INVESTIGATION 5

PHOTOSYNTHESIS

What factors affect the rate of photosynthesis in living leaves?

BACKGROUND

Living systems require free energy and matter to maintain order, to grow, and to reproduce. Energy deficiencies are not only detrimental to individual organisms, but they cause disruptions at the population and ecosystem levels. Organisms employ various strategies that have been conserved through evolution to capture, use, and store free energy. Autotrophic organisms capture free energy from the environment through photosynthesis and chemosynthesis, whereas heterotrophic organisms harvest free energy from carbon compounds produced by other organisms. In multicellular plants, photosynthesis occurs in the chloroplasts within cells.

The process of photosynthesis occurs in a series of enzyme-mediated steps that capture light energy to build energy-rich carbohydrates. The process is summarized by the following reaction:

$$2\ H_2O + CO_2 + \text{light} \rightarrow \text{carbohydrate } (CH_2O) + O_2 + H_2O$$

To determine the net rate of photosynthesis, one could measure one of the following:

- Production of O_2
- Consumption of CO_2

The difficulty related to measuring the production of oxygen is compounded by the complementary process of aerobic respiration consuming oxygen as it is produced. Therefore, measuring oxygen production is equivalent to measuring net photosynthesis. A measurement of respiration in the same system allows one also to estimate the gross production.

Generally, the rate of photosynthesis is calculated by measuring the consumption of carbon dioxide. However, equipment and procedures to do this are generally beyond the reach of most introductory laboratories.

In Getting Started, students conduct prelab research on the process of photosynthesis and review concepts they may have studied previously — particularly concepts about the properties of light.

In the first part of the lab, students learn how to measure the rate of photosynthesis indirectly by using the floating leaf disk procedure to measure oxygen production. Alternatively, they could explore how to measure the rate of photosynthesis using various probes interfaced to computers.

In the floating leaf disk procedure, a vacuum is used to remove trapped air and infiltrate the interior of plant (leaf) disk samples with a solution containing bicarbonate ions that serve as a carbon source for photosynthesis. The infiltrated leaves sink in the

bicarbonate solution. When placed in sufficient light, the photosynthetic processes then produce oxygen bubbles that change the buoyancy of the disk, eventually causing them to rise.

Students should develop the skills necessary to implement the selected procedure so that they can explore their own questions about photosynthesis in Designing and Conducting Your Investigation. Procedure serves as a structured inquiry that is a prerequisite for open inquiry into the variables that may affect photosynthesis.

First, during class discussions, students consider a number of variables that might affect the rate of photosynthesis in plants — both physical variables and biotic variables. Likewise, students consider variables that might affect the floating disk procedure itself. These variables are compiled and categorized to serve as a guide for student questions and experimental design, as illustrated in Table 1.

Table 1. Variables Affecting Rate of Photosynthesis

Environmental Variables	Plant or Leaf Variables	Method Variables (These variables may not affect photosynthesis but are still important to investigate.)
• Light intensity (brightness) • Light color (How can students explain that plants are green and that chlorophyll does not absorb green light?) • Temperature • Bicarbonate concentration (CO_2 source) • Direction of incoming light • pH of solution	• Leaf color (chlorophyll amount) • Leaf size • Stomata density • Stomata distribution • Light-starved leaves vs. leaves kept in bright light • Type of plant • Leaf age • Leaf variegation • Role of respiration in plants along with photosynthesis — measuring gross photosynthesis	• Size of leaf disk • Depth of bicarbonate solution • Methods of cutting disks • Leaf disk overlap • Soap amount • How many times can the procedure be repeated with the same disks? • How long can the disks remain sunk in the solution — can they be stored overnight? • Method of collecting data

Once students learn how to measure the rate of photosynthesis and have discussed a number of variables that might be measured, questions should emerge about the process that leads to independent student investigations.

One advantage of the floating disk technique is that the equipment and supplies required are inexpensive, so nearly every classroom environment can provide ample supplies for individual student investigations.

Finally, students design and conduct an experiment(s) to investigate one or more questions that they raised in Procedure. Their exploration will likely generate even more questions about photosynthesis.

For students who try but are unable to develop questions of their own, consider the following supplemental prompts:

- What makes plants stop growing? Could any of these affect photosynthesis?
- Do all leaves look the same? What is different? Could these differences affect photosynthesis?

The lab also provides an opportunity for students to apply, review, and/or scaffold concepts that they have studied previously, including the relationship between cell structure and function (chloroplast); enzymatic activity (especially rubisco, if temperature as a variable is explored); strategies for capture, storage, and use of free energy; diffusion of gases across cell membranes; behavior of gases in solution; evolution of plants and photosynthesis (including an explanation of why plants don't absorb green light); and the physical laws pertaining to the properties of buoyancy.

Note About Light Sources: A strong light source is necessary for success in this procedure. Some of the best results have been obtained when placing the cups of leaf disks on the bed of an overhead projector. Another inexpensive light source is the "work spotlights" that you can purchase from various retail stores, coupled with 100-watt equivalent compact fluorescent bulbs.

PREPARATION

Materials and Equipment

- Baking soda (sodium bicarbonate)
- Liquid soap (approximately 5 mL of dishwashing liquid or similar soap in 250 mL of water)
- 2 plastic syringes without needles (10 mL or larger), available from biological and scientific supply companies or rather cheaply at large chain drugstores (ask for 10 mL oral medicine dispensers). It is a good idea to have extra syringes on hand, as some students may need more than two for their independent investigations.
- Living leaves [spinach, especially baby spinach from the produce section of the grocery story, or ivy (*Hedera helix*), which is perennially green and naturalized throughout the country]
- Hole punch
- 2 clear plastic cups
- Timer
- Light source (Inexpensive light sources include the clamp lights purchased at big-box stores coupled with 100-watt equivalent compact fluorescent bulbs. These lights do a great job of producing the low-heat, high-intensity light needed for this work.)
- Students invariably underestimate the various light parameters in this procedure. An important piece of equipment to include in any classroom when studying photosynthesis is a PAR meter (photosynthetically active radiation). A PAR meter counts photons in the PAR spectrum. A PAR meter will greatly facilitate experimental design. The sample graphs included in this lab investigation measured light intensity with an outdated measurement, the foot candle, which is a subjective measure of luminance not closely related to PAR flux.

Timing and Length of Lab

The prelab questions and online preparation and review activities suggested in Getting Started can be assigned for homework.

The first part of the investigation requires one lab period of about 45 minutes to introduce the methods of either procedure. The second part, Designing and Conducting Your Investigation, requires approximately two lab periods of about 45 minutes each for students to conduct their own investigations. If interfaced sensors are available and students know how to use them, students can begin working on the procedure outlined in the first part. Another suggestion is to have students design their experiment(s) as a homework assignment; lab groups can communicate through various social networking sites or by email. Teachers also should dedicate a third lab period for students to share their results and conclusions with the class by appropriate means, such as a mini-poster session, an oral presentation, or a traditional lab report.

Students can work as pairs, trios, or small groups to accommodate different class sizes and equipment availability.

Safety and Housekeeping

The primary safety issues in this lab have to do with solutions near electric lights. Caution students to observe proper care with solutions near lights. Because students will be working in close proximity to exposed lightbulbs, be sure to require eye protection in the form of safety goggles. Moreover, some high-intensity light sources get extremely hot. If you are using these, advise students not to drip water on them (shatter hazard) or to lean against a light (burn hazard). Most but not all syringes are capable of withstanding the vacuum created in this procedure without failure. However, you should test the syringes beforehand.

ALIGNMENT TO THE AP BIOLOGY CURRICULUM FRAMEWORK

This investigation can be conducted during the study of concepts pertaining to cellular processes (big idea 2), specifically, the capture, use, and storage of free energy, or interactions (big idea 4). In addition, some questions students are likely to raise connect to evolution (big idea 1). As always, it is important to make connections between big ideas and enduring understandings, regardless of where in the curriculum the lab is taught. The concepts align with the enduring understandings and learning objectives from the AP Biology Curriculum Framework, as indicated below.

Enduring Understandings

- 1B1: Organisms share many conserved core processes and features that evolved and are widely distributed among organisms today.
- 2A1: All living systems require constant input of free energy.
- 2A2: Organisms capture and store free energy for use in biological processes.

- 2B3: Eukaryotic cells maintain internal membranes that partition the cell into specialized regions (e.g., chloroplasts).
- 4A2: The structure and function of subcellular components, and their interactions, provide essential cellular processes.
- 4A6: Interactions among living systems and with their environment result in the movement of matter and energy.

Learning Objectives

- The student is able to describe specific examples of conserved core biological processes and features shared by all domains or within one domain of life, and how these shared, conserved core processes and features support the concept of common ancestry for all organisms (1B1 & SP 7.2).
- The student is able to justify the scientific claim that organisms share many conserved core processes and features that evolved and are widely distributed among organisms today (1B1 & SP 6.1).
- The student is able to justify the scientific claim that free energy is required for living systems to maintain organization, to grow, or to reproduce, but that multiple strategies exist in different living systems (2A1 & SP 6.1).
- The student is able to use representations to pose scientific questions about what mechanisms and structural features allow organisms to capture, store, and use free energy (2A2 & SP 1.4, SP 3.1).
- The student is able to use representations and models to describe differences in prokaryotic and eukaryotic cells (2B3 & SP 1.4).
- The student is able to construct explanations based on scientific evidence as to how interactions of subcellular structures provide essential functions (4A2 & SP 6.2).
- The student is able to apply mathematical routines to quantities that describe interactions among living systems and their environment, which result in the movement of matter and energy (4A6 & SP 2.2).

ARE STUDENTS READY TO COMPLETE A SUCCESSFUL INQUIRY-BASED, STUDENT-DIRECTED INVESTIGATION?

Before students investigate photosynthesis, they should demonstrate an understanding of the following concepts related to the physical properties of light. The concepts may be scaffolded according to level of skills and conceptual understanding.

- Measuring light intensity
- The inverse square law
- The wave nature of light (visible light spectrum, i.e., colors)
- Light as energy

This investigation reinforces the following skills:

- Preparing solutions
- Preparing a serial dilution
- Measuring light intensity
- Developing and applying indices to represent the relationship between two quantitative values (e.g., an ET_{50} Index)
- Using reciprocals to modify graphical presentations
- Utilizing medians as a measure of central tendencies
- Constructing data tables and graphs
- Communicating results and conclusions

Skills Development

Students will develop the following skills:

- Applying the floating disk assay procedure to study photosynthesis or dissolved oxygen or carbon dioxide sensors with computer interface
- Measuring/calculating rates of photosynthesis

Potential Challenges

Students often come to biology with the misconception that plants undergo photosynthesis (only) and animals undergo cellular respiration. Students often forget that most plant cells also possess mitochondria and respire. In the final part of this investigation, students can explore the combined role of respiration and photosynthesis with experiments of their own design. For example, if a student places disks that have floated under light into a dark environment, plant respiration will consume the oxygen bubbles causing the disks to re-sink.

Students have a difficult time understanding the properties of light and how these properties can affect photosynthesis. The instructor may want to include a quick demonstration of the inverse square law and another quick demonstration on light absorbance.

If students have a solid understanding of the aforementioned concepts, they should be able to pose scientific questions about photosynthesis and design an experiment(s) around the effects of variables on the rate of photosynthesis. The skills and concepts may be taught through a variety of methods in an open-inquiry investigation, and photosynthetic rates may be measured by several means. Only the floating disk technique is described in the Student Manual, and alternative procedures may be equally and successfully substituted. For example, in the procedures outlined in the Student Manual, production of O_2 gas in photosynthesis is measured, but students also can measure the production of CO_2, or even simultaneous changes in volumes of both gases, depending on available equipment (e.g., gas sensor probes with computer interface).

Measuring the rate of photosynthesis is a challenge in a high school laboratory. Because the purchase of appropriate sensors or instrumentation is expensive, the floating disk system described in the Student Manual provides an easier, cheaper, and more reliable method to study both photosynthetic rates as well as rates of respiration. The cost of materials and equipment is under $0.50 per student (exclusive of light sources or meters). A video outlining the method can be found at ***http://www.kabt.org/2008/09/29/video-on-sinking-disks-for-the-floating-leaf-disk-lab/***.

The steps in the first part of the lab require teacher direction to familiarize students with the floating disk system or computer-based sensors. The final part of the investigation requires less teacher direction and instruction, the degree to which depends on conceptual understanding and the skill level of the students.

If students are to be successful in the final part, in which they design and conduct their own investigations, it is essential that they have success in sinking their leaf disks. Attention to this task generally is the deciding variable that points to positive student outcomes.

THE INVESTIGATIONS

Getting Started: Prelab Assessment

Investigating biology requires a variety of skills. The skills reinforced and introduced vary across the laboratories in this manual. The skills emphasized in a laboratory dictate whether a prelab assessment is appropriate.

This particular investigation provides a lab environment, guidance, and a problem designed to help students explore various parameters that can affect the rate of photosynthesis along with aspects of experimental design. Very little background knowledge is required to begin this work, but exploring some parameters deeply might require further research. For example, when students begin this procedure, they generally are not familiar with either the properties of light or the chemistry of dissolved carbon dioxide and bicarbonate ions. Students can begin asking and answering their own questions without this knowledge. As they work through the lab, students may be motivated to do additional research on photosynthesis.

Data Tables

The analysis and presentation of data are difficult challenges for most students. Following is an example of a graph of results that a student might present:

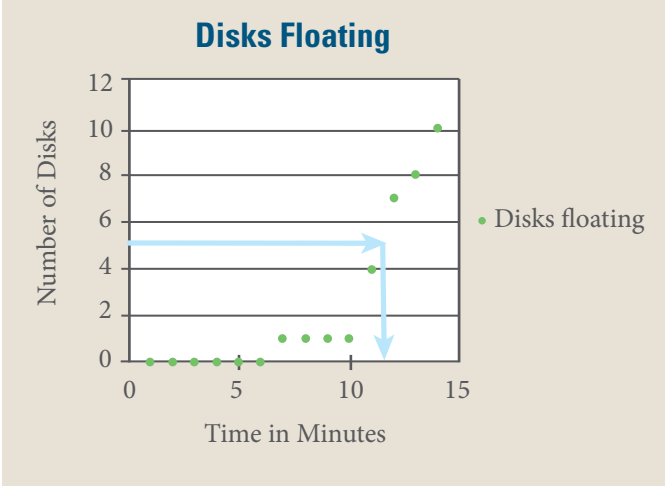

Figure 1. Disks Floating

The following method of data collection is suggested for students, although others work as well. In this case, the disks floating are counted at the end of each time interval. The median is chosen over the mean as the summary statistic. For most student work, the median will generally provide a better estimate of the central tendency of the data because, on occasion, a disk fails to rise or takes a very long time to do so. Consequently, for this sample, the median time for five disks to rise is somewhere between 11 and 12 minutes. A term coined by G. L Steucek and R. J Hill (1985) for this relationship is ET_{50}, the estimated time for 50% of the disks to rise. That is, rate is a change in a variable over time. The time required for 50% of the leaf disks to float is represented as Effective Time = ET_{50}.

Figure 2 is a sample graph of a photosynthesis light response curve utilizing the ET_{50} concept.

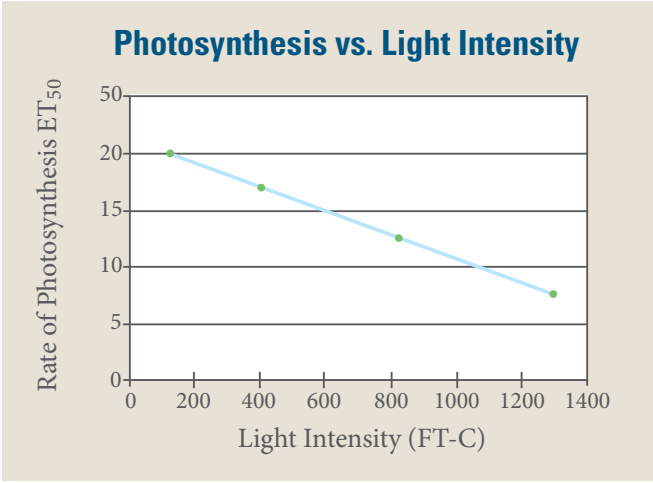

Figure 2. Photosynthesis vs. Light Intensity [Source: Steucek and Hill, 1985]

Note that the shape of this curve is not the expected curve that rises and levels off. This is because the times to float are the inverse of the rate of photosynthesis. Taking the reciprocal of ET_{50}, $1/ ET_{50}$ allows the graphic presentation to more closely express the physical phenomenon, as shown in Figure 3.

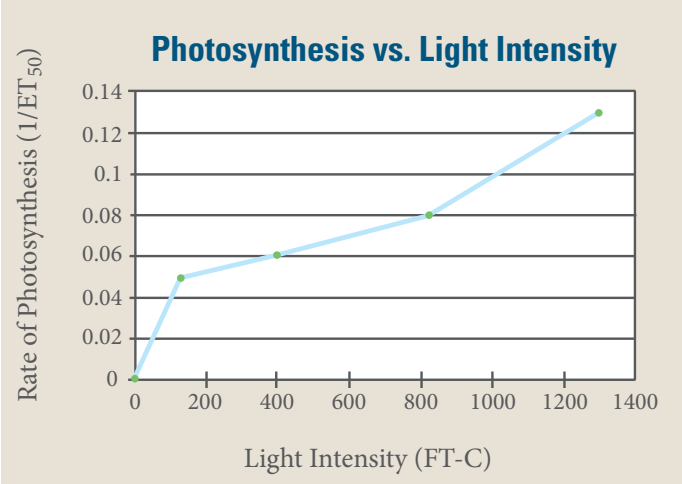

Figure 3. Photosynthesis vs. Light Intensity (1/ ET_{50}) [Source: Steucek and Hill, 1985]

This procedure is particularly useful for comparing photosynthetic rates between C4 and C3 plants. This procedure is also very useful for exploring the connection between photosynthesis and cellular respiration. Once the infiltrated disks have floated because of photosynthesis, the rate of cellular respiration can be determined by placing the systems in a dark environment. If the disks are still swirled after each minute, the process of cellular respiration will consume the oxygen bubbles in the mesophyll spaces, causing the disks to sink again. This phenomenon is illustrated in Figure 4.

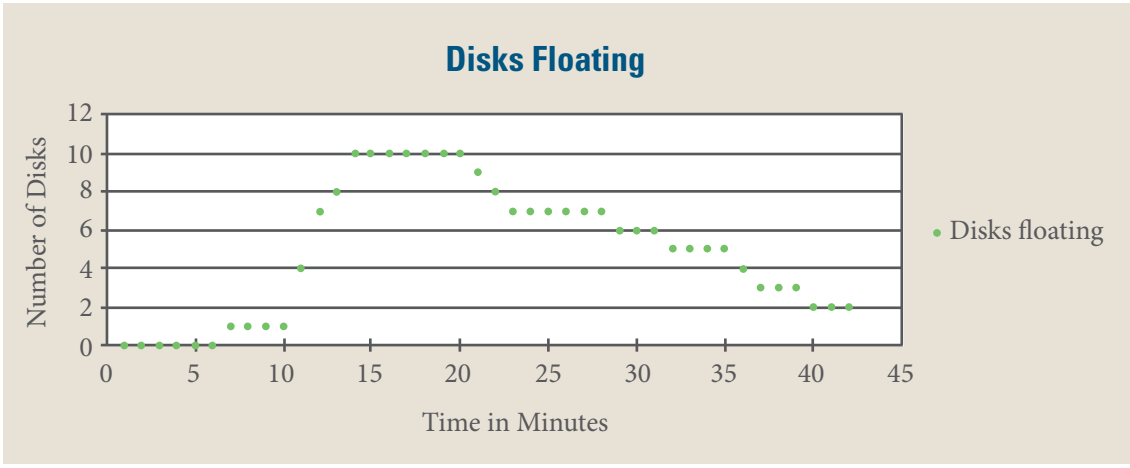

Figure 4. Disks Floating

(In this case, the cup with floating disks was placed under a cardboard box with no light at the 14-minute mark. Note that the slope of the sinking rate is less than that of the floating rate.)

There are many research papers on the Internet that explore photosynthesis. These studies can serve as guides to the kinds of questions that students can ask. For example, if you put the terms "photosynthesis light response curve" into your search engine, you will find myriad ideas for student questions and experimental designs.

Figure 5 shows a sample light response curve as an example of the type of work students can do with this technique. The total time required was about one hour of laboratory work per student. The plant is deep shade English Ivy grown at 25° C, with

excess bicarbonate solution. All of the leaf disks came from a single leaf. The technique was modified by placing the infiltrated disks in petri dishes with 30 mL of bicarbonate solution each. This created a very shallow solution depth in which the leaf disks rose more quickly.

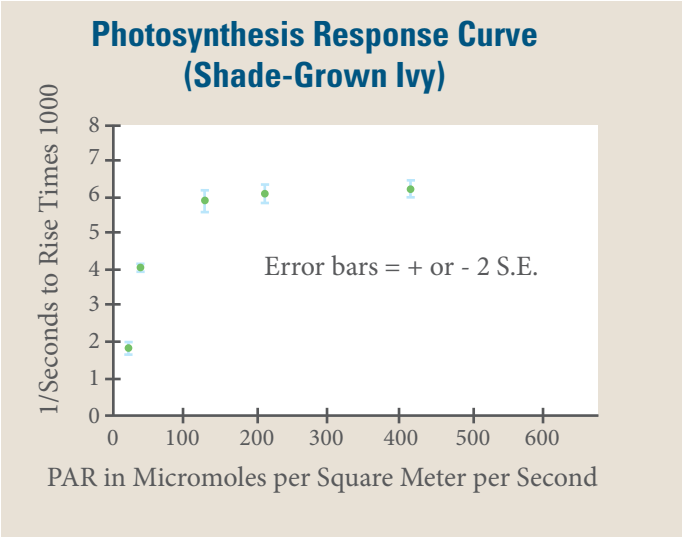

Figure 5. Photosynthesis Response Curve

In this example, the time for each disk to rise was measured in seconds (difficult to do accurately by oneself but relatively easy to do and much more precise with a digital video camera or a group of students). In this case, a PAR meter was used to measure PAR flux, and a shop light with an 8-inch reflector and a 100-watt equivalent compact fluorescent bulb created the light source. Since the rise of each disk was measured (not the ET_{50} method), an estimated Standard Error could be calculated, although the reciprocal of time for each leaf disk to rise was still plotted.

There is one data point that was excluded — for very bright light (>1,000 micromoles per square meter per second). The disks were so close to the bulb that the temperature of the water rose, affecting the results. To avoid this problem, consider introducing the idea of a water heat filter to students investigating similar variables.

Designing and Conducting Independent Investigations

Once students have mastered the floating disk technique, they will design an experiment to test another variable that might affect the rate of photosynthesis. Possible questions generated from students' observations include the following. However, it is suggested that students generate their own questions to explore.

- What environmental variables might affect the net rate of photosynthesis? Why do you think they would affect it? How do you predict they would affect it?
- What features or variables of the plant leaves might affect the net rate of photosynthesis? How and why?

- Could the way you perform the procedure affect the outcome? If the outcome changes, does it mean the net rate of photosynthesis has changed? Why do you think that?

If students are truly stumped, you can give them some guidance. Tell students that leaves with hairy surfaces should be avoided and that ivy and spinach are among the plants that work well. Differences between plants may be one of the ideas that students want to investigate.

Summative Assessment

A particularly effective method of assessment involves the use of peer-reviewed laboratory notebooks and mini-posters (described in Chapter 6). With an appropriate lab investigation rubric, students can deliver feedback to each other that is not graded, providing valuable formative feedback during and after their investigations. The advantage of peer-review is that revisions can be encouraged before a grade is determined.

For this investigation the mini-poster has proven to be a very effective tool to evaluate individual or group work. The following are suggested as guidelines to assess students' understanding of the concepts presented in the investigation, but you are encouraged to develop your own methods of postlab assessment:

1. Revisit the learning objectives. Based on students' answers to the analysis questions, do you think students have met the objectives of the laboratory investigation?
2. Have students develop a list of common misconceptions or concepts that they had difficulty understanding about the process of photosynthesis before conducting their investigations.
3. Did students have sufficient mathematical skills to calculate the rate(s) of photosynthesis?
4. Released AP Exams have several multiple-choice and essay questions based on the concepts studied in this investigation. These could be used to assess your students' understanding.

SUPPLEMENTAL RESOURES

Prelab Activities

http://mw2.concord.org/tmp.jnlp?address=http://mw2.concord.org/public/part2/photosynthesis/index.cml

This resource provides an interactive tutorial on the process of photosynthesis and the interaction with light.

Procedural Resources

AP Biology Lab Manual, Lab 4: Plant Pigments and Photosynthesis, The College Board, 2001.

Although this laboratory protocol is teacher directed, students can use the resource to glean information about the process of photosynthesis as they design experiments to investigate factors that affect photosynthesis.

http://www.kabt.org/2008/09/29/video-on-sinking-disks-for-the-floating-leaf-disk-lab/

This video demonstrates the floating leaf disk technique.

http://www.elbiology.com/labtools/Leafdisk.html

This resource describes the leaf disk technique.

The following resources either offer variations of the floating disk technique or used the technique to provide evidence for research. All offer good ideas that can be adapted for student research. Try to obtain a copy of the Wickliff and Chasson (1964) paper. It is the earliest paper of which this author is aware that describes this technique, and it is perhaps the best. There are many ideas that can lead to good student projects.

W. K. Vencill and C. L. Foy, "Distribution of triazine-resistant smooth pigweed (*Amaranthus hybridus*) and common lambsquarters (*Chenopodium album*) in Virginia," Weed Science 36, no. 4 (1988): 497–499.

F. Juliao and H. C. Butcher IV, "Further Improvements to the Steucek & Hill Assay of Photosynthesis," *The American Biology Teacher* (1989): 174–176.

J. L. Wickliff and R. M. Chasson, "Measurement of photosynthesis in plant tissues using bicarbonate solutions," *BioScience* 14, no. 3 (1964): 32–33.

G. L. Steucek and R. J. Hill, "Photosynthesis: I: An Assay Utilizing Leaf Disks," The *American Biology Teacher* (1985): 96–99.

R. J. Hill and G. L. Steucek, "Photosynthesis: II. An Assay for Herbicide Resistance in Weeds," The American Biology Teacher 47, no. 2 (1985): 99–102.

INVESTIGATION 5

PHOTOSYNTHESIS

What factors affect the rate of photosynthesis in living leaves?

BACKGROUND

Photosynthesis fuels ecosystems and replenishes the Earth's atmosphere with oxygen. Like all enzyme-driven reactions, the rate of photosynthesis can be measured by either the disappearance of substrate or the accumulation of product (or by-products).

The general summary equation for photosynthesis is

$$2\ H_2O + CO_2 + \text{light} \rightarrow \text{carbohydrate } (CH_2O) + O_2 + H_2O$$

What could you measure to determine the rate of photosynthesis?

- Production of O_2 (How many moles of O_2 are produced for one mole of sugar synthesized?)

  or

- Consumption of CO_2 (How many moles of CO_2 are consumed for every mole of sugar synthesized?)

In this investigation, you will use a system that measures the accumulation of oxygen.

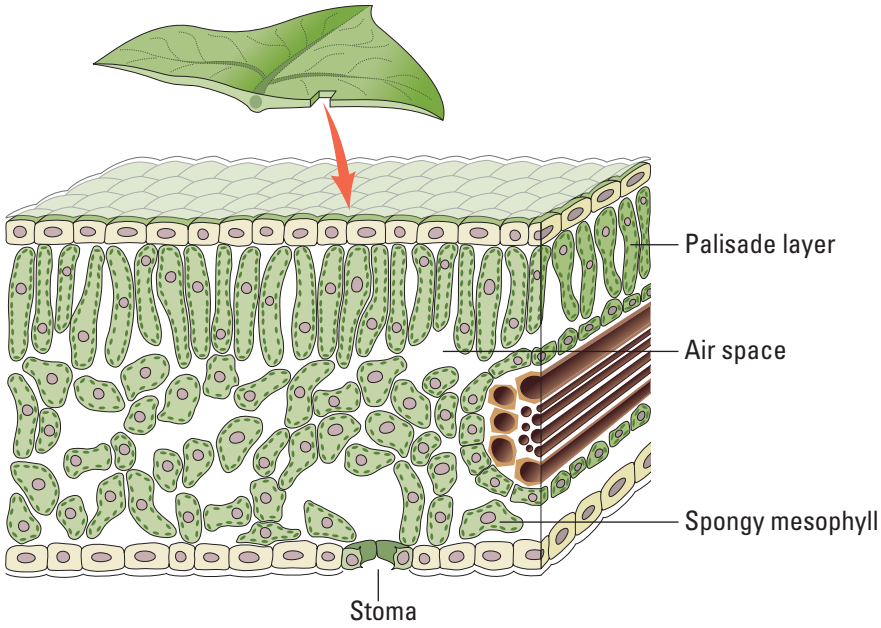

Figure 1. Leaf Anatomy

Because the spongy mesophyll layer of leaves (shown in Figure 1) is normally infused with gases (O_2 and CO_2), leaves — or disks cut from leaves — normally float in water. What would you predict about the density of the leaf disk if the gases are drawn from the spongy mesophyll layer by using a vacuum and replaced with water? How will that affect whether or not the leaf floats? If the leaf disk is placed in a solution with an alternate source of carbon dioxide in the form of bicarbonate ions, then photosynthesis can occur in a sunken leaf disk. As photosynthesis proceeds, oxygen accumulates in the air spaces of the spongy mesophyll, and the leaf disk will once again become buoyant and rise in a column of water. Therefore, the rate of photosynthesis can be *indirectly* measured by the rate of rise of the leaf disks. However, there's more going on in the leaf than that! You must also remember that cellular respiration is taking place at the same time as photosynthesis in plant leaves. (Remember that plant cells have mitochondria, too!) What else could be going on that might affect this process? Aerobic respiration will consume oxygen that has accumulated in spongy mesophyll. Consequently, the two processes counter each other with respect to the accumulation of oxygen in the air spaces of the spongy mesophyll. So now you have a more robust measurement tool — the buoyancy of the leaf disks is actually an indirect measurement of the *net* rate of photosynthesis occurring in the leaf tissue.

Learning Objectives

- To design and conduct an experiment to explore the effect of certain factors, including different environmental variables, on the rate of cellular photosynthesis

- To connect and apply concepts, including the relationship between cell structure and function (chloroplasts); strategies for capture, storage, and use of free energy; diffusion of gases across cell membranes; and the physical laws pertaining to the properties and behaviors of gases

General Safety Precautions

You must wear safety goggles or glasses, aprons, and gloves because you will be working in close proximity to exposed lightbulbs that can easily shatter.

Be careful to keep your solutions away from the electrical cord of your light source. Follow your teacher's instructions.

If you investigate temperature as a variable in Designing and Conducting Your Investigation, there is no need to heat any solution beyond 50–60°C.

Most but not all syringes are capable of withstanding the vacuum created in this procedure without failure. However, you should test the syringes beforehand.

THE INVESTIGATIONS

Getting Started

To study photosynthesis, review the properties of light and how it interacts with matter. In addition to your textbook, the Concord Consortium has a Java-based Web activity that will review the properties of light and the ways in which visible light interacts with matter in the process of photosynthesis. This multistep activity uses visualizations, animations, and a molecular modeling engine that does an excellent job of making abstract concepts understandable. To explore this activity, enter these terms in your search engine: "concord consortium molecular workbench photosynthesis."

While going through this activity, record any questions in your laboratory notebook. These questions and others that occur to you while working through the steps in Procedure can serve as a basis for your own investigation in Designing and Conducting Your Investigation.

Procedure

In this part of the lab, you will learn how the floating leaf disk technique can measure the rate of photosynthesis by testing a variable that you know affects photosynthesis. Later, you will apply this technique (or computer-based probes) to test a variable that you choose. It is important for you to develop a few skills during this part of the investigation in order to carry out your own investigation. For the floating disk technique, the most challenging skill is getting the disks to sink. Don't just watch someone do this; make sure you can get the disks to sink as well.

Materials

- Baking soda (sodium bicarbonate)
- Liquid soap (approximately 5 mL of dishwashing soap in 250 mL of water)
- 2 plastic syringes without needle (10 mL or larger)
- Living leaves (spinach, ivy, etc.)
- Hole punch
- 2 clear plastic cups
- Timer
- Light source

Figure 2. Materials

When immersed in water, oxygen bubbles are usually trapped in the air spaces of the spongy mesophyll in the plant leaf. By creating a vacuum in this experimental procedure, the air bubbles can be drawn out of the spongy mesophyll, and the space is refilled by the surrounding solution. This allows the leaf disks to sink in the experimental solution. If the solution has bicarbonate ions and enough light, the leaf disk will begin to produce sugars and oxygen through the process of photosynthesis. Oxygen collects in the leaf as photosynthesis progresses, causing the leaf disks to float again. The length of time it takes for leaf disks to float again is a measure of the net rate of photosynthesis. This process is shown in Figure 3.

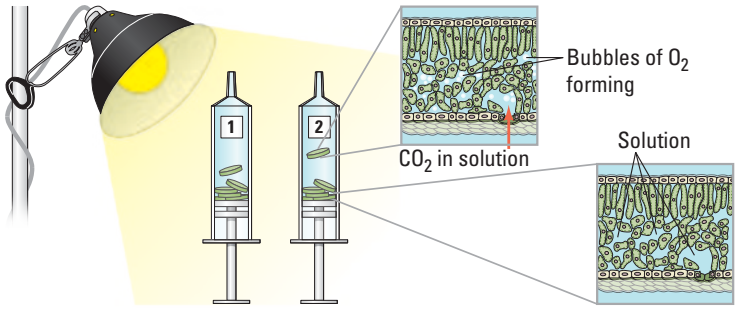

Figure 3. Photosynthesis at Work

Question: If the leaf disks are treated in a way you know increases the net rate of photosynthesis, should they start to float faster or slower? Why?

Step 1 Prepare 300 mL of 0.2% bicarbonate solution for each experiment. The bicarbonate will serve as a source of carbon dioxide for the leaf disks while they are in the solution.

Step 2 Pour the bicarbonate solution into a clear plastic cup to a depth of about 3 cm. Label this cup "With CO_2." Fill a second cup with only water to be used as a control group. Label this cup "Without CO_2." Throughout the rest of the procedure you will be preparing material for both cups, so do everything for both cups simultaneously.

Step 3 Using a pipette, add one drop of a dilute liquid soap solution to the solution in each cup. It is critical to avoid suds. If either solution generates suds, then dilute it with more bicarbonate or water solution. The soap acts as a surfactant or "wetting agent" — it wets the hydrophobic surface of the leaf, allowing the solution to be drawn into the leaf and enabling the leaf disks to sink in the fluid.

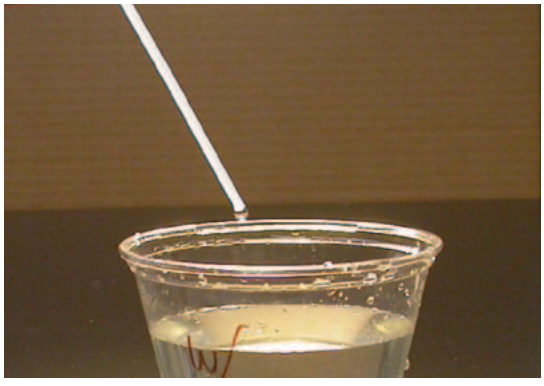

Figure 4. Dilute Liquid Soap Solution Added to Cup

Step 4 Using a hole punch, cut 10 or more uniform leaf disks for each cup. Avoid major leaf veins. (The choice of plant material is perhaps the most critical aspect of this procedure. The leaf surface should be smooth and not too thick.)

Figure 5. Leaf Disks

Step 5 Draw the gases out of the spongy mesophyll tissue and infiltrate the leaves with the sodium bicarbonate solution by performing the following steps:

a. Remove the piston or plunger from both syringes. Place the 10 leaf disks into each syringe barrel.

b. Replace the plunger, but be careful not to crush the leaf disks. Push in the plunger until only a small volume of air and leaf disk remain in the barrel (<10%).

c. Pull a small volume (5 cc) of sodium bicarbonate plus soap solution from your prepared cup into one syringe and a small volume of water plus soap into the other syringe. Tap each syringe to suspend the leaf disks in the solution. Make sure that, with the plunger inverted, the disks are suspended in the solution. Make sure no air remains. Move the plunger to get rid of air from the plunger before you attempt Step d.

d. You now want to create a vacuum in the plunger to draw the air out of the leaf tissue. This is the most difficult step to master. Once you learn to do this, you will be able to complete the entire exercise successfully. Create the vacuum by holding a finger over the narrow syringe opening while drawing back the plunger (see Figure 6a). Hold this vacuum for about 10 seconds. While holding the vacuum, swirl the leaf disks to suspend them in the solution. Now release the vacuum by letting the plunger spring back. The solution will infiltrate the air spaces in the leaf disk, causing the leaf disks to sink in the syringe. If the plunger does not spring back, you did not have a good vacuum, and you may need a different syringe. You may have to repeat this procedure two to three times in order to get the disks to sink. **(If you have any difficulty getting your disks to sink after three tries, it is usually because there is not enough soap in the solution. Try adding a few more drops of soap to the cup and replacing the liquid in the syringe.)** Placing the disks under vacuum more than three times can damage the disks.

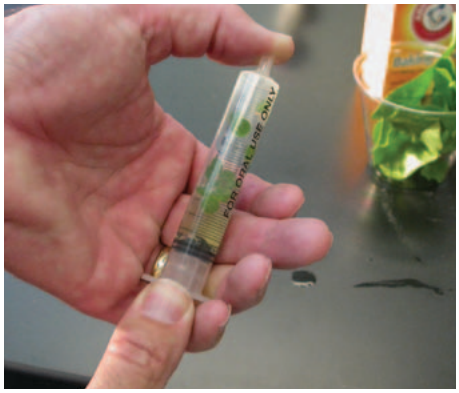

Figure 6a. Creating a Vacuum in the Plunger

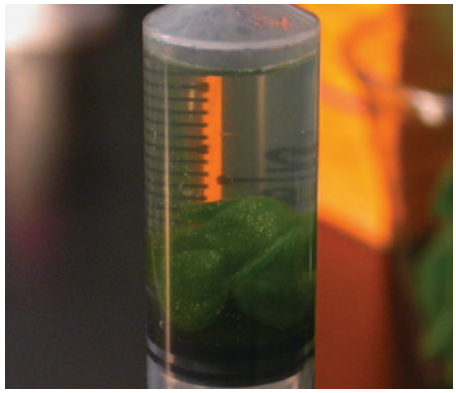

Figure 6b. Sinking Leaf Disks

Step 6 Pour the disks and the solution from the syringe into the appropriate clear plastic cup. Disks infiltrated with the bicarbonate solution go in the "With CO_2" cup, and disks infiltrated with the water go in the "Without CO_2" cup.

Step 7 Place both cups under the light source and start the timer. At the end of each minute, record the number of floating disks. Then swirl the disks to dislodge any that stuck against the side of the cups. Continue until all of the disks are floating in the cup with the bicarbonate solution.

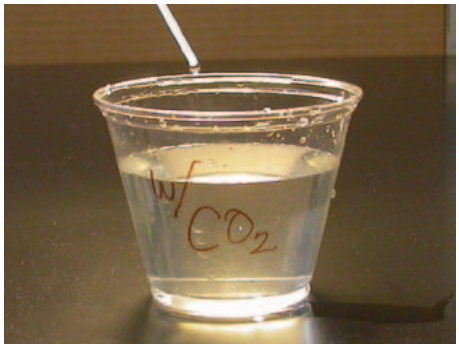

Figure 7a. Cup Under Light Source

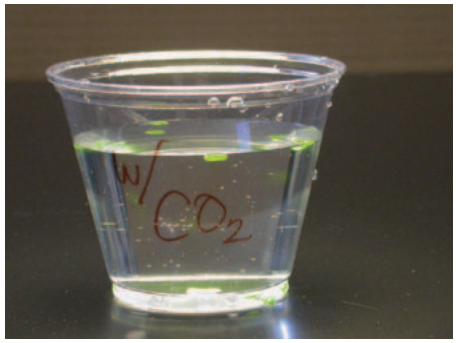

Figure 7b. Disks Floating in Cup with Bicarbonate Solution

Step 8 To make comparisons between experiments, a standard point of reference is needed. Repeated testing of this procedure has shown that the point at which 50% of the leaf disks are floating (the median or ET_{50}, the Estimated Time it takes 50% of the disks to float) is a reliable and repeatable point of reference for this procedure.

Step 9 Record or report findings.

Designing and Conducting Your Investigation

What factors affect the rate of photosynthesis in living plants?

1. Once you have mastered the floating disk technique, you will design an experiment to test another variable that might affect the rate of photosynthesis. Some ideas include the following, but don't limit yourself to just these:

- What environmental variables might affect the net rate of photosynthesis? Why do you think they would affect it? How do you predict they would affect it?
- What features or variables of the plant leaves might affect the net rate of photosynthesis? How and why?
- Could the way you perform the procedure affect the outcome? If the outcome changes, does it mean the net rate of photosynthesis has changed? Why do you think that?

Note: If you are truly stumped, your instructor can give you some guidance. Keep in mind that leaves with hairy surfaces should be avoided. Ivy and spinach work well, but many others do as well. Differences between plants may be one of the ideas that you want to investigate.

2. Use your results to prepare a lab report/mini-poster for a classroom peer review presentation. See Chapter 2 for guidance on this.

Additional Guidelines

1. Consider combining variables as a way to describe differences between different plants. For instance, if you investigate how light intensity affects the rate of photosynthesis, you might generate a "photosynthesis light response curve"—the rate of photosynthesis at different light intensities. The shape of this curve may change for different plants or plants in different light environments. The "light response curve" is a form of measurement itself. How do you think a light response curve (the first variable) for a shade-grown leaf compares to that of a sun-grown leaf? In this situation, sun versus shade is the second variable. Comparing light response curves is a standard research technique in plant physiological ecology.

2. When you compare the ET_{50} across treatments, you will discover that there is an inverse relationship between ET_{50} and the rate of photosynthesis — ET_{50} goes down as rate of photosynthesis goes up, which plots a graph with a negative slope. This creates a seemingly backward graph when plotting your ET_{50} data across treatments, as shown in Figure 8a. To correct this representation and make a graph that shows increasing rates of photosynthesis with a positive slope, the ET_{50} term can be modified by taking its inverse, or $1/ET_{50}$. This creates a more traditional direct relationship graph, as shown in Figure 8b.

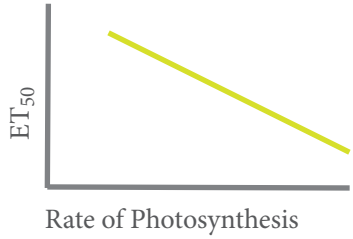

Figure 8a. Inverse Relationship

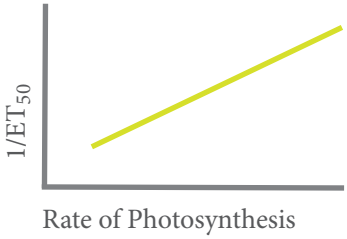

Figure 8b. Direct Relationship

3. Don't forget to include other appropriate data analyses as you prepare and study your discussion and conclusions. In particular for this investigation, you should somehow indicate the variability in your data. The ET_{50} measurement is calculated from the median. To indicate the spread of your data, you could use error bars around the ET_{50} point that express that variation, or you might consider using "box and whisker" plots.

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