Leaf Structure and Pigments

The objectives of this lab exercise are that you:

- Learn about the roles of pigments in photosynthesis and other functions of plants.
- Understand the basic principles of paper chromatography.
- Learn about basic leaf structure and how it relates to environmental adaptation
- Use the results of the pigment exercise for the writing of a lab report to improve your writing skills and ability to convey information accurately and precisely.

I. Introduction to Leaf Pigments

This part of the lab exercise will be the basis for writing the next lab report.

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

A leaf has evolved, chemically and structurally, to optimize photosynthesis (Greek: photo = light). The overall function of the biochemical process of photosynthesis is to absorb light energy and convert it into chemical bond energy that is then useable by the plant; this chemical bond energy is within the glucose sugar which is synthesized by the photosynthetic process. Thus, it is sometimes said that a plant gets its “food” (glucose) from sunlight. The “inputs” required by photosynthesis are light, carbon dioxide and water, and the “outputs” produced are glucose and oxygen. Your textbook provides greater detail of the biochemical process involved in photosynthesis.

\[
\text{Photosynthesis} \quad \begin{array}{c}
6\text{CO}_2 + 6\text{H}_2\text{O} + \text{light} \\
\rightarrow \\
\text{C}_6\text{H}_{12}\text{O}_6 + 6\text{O}_2
\end{array}
\]

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), and plants possess pigments that can absorb light in specific regions of the spectrum (see Figure 1). One of the green pigments that absorbs light for use in photosynthesis is called “chlorophyll a”; it readily absorbs violet/blue and red light but not much of the lighter blue, and green and yellow light. “Chlorophyll b” is structurally only slightly different from chlorophyll a, but its absorption spectrum is somewhat different. Chlorophyll b absorbs more in the blue and orange-red ranges. Thus, chlorophylls appear green because the pigments absorb light in all of the other color ranges, and only green is transmitted to our eyes. Due to the slightly different absorption spectra, chlorophyll a looks bluish green, while chlorophyll b looks yellowish green.
In addition to producing chlorophyll, leaves have evolved to produce several other pigments, collectively termed accessory pigments, which absorb solar energy for photosynthesis. Why bother having accessory pigments? Accessory pigments absorb wavelengths of light that chlorophyll cannot absorb effectively, enabling the plant to use more of the sun’s energy. One family of accessory pigments is called carotenoids. As shown in Figure 1, carotenoids absorb light from violet into the greenish-blue range; as a result carotenoids appear in various shades of yellow or yellow-orange to our eyes.

A third class of pigments is the anthocyanins. Unlike the chlorophylls and carotenoids, anthocyanins do not participate in photosynthesis and may appear red, purple, or blue. Anthocyanins occur widely among higher plants, and are the pigments that generally give color to flowers, but also occur in leaves and fruits. In leaves, these pigments often help to protect against excessive sunlight that can damage some leaf tissues. This is one reason why a young, newly developing leaf is often redder than when it reaches its mature size.

**Paper Chromatography**

Paper chromatography can be used to separate the components of a mixture of molecules, such as a mixture of pigments or a mixture of amino acids. When performed for leaf pigments, the result is a series of bands that are colored and visible directly (Figure 1). To perform the procedure, the mixture is spotted onto a strip of paper, and then the paper’s end is put into a solvent. The solvent travels up the paper, and 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 paper, and 2) the molecule’s solubility in that solvent. At the end of a chromatography run, the mixture will have separated 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 will end up in the same position on the completed chromatogram. Alternatively, if a pigment is not soluble in the solvent used or binds tightly to the paper, it will not move.

For the lab report:
- **Experimental purpose:** to determine the types and relative abundance of pigments in leaves from four different plants.
- **Hypothesis:** All of the plant tissues tested will contain photosynthetic pigments.
II. Analysis of plant pigments in leaves from different plants

Procedures: (Work in groups of two today)

Note: Two organic solvents are used in this lab exercise, acetone and petroleum ether. While these solvents are not generally considered to be toxic, they are volatile, and you should avoid breathing the fumes and keep the solvents away from heat and other ignition sources.)

A. Preparing the chromatography tubes.
1. Prepare 8 test tubes by putting 3 ml of the solvent solution (12% acetone, 88% petroleum ether) into each tube using a pipet and pipet pump. Loosely fit the rubber stoppers onto the tubes. Place the tubes on your bench to allow the solvent fumes to fill the tubes.

B. Preparing the paper strips and test-tubes (each student should prepare a set of strips for each plant type)
1. Get eight strips of chromatography paper. Cut the bottom of each strip to a point and using a pencil lightly draw a line across each strip 3 cm from the point and 2 cm from the top as shown in Figure 3.
2. Write the plant name at the very top of one of the paper strips. Place a few layers that leaf type under the pigment loading line of the chromatogram, and using a hard edge (e.g., scissor edge, coin), press down hard on the paper along the pencil line to crush the leaf and allow the pigments to be absorbed into the paper.
3. Repeat step 2 for each of the other three leaf types.
4. Allow the chromatograms to dry (~ 5 minutes).
5. Repeat steps 2 - 4 to obtain a dark but thin line of pigment on each of the chromatograms.

During the drying times, read the introduction section about leaf structure and complete the activities on

C. Running the chromatograms
1. When all strips have dried, insert each strip into a 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!
2. In each tube, loosely place a stopper.
3. Let the tubes stand undisturbed and vertically in a rack.
4. When the solvent front reaches the top line (about 20 minutes), remove each strip (now called a chromatogram) and lay it on a paper towel to dry.

D. Solvent disposal
1. Carefully pour any remaining solvent from your test tubes into the container designated for this purpose.
2. Leave the test tubes to dry in the hood, and return the rubber stoppers to your work bench.

Figure 3. Where to draw lines on chromatogram.
E. Mounting the chromatograms and data collection

1. **Tape the strips** by their tops and bottoms onto the chromatogram sheet on the next page. Write the names of each plant above each chromatogram, and **number the positions** of the pigments from 1 (at load line) to 6 (at solvent front).

2. During the lab period **draw an outline** around the region of each pigment spot, and summarize the information about the leaf pigments in Tables 1 – 4.

*** If neatly prepared, the Tables can be included directly in your report; however, messy Tables will not be acceptable and must be retyped.

Describe all of the **common features** of the chromatograms you mount in Figure 1:
Figure 1. Pigment Chromatograms from leaves of four plant species.

<table>
<thead>
<tr>
<th>Pigment band number</th>
</tr>
</thead>
<tbody>
<tr>
<td>_______</td>
</tr>
<tr>
<td>_______</td>
</tr>
<tr>
<td>_______</td>
</tr>
<tr>
<td>_______</td>
</tr>
</tbody>
</table>
Table 1. Pigments found in Spinach leaf.

Describe the visual coloration of this leaf: _______________________________________

<table>
<thead>
<tr>
<th>Pigment</th>
<th>Color</th>
<th>Pigment Type*</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 (at top)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 (at bottom)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Describe the distinctive features of the chromatogram and the types of pigments found in this leaf

Table 2. Pigments found in __________ leaf

Describe the visual coloration of this leaf: _______________________________________

<table>
<thead>
<tr>
<th>Pigment</th>
<th>Color</th>
<th>Pigment Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 (at top)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 (at bottom)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Describe the distinctive features of the chromatogram and the types of pigments found in this leaf
Table 3. Pigments found in ________________ leaf

Describe the visual coloration of this leaf: ____________________________________________

<table>
<thead>
<tr>
<th>Pigment</th>
<th>Color</th>
<th>Pigment Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 (at top)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 (at bottom)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Describe the distinctive features of the chromatogram and the types of pigments found in this leaf

Table 4. Pigments found in ________________ leaf

Describe the visual coloration of this leaf: ____________________________________________

<table>
<thead>
<tr>
<th>Pigment</th>
<th>Color</th>
<th>Pigment Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 (at top)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 (at bottom)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Describe the distinctive features of the chromatogram and the types of pigments found in this leaf
III. Introduction to Leaf Structure

Leaf structure has evolved to optimize photosynthesis in its cells. **What are the functions of different parts of the leaf?**

- **Mesophyll cells.** The mesophyll cells are the photosynthetic cells of the leaf, capturing sunlight and using the energy to convert CO$_2$ into carbohydrates.

- **Epidermis and cuticle.** These are the protective layers on the surfaces of the leaf. The epidermis is composed of cells, and the cuticle is a waxy layer on the surface that minimizes water loss.

- **Stomata** (singular = ‘stoma’). These are pores through which gases (O$_2$, CO$_2$, and H$_2$O) can move into and out of the leaf. They are straddled by a pair of **guard cells** that can open or close the pore under different conditions.

- **Vascular tissue.** This is the tissue through which water and nutrients pass into and out of the leaf to other parts of the plant. Cells of the xylem carry water and nutrients up from the roots, whereas carbohydrates produced in the leaves are transported out in the phloem.

- **Fibers.** Fibers are bundles of long, thick-walled cylindrical cells that provide rigidity and support to leaves of some plants.

**What are some of the characteristics of plants living in different environments?**

Plants living in different physical environments have leaves with specialized adaptations that promote survival under those conditions. Of particular importance is the **availability of water.**

- **Mesophytic plants** live in environments with a moderate annual rainfall.

- **Aquatic (hydrophytic) plants,** such as Elodea, grow submerged in water, whereas other hydric plants like water-lilies float their leaves on the water surface.

- **Xerophytic plants,** such as Yucca, that grow in the desert and have other adaptations to promote survival in this difficult xeric (dry) environment.

**What are some adaptations of leaves to different water availability?**

- **Leaf thickness:** thin leaves maximize sunlight exposure and gas-exchange for photosynthetic cells; whereas thick leaves contain specialized cells used for water storage.

- **Thickness of the cuticle:** generally thicker in drier environments to reduce water loss.

- **Abundance of fibers:** may be needed to prevent ‘wilting’ under dry conditions and to discourage herbivores.

- **Other specializations:** such as unusually large air spaces, in the mesophyll, for floatation.
Where do the pigments occur in plant cells?

- **Mesophyll cells** possess specialized structures (or ‘organelles’) called **chloroplasts** where photosynthesis and the photosynthetic pigments are located.
- The **vacuole** usually is the location of pigments not involved in photosynthesis. The vacuole a large cellular structure that also serves as a storage place for water and nutrients. The vacuole often fills much of the internal volume of the cell, pushing the rest of the cytoplasm to the outer edge.

Some additional tidbits about fibers …

Fibers from different plants have different properties. Your dollar bill is made of about 75% **cotton** (long cells on the seed coat) and about 25% **linen** (fibers in the stem of flax plants). Flax fibers are two to three times as strong as cotton fibers (Simpson and Ogorzaly, 1986, 490) and enhance 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 synthetic fibers but prior to WWI were silk. You may know that silk is produced by the silk glands of a silk worm caterpillar of the silk moth. Crane & Company has continuously supplied the paper for U.S. paper money since 1879.

**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. The North American Industrial Hemp Council claims on its web site that 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 a preferred paper for Bibles.

IV. Laboratory investigation of leaf structure

Summarize the functions of the different parts of a leaf

<table>
<thead>
<tr>
<th>Leaf Structure</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>A.</td>
<td></td>
</tr>
<tr>
<td>B.</td>
<td></td>
</tr>
<tr>
<td>C.</td>
<td></td>
</tr>
<tr>
<td>D.</td>
<td></td>
</tr>
<tr>
<td>E.</td>
<td></td>
</tr>
</tbody>
</table>

Identify the types of plants that live in different environmental regions:

<table>
<thead>
<tr>
<th>Name</th>
<th>Environment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td></td>
</tr>
</tbody>
</table>
In-Class Comparison of Leaf Structures

As you look at the prepared leaf cross-sections (or living *Elodea*) answer the questions on the lab exercise pages.

**A. Lilac Leaf (*Syringa*) - a mesophyte ("phyte" means plant)**

1. While looking at the Lilac leaf cross-section under the microscope, find all of the structures labeled A-E in the diagram to the right. **Add the names of the structures to the diagram.**

2. The ________________ of the leaf consists of a single layer of cells that protect the internal parts of the leaf. On the outer surface of this layer in lilac leaves, a waxy ___________ layer is present but may be too thin to see clearly.

3. Note the pores in the lower epidermis. These pores are called ____________, and each is surrounded by a pair of ______________ that can change shape and close the pore when the leaf is losing too much moisture. These pores are on the bottom side of the leaf. **Why does this location help the plant conserve water?**

4. There are two layers of photosynthetic ________________ cells in this leaf; those near the ________________ (upper / lower) surface are **tightly packed** whereas those near the ________________ (upper / lower) are **loosely arranged**; this layer is full of air spaces to allow the gases carbon dioxide and oxygen to diffuse freely.

5. Note the leaf veins, with the vascular tissue. Cells of the __________ carry water into the leaf. These cells have a large diameter and thick cell walls. Cells of the __________ conduct sugars to other parts of the plant. These have a smaller diameter and thin cell walls. **Label these two regions of the vascular tissue in the diagram to the right.**
B. The Water-lily Leaf (*Nymphaea*) - Leaves floating on water surface.

1. **Look at the prepared slide of the cross section of a Water Lily leaf** and compare the leaf structure to that of the Lilac leaf (which is surrounded by air).

2. On which side of the water lily leaf are the stomata located? 
   
   Why is this different than for the lilac leaf?

3. Why do you think this plant has such large air spaces in the spongy mesophyll?

4. In the diagram to the right, label the mesophyll, air chambers and vascular bundle.

C. The *Elodea* leaf (Water-weed) is a submerged hydrophyte

   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 plants have lost some terrestrial adaptations over evolutionary time.

1. **Look at the permanently mounted leaf cross-section.** Notice that there is no cuticle layer. Why does this make sense for *Elodea*?

2. Notice that there are no thick-walled wide-bore cells (xylem) in the leaf vein. Considering the function of xylem discussed above, **why does its absence in leaves of *Elodea* make sense?**

3. Make a wet mount of an *Elodea* leaf and observe it under 10X and 40X objective lenses. Under the 40X objective find a cell in which you can clearly see how the **chloroplasts** are distributed. ‘Twiddle’ the fine focus up and down and notice that sometimes the cell looks like Figure ‘A’ and sometimes like Figure ‘B’. Read about the vacuole above, and then explain why the **distribution of the chloroplasts seems to change**: 

   (Also, look for movement of the Chloroplasts – this is called ‘cytoplasmic streaming’.)
D. The *Yucca* Leaf - a xerophyte

1. Look at a prepared slide of the cross section of a *Yucca* leaf under a compound microscope. Find and identify the waxy cuticle? Of the above four plants observed, Yucca has the thickest cuticle. **Why does this make sense?**

2. Look at the vascular bundles scattered throughout the leaf section. Locate the cluster of fibers (thick-walled, small-bored cells) fibers on either side of each vascular bundle. See also the other bundles of fibers scattered through the leaf. (Note: Native Americans extracted bundles of yucca fibers and used them to make cords, rope, nets, baskets, etc.) **How do these long stiff fibers benefit the Yucca?**

3. Notice the arrangement of the photosynthetic cells. **Why are they located just below the epidermis and not distributed throughout the leaf?**

4. There are numerous large cells in the center region of the leaf that are specialized for holding water. Why are these found in the Yucca leaf but not the Lilac leaf?

5. In the image to the right, label the epidermis, stoma, cuticle, vascular bundles, fiber bundles and mesophyll.

E. Summarize your findings

Which plant species has leaves with:

<table>
<thead>
<tr>
<th>Plant name</th>
<th>Habitat</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Abundant fibers:</td>
<td></td>
</tr>
<tr>
<td>2. Air spaces for flotation:</td>
<td></td>
</tr>
<tr>
<td>3. Cuticle that is</td>
<td></td>
</tr>
<tr>
<td>Thick:</td>
<td></td>
</tr>
<tr>
<td>Thin:</td>
<td></td>
</tr>
<tr>
<td>Absent:</td>
<td></td>
</tr>
<tr>
<td>4. Water storage cells:</td>
<td></td>
</tr>
</tbody>
</table>
Lab report for the leaf pigment lab exercise

For this report you will turn in:
1. Results (Description of Results and the Figure)
2. Discussion (Conclusion, Explanation of Results, Future Experiment)
3. Literature Cited

Results
Describe the results of your chromatogram in paragraph form. Expected length is 2-3 full paragraphs.

In the “Description of results” section:
• A description the order in which the spots appear on the chromatograms (1 to 6, from bottom to top), the colors of each spot, and which pigments corresponded with each spot. (Base this upon the description of common features you wrote for Figure 1.)
• Describe the appearance of each leaf and the distinctive features of its chromatogram. Which pigment(s) occurred in each leaf type, and which types of pigments were most abundant? (Base this upon your summary of the results for Tables 1 – 4)
• Describe any other observations of the chromatograms that has importance to the interpretation of the results.
• When describing results, be sure to the Figures and tables in which the results are presented. (e.g., “… as shown in Figure 1…”).

Figure and Tables
Include the page with your chromatogram securely taped in place and with the plant names and pigment names labeled. You can use the Tables on the other side if they are neatly written; if not retype them (or download a clean copy from the Biol 105 web page).

Discussion section
The Discussion section will discuss the results in context of broader meanings and in context of information from literature sources. Expected length is about 2 full pages. Your discussion should include the following sections and cover the following topics:

Conclusion: Assuming that the hypothesis of the exercise was that “All leaves contain photosynthetic pigments,” do your results support the hypothesis?
The “Explanation of Results” section you should discuss:
• What are the functions of chlorophyll, carotenoid, and anthocyanin type pigments? Cite and reference literature source(s) for this information.
• Which spots correspond to which types of pigments? Cite in “(author(s), year, pg #)” format and reference literature source(s) that supports this interpretation.
• Which leaves appeared to be capable of carrying on photosynthesis, or not? What is the evidence for this? If the tissue is not photosynthetic, what other function may it be serving? Cite and reference a literature source that supports these interpretations.
• Why might leaves of different plants have different amounts of pigments; how might this relate to their growth environment or other evolutionary adaptations? (Cite source information.)

Future experiment: What new question do your results raise in your mind; and what would be a follow-up experiment that could help answer that question? Merely repeating with more samples is not sufficient.
**Literature Sources**

A minimum of three outside (non-text book & non-lab manual) sources should be used, including one from each of the three categories listed below. Over-reliance on “inside” sources will detract from the final grade. Include references to all cited literature sources in the formats described in the Lab Report Guidelines.

**Materials on reserve in the library:**

***These references are intentionally incomplete and must be rewritten with additional information and properly formatted in your lab report***

**Photosynthesis in general**


Chapter 12 is on photosynthesis is nicely illustrated. As an advanced text it goes into more detail that you need for this report, but it has a nice overview of the photosynthetic process and the photosynthetic pigments. (Complete this reference this as a chapter in a book.)


Chapter 4 provides a nice general overview of photosynthesis and use of solar energy. (Since the chapters do not have separate authors, so complete this reference as a book. Note: Govindjee has no first name, sort of like Usher.)


Provided is the beginning of Chapter 6 on photosynthesis; a good general introduction to the topic. (Since chapters do not have separate authors, complete this as reference to a book.)

**Carotenoids**


This paper tends to focus on structure and synthesis of carotenoids, it does have a nice section on the “Function of Carotenoids”.  (Complete this reference this as an article from a journal)


Section B has an overview of the functions of carotenoids that are related to light absorption or not related to light. Verlag (Complete this as a reference to a chapter in a book.)

**Anthocyanins**


(Complete this reference this as an article from a journal)


This chapter provides a lot of useful information about the roles of anthocyanins. (Complete this reference as a chapter in a book edited by Gould and Lee.)


Each of the subsections (beginning with 1.2) each begins with a synopsis of the different functions of anthocyanins. (Complete this as a reference to a chapter in a book.)