BIOLOGY 1101
LAB 3: PHOTOSYNTHESIS

READING: Please read chapter 7 in your textbook prior to lab.

INTRODUCTION: Photosynthesis is the process by which certain organisms use energy from sunlight and carbon from carbon dioxide (CO_2) to make simple carbohydrates like glucose, which can be used as an energy source or converted to starch and stored in cells. Because of this capability, photosynthetic organisms are said to be **autotrophic** ("auto" = self, "trophe" = food; make their own food). While the best known photosynthetic organisms are plants, there are others including some bacteria and a variety of protists (such as green algae). The evolution of photosynthesis had a great impact on life on Earth. The first photosynthetic organisms (**cyanobacteria**) appeared on Earth about 2.7 billion years ago and over the next billion years, oxygen gas (O_2) released as a byproduct of photosynthesis built up in the atmosphere. This favored the evolution of **cellular respiration**, an oxygen-requiring process that all higher organisms depend on to break down glucose for energy, and resulted in a much greater diversity of unicellular and multicellular life. By 500 million years ago, plants were making the move to land, and animals followed soon thereafter. Photosynthetic organisms (plants) are now the dominant form of life on Earth and provide the food (energy) that supports most animal life, either directly or indirectly. We will focus on photosynthesis in plants for the remainder of the lab.

During photosynthesis, light energy is used to convert carbon into **glucose** ($C_6H_{12}O_6$), which is a simple sugar, or **monosaccharide.** Glucose molecules can later be bonded together using **dehydration synthesis** to form **polysaccharides** (complex carbohydrates) like starch or cellulose. The summary reaction of photosynthesis is:

$$\mathbf{6\ CO_2 + 6\ H_2O + \text{light energy} \rightarrow C_6H_{12}O_6 + 6O_2}$$

The “reaction” above occurs in two stages inside the chloroplasts of plant cells. The first stage is the **light-dependent reaction**, (see below) during which energy from sunlight is captured and converted to chemical energy, in the form of ATP and NADPH. In this step, water molecules (H_2O) are broken apart, releasing electrons and hydrogen ions for use in stage two of photosynthesis, and releasing oxygen as a waste gas. Oxygen then diffuses out of leaf tissue through **stomata** (singular: stoma), or pores, in the leaf surface that allow gas exchange across the waxy, protective cuticle. The overall light-dependent reaction is:

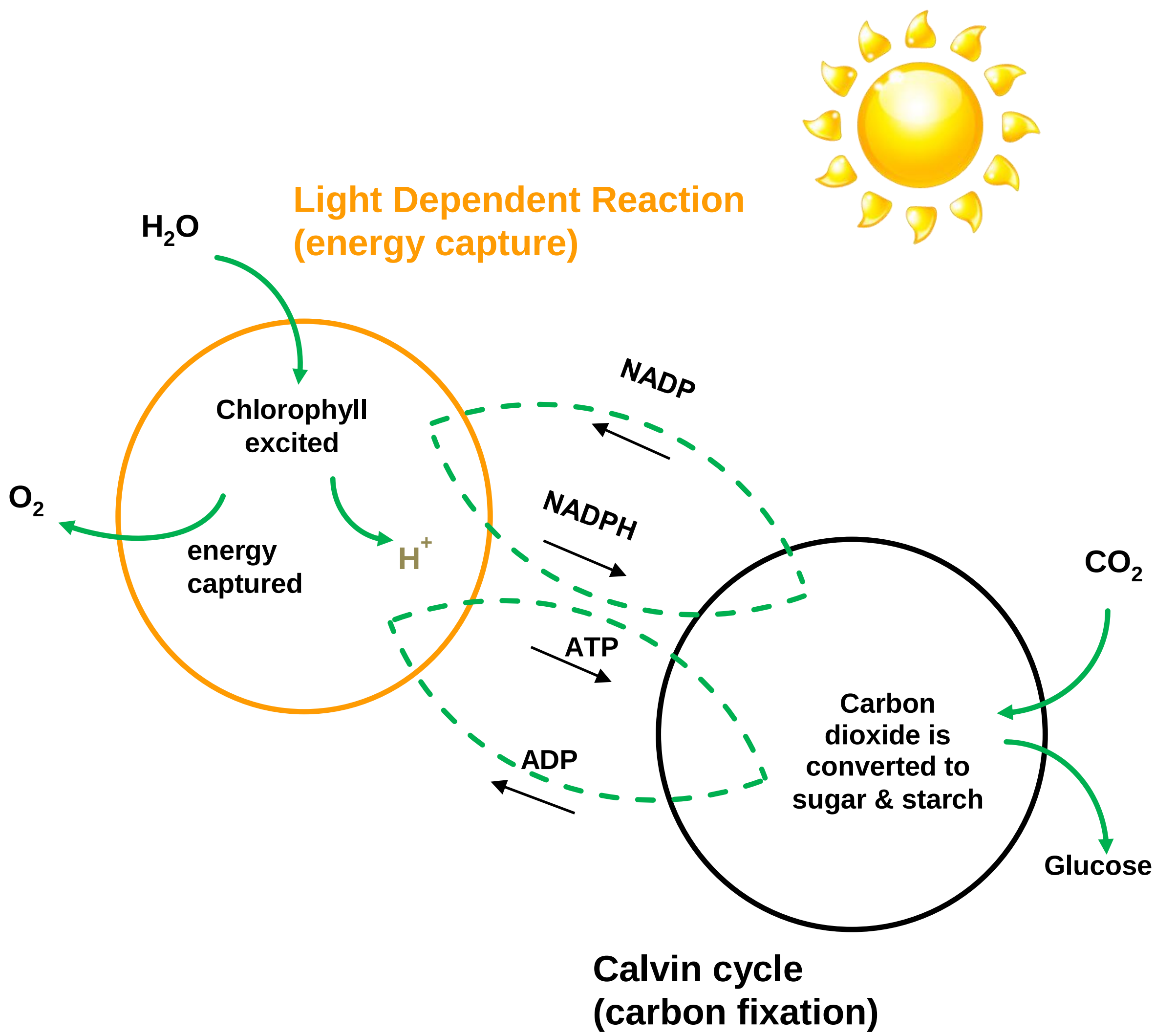
$$12\ H_2O + 12\ NADP^+ + 18\ ADP + 18\ P \rightarrow 6\ O_2 + 12\ NADPH + 18\ ATP$$

The energy stored in the chemical bonds of ATP and NADPH is used to power the second stage of photosynthesis, called the **<u>Calvin cycle</u>** (or **<u>light-independent reactions</u>**). In this stage, carbon is "fixed" in the biosphere when it is converted from an inorganic form (CO_2) to an organic form (simple sugars). CO_2 molecules diffuse into leaf tissue through the stomata at the same time that O_2 diffuses out. The overall Calvin cycle reaction is:

$12\ NADPH + 18\ ATP + 6\ CO_2 \rightarrow C_6H_{12}O_6 + 12\ NADP^+ + 18\ ADP + 18\ P + 6\ H_2O$

Glucose produced during photosynthesis can later be broken down to provide energy through the process of **<u>cellular respiration</u>**, a chemical reaction that is essentially the reverse of photosynthesis. Cellular respiration requires oxygen, and CO_2 is released as a byproduct. All higher organisms (including plants and animals) respire. The overall respiration reaction is the opposite of photosynthesis:

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



LABORATORY OBJECTIVES: The purpose of this laboratory exercise is to introduce you to the process of photosynthesis. You will have a better understanding of the two main reactions of photosynthesis. You should be familiar with the reactants and products of each step in the process and understand how we can measure photosynthesis indirectly through changes in leaf tissue buoyancy. You will also become familiar with leaf structures such as stomata, which allow for the diffusion of O_2, CO_2, and H_2O in and out of leaf tissue, and mesophyll cells, which house the plant's photosynthetic organelles.

EXERCISES:

Part 1. You will look for evidence that photosynthesis is taking place in spinach leaves that have been cut into small disks and placed in a bicarbonate solution. When CO_2 is dissolved in water, it may convert to bicarbonate and carbonate ions:

$$CO_2 + H_2O \rightarrow \underset{\text{bicarbonate}}{HCO_3^{-}} + H^+ \rightarrow \underset{\text{carbonate}}{CO_3^{2-}} + 2H^+$$

You will use a bicarbonate solution to ensure there is plenty of inorganic carbon available for photosynthesis.

Each group will treat spinach leaf disks to remove air from intracellular spaces (see below). The spinach leaves will be placed in bicarbonate solution in a plastic cup and set in front of a light source. As the disks photosynthesize, oxygen, which is produced as a byproduct, will fill the intracellular spaces and make the leaf tissue buoyant. Groups will check the spinach leaves at 1-minute intervals and record the number of disks that have risen to the top of the cup at each interval.

<u>Materials</u>

- Beaker with ~300ml sodium bicarbonate solution
- Liquid soap
- 2 plastic syringes
- 2 rubber pipette bulbs
- 2-3 spinach leaves
- Hole punch
- Paper towels
- 2 plastic cups
- Timer
- Lamp

<u>Procedure</u> (Read the entire section before you start):

1. Work in groups of 3-4 students.
2. Using the hole-punch, gently punch out 24 leaf disks from the spinach leaves, avoiding large veins. Replace any disks that are torn or incomplete.

3. Remove the plungers from the syringes and place 12 leaf disks in each syringe. Replace the plunger and slowly press the plunger down until it is near the bottom of the syringe, taking care not to damage the leaf disks.
4. Add one *small* drop (think half-drop) of liquid soap to the sodium bicarbonate solution (the soap helps wet the leaf and allow gas and liquid to penetrate the leaf disks). Submerge the first syringe in the sodium bicarbonate solution and draw about 3cm of solution into the syringe. Hold the syringe upright (plunger facing down) and swirl the contents until all the leaf disks are floating in solution.
5. Place a rubber pipette bulb securely over the syringe tip. Pull the plunger down to create a strong vacuum. Hold this position for 10 seconds, then slowly let go, to break the vacuum.
6. Swirl the contents; you should start to see some leaf disks begin to sink (when you create a vacuum, you pull all the air out of the leaf tissue, and the disks lose their buoyancy). Repeat step 5 until all leaf disks have sunk to the bottom of the solution. If you have trouble getting the leaf disks to sink, you may need to pull harder on the plunger to create a stronger vacuum.
7. Repeat steps 4-6 with the second syringe.
8. Once all leaf disks have sunk to the bottom of the each syringe, carefully remove the plungers and pour the contents into separate clear plastic cups. Add bicarbonate solution to the cups until they are about half full.
9. Place one cup in a bench drawer away from all light. Place the second cup in front of the lamp, about 5 cm away from the bulb. Start the timer.
10. Every minute, record how many leaf disks have risen to the top of the solution and are floating on the surface.
11. Record the length of time it takes for all leaf disks to become completely buoyant in the table below.

Minutes	**# disks - light**	**# disks - dark**	**Minutes**	**# disks - light**	**# disks - dark**
1			11		
2			12		
3			13		
4			14		
5			15		
6			16		
7			17		
8			18		
9			19		
10			20		

12. Compare your results with those of other groups. Are they similar or different? What variables might have caused groups to obtain different results?

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13. What do you think would happen to the floating leaf disks if you removed the light source? Why?

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Part 2a. Plants open their **stomata** (pores) to allow CO_2 molecules to diffuse into the cells for use in photosynthesis. When stomata are open, H_2O molecules simultaneously diffuse out of the cells into the surrounding environment. Water loss is only an issue when plants are growing in arid environments where there is not much water in the soil to replace what is lost from leaves. Consequently, some plants have adapted structures or mechanisms that aid in maintaining **turgor** (water pressure within the plant cells).

You will examine leaf tissue under a microscope to identify certain leaf structures and describe their role in photosynthesis. You will compare leaf tissue of *Coleus* sp., a C_3 tropical dicot to that of *Dracaena* sp., a C_3 monocot that is adapted to arid and semi-arid environments. The goal is to see whether you can identify any differences in the stomata that might be associated with the environment to which they are adapted.

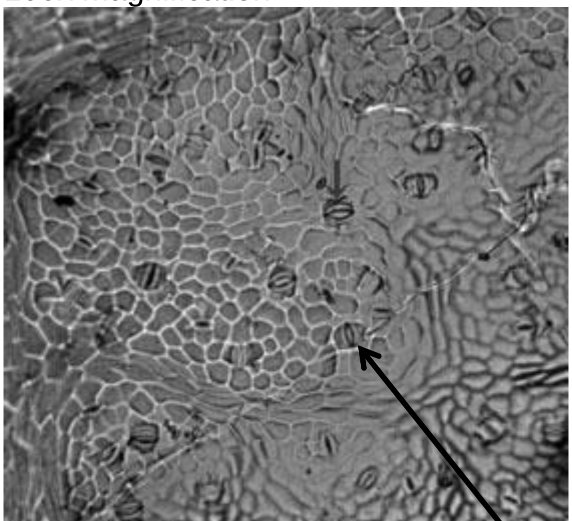
Materials

- A compound microscope
- Packet of lens paper
- Access to glass cleaner (ask your instructor)
- 2 clean slides
- A piece of ***Coleus*** leaf
- A piece of ***Dracaena*** leaf
- 1 set of forceps and scissors
- Distilled water bottle
- A prepared slide of plant leaf tissue

1. **If necessary, review the instructions for microscope use beforehand.** Start with live leaf tissue. Cut a piece of *Coleus* leaf about $1cm^2$ in area (*note:* avoid using the lavender/white center portion of the leaf). Place the *Coleus* leaf on the slide with the **underside** (leaf bottom) facing upwards and mount it on the stage. Starting with the lowest power (50x magnification), bring the leaf cells into focus. Switch to 100x, and then 400x magnification, using the fine focus knob to resolve the image.
2. Try to identify stomata (pores) in the leaf epidermis. Use the photos at the top of the next page as a guide:

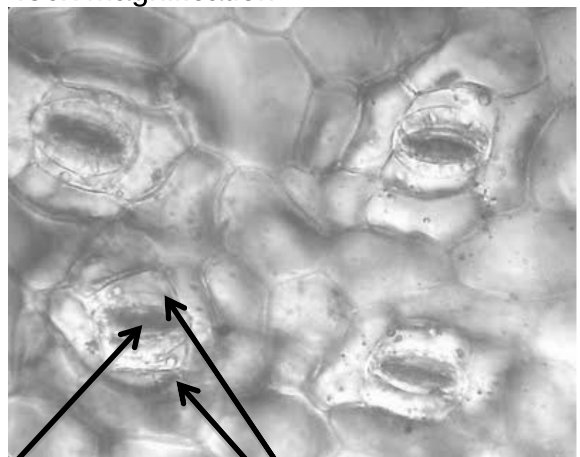
Leaf Epidermis and stomata

100x magnification



Courtesy University of California at Davis

450x magnification



Stoma at 100x (left) and 450x (right)

Guard cells (2 per stoma)

3. *Draw what you see under the microscope*:

100x:

400x:

4. Count the number of stomata in the viewing frame at three different places on the leaf tissue*. Record your counts and calculate an average:*

Count 1 __________

Count 2 __________

Count 3 __________ Average ____________

5. Repeat steps 1-3 using *Dracaena* sp. **NOTE**: Cut across the Dracaena leaf and then cut along each edge of the raised central vein to so each piece will lay flat on the slide. If necessary, place a second slide on top to keep the tissue flattened. Mount the *Dracaena* leaf so that the top side is what you view under the microscope. *Draw what you see:*

100x: 400x:

6. Count the number of stomata in three places, as before. *Record counts and calculate an average:*

Count 1 ___________

Count 2 ___________

Count 3 ___________ Average _____________

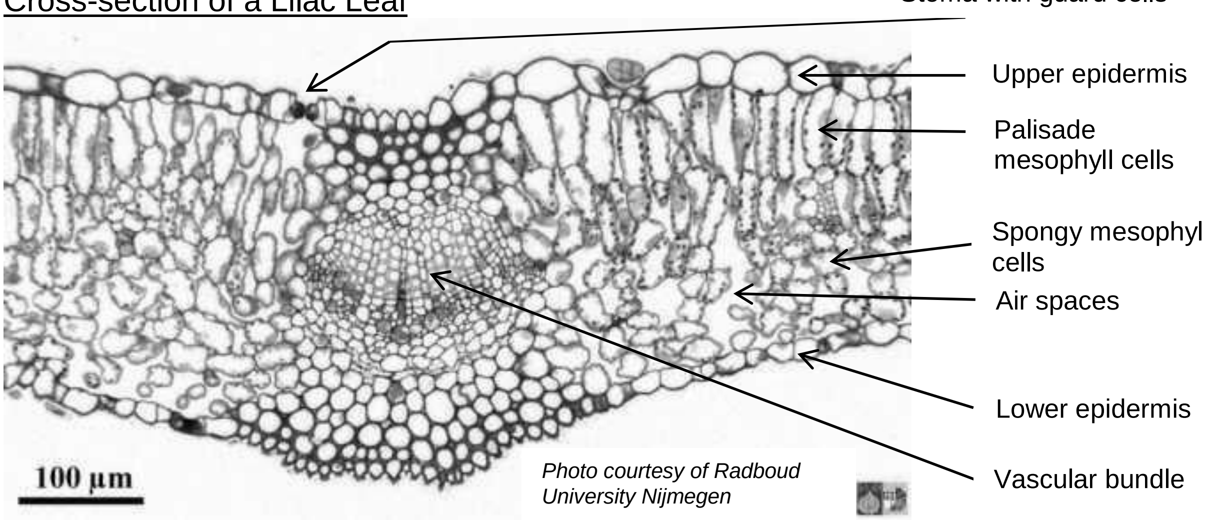
7. *How do the two species compare in terms of the number of stomata per unit area? Does the species adapted to a dry environment have more, less, or similar numbers of stomata as the species adapted to a moist environment? Why do you think this is the case?*

Part 2b. Some plants produce both “sun” leaves and “shade” leaves. Sun leaves receive much more sunlight and, consequently, are susceptible to greater water loss (warmer temperatures increase evaporation rates). Shade leaves are limited by the amount of sunlight they receive, and so must maximize their ability to capture light energy. Sun leaves tend to be smaller and thicker, with a lower surface-to-volume ratio, to minimize water loss. Shade leaves tend to be broad and thin, with a high surface-to-volume ratio, to capture as much energy as possible in low-light conditions.

You will examine a prepared slide of cross-sections of pear leaves (*Pyrus* sp.). Each slide contains four cross-sections: two from a shade leaf and two from a sun leaf. You will observe differences at the cellular level between the two leaf types. Try to determine which cross-section is from which leaf type.

1. Mount the prepared slide on the microscope stage. Start with the lowest magnification (50x) and bring the leaf cross-section into focus. Switch to 100x magnification. Compare what you to see to the following photograph:

<u>Cross-section of a Lilac Leaf</u>



8. Observe all four cross-sections on your prepared slide. Two are from a sun leaf and two are from a shade leaf. Can you identify what's different between them? *Draw what you see:*

100x Sun leaf cross-section

100x Shade leaf cross-section

9. *What are the major differences in cellular structure between sun and shade leaves? How do these differences relate to the environmental conditions that sun and shade leaves experience (e.g., heat and low light levels, respectively)?*

Comprehension Questions

Make sure you understand and can answer the following questions, as the concepts may appear on a lab exam. You do not need to answer them during the lab period.

1. What does it mean for an organism to be called "autotrophic"? What are some types of autotrophic organisms?

2. What important changes did photosynthesis bring about that influenced the evolution of higher organisms (single and multicelled eukaryotes) about 1 billion years later?

3. Plants capture light energy and use it to convert carbon in CO_2 to glucose, a simple carbohydrate, called a ________________________. Later, glucose can be converted to more complex carbohydrates like starch. Complex carbohydrates are known as ____________________________.

4. During the light-dependent reaction, light energy is converted to two types of chemical energy, __________ and ___________.

5. Plants open stomata to allow CO_2 molecules to diffuse into leaf cells for use in photosynthesis. Why are open stomata potentially detrimental to plants growing in arid (dry) or semi-arid environments?

6. Plants in arid and semi-arid environments have evolved adaptations to prevent excessive water loss. What is one such adaptation, based on your observations of *Dracaena*? Can you think of other possible adaptations to arid conditions that might be found in other plant species?

7. Leaves on the same plant often encounter different environmental conditions, such as sun and shade, and consequently are structurally different. What are the characteristics of sun and shade leaves that enable them to maximize photosynthetic ability while minimizing water loss?

Extra space for drawings or notes: