

LEAVES AND PIGMENTS

BACKGROUND

Green plants have green leaves, and the leaves are green because of the green pigment called chlorophyll that is involved in Photosynthesis. Well, yes, but it's really more complex than just this.

A leaf is designed, structurally and chemically, to optimize photosynthesis. **Photosynthesis** is the biochemical process that absorbs light energy and converts it into chemical bond energy; this chemical bond energy is within the glucose sugar that is synthesized as part of the photosynthetic process. The process of photosynthesis requires light, water and carbon dioxide (inputs), and produces glucose and oxygen (outputs). Thus it is sometimes said that a plant gets its "food" (glucose) from sunlight.

Let's focus on **LIGHT** and its capture by a cell. The visible light spectrum ranges from red (the longest wavelength) through orange, yellow, green, blue, indigo, and finally violet (the shortest wavelength). See Figure 19.12(a) of your text (Levine and Miller, 1994, 385). The green pigment that absorbs light and is directly involved in photosynthesis is called "**chlorophyll a**". As you can see in figure 19.12(a), chlorophyll a absorbs violet/blue and red light very readily but not much of the lighter blue, and green and yellow light. The lighter blue, green and yellow light that is not well absorbed by chlorophyll a is reflected back to your eye and that is why chlorophyll a looks bluish green to us!

Besides chlorophyll a, a leaf has several other pigments collectively termed **accessory pigments**; accessory pigments also absorb solar energy but they then pass this absorbed energy over to chlorophyll a for photosynthesis. "**Chlorophyll b**" is structurally only slightly different from chlorophyll a, but its absorption spectrum is somewhat different. As Figure 19.12(a) shows, chlorophyll b absorbs more of the blue range along with the red; thus chlorophyll b looks to our eyes more yellow-green. There are also a variety of accessory pigments that are collectively called **carotenoids**; these carotenoid pigments absorb a great deal in the blue and green range, and appear to our eyes as various shades of yellow or yellow-orange. Why bother having accessory pigments? These accessory pigments can absorb wavelengths of light that chlorophyll a cannot absorb effectively, and thus the plant is able to use more of the sun's energy than it could with chlorophyll a alone.

The **STRUCTURE** of a leaf is also designed to optimize photosynthesis in its cells. Leaves are **thin** so that the light can penetrate and reach all the photosynthetic cells within the leaf. Leaves of land plants are covered with a waxy **cuticle** layer to prevent water loss, but the cuticle also has the negative effect of preventing gas exchange. Therefore, the outer cell layer (epidermis) also has pores called **stomata** through which gas exchange can occur by "diffusion". Diffusion is the movement of molecules from a region of higher concentration to a region of lower concentration. (The smell of skunk diffuses very well through the air.) Carbon dioxide travels by diffusion through stomata into the leaf and excess oxygen travels by diffusion out of the leaf. Another structural feature is that the internal cells of the leaf are not all tightly packed together, but rather there are some **air spaces**, as you will see, that permit diffusion of gases through the interior spaces of the leaf to reach all the cells. Leaves also have veins of **vascular tissue** that conduct water to all the leaf cells and conduct excess sugar from the photosynthetic cells to other parts of the plant for use or storage. If glucose molecules are to be stored for later use as an energy source, they are chemically bonded to each other and the resulting structure is called **starch**. We get energy from eating starchy French fries by breaking down the large starch grains within the potato cells.

Today you will first work to set up a chromatography experiment of ground up spinach leaves. During the waiting times, you will look at several different prepared microscope slides of leaf cross sections. You will also conduct an iodine staining procedure to test for the presence of starch in leaves.

Objectives:

1. Understand the technique of paper chromatography and how it can be affected by different solvents.
2. Become familiar with the array of pigments in a green leaf, and why they are there.
3. Become familiar with the microscopic structure of a typical Angiosperm leaf (Lilac).
4. Become familiar with plant adaptations: the structural modifications found in plant leaves that exist under water (*Elodea*), that exist on top of water (Water-lily), and that live in a desert (*Yucca*).

CHROMATOGRAPHY OF AN EXTRACT OF SPINACH LEAVES**Background:**

The method of paper chromatography can be used to separate the components of a mixture of molecules—a mixture of pigments or a mixture of amino acids, for example. The mixture is spotted onto a solid support (paper); the paper's end is put into a solvent; as the solvent travels up the paper, the different types of molecules of the mixture are carried along at a rate that is determined by (1) the molecule's affinity for the solid (paper), AND (2) the molecule's solubility in the solvent. At the end of a chromatography run, the mixture will have separated out into a series of spots IF the different components of the mixture differ in their affinity and solubility properties. Note that if two different pigments happen to have the same affinity and solubility properties, then they travel at the same rate and will be in the same spot on the completed chromatogram. Chromatography of leaf pigments results in an array of spots that are colored and visible directly; chromatography of amino acids, however, results in spots that are invisible on the chromatogram and must first be dried and sprayed with a reagent that reacts with the amino acids to produce color.

In this **experiment** you will make a spinach extract, prepare seven paper strips for chromatography, and then use seven different solvents in the tubes for the running of the chromatography. The **purpose** is to determine what effect if any the different solvents have on the outcome, and to determine what would be the best solvent solutions to use for optimal pigment separation.

Procedure: (work in groups of two today)

1. Rip up several spinach leaves and then use scissors to snip the pile into tiny bits, into a mortar.
2. Just wet the pile of leaf bits with distilled water (using acetone instead also works but it is very smelly). Do not add too much liquid or the extract will be too dilute.
3. Dust with quartz sand, and use a pestle to grind the leaf bits into a pulpy soup. The liquid extract should be dark green. If the liquid extract is not dark green, then add more leaf material and grind it up.
4. Cut seven strips of chromatography paper so that they are somewhat longer than the test tubes; cut the bottom to a point and lightly draw a pencil line across each strip, about 3 cm from the point (see Fig. 1).

Fig. 1: Chromatography Strip

5. Put a Pasteur pipet into your extract, firmly put your index finger over the wide end of the pipet, lift the pipet out of the extract, and pull the tip over the pencil line—all the while keeping your index finger firmly over the wide end of the pipet. You want a line of extract that is **as narrow as possible** on the paper, and a line **not touching the edges** of the paper strip.
6. Let the line dry; apply another line of extract exactly on top of the first line and let dry; repeat until there are **at least half a dozen** applications of pigment extract on top of the pencil line. The final line should look dark green.
7. While waiting for the lines to dry, prepare your seven test tubes by putting 5 ml of solution into each tube using separate pipets and pipet pumps, and label the tubes as indicated. **(Do not breathe the fumes, and keep the solvents away from heat.)**
 - Tube 1: distilled water
 - Tube 2: 0% acetone, 100% petroleum ether
 - Tube 3: 4% acetone, 96% petroleum ether
 - Tube 4: 8% acetone, 92% petroleum ether
 - Tube 5: 12% acetone, 88% petroleum ether
 - Tube 6: 16% acetone, 84% petroleum ether
 - Tube 7: 20% acetone, 80% petroleum ether
8. When all strips have dried, label each strip with one of the solvent names and insert each strip into its corresponding solvent tube; double check the labels! The bottom tip of each strip must be immersed but be sure that **the line of pigments is never immersed in the solution!**
9. Fold the top of the strip over the edge of each tube and put a stopper on each tube.
10. Let the tubes stand **undisturbed and vertically** in a rack.
11. When the solvent front reaches the top (about 45 minutes), remove each strip (now called a chromatogram), lay it on a paper towel to dry, and outline each pigment spot with pencil.
12. Tape the strips by their tops and bottoms onto your group's hand-in sheet (pg. 7 & 8 of this lab).
13. Label each pigment spot as to whether it is **chlorophyll a** (blue-green), **chlorophyll b** (yellow-green), or **carotenoids** (yellow or yellow-orange).
14. Did the type of solvent make any difference? What is the optimal solvent for separating spinach leaf pigments? At least how many pigments are in spinach leaves? Could there be more; why? Answer on the Hand-in, which starts on pg. 7 of this lab handout.

DURING WAITING TIMES, DO THE FOLLOWING:

STARCH IN PLANTS

Background

The excess glucose sugars produced by photosynthesis are bonded together to form complexes called starch. The starch molecules form starch grains in the leaves. Sugars also get transported elsewhere via the vascular tissue called phloem; that's how potatoes under the ground end up full of starch.

Deciduous plants drop all their leaves at the start of the inhospitable season (before winter in temperate regions, before the dry hot summer in desert regions). This may seem like a terrible waste, after the plant spent all that energy making the leaves. It's not, however, because the plant breaks down whatever it can within the leaf and transports it into the twigs and stems before dropping the leaf. (Sort of like stripping a car before dumping it.) Thus the chlorophyll gets broken down, revealing the underlying carotenoids in the colorful autumn leaves, and starch also gets broken down and removed before leaf fall.

--Starch in Potatoes

1. slice an extremely thin wedge of tissue from a white potato, place it in a drop of water on a microscope slide, cover with a cover glass, and look at it with a compound microscope.
2. Look for transparent, egg-shaped starch grains that have spilled out of ruptured cells. (Other kinds of plants have other shapes of starch grains.)
3. Then add a drop of iodine solution (IKI solution) to one edge of the cover glass; iodine stains starch a deep purplish black. (NOTE: Iodine also stains clothes, so be careful!)
4. Look through the microscope and watch the clear egg-shaped starch grains turn purple as the iodine solution reaches them.

--Starch in green vs. autumn leaves

1. Get a green leaf and a yellow or orange autumn leaf.
2. Boil the leaves in a boiling water bath on a hot plate for several minutes to break open the cell walls and membranes.
3. With forceps, CAREFULLY transfer the leaf to a boiling alcohol bath sitting in a pan of water on a hot plate. Boil in alcohol for several minutes to extract leaf pigments.
4. With forceps, CAREFULLY transfer the leaf to a petri dish containing iodine solution. Areas with starch will turn purplish black. You can remove excess stain by placing the leaf in a petri dish of water.
5. Did your green leaf and autumn leaf show the same staining? Explain on the Hand-in.

LEAF STRUCTURE and ADAPTATIONS

There is a typical leaf structure for many flowering plants that optimizes success in mesic (moderate) terrestrial environments. Plants in other environments have evolved adaptations that promote survival in those other conditions. Some flowering plants such as *Elodea* have returned to a submerged aquatic (hydric) lifestyle. Other hydric plants like Water-lilies float their leaves on the water surface. Even other plants such as *Yucca* grow in the desert and have additional features to promote survival in this difficult xeric (dry) environment.

You will look at the following prepared leaf cross-sections, answering the questions on the Hand-in:

- Lilac (*Syringa*) is a mesophyte (“phyte” means plant) ;
- Water-weed (*Elodea*; old name *Anacharis*) is a submerged hydrophyte;
- Water Lily (*Nymphaea*; old name *Castalia*) is mostly submerged except for the emergent flowers and floating leaf blades.
- Yucca (*Yucca*) is the xerophyte.

Lilac Leaf cross section

1. Refer to Figure 30.22 of your text (Levine and Miller, 1994, 617)
2. Note the colorless **upper and lower epidermis** of the leaf; its function is simply protection, not photosynthesis. The waxy **cuticle** layer on the outside of the epidermal cells is generally so thin on a mesophyte such as Lilac that it is hard to see.
3. Note the pores (**stomata**; plural of stoma) in the lower epidermis; each stoma is surrounded by a pair of **guard cells** that can change shape and close the pore when the leaf is losing too much moisture.
4. Note the tightly packed **palisade mesophyll** layer just beneath the upper epidermis; it is tightly packed in order to capture as much of the incoming light as possible.
5. Note the **spongy mesophyll** layer in the lower half of the leaf; this layer is full of air spaces to allow the gases carbon dioxide and oxygen to diffuse freely.
6. Note the leaf veins, with the vascular tissue: the thick-walled wide-bore xylem cells carry water into the leaf; the thin-walled smaller phloem cells conduct sugars to other parts of the plant.

The *Elodea* leaf

--Structure

For flowering plants that have returned to an aquatic habitat, the several structural features that promote survival on land are of no benefit in water and thus a waste of energy to make. Thus aquatic flowering plants have lost the terrestrial adaptations over evolutionary time.

Answer the questions on your Hand-in sheet.

1. Is there any waxy cuticle layer visible? Why does this make sense?
2. Can you see thick-walled wide-bore cells of xylem in the leaf vein? Why does this make sense?
3. Compare the thickness of the *Elodea* leaf to the Lilac leaf.
4. Do you see any fiber cells (very thick-walled & small-bore)? Why does this make sense?

--Chloroplasts and Cytoplasmic Streaming in *Elodea*

1. Place an *Elodea* leaf in a drop of water on a microscope slide, and cover with a cover glass. Examine the leaf cells under a compound microscope.
2. Within each cell, look for the green pill-shaped discs that are the organelles called the **chloroplasts**. The chloroplasts are specialized for performing **photosynthesis**. ...Where do you suppose the chlorophyll molecules are located in a plant cell??
3. Watch the chloroplasts for several minutes as the leaf is exposed to the bright microscope light. Do you see the chloroplasts very slowly moving around in the cell? [You may be able to see this most clearly in cells located at the edge of the leaf, where the leaf is only one cell layer thick.] This slow movement is called **cytoplasmic streaming**; the **cytoplasm** is the liquid portion of the cell; the plant cell's cytoplasm streams, carrying the chloroplasts along with it.
4. Do the chloroplasts stream freely throughout the volume of the cell or is the streaming restricted to a certain region? Note that the center of a plant cell is occupied by a large clear membrane-bound bag-like organelle called the **vacuole**. The vacuole is the cell's toxic waste storage and recycling center. The chloroplasts are in the cytoplasm surrounding this large centrally located vacuole.

The Water-lily Leaf

1. Look at the prepared slide of the cross section of a Water Lily leaf (which floats on the water surface) and compare the leaf structure to that of the Lilac leaf (which is surrounded by air).
2. Where are the stomata on the water lily leaf and in the lilac leaf? Does the location make sense for each? Explain.
3. Which of these two kinds of leaves has the largest air spaces in the spongy mesophyll? How would this be an adaptation to its environment?

The Yucca Leaf

1. Look at the prepared slide of the cross section of a Yucca leaf under a compound microscope.
2. Can you see the waxy cuticle? Which of the above four plants had the thickest cuticle? Why does this make sense?
3. Look at the vascular bundles scattered throughout the leaf section. Locate the cluster of very thick-walled, small-bored fibers on either side of each vascular bundle. How could these long stiff leaf fibers be of benefit to the Yucca, and why did the other plants examined not have them?

Note: Native Americans extracted these bundles of fibers and used them as string. (Moerman, 1999)

☛Some additional tidbits:...

FIBERS from different plants have different properties. According to Crane & Company which has continuously supplied the paper for U:S paper money since 1879, your **Dollar bill** is made of recovered **cotton** (long cells on the seed coat) and **flax** (fibers in the stem). (Anonymous, 1999) Flax is what “linen” is made of. Flax is two to three times as strong as cotton fibers (Simpson and Ogorzaly, 1986, 490) and enhances the paper currency’s resistance to tearing and deterioration. (Put a dollar bill and a piece of regular paper made of wood pulp (xylem) in your jeans and toss them in the washer; which one survives intact?) . The scattered little red and blue strands in paper money are silk—from silk glands (modified salivary glands) of a silk worm (caterpillar of the silk moth).

Industrial Hemp is a variety of *Cannabis sativa* that was selectively bred for maximal stem fibers and minimal THC, while a different variety called **marijuana** is from the same plant species but it was selectively bred for high levels of the psychoactive chemical THC. According to the North American Industrial Hemp Council’s web site (Anonymous, 1999), hemp fibers are longer, stronger and more mildew-resistant than cotton; in addition, hemp paper is very long-lasting and doesn’t yellow, and thus is preferred for Bibles. The American forefathers widely grew hemp (for fibers)!

Literature Cited

- Anonymous Crane & Company “Continuum Paper: Old Money” (<http://www.crane.com/products/continuum/old-money.html>) 1999
- Anonymous North American Industrial Hemp Council, Inc. “The Comprehensive Information Source for the Hemp Industry” (<http://www.naihc.org>) 1999.
- Levine, J. and Miller, K. (1994) Biology: Discovering Life, Second Edition. D. C. Heath and Company, Lexington, MA.
- Moerman, Dan (Department of Anthropology at The University of Michigan--Dearborn) “Native American Ethnobotany Database” (<http://www.umd.umich.edu/cgi-bin/herb>) August, 1999.
- Simpson, B. and Ogorzaly, M. (1986) Economic Botany: Plants in Our World. McGraw-Hill Book Company, New York, NY.

HAND-IN (DUE at end of lab period)

NAMES of group members:

Below, tape your seven labeled chromatograms **in order**; then label each pigment spot (**chl a**, **chl b**, **carotenoid**)

Over →

1. Did the type of solvent make any difference in the separation of the leaf pigments? ____ What is the optimal solvent for separating spinach leaf pigments? _____
2. At least how many pigments are in spinach leaves? ____ Could there be more; why?
6. Did your green and autumn leaves show the same staining? Explain why.

Elodea

7. Is there any waxy cuticle layer visible? Why does this make sense?
8. Can you see thick-walled wide-bore cells of xylem in the leaf vein? ____ Why does this make sense?
9. Compare the thickness of the *Elodea* leaf to the Lilac leaf. How is this beneficial to the *Elodea*?
10. Do you see any very thick-walled, very thin-bore fiber cells? ____ Why does this make sense?
11. Draw a leaf cell from *Elodea* (at right), showing and labeling the chloroplasts and vacuole.

Water-lily

12. Where are the stomata on the water-lily leaf and in the lilac leaf? Does the location make sense for each? Explain.
13. Does a lilac or a water lily leaf have the largest air spaces in the spongy mesophyll? How would this be an adaptation to its environment?

The Yucca Leaf

14. Which of the four different kinds of leaves had the thickest cuticle? _____
Why does this make sense?
15. How could the bundles of long stiff leaf fibers be of benefit to the xerophyte *Yucca*, and why did the other plants examined not have them?