

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.



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). The green pigment 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.

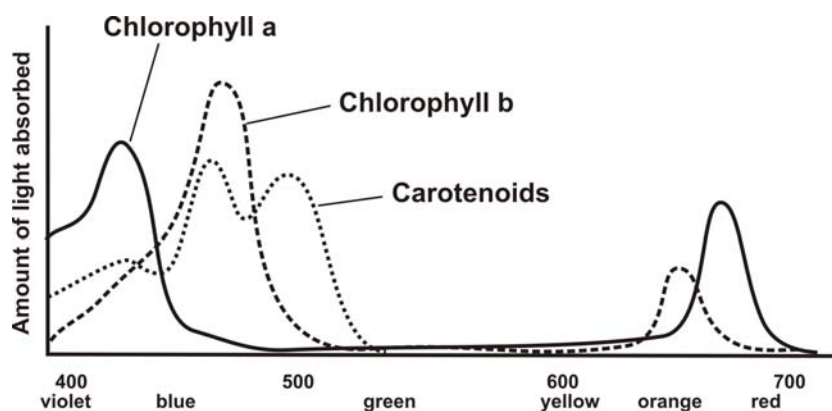


Figure 1. Absorbance spectrum of different photosynthetic pigments.

In addition to producing chlorophyll, leaves have evolved to produce several other pigments collectively, termed **accessory pigments**, that 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, carotenoid 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 are 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 allowed to dry, 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.

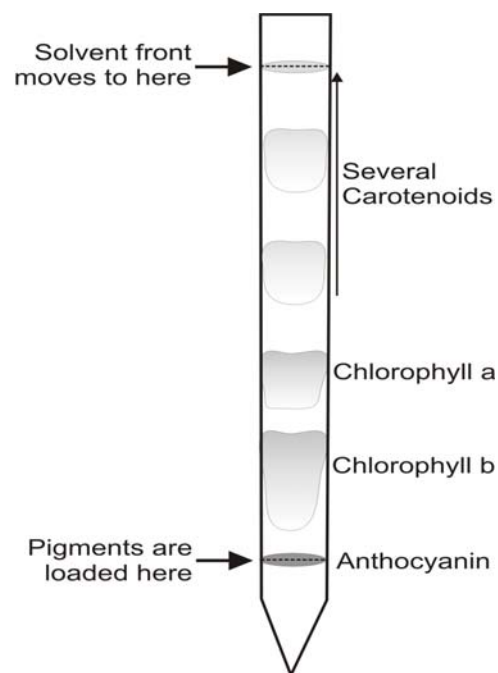


Figure 2. Completed paper chromatogram.

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)

A. 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 3 cm from the top as shown in Figure 3.
2. Cut a leaf into a strip slightly narrower than the width of the chromatography paper. Avoid the center vein and any other unpigmented regions.
3. Write the plant name at the very top of the paper strip. Using a hard edge (e.g., scissor edge, coin), press down and crush the leaf along the pencil line to transfer pigments to the paper. Repeat 4-8 times to obtain a **dark but thin line of pigment** on the paper.
4. Repeat steps 2 & 3 for each of the other three leaf types.
5. Allow the pigment lines to air dry.
6. While waiting for the lines to dry, 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 pumps. Loosely fit the rubber stoppers onto the tubes. (**Do not breathe the fumes, and keep the solvents away from heat and other ignition sources.**)

B. 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), lay it on a paper towel to dry.

During waiting time, read the introduction section about leaf structure

C. 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.

D. 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 a circle** 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.

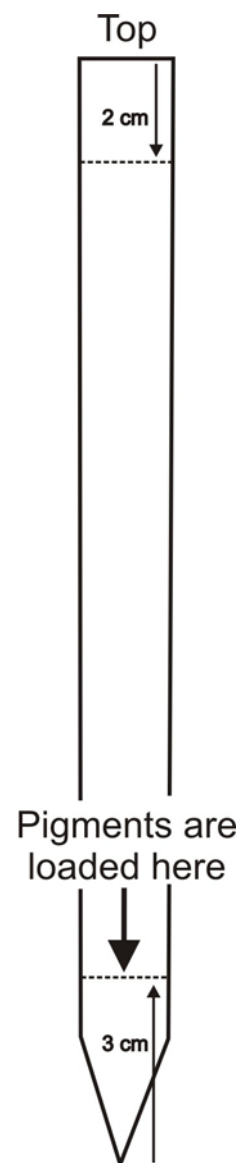
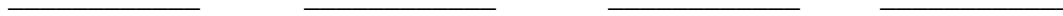


Figure 3. Where to draw lines on chromatogram.

Figure 1. Pigment Chromatograms from leaves of four plant species.



Pigment
number

Table 1. Pigments found in Spinach leaf

Pigment	Color	Pigment Type
6 (at top)		
5		
4		
3		
2		
1 (at bottom)		

Table 2. Pigments found in _____ leaf

Pigment	Color	Quantity*	Pigment Type
6 (at top)			
5			
4			
3			
2			
1 (at bottom)			

*compared to Spinach: record as Absent, Less, Same, More

Table 3. Pigments found in _____ leaf

Pigment	Color	Quantity*	Pigment Type
6 (at top)			
5			
4			
3			
2			
1 (at bottom)			

*compared to Spinach: record as Absent, Less, Same, More

Table 4. Pigments found in _____ leaf

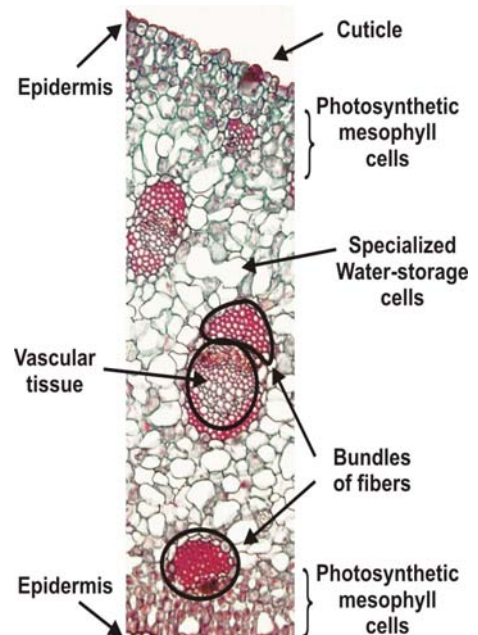
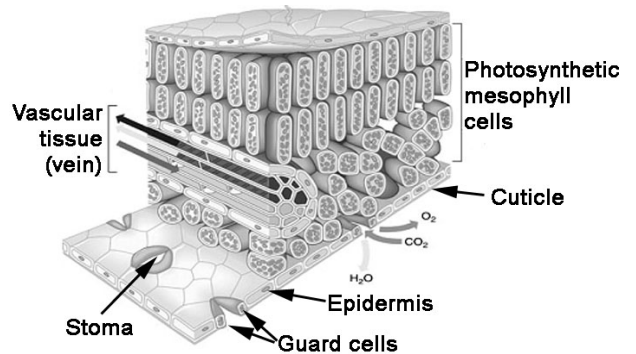
Pigment	Color	Quantity*	Pigment Type
6 (at top)			
5			
4			
3			
2			
1 (at bottom)			

*compared to Spinach: record as Absent, Less, Same, More

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_2O) 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 cylindrical cells that provide rigidity and support to leaves of some plants.



Cross-section of a leaf from a xerophytic plant.

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**.

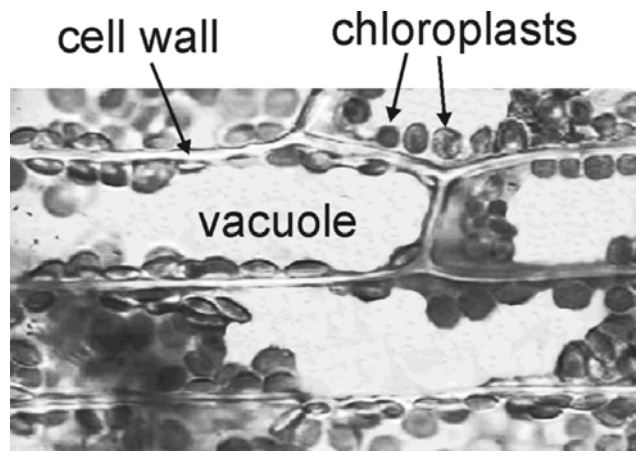
- **Mesophytic plants** live in environments with a moderate annual rainfall.
- **Aquatic (hydrophytic) plant**, such as *Elodea* grow submerged in water, whereas other hydric plants like water-lilies float their leaves on the water surface.
- **Xerophytic plants**, such as *Yucca*, that grow in the 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 maximum 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 air spaces 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.

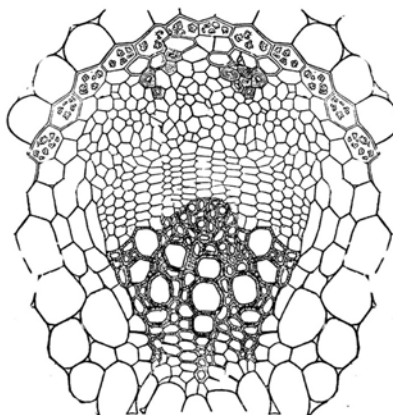
Lewis R, Parker B, Gaffin D, Hoefnagels M. 2007. Life, 6th ed. Boston (MA): McGraw-Hill; 1012p.
Simpson B, Ogorzaly M. 1986. Economic botany: plants in our world. New York (NY): McGraw-Hill.

IV. 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. The _____ of the leaf consists of a single layer of cells that protect the internal parts of the leaf. In lilac leaves the waxy _____ layer is present but may be too thin to see clearly.
2. 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?**
3. 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.
4. 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.

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).

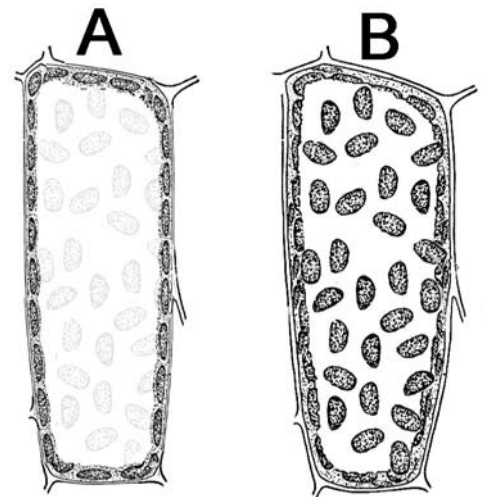
1. On which side of the water lily leaf are the stomata located? _____ Why is this different than for the lilac leaf?
2. Why do you think this plant has such large air spaces in the spongy mesophyll?

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. First look at the permanently mounted leaf cross-section.

1. 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. Can you see 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?

E. Summarize your findings

Which plant species has leaves with:

	<u>Plant name</u>	Habitat
1. Abundant fibers:	_____	_____
2. Air spaces for floatation:	_____	_____
3. Cuticle that is		
Thick:	_____	_____
Thin:	_____	_____
Absent:	_____	_____
4. Water storage cells:	_____	_____

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. Your description of results should include:

- A description of the colors of each pigment type.
- The types of pigments found in leaves from the different plants.
- Which pigment(s) tended to be most abundant in each leaf type?
- Significant differences in the quantity of the pigment between leaf types .
- Be sure to cite your Figure at some point (e.g., "... as shown in Figure 1...").

Figure

Include the page with your chromatogram securely taped in place and with the plant names and pigment names labeled.

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 cover the following topics:

Conclusion: Do the results support the hypothesis?

In the "Explanation of Results" section you should discuss:

- Did all of the leaves contain the same types of pigments? What is the evidence for identifying each pigment type? Cite a literature source that supports this interpretation in "(author(s), year, pg #)".
- Which leaves appeared to be capable of carrying on photosynthesis, or not? What is the evidence for this; which pigments are used (or not) for photosynthesis? Cite a literature source that supports this interpretation in format as described in the lab report guidelines.
- Among those leaves that can carry out photosynthesis, do they all appear to use the same wavelengths of light? What is the evidence for this? Cite a literature source that supports this interpretation.
- Do all of the leaves possess non-photosynthetic pigments? What is the evidence that these are non-photosynthetic? Why might some leaves contain non-photosynthetic pigments? Cite a literature source that supports your interpretation.

Future experiment: What new question do these 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-lab manual) sources should be used, including both articles and books available in the library. Over-reliance on the lab manual will detract from the final grade. Include references to all cited literature sources in the formats described in the Lab Report Guidelines.

Literature sources available for the Leaf pigment lab report

(Note: never cite information in abstracts; cite information from the body of the article.)

Books on reserve in the library (do not check these out)

Buchanan BB, Gruissem W, Jones RL. 2000. Biochemistry and molecular biology of plants. Rockville (MD): American Society of Plant Physiologists; 1367p

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.

Gould KS, Lee DW, eds. 2002. Anthocyanins in leaves. Vol. 37 of Advances in Botanical Research. Amsterdam (The Netherlands): Academic Press.

The chapters by Lee and Gould “Anthocyanins in leaves and other vegetative organs: an introduction” and by Lee “Anthocyanins in leaves: distribution, phylogeny and development.” provide a lot of useful information about the roles of anthocyanins. Note: these sources must be referenced as a chapter of an edited book, and described in the lab report guidelines.

Rabinowitch E, Govindjee. 1969. Photosynthesis. New York (NY): John Wiley & Sons, Inc; 273p.

Chapter 4 provides a nice general overview of the photosynthesis and use of solar energy. (Note: Govindjee has no first name, sort of like Madonna.)

Articles available on-line

Bartley GE, Scolnik PA. 1995. Plant carotenoids: pigments for photoprotection, visual attraction, and human health. *The Plant Cell*, 7: 1027-1038.

Available through JSTOR; this paper tends to focus on structure and synthesis of carotenoids, it does have a nice section on the “Function of Carotenoids”.

Kong J-M, Chia L-S, Goh N-K, Chia T-F, Brouillard R. 2003. Analysis and biological activities of anthocyanins. *Phytochemistry* 64: 923-933.

Available through the Electronic Journal Center; these sections should be particularly useful

3. Functions of anthocyanins
4. Biological activities of anthocyanins

Nishio JN. 2000. Why are higher plants green? Evolution of the higher plant photosynthetic pigment complement. *Plant, Cell and Environment*. 23: 539-548.

Available through the Electronic Journal Center; although this paper gets rather technical quickly, the introduction contains information pertinent to the roles of photosynthetic pigments.