses with ")" and hit enter.

C. Mode. The mode for a data set is the most frequently occurring value. For the same set of data as above (10, 8, 11, 9, 22), there is no mode since not a single value is repeated. However, if we had an additional "11" in this dataset, then the mode would be 11 since 11 would be found twice and all other values would be found only once.

> **In Excel:** compute the mode by clicking on an empty cell and typing "=mode(" and then select the range of cells with data for which you want to compute the mode, and then close parentheses with ")" and hit enter.

II. Measures of Dispersion

It is generally insufficient to describe a data set using a measure of central tendency alone, because such measures say nothing about the variability (dispersion) in the data. This is particularly so in biology, since a fundamental characteristic of biological diversity is the existence of variation. Other sources of dispersion in your data include sampling and measurement error. Common measures of dispersion include the range, variance, and standard deviation.

A. Range (and minimum and maximum). The range is the interval between the minimum and maximum value in your data set. The range indicates the spread in your data and is shown by the following equation:

$$\textit{range} = \text{maximum} - \text{minimum}$$

For example, using the sample data above (10, 8, 11, 9, 22), we would calculate the range to be 14, since the minimum value was 8 and the maximum value was 22. Often biologists will describe a range indirectly by describing the minimum and maximum values. Doing so tells the reader the full range of possible values that a given sample is known to fall in and, as such, is more informative than describing the magnitude of the interval only. Taxonomists, in particular, rely heavily on the ranges of variation within species since non-overlapping ranges allow them to distinguish between species. In taxonomic keys and descriptions, it is common for taxonomists to report the minimum and maximum values of particularly dimensions for species (rather than simply the numeric spread), since these can be used by readers to tell species apart.

One issue with the range statistic is that it is susceptible to misrepresentation of the actual variation in a population if there are uncharacteristically large or small values observed in your sample data. For example, if 99.9% of plants in a given species or sample have fruits between 5 and 10 cm long and 0.1% have fruits 100 cm long, then the reported range would be 5-100 cm without any indication of just how atypical 100cm-long fruits in that species were.

> **In Excel:** compute the maximum value by clicking on an empty cell and typing "=max(" and then select the range of cells with data for which you want to compute the max, and then close parentheses with ")" and hit enter.
>
> For minimum value, use "=min()".
>
> For range, use "=max()-min()", which is the equation subtracting the minimum value from the maximum value.

B. Variance. The sample variance ($\mathbf{s^2}$) is the average squared distance (deviation) of observations from the mean within a sample (e.g., one group). Large variances indicate that the individual scores deviate considerably from the sample mean, whereas small variances reflect little deviation from the mean. The formula for sample variance is

$$\mathbf{s^2 = \frac{\sum (X - \bar{X})^2}{n-1}}$$

Where:

- Σ = the Greek capital letter sigma, meaning here "the sum of…"
- $\bar{X}$ = the mean of sample (group)
- X = one sample value
- n = the number of replicates in (sample size of) group
- n-1 is used instead of n in the denominator because it provides a better estimate of the true population variance.

As an example, the variance for the sample including 10, 8, 11, 9, and 22 is 32.5, which is calculated as follows:

$$S^2 = \frac{(10-12)^2 + (8-12)^2 + (11-12)^2 + (9-12)^2 + (22-12)^2}{5-1}$$

$$S^2 = \frac{4 + 16 + 1 + 9 + 100}{5-1}$$

$$S^2 = 32.5$$

> **In Excel:** compute the variance by clicking on an empty cell and typing "=var(" and then select the range of cells with data for which you want to compute the variance, and then close parentheses with ")" and hit enter.

C. Standard Deviation. The sample standard deviation (s) is calculated as the square root (√) of the sample variance (s^2).

$$\mathbf{s = \sqrt{s^2}}$$

The standard deviation is commonly used in tandem with mean and *n* value to describe a data set. The standard deviation of the above data set (10, 8, 11, 9, 22) is

$$S = \sqrt{32.5}$$
$$S = 5.7$$

In Excel: compute the sample standard deviation by clicking on an empty cell and typing "=stdev(" and then select the range of cells with data for which you want to compute the variance, and then close parentheses with ")" and hit enter.

Credits: This appendix was developed by C.R. Hardy in August 2015.

Appendix 02. Tables

Tables are important tools for the collection of raw data and may also provide alternatives to figures (Appendix 3) for summarizing results of a study. This appendix emphasizes the latter.

I. Elements of Tables

All of the following considerations and table elements must be included in the tables you prepare this semester. Treat this section as a checklist to follow when you prepare your own tables.

A. Information Content. Tables of raw, unprocessed data generally do not make it into a professional scientific publication, although they certainly belong in your lab notebook. Rather, tables that do make it in reports or publications are those providing summaries of results and are those which help convince the reader of the conclusions of your research. Tables are not meant to duplicate the content of figures; rather they should complement any figures.

B. Columns & Rows. Table layout requires careful consideration in order to maximize a table's utility. Think carefully about which data or statistics should be arranged in columns (vertically) vs. rows (horizontally) in order to maximize readability.

C. Headers & Units. Columns or rows should be labeled descriptively with headers where appropriate. After the header title, units (e.g., seconds, grams, centimeters; often in parentheses) are indicated. It is customary to abbreviate such units with internationally recognized standard abbreviations such as sec, g, and cm. When the units are included in the header for a column or row, there is no need to repeat units over and over again with the values of individual cells.

D. Table Captions. Tables are presented with a descriptive **table caption** above the table. The caption begins with a table heading (e.g., "Table 1"), followed by a period and then a single-sentence or single-phrase descriptive title. Each table is labeled by a unique heading and in the order that they are referenced and appear in the report (e.g., "Table 1" appears first and "Table 2 appears second, etc.). Titles can be **purely descriptive** or can convey the conclusion or "**take-home message**" that is to be drawn from the table.

E. Neatness & Clarity. Tables should be neat and clearly show the results. Manually constructed tables should have borders drawn using a ruler.

F. P-values, Test Statistics & n-values

In tables in which descriptive statistics such as means and others (Appendix 1) are presented, other statistics that more fully describe the data should be included.

First and foremost of this additional information is the sample size on which each mean, for example, is based (the so-called ***n*-value**). The *n*-value gives the reader a rough idea of the reliability of the mean and how likely it is that your sample mean is close to the true population mean. Larger n's evoke greater confidence in your conclusions than smaller n's.

P-values, **chi-square** and ***t*-test** test statistics should be presented in a table whenever appropriate and whenever this same information is not already had in a figure.

Credits: This appendix was developed by C.R. Hardy in August 2015.

Appendix 03. Graphing

Graphs are utilized to summarize data in a form that clearly conveys to the reader the findings of your research. They are far superior to merely presenting your raw data in a table. The following is a description of the three types of graphs that you may be asked to use this semester.

I. Elements of Graphs

All of the following considerations and graph elements must be included in the graphs you prepare this semester. Treat this section as a checklist to follow when you prepare your own graphs. Each of these items will be exemplified later in this appendix with real graphs.

A. Information Content. Your graphs should be chosen to tell a story, to communicate your findings and conclusions from your research. If your experiment was meant to compare two or more treatment groups for a given variable (e.g., some measure of plant growth in response to contrasting growing conditions), then you should plot the results from all treatment groups into the same graph space to allow that comparison. One mistake students sometimes make is to plot their control group onto one graph, and their experimental group onto separate graph, and this confounds any comparison.

B. Axis Designation. The two axes of a graph represent two variables. When graphing the results of an experiment, the independent variable (that variable whose values were established and manipulated by the experimenter at the start of the experiment) goes on the x-axis (horizontal axis), and the dependent variable (i.e., that which is measured during the experiment and whose values may be dependent upon the independent variable) goes on the y-axis (the vertical axis).

C. Axis Labels & Units. Each axis must 1) be clearly labeled with the precise variable name (e.g., Mean Weight), 2) have units indicated (e.g., seconds, grams, centimeters; often in parentheses), and 3) be graduated (in proportional intervals and/or ordinal manner) where appropriate. When axes reflect quantitative or ordinal variables, it is standard to order them so that values are increasing from left-to-right on the x-axis and from bottom-to-top on the y-axis.

D. Data Symbols. The datum points plotted (e.g., each x & y combination) should be clearly and precisely represented in graph space by symbols (e.g., small black or white circles or squares) that are not so large as to be obtrusive or so small that they are difficult to see. When multiple treatment groups are plotted on the same graph, then the data points, lines, and/or bars representing the different groups should be differentially colored, filled or otherwise distinguished graphically and this distinction should be clearly depicted in a key or legend somewhere next to the graph or, ideally for compact display, in unused graph space.

E. Figure Captions. Graphs are prepared as "figures". The proper figure format in this class will be to have a descriptive **figure caption** below the figure, which begins with a figure header (e.g., "Figure 1"), followed by a single-sentence or single-phrase descriptive title and then a brief explanation of how the data were generated and what any symbols or error bars in your graph signify. Each figure is labeled by a unique header and in the order that they are referenced and appear in the report (e.g., "Figure 1" appears first and "Figure 2 appears second, etc.). Titles can be **purely descriptive** (e.g., Fig 1) or can convey the conclusion or "**take-home message**" that is to be drawn from the figure (e.g., Fig 3).

F. Neatness, Clarity, and Optimal Usage of Graph Space

Graphs should be neat and clear. Manually constructed graphs should be done using graph paper and a ruler. Optimal usage of graph space means that the graph is not crammed into one small corner of the available graph space, nor are data points or other graph elements sloppily placed outside of the available graph space or crammed into a margin of the graph paper.

G. Error Bars, P-values, & n-values

It is good practice to include more information than just raw values or means with your graph. The example graphs that follow in this appendix will show how to do this.

***n* values** inform the reader about the sample size on which each mean is based. Larger n's evoke greater confidence in your conclusions than smaller n's. When used, *n* values can be show on the graphs themselves or stated in the caption.

Errors bars are commonly used in graphs. Errors bars can represent ranges or standard deviations to indicate dispersion in your data, or standard errors and confidence intervals to allow inferences of statistical significance of your findings.

P-values may also be used to indicate your findings from a statistical test for significance. An indication of how to do this on a graph after a Chi-square or *t*-test is provided in the separate appendices on these tests.

R^2 values (see appendix on Simple Regression) are commonly used to indicate how well a best-fit line in a scatterplot matches the data (or how well the data match the best-fit line).

II. Some Graph Types to be Encountered in this Course

A. Bar Graph. A bar graph (sometimes called a column graph when bars are vertical) shows the relationship between some dependent variable on the y-axis and either a categorical or, when ≤ 2 values, quantitative independent variable on the x-axis.

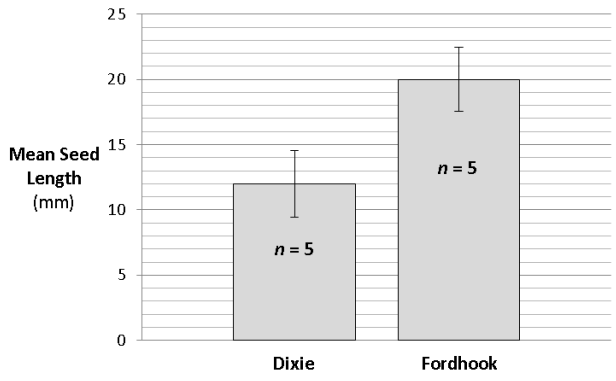


Figure 1. Comparison of mean seed lengths between Dixie and Fordhook cultivars of the Lima bean, *Phaseolus lunatus*. Error bars depict standard error of the mean. Samples of five seeds from each cultivar were selected at random from 16 oz bags of the beans purchased from John Herr's Supermarket in Millersville, PA, on August 10, 2015. Measurements of each seed were made separately using a ruler.

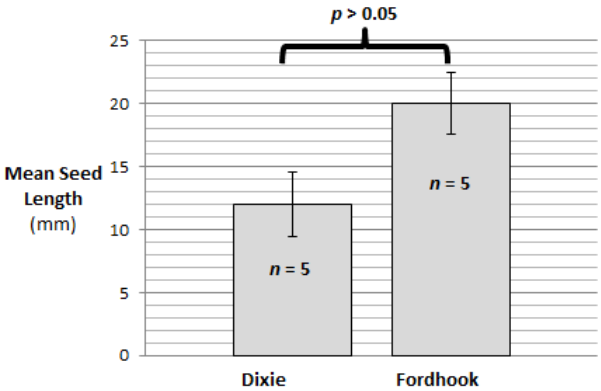


Figure 2. Alternate version of Figure 1 depicting the p-value finding from a *t*-test. The type of test on which the p-value was based would be indicated in the caption somewhere after the title. The p-value from a t-test could be used in lieu of standard error bars.

B. Scatterplot. A scatterplot shows the relationship between two quantitative variables. Such graphs are good at showing correlations or relationships between two variables or the response of a dependent variable to an independent variable. A **best-fit line** is a linear or curvilinear line that shows the general trend that the data seem to follow. It is used instead of simply connecting the dots and can be employed in interpolating or extrapolating to variable values not measured.

Note that while while R^2 values are standard for scatterplots with best-fit lines, other information such as error bars and *n* values are not always displayed on such graphs not only because they may clutter the graph but also because the robustness of the graph is conveyed to the audience more by the slope of the line and the R^2 value.

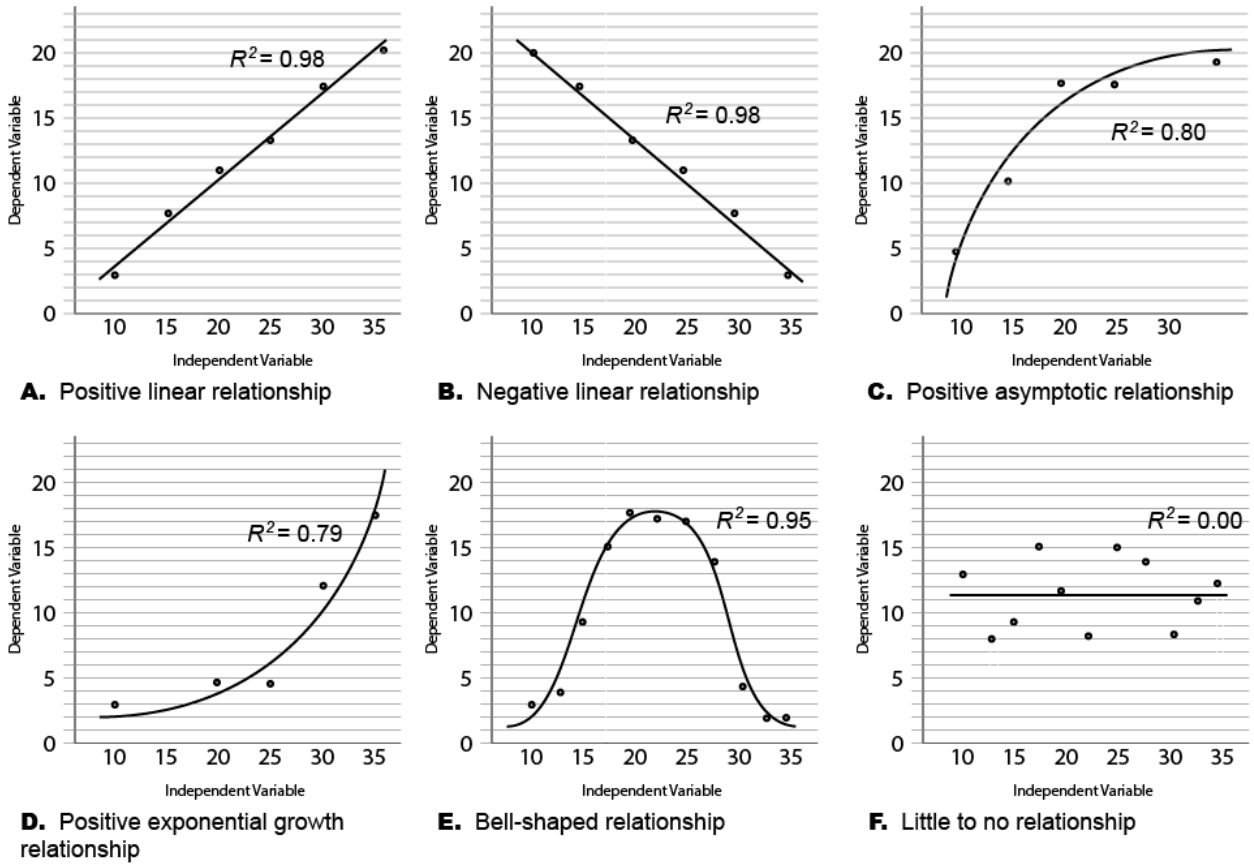


Figure 3. Scatterplots with best-fit lines are useful for revealing the relationship or response of a dependent variable to an independent variable. **A and B** show linear relationships that are positive (positively sloped) and negative (negatively sloped), respectively. **C, D & E** show curvilinear relationships, where the rate of change in the dependent variable is not constant across the scale of the independent variable. **F** shows little to no relationship, thus the slope of a straight line would be zero and the R^2 would be at or near to zero.

III. Calculating Slope from a Best-Fit Line

The slope of a straight line fit to the data in a scatterplot is useful to determine for a variety of purposes. Firstly, the magnitude of the slope provides information on how strong a positive or negative relationship between two variables is. Quantifying the magnitude of the slope in a graph depicting photosynthetic response of a plant to one wavelength of light, for example, allows its comparison to the response of that plant to another wavelength of light. By doing things like this, plant physiologists have proven that photosynthesis proceeds more rapidly under some wavelengths (e.g., red wavelengths of light) than others (e.g., green wavelengths). Ultimately, determining the slope of a line provides a rather rigorous way of calculating the rate of the process that is being measured by the experiment and graph.

As illustrated in Figure 4, the slope of a line is determined by picking any two points along that line and plugging those x and y values at those two points into the following formula:

$$\textbf{Slope} = (y_2 - y_1) / (x_2 - x_1)$$

Doing so yields the change in *y* over the change in unit *x*. In this case, we have the CO_2 produced (exhaled) per min by person, which reflects a rate of respiration. Based on the graph below, we would calculate the rate of respiration for this organism to be 0.7 ppm/min as follows:

Rate of Respiration = (20.4 – 6.4 ppm) / (35.0 – 15.0 min)
Rate of Respiration = 14.0 ppm / 20 min
Rate of Respiration = 0.7 ppm / min

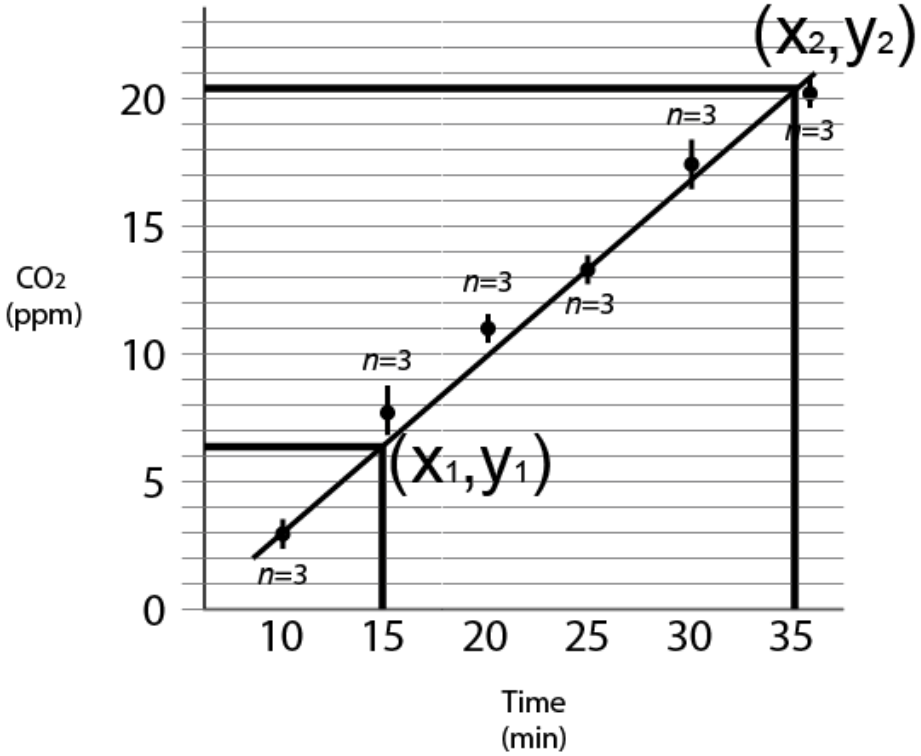


Figure 4. Illustration depicting how one would select two points along a line depicting the the hypothetical rate of CO_2 exhalation by a human at rest. Using this pair of x and y values in the equation for a slope provides an estimate of the rate of respiration for this human at rest.

Credits: This appendix was developed by C.R. Hardy in January 2016.

Appendix 4. Standard Error

The standard error of the mean, also simply called the standard error, is an inferential statistic that provides a measure of how variable the sample mean will be if the study is repeated many times. This information allows one to make inferences regarding 1) how close the sample mean is to the true population mean and 2) whether the means of two independent samples are statistically significantly different. Given this utility, it is commonly used to construct error bars on graphs.

I. Calculation

Standard error (SE) is calculated as the sample standard deviation (*s*) divided by the square root of the sample size (*n*) for a given group.

$$SE = \frac{s}{\sqrt{n}}$$

Because the sample size (*n*) is in the denominator, standard error decreases as sample size increases (which is a good property). The formula for standard deviation is available in Appendix 1 on Descriptive Statistics. Standard deviation itself is calculated as the square root of the variance, also described in Appendix 1.

To illustrate SE calculation, let us use the hypothetical data on seed length of two different cultivated varieties of Lima beans (Table 1). The question that would have guided this study might have been "Are the seed lengths of these different cultivated varieties different?" or "Are Fordhook Lima beans larger than Dixie Lima beans?"

Table 1. Hypothetical data for seed length for Dixie and Fordhook cultivated varieties of Lima beans. Mean, variance, and standard deviation (based on formulas given in Appendix 1) are also presented here for convenience of having statistics that are used in the derivation of SE.

	Dixie Length (mm)	**Fordhook Length (mm)**
Sample 1	10	18
Sample 2	8	21
Sample 3	11	17
Sample 4	9	15
Sample 5	22	29
Mean	12	20
Variance	32.5	30.0
Standard Deviation	5.7	5.5

Using this equation, the SEs for the Dixie (group a below) and Fordhook (group b below) Lima beans in Table 1 are 2.5 and 2.4 mm, respectively, as follows (see Appendix 1 for the equation for standard deviation).

$$SE_a = \frac{s_a}{\sqrt{n}} \qquad SE_b = \frac{s_b}{\sqrt{n}}$$

$$SE_a = \frac{5.7}{\sqrt{5}} \qquad SE_b = \frac{5.5}{\sqrt{5}}$$

$$SE_a = 2.5\ \text{mm} \qquad SE_b = 2.4\ \text{mm}$$

Where
- *s* = the sample standard deviation
- n = the number of samples
- The subscripts *a* and *b* merely represent the control and experimental treatment groups, respectively.

II. Standard Error Bars & Estimating Significance from Your Graph

A. Fitting Standard Errors Bars to Your Graph. Figure 1 depicts the mean values from Table 1 with error bars extending one SE above and below each mean. Error bars may also be used in scatterplots and line graphs.

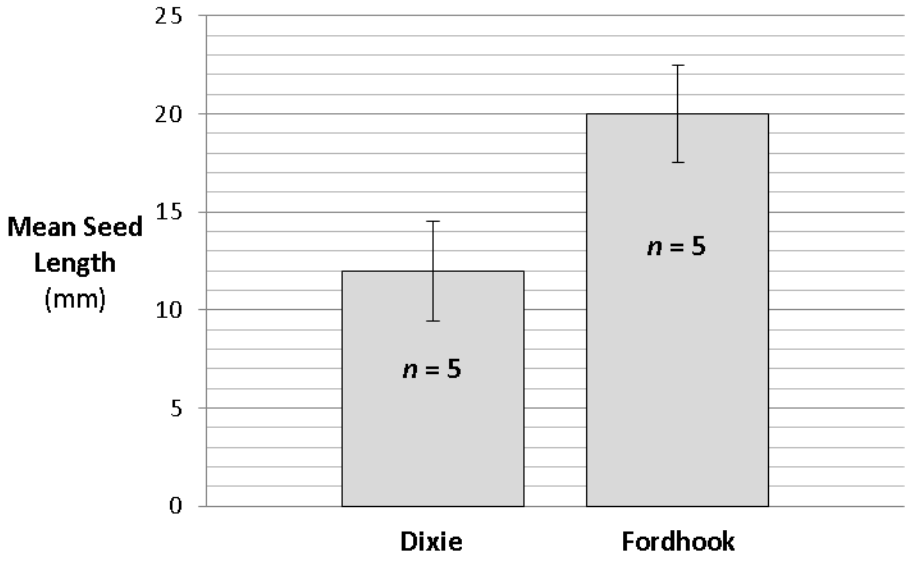


Figure 1. Comparison of mean seed length between Fordhook and Dixie cultivars of Lima bean (*Phaseolus lunatus*). Error bars reflect standard errors of the mean of 2.5 mm and 2.4 mm for Dixie and Fordhook cultivars, respectively.

B. Inferring Statistical Significance. Your inference of statistical significance must be grounded by some basic statistical theory. Firstly, regardless of which bean variety you hypothesized to be larger or longer, statistical inference will be based on accepting or rejecting the statistical null hypothesis (H_0).

H_0: $\mu_a = \mu_b$

Where:
μ_a = mean of population a
μ_b = mean of population b
H_0 = the null hypothesis (no difference)

We further wish to generalize your hypothesis to the statistical alternative hypothesis (H_A) for which support is found by rejecting H_0:

H_A: $\mu_a \neq \mu_b$

The degree of separation between your standard error bars will enable an estimation of the probability that H_0 is true, a so-called p-value. It is standard in experimental biology to set the threshold p-value to 0.05 (this threshold p-value is known as your alpha level; in this case $\alpha = 0.05$).

When the p inferred from your error bars is $p = 0.05$, you have a 5% chance that the differences between your means is not real, but rather obtained by chance, and you'd be rejecting H_0 in error (which is known as Type I error).

When the p inferred from your error bars is $p < 0.05$, you have less than a 5% chance of observing a difference by chance, and you'd be rejecting H_0 in error.

Setting $\alpha = 0.05$ signifies that you are willing to accept this level of error as follows:

When $p \leq 0.05$, reject H_0 and conclude that the means are significantly different.

When $p > 0.05$, accept H_0 and conclude that the evidence for a difference in means is insufficient.

A *t*-test (Appendix 5) could be performed to determine this p-value, but the following guidelines (Figure 2) regarding the separation of standard error bars can be used in lieu of a *t*-test. Read the figure caption fully for these rules. (CAVEAT: Statistical inferences become significantly more reliable with sample sizes of 10 or more; Cumming et al. 2007 and Vaux et al. 2014).

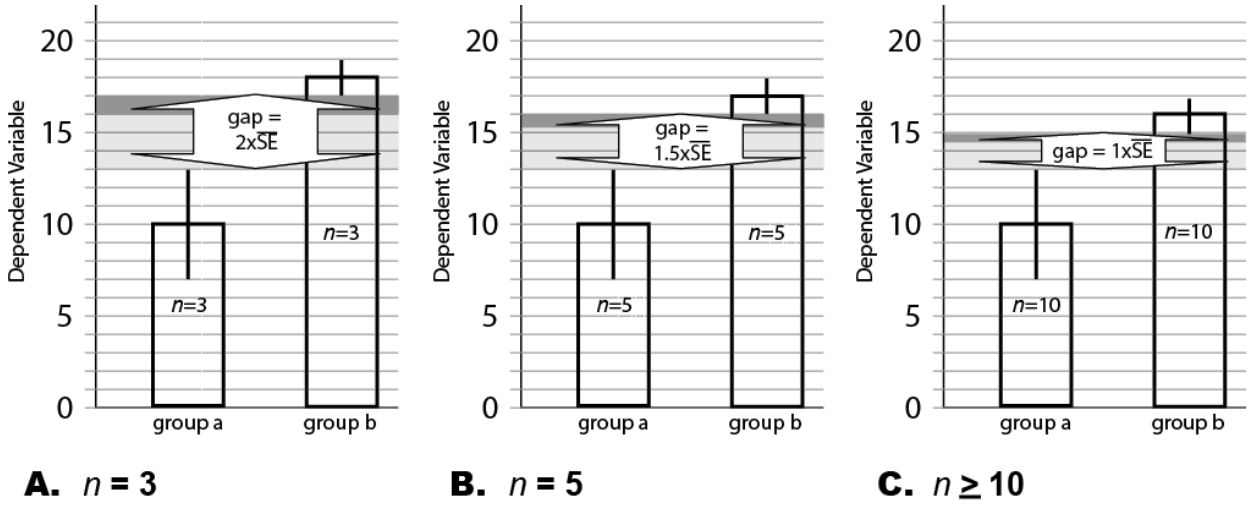


Figure 2. Estimating statistical significance using the overlap rule for standard error bars (drawn based on the rules outlined by Cumming et al. 2007, Vaux 2014 and Vaux, personel communication). When standard error bars overlap, it is a given that $p > 0.05$ and so the difference between the means is not significant. Depending on the *n*-value, the threshold for determing significance based on the gap between error bars varies. For small sample sizes such as $n = 3$ and $n = 5$, $p \approx 0.05$ when the gap is 2 times and 1.5 times, respectively, the average of the two standard errors. Greater gaps imply $p < 0.05$. For $n \geq 10$, the minimum gap is 1 times the standard error.

C. Reporting the results of your statistical inference

When you are reporting your conclusions regarding intergroup differences and statistical significance (e.g., in the Results section of a report), the goal is to give the reader everything they will need to accept your conclusions, and to do this succinctly but thoroughly. Furthermore, it is important provide

discussion that identifies possible underlying reasons for this finding and you should attempt to identify a future study that could help resolve outstanding questions. When identifying reasons for a negative results, you should not rely on "experimental error" explanations: you must at least also explore biological explanations. As example, the conclusion that you would base on the hypothetical data presented above in Figure 1 could be constructed as follows. Unless otherwise noted by your instructor, we expect that you will follow this format in all of your work in this course:

> "Fordhook Lima beans averaged 20 mm in length (*n* =5, SE = 2.4 mm; Fig 1) whereas Dixie Lima beans averaged 12 mm in length (*n* =5, SE = 2.5 mm; Fig 1). Using standard error gap guidelines of Vaux (2014), however, these differences were not significant ($p > 0.05$) and thus there is insufficient evidence for the hypothesis that Fordhook beans are longer than Dixie beans. This conclusion may have been disproportionately affected by the high degree of dispersion in our limited sample of 5 beans per cultivar, which resulted in moderately large variances. For example, whereas 4 of the 5 Dixie beans ranged between just 8 and 11 mm long, one was found to be 22 mm long, which placed it well within the range observed for Fordhook beans (Table 1). Other than size, Dixie and Fordhook beans are indistinguishable morphologically; thus, one possibility for these results was that the 22 mm long bean in the Dixie sample was a Fordhook bean misplaced upon packaging. Alternatively, this 22 mm long Dixie bean may simply have been atypical. A future study should be conducted with a greater sample size."

III. Literature Cited

Cumming G, F Fidler, DL Vaux. 2007. Error bars in experimental biology. *The Journal of Cell Biology* 177: 7-11.

Vaux DL. 2014. Basic statistics in cell biology. *Annual Review of Cell & Developmental Biology* 30: 23-37.

Credits: This appendix was developed by C.R. Hardy in August 2015.

Appendix 05. *t*-test

I. Explanation and Procedure

The *t*-test is a statistical approach to comparing the **means** of two populations based on independent samples from each. The objective is to determine if the mean for some variable (e.g., height or weight) in one population is different than that of the other population. A taxonomist or plant breeder, for example, can use a *t*-test to determine if a putative new species or cultivar produces seeds that are significantly larger or smaller than another closely related species or cultivar. A botanist may use the *t*-test to test whether or not some treatment has an effect on crop plant growth or yield. The description below is how you would manually compute a t-test. If your instructor allows, you may automate most of this *t*-test using Excel or Graph Pad Software's online tool at http://graphpad.com/quickcalcs/ttest1.cfm. You would need to be careful about which version of the *t*-test you select to use at this site.

A. Determine the null hypothesis

The *t*-test is designed to test the **null hypothesis** ($\mathbf{H_0}$). The null hypothesis is that there is no difference between the two groups or that there will be no effect of a treatment, and so the means of the two treatment groups (a and b) will be the same (i.e., not significantly different). Although scientists typically initiate an experiment with an **alternative hypothesis** ($\mathbf{H_A}$) that there *will* be some difference or effect, they ultimately find support for H_A indirectly by rejecting H_0.

H_0: $\mu_a = \mu_b$
H_A: $\mu_a \neq \mu_b$

Where:
μ_a = mean of population a
μ_b = mean of population b
H_0 = the null hypothesis (no difference)
H_A = the alternative hypothesis (some difference)

B. Sample and calculate the sample mean and sample variance for each group

The sample variance ($\mathbf{S^2}$) is a measure of the variability within a population sample (e.g., one group). A large variance indicates that the individual scores deviate considerably from the sample mean, whereas a small variance reflects little deviation from the mean. The formula for variance of group a ($\mathbf{S_a^2}$) is

$$S_a^2 = \frac{\sum (X_{i,a} - \overline{X}_a)^2}{n_a - 1}$$

Where:
$\overline{X}_a$ = the mean of sample (group) a
$X_{i,a}$ = variable value of the i^{th} replicate in group a
n_a = the number of replicates in (sample size of) group a

Note that $\overline{X}_a$ is the sample mean, which is only an estimate of the true population mean (μ_a), the latter of which we cannot know unless we sampled every single individual from population a.

C. Use means and variances to calculate the value of the *t* statistic

The *t*-test statistic can be calculated using the following formula*:

$$t = \frac{\bar{X}_a - \bar{X}_b}{\sqrt{\frac{S_a^2}{n_a} + \frac{S_b^2}{n_b}}}$$

Where:

$\bar{X}_a$ = mean of sample a

$\bar{X}_b$ = mean of sample b

S_a^2 = variance of sample group a

S_b^2 = variance of sample group b

n_a = sample size of sample a

n_b = sample size of sample b

*This follows that of the Welch's *t*-test which does not assume equal variances. Ruxton (2006) compares both the Welch's and Student's t-test, which assumes equal variances: he favors Welch's in all cases.

D. Determine the degrees of freedom for your test

A **simple** degrees of freedom formula often used is

$$df = (n_a - 1) + (n_b - 1)$$

A **more complex** formula favored by Ruxton (2006), particularly where equal variances are not assumed, is as follows. It generally has the effect of producing lower estimates of df, which makes your t-test more conservative (more cautious) in rejecting the null hypothesis. Unless instructed otherwise by your instructor, you may use the simple form (above) when conducting manual calculations since the simple formula is easier to compute. If using the complex formula below, however, do know that, unlike the simple formula above, the value obtained from the complex formula will generally be a non-integer value and it is customary to round down to the nearest integer. Thus, if you have a raw df value of 6.76, you will round down (not up) to 6.

$$df = \frac{\left(\frac{S_a^2}{n_a} + \frac{S_b^2}{n_b}\right)^2}{\frac{\left(\frac{S_a^2}{n_a}\right)^2}{n_a - 1} + \frac{\left(\frac{S_b^2}{n_b}\right)^2}{n_b - 1}}$$

E. Use this df value to determine the critical t value for an alpha level (α) of 0.05.

$t_{critical}$ = the value obtained from a two-tailed critical t value table (given in section III on the last page of this appendix) for the chosen alpha level and your degrees of freedom.

An alpha level of 0.05 is a standard threshold for determining statistical significance: it allows you to reject or accept your hypothesis with greater than or equal to 95% confidence. The flip side of this is that you will have a 5% chance or less of rejecting or accepting your hypothesis in error.

F. Compare the absolute value of your calculated t with the critical t value to determine significance

When $|t| \geq t_{critical}$, reject H_0, accept H_A (i.e., there is a significant difference).

If the $|t| = t_{crit}$, then you reject with 95% confidence, that the chance of you observing such differences in mean by chance alone is 5%. That is, there is a 5% chance that you have made a **Type 1 error** (having rejected H_0 even though it is true or having detected a difference even though there is none).

If the $|t| > t_{crit}$, then you reject with greater than 95% confidence, that the chance of you observing such differences in mean by chance alone is less than 5%. That is, there is *less than* a 5% chance that you have made a **Type 1 error**.

When $|t| < t_{critical}$, accept H_o (i.e., there is not a significant difference).

At $\alpha = 0.05$, you are making this conclusion with greater than 95% confidence, but there is still a less than 0.05 probability (5% chance) of having accepted H_0 (no difference) when it is not true. This error is called **Type 2 error** by statisticians.

G. Reporting the results of your *t*-test

When you are reporting the results of your *t*-test (e.g., in the Results section of a report), the goal is to give the reader everything they will need to accept your conclusions, and to do this succinctly. Take the following hypothetical example. We expect that you will follow this format in all of your work in this course, unless otherwise noted by your instructor.

"This study found evidence for sexual dimorphism in groundhogs (*Marmota monax*), with males being significantly heavier (mean = 3.90 kg, *n*=16) than females (mean = 3.14 kg, *n* = 12; *t*=2.71, *df*=26, *p*<0.05; see Table 1 and Fig 2)."

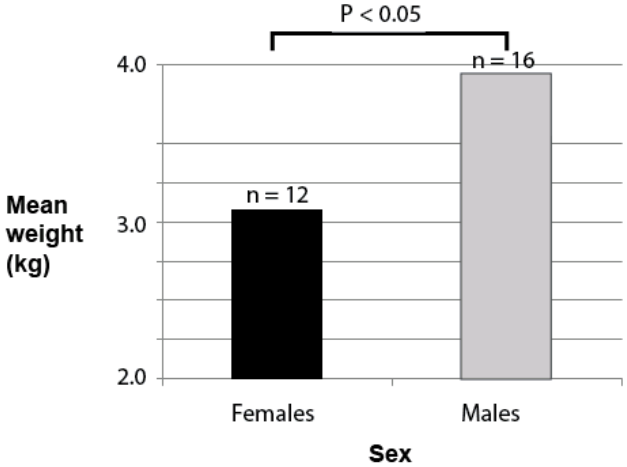


Fig 2. Male groundhogs weigh more, on average, than female groundhogs (*t*=2.71, *df*=26, *p*<0.05). Male and female groundhogs were captured and weighed in June 2014 followed by their release back into the wild."

Note that for each group in the above hypothetical study, the mean and sample size was given in parentheses following the mention of each group. It is always important to indicate the sample size on which a descriptive statistic such as a mean is based. Afterwards, the t-test statistic value you calculated is given (t = 2.71), along with the degrees of freedom (df = 26) that determined the appropriate critical value (t_{crit} = 2.0555) on which to assess your significance, and then an indication that the p value was less than your threshold alpha level of 0.05 for determining significance. The p value indicates the probability of obtaining a result like this by chance alone: in this case, there is less than a 0.05 (i.e., less than a 5 %) chance of you finding a difference in mean when there actually is not (Type 1 error). We can say less than because the t test statistic value was greater than the t critical value of 2.06 for 26 degrees of freedom at this alpha level. If your test statistic value was exactly 2.06, then you would have reported "p = 0.05" instead. Finally, it is appropriate to refer your readers to a table and/or a figure that presents the underlying data ("Table 1") or summarizes your results graphically ("Fig 2").

Alternatively, one could report the result of no significant difference as follows:

> "This study failed to find evidence of sexual dimorphism in groundhogs (*Marmota monax*). Whereas the average male weighed more (mean 3.55 kg, n = 16) than the average female (mean = 3.33 kg, n = 12), this difference was not significant (t = 1.92, df = 26, p > 0.05; see Table 1 and Fig 2)."

H. Advanced Reading About *t*-test Variants

The Student's *t*-test is a commonly used version of this test that was named after its author who, in 1908, described it in a journal article under the pen name "Student" (Student 1908, Box 1987). However, Student's *t*-test assumes that the variances of both groups are the same and yet that assumption will not always be valid. A version of the *t*-test that does not assume equal variances is Welch's *t*-test, also known as the unequal variance *t*-test (Welch 1938, Ruxton 2006). Studies have shown the unequal variance *t*-test to be as good as or superior to the Student's *t*-test at controlling **Type I** and **Type II** error rates when the assumption of both tests—that the underlying distributions are normal—is met (Ruxton 2006, Moser and Stevens 1992). **Type I error** is when you detect a difference in mean when there actually is none. **Type II error** is when you fail to detect a difference in mean when there actually is one. The procedure for Welch's t-test is as follows.

I. References

Box, JF. 1987. Guinness, Gossett, Fisher, and small samples. *Statistical Science* 2: 45-52.

Moser, BK, and GR Stevens. 1992. Homogeneity of variance in the two-sample means test. *American Statistician* 46: 19-21.

Ruxton, GD. 2006. The unequal variance *t*-test is an underused alternative to Student's *t*-test and the Mann-Whitney *U* test. *Behavioral Ecology* 17: 688-690.

Student. 1908. The probable error of a mean. *Biometrika* 6: 1-25.

Welch, BL. 1938. The significance of the difference between two means when the population variances are unequal. *Biometrika* 29: 350-362.

II. Practice

A. Practice Problem

The authors hypothesized that moose in Sweden weighted less than those in Finland. Is there statistical support for this? Conduct a t-test.

Table 1. Weight and locations of male moose sampled from Swedish and Finish populations between 2013-2014.

	Sweden		Finland	
Specimen Number	**Weight (kg)**	**Location (latitude, longitude)**	**Weight (kg)**	**Location (latitude, longitude)**
1	184	58.0 N, 14.0 E	198	60.0 N, 23.0 E
2	190	58.5 N, 16.5 E	200	60.5 N, 22.0 E
3	194	57.5 N, 13.5 E	211	61.0 N, 27.5 E
4	212	62.0 N, 15.5 E	220	61.5 N, 23.0 E
5	230	63.5 N, 18.0 E	230	65.0 N, 27.0 E

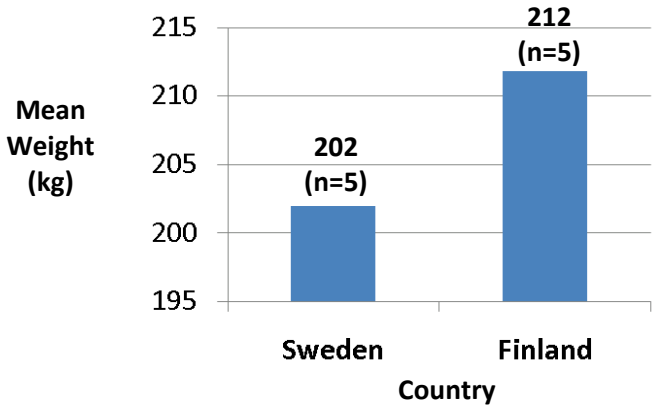


Fig 1. Mean weights for moose from Sweden and Finland.

B. Answers to practice problem

Note that the following table merely presents the numerical answers to the various calculations that you made above. If you were presenting this formally for a grade, you would need to follow the instructions for reporting the results of your *t*-test in Section I, G above.

	Swedish moose	Finnish moose
n	5	5
Mean weight (kg)	202.0	211.8
Variance	354.0	182.2
t	0.95 (absolute value)	
df (simple)	8	
df (complicated)	7 (rounded from 7.26)	
$t_{critical}$	2.36 (from critical values table)	

III. Critical values of the *t* distribution for < 28 degrees of freedom and various alpha levels

α (2 tail)	0.1	0.05	0.02	0.01	0.005	0.002	0.001
df							
1	6.3138	12.7065	31.8193	63.6551	127.3447	318.4930	636.0450
2	2.9200	4.3026	6.9646	9.9247	14.0887	22.3276	31.5989
3	2.3534	3.1824	4.5407	5.8408	7.4534	10.2145	12.9242
4	2.1319	2.7764	3.7470	4.6041	5.5976	7.1732	8.6103
5	2.0150	2.5706	3.3650	4.0322	4.7734	5.8934	6.8688
6	1.9432	2.4469	3.1426	3.7074	4.3168	5.2076	5.9589
7	1.8946	2.3646	2.9980	3.4995	4.0294	4.7852	5.4079
8	1.8595	2.3060	2.8965	3.3554	3.8325	4.5008	5.0414
9	1.8331	2.2621	2.8214	3.2498	3.6896	4.2969	4.7809
10	1.8124	2.2282	2.7638	3.1693	3.5814	4.1437	4.5869
11	1.7959	2.2010	2.7181	3.1058	3.4966	4.0247	4.4369
12	1.7823	2.1788	2.6810	3.0545	3.4284	3.9296	4.3178
13	1.7709	2.1604	2.6503	3.0123	3.3725	3.8520	4.2208
14	1.7613	2.1448	2.6245	2.9768	3.3257	3.7874	4.1404
15	1.7530	2.1314	2.6025	2.9467	3.2860	3.7328	4.0728
16	1.7459	2.1199	2.5835	2.9208	3.2520	3.6861	4.0150
17	1.7396	2.1098	2.5669	2.8983	3.2224	3.6458	3.9651
18	1.7341	2.1009	2.5524	2.8784	3.1966	3.6105	3.9216
19	1.7291	2.0930	2.5395	2.8609	3.1737	3.5794	3.8834
20	1.7247	2.0860	2.5280	2.8454	3.1534	3.5518	3.8495
21	1.7207	2.0796	2.5176	2.8314	3.1352	3.5272	3.8193
22	1.7172	2.0739	2.5083	2.8188	3.1188	3.5050	3.7921
23	1.7139	2.0686	2.4998	2.8073	3.1040	3.4850	3.7676
24	1.7109	2.0639	2.4922	2.7970	3.0905	3.4668	3.7454
25	1.7081	2.0596	2.4851	2.7874	3.0782	3.4502	3.7251
26	1.7056	2.0555	2.4786	2.7787	3.0669	3.4350	3.7067
27	1.7033	2.0518	2.4727	2.7707	3.0565	3.4211	3.6896
28	1.7011	2.0484	2.4671	2.7633	3.0469	3.4082	3.6739

Credits: This appendix was developed by C.R. Hardy in January, 2016. The author appreciates Larry Reinking and Rachel Fogle for his careful review of an earlier draft of an earlier version of this appendix.

Appendix 06. The Chi-square Test

I. Explanation and Procedure

The Chi-square test, also referred to as the Chi-squared test, is useful for testing for significant differences between your observed categorical count data and some expected counts based on either the null hypothesis or some other hypothesis. Percentages, averages, etc. cannot be used: Only actual counts.

A. Identify the hypothesis to be tested

The chi-square test can be used to test any hypothesis that can be expressed in terms of counts for discrete categorical outcomes of some empirical study. In the first lab of BIOL 101, for example (see Lab 1 – Laboratory Skills), we asked you to determine whether or not peaberry coffee beans occurred naturally at the same frequency as regular coffee beans. You might even have surmised that peaberries occurred at some unknown lower frequency than regular coffee beans based on a quick, superficial count. But without any prior and precise knowledge on relative frequencies of the two categories of coffee beans, the best hypothesis to test was the **null hypothesis** (**H_0**)—that there is no difference in frequency of occurrence between the two types of beans. The **alternative hypothesis** (**H_A**) is that there *is* some unspecified difference in the frequency of occurrence between the two types, but support for H_A would still be found by explicitly test and rejecting H_0.

B. Determine your observed and expected values

Determine the number of observations (counts) for each different type from a random sample. These are your **observed values**. For example, a hypothetical random sample of 90 beans may have found 56 regular coffee beans and 34 peaberries. Your **expected values** depend on your hypothesis, but the null hypothesis translates into an expected 45 regular (0.5 x 90) and 45 peaberry (0.5 x 90 beans) coffee beans. Instead of equal frequencies, you might have had information for a more precise hypothesis of 2 regular beans for every 1 peaberry (i.e., that regular beans were twice as abundant as peaberries), and that would have translated into an expected 60 regular (0.67 x 90) and 30 peaberry (0.37 x 90) coffee beans from that same sample of 90.

C. Calculate your chi-square test statistic.

The summary formula to calculate the chi-square statistic, X^2, is as follows.

$$X^2 = \sum \frac{(o - e)^2}{e}$$

Where o=observed and e=expected values.

From the hypothetical data above, you might choose to organize a simple table of observed and expected values for both types of beans under the null hypothesis as follows:

	Obs	Exp
Regular	56	45
Peaberry	34	45
totals	90	90

Your calculation would then be as follows

$$X^2 = \frac{(56 - 45)^2}{45} + \frac{(34 - 45)^2}{45} = 5.38$$

D. Determine the number of *degrees of freedom* (df) as follows:

df = n – 1, where n = the number of categories.
In the above example there are two categories,
regular and peaberry, and thus 1 degree of freedom.

E. Determine the critical value

Use your calculated degrees of freedom to determine what statisticians have determined to be the Chi-square ***critical value*** for our test from the critical values table in Section II that follows. The critical values in this table are for an alpha level (α) of 0.05.

$X^2_{critical}$ is found in Section II of this appendix.

For this hypothetical example, $X^2_{critical}$ for df =1 and alpha = 0.05 is 3.84

F. Compare your calculated X^2 with the critical X^2 value to determine significance

When $X^2 \geq X^2_{critical}$, reject your hypothesis (expected values) because there is a significant difference between your observed and expected values.

If the $X^2 = X^2_{crit}$, then you reject with 95% confidence, that the chance of you observing such differences by chance alone is 5%.

If the $X^2 > X^2_{crit}$, then you reject with greater than 95% confidence, that the chance of you observing such differences by chance alone is less than 5%.

When $X^2 < X^2_{crit}$, accept your hypothesis (i.e., there is not a significant difference).

In our hypothetical example, your calculated X^2 = 5.38, which is greater than X^2_{crit} of 3.84. Thus, you would reject your hypothesis, in this case the null hypothesis.

G. Reporting the results of your X^2 test

When you are reporting the results of your X^2 test (e.g., in the Results section of a report), the goal is to give the reader everything they will need to accept or at least follow the reasoning of your conclusions, and to do this succinctly. For our hypothetical example, one could present it as follows. We expect that you will follow this format in all of your work in this course, unless otherwise noted by your instructor:

> "Peaberries were found to occur at a significantly lower frequency (0.38) than regular, flat sided coffee seeds (0.62), $X^2 = 5.38$, $n = 90$, $df = 1$, $p < 0.05$ (Fig 2)."

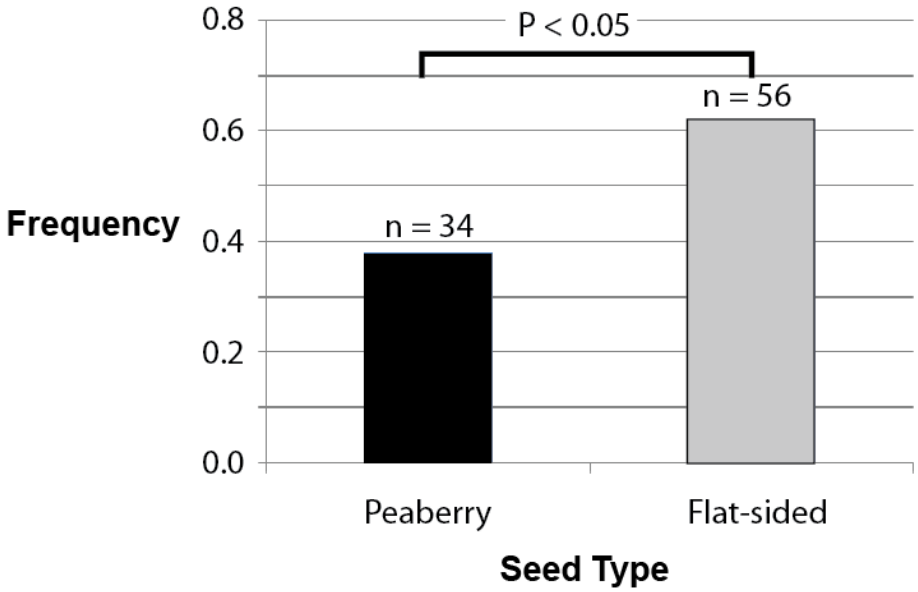


Fig 2. The relative frequencies of peaberry and flat-sided coffee seeds in a mixed bag of coffee. These frequencies out of 1.00 are based on a random sample of 90 coffee seeds. A chi-square analysis found peaberries to be significantly less abundant than flat-sided seeds.

Note that for each category of seeds, the frequency (a number out of 1, or the proportion) was given in parentheses following the mention of each group. Even though we used actual counts for the test, a frequency or proportion value out of 1.0 is easier to interpret when discussing the relative abundance of one type of thing to the other. The actual counts can be left to the table or, in this case, the figure suffices since the data are relatively simple. Afterwards, the X^2 test statistic value of 5.38 is given, as well your sample size (here, 90, the total number of seeds counted), the degrees of freedom on which your critical value for significance is determined, followed by an indication that the *p* value was less than your threshold alpha level of 0.05 for determining significance. The *p* value indicates the probability of obtaining a result like this by chance alone: in this case, there is less than a 0.05 (i.e., less than a 5 %) chance of you finding a difference in frequency when there actually is not. We can say less than because the X^2 test statistic was greater than the X^2 critical value of 3.84 for one degree of freedom. If your test statistic value was exactly 3.84, then you would have reported "$p = 0.05$" instead. Finally, it is appropriate to refer your readers to a table and/or a figure such as a graph ("Fig 2" in the hypothetical example) that provides the underlying data and summarizes your results graphically ("Fig 2"). Note that

the figure indicates the P value and conveys that there was a significant difference between the two counts.

An alternative hypothetical result of no difference in frequency could be presented as follows:

> "Although fewer peaberries than regular coffee beans were counted in this study, the peaberry frequency (0.49) was not found to be significantly different than that of regular, flat sided coffee beans (0.51), $X^2 = 0.04$, $n = 90$, $df = 1$, $p > 0.05$ (Fig 2)."

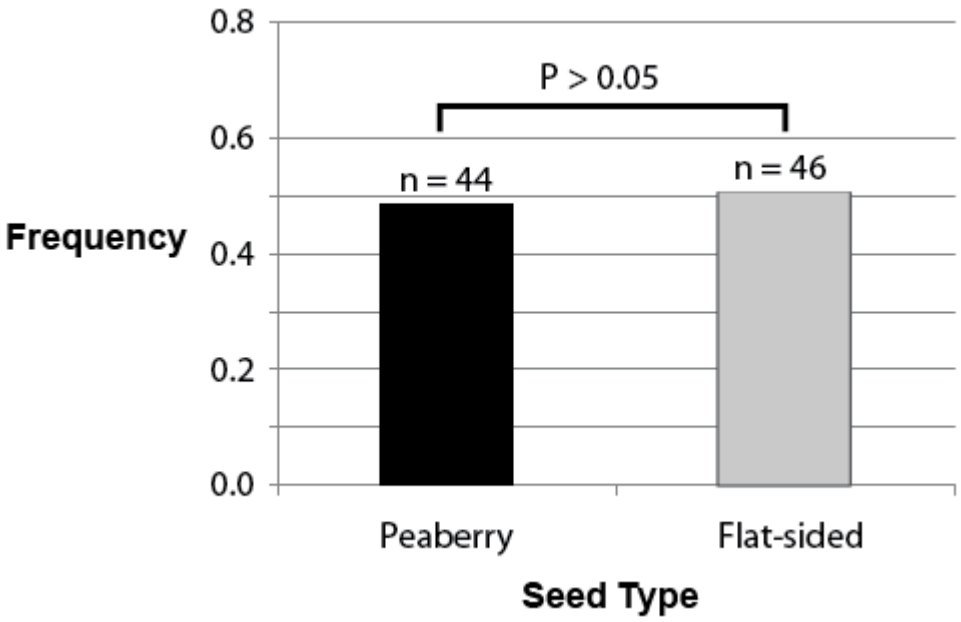


Fig 2. The relative frequencies of peaberry and flat-sided coffee seeds in a mixed bag of coffee. These frequencies out of 1.00 are based on a random sample of 90 coffee seeds. A chi-square analysis found that peaberry abundance was not significantly different than that of flat-sided seeds.

II. Critical Chi-square Values for up to Six Degrees of Freedom and an Alpha Level of 0.05

Degrees of Freedom (n-1)	Critical Value
1	3.84
2	5.99
3	7.81
4	9.49
5	11.07
6	12.59

Credits: This appendix was developed by C.R. Hardy in January 2015.

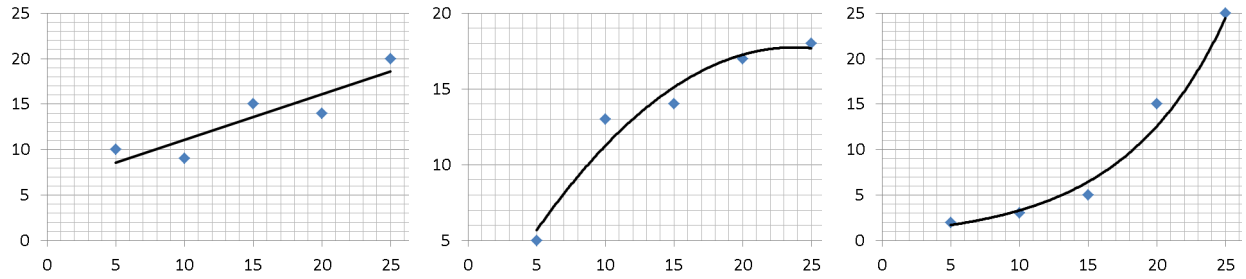
Appendix 07. Simple Regression Analysis

I. Explanation and Procedure

Simple regression analysis is a way to determine the degree to which variation in some independent variable can explain (or cause) variation in a dependent variable. It is different than Pearson's and Spearman's Correlation analyses in that, rather than assuming simply a correlation between two variables, it explicitly assumes that the one variable (dependent variable) on the y-axis is dependent upon the other variable (independent variable) on the x-axis. Computer programs such as Microsoft Excel and SPSS employ sophisticated graphing and mathmatical algorithms to do this, but the following procedure describes the fundamental approach to simple regression analysis that is easy to apply manually and to all types of linear and curvilinear or non-linear relationships that you are likely to encounter.

A. Graph your data using a scatterplot and line of best -fit.

Making a scatterplot is the first step in regression analysis because this gives you a coarse undestanding of the relationship between the two variables: i.e., whether or not the relationship is positive or negative, for example, and whether it is linear or some curvilinear or non-linear relationship (Fig 1). A line of best-fit (a "regression line") is then fitted to the data in the scatterplot to better illustrate this relationship (Fig 1). A line of best-fit is the relationship between x and y that is suggested by the data.



A. A positive linear relationship **B.** A positive asymptotic relationship **C.** A positive exponential relationship

Fig 1. Three of many possible relationships between two variables in biology.

B. Calculate the Coefficient of Determination, R^2.

Once a best-fit line is fitted to the data, we now quantify how closely y varies with x along that line. This is done with the Coefficient of Determination (R^2, Equation 1), in which a maximum value of 1 indicates a perfect covariance and a value of 0 indicates no covariance.

$$R^2 = 1 - \frac{\Sigma (y_{obs} - y_{line})^2}{\Sigma (y_{obs} - \bar{y}_{obs})^2}$$

Equation 1, where

- **Σ** = the formulaic expression "the sum of.."
- $(y_{obs} - y_{line})^2$ = the squared difference between one observed y value and the y value predicted by the line for a given x value.
- $(y_{obs} - \bar{y}_{obs})^2$ = the squared difference between one observed y and the mean of of all observed y's.

As an example, take the following sample graph with a best-fit line that suggests a positive, linear relationship between light bulb strength (in Watts) and photosynthetic rate (in parst per million of CO_2 consumed per minute) for some experimental setup:

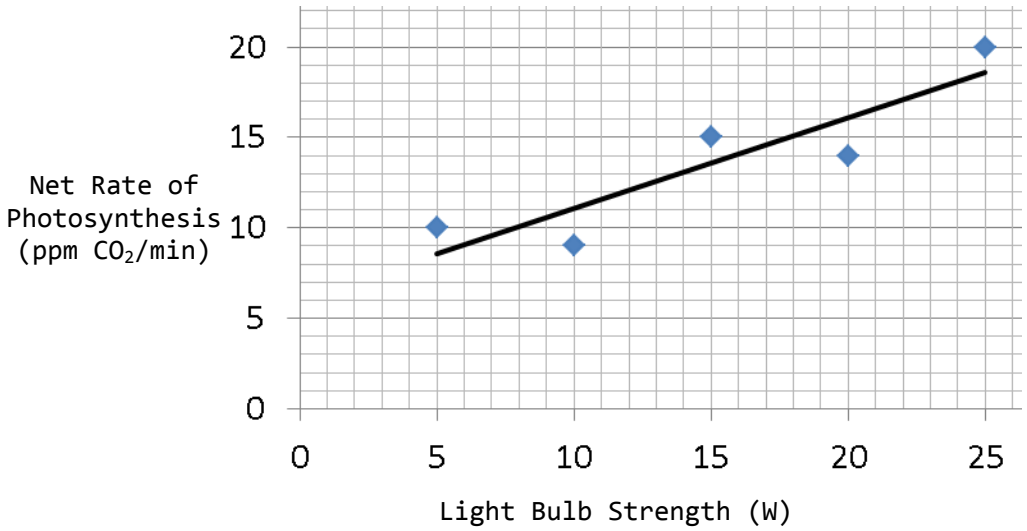


Fig 2. Sample graph of data used to demonstrate an R^2 calculation.

- Where the observed x values ($\mathbf{x_{obs}}$) were 5.0, 10.0, 15.0, 20.0, and 25.0.
- Where the observed y values ($\mathbf{y_{obs}}$) were 10.0, 9.0, 15.0, 14.0, and 20.0, with a mean of 13.6.
- Where the y values suggested by the line ($\mathbf{y_{line}}$) are 8.6, 11.1, 13.6, 16.1 and 18.6.
- Then, solving for R^2, we have:

$$R^2 = 1 - \frac{\Sigma (y_{obs} - y_{line})^2}{\Sigma (y_{obs} - \bar{y}_{obs})^2} = 1 - \frac{(10.0-8.6)^2+(9.0-11.1)^2+(15.0-13.6)^2+(14.0-16.1)^2+(20-18.6)^2}{(10.0-13.6)^2+(9.0-13.6)^2+(15.0-13.6)^2+(14.0-13.6)^2+(20.0-13.6)^2}$$

$$\mathbf{R^2 = 0.81}$$

C. Interpreting and presenting results.

The R^2 value of 0.81 indicates that 81% of the variation in y could be explained by variation in x. Generally, R^2 values of >0.7 indicate a strong, 0.4 - 0.7 a moderate, and <0.4 a weak if any relationship between the two variables. R^2 values of 1.0 are unlikely in an experiment due to 1) natural biological variation (e.g., the different plants used as replicates in this experiment would have had varying photosynthetic capacities), 2) experimental artifact (e.g., variation in the output of bulbs labeled as 5 vs. 10 Watts, etc.) and 3) experimental error (e.g., error in our ability to precisely measure the process of photosynthesis, or unseen damage to one or more plants during experimental setup). The following describes the steps you should now take in presenting and discussing your results.

1. Describing your results.
In the Results section of a report, a written description of your findings should be given that cites relevant figures or tables and might also provide the R^2 value which indicates the strength of your findings. For example,

> "Photosynthetic rate in a pea plant was found to increase in a linear fashion with increasing Wattage of the light bulb used (Fig 3, R^2 = 0.81)."

2. Visualizing your results.
Your R^2 value should be presented on your graph and your graph should be presented as formal figure that accompanies your description in the Results section of your assignment or report as follows:

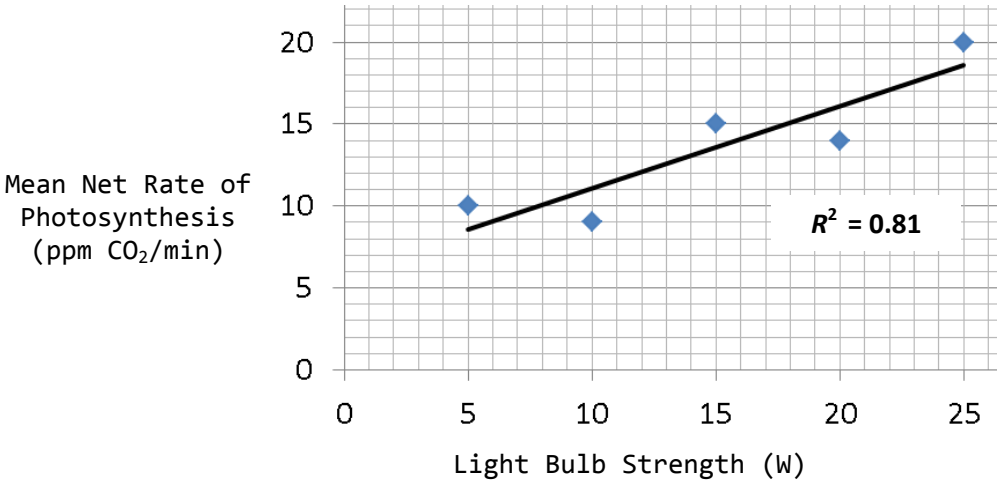


Fig 3. Photosynthetic rate in a pea plant grown using artificial light increases the Wattage of the bulb.

3. Discussing your results.
After describing your results with both written text and a figure, you must discuss potential biological explanations for your results and make conclusions from your experiment. This discussion should again cite, where appropriate, the relevant data or figures that support your conclusion(s). Your discussion should also cite literature that contains information directly relevant to and can bolster your explanation(s). The cited literature would appear in a literature cited section (not shown here). For example,

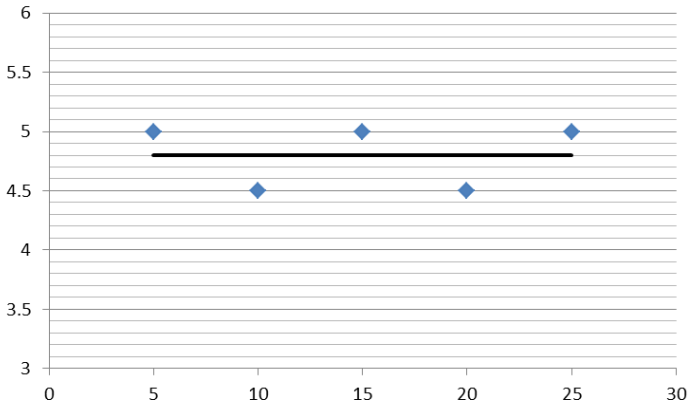
> "This study provides strong support for the hypothesis that increasing light intensity has a positive effect on the rate of photosynthesis, at least over the range of bulb Wattages for incandescent bulbs tested in this study (Fig 3). These results can be explained by the fact that as the Watts of electricity used by a bulb increases, so also increases the output of light which can then be used to drive higher rates of photosynthesis (Hoefnagels 2013). This generalization, of course, will depend on the nature of the bulb. Whereas incandescent bulbs were used in this study, fluorescent and mercury-vapor bulbs now on the market generally produce different wavelengths of visible light in different quantities and they generally utilize fewer Watts to produce that light (General Electric 2015)."

II. Practice Problems

Practice your R^2 calculations with the following sample data and graphs. You will probably want scrap paper on which to perform your calculations. Your final calculated value may not match the actual value due to you inability to precisely estimate y_{line} from the graphs. However, you should be within 0.1 to 0.01 of the actual answer. (Answers on the last page of this appendix.)

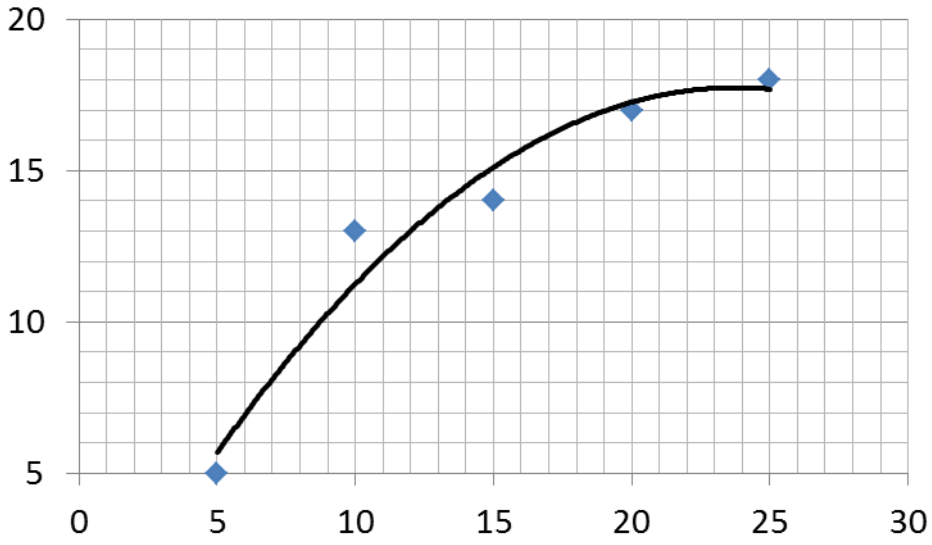
A. Sample 1. Calculate R^2 given the following data which were used to make the following graph.

x	Y
5	5
10	4.5
15	5
20	4.5
25	5



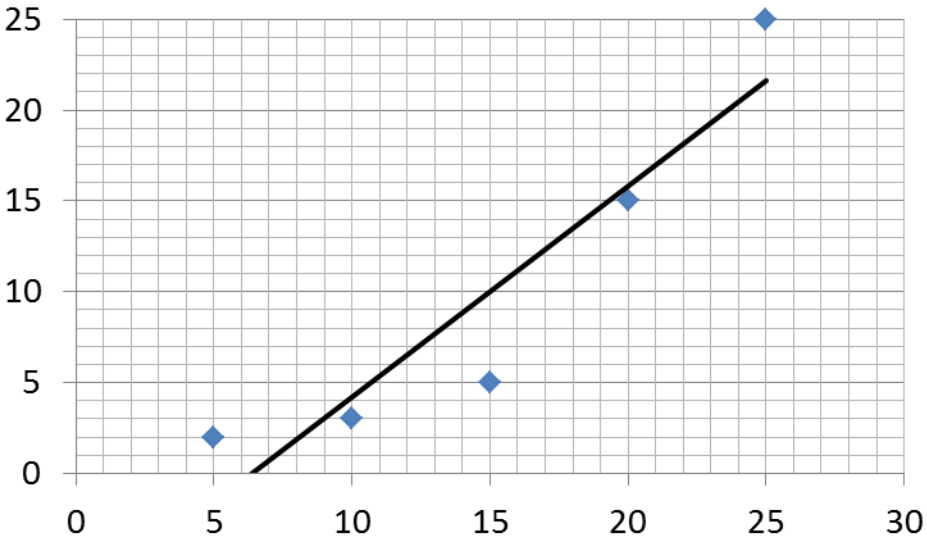
B. Sample 2. Calculate R^2 given the following data which were used to make the following graph.

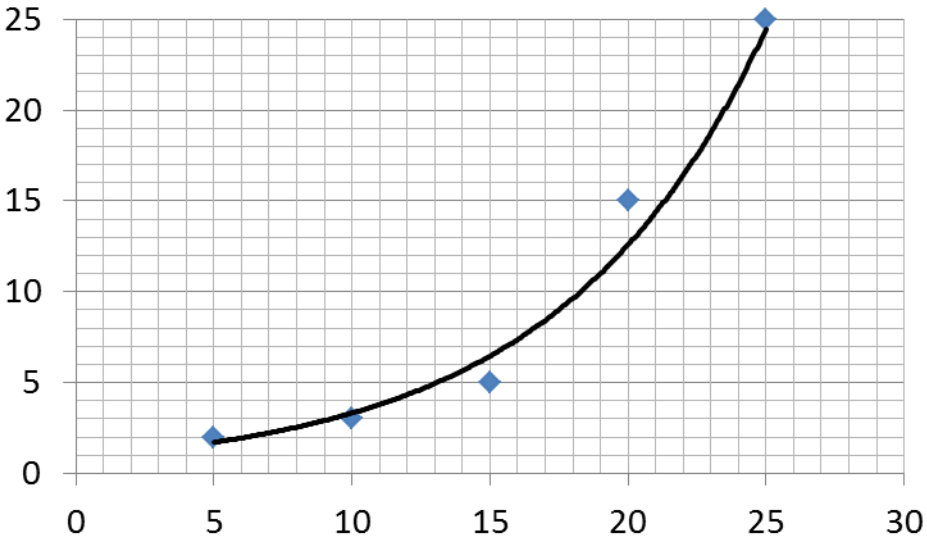
x	Y
5	5
10	13
15	14
20	17
25	18



C. Sample 3. Use R^2 to determine if the relationship between X and Y is linear (top graph) or exponential (lower graph). The line that yields the higher R^2 fits the data better.

x	Y
5	2
10	3
15	5
20	15
25	25





Credits: This appendix was developed by C.R. Hardy in January 2016.

Answers to practice problems:
A, R^2= 0.00; although the points are all very close to the line, the regression line has a slope of zero and so there is zero influence of X on Y.

B, R^2= 0.95.

C, although both graphs accurately show a positive relationship, the linear line in the top graph yields R^2= 0.87 whereas the exponential curve of the lower graph yields R^2= 0.97 with the same data. Thus, the exponential curve is a better representation of the data.