Tested Studies for Laboratory Teaching
Proceedings of the Association for Biology Laboratory Education
Vol. 33, 1–7, 2012

Studying Photosynthesis and Respiration in *Hedera helix*

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This exercise presents a method for studying photosynthesis using the floating leaf disk assay (FLDA). It utilizes the rate at which oxygen is produced or consumed as a measure of the processes of photosynthesis and respiration, respectively. After disks of leaf tissue are vacuum-infiltrated, they sink. As photosynthesis occurs, the oxygen makes the disks float. When the disks are placed in the dark, oxygen is consumed and the leaf disks sink. This exercise explains how to carry out the FLDA. It also explains how to graph the data in Excel, and how to calculate the rates of photosynthesis and respiration.

Keywords: Floating leaf disk assay, photosynthesis, cell respiration

Introduction

Although photosynthesis is arguably one of the most crucial metabolic pathways carried out on earth, it is difficult to find a reliable protocol that can be used by students to study it. The method presented here is a compilation of aspects of the floating leaf disk assay (FLDA) from at least three sources (Armstrong, 1995; Pitkin, 2004; Steucek and Hill, 1985). The goal of this compilation was streamlining the protocol, eliminating problematic steps (e.g., aspirators to produce the vacuum and the need for multiple solutions) and developing a procedure that consistently and quickly produced reliable data.

The FLDA utilizes the rate at which oxygen is produced or consumed as a measure of the processes of photosynthesis and respiration, respectively. Disks of leaf tissue are vacuum-infiltrated to replace intercellular air with liquid. After infiltration, the disks sink. As photosynthesis takes place and oxygen is produced, the gas imparts buoyancy to the leaf disks so that they float. Conversely, oxygen is consumed as respiration occurs in the dark, and the floating leaf disks sink. A series of calculations results in the rates of both processes: photosynthesis and respiration. This exercise explains how to carry out the FLDA with leaves from *Hedera helix* (English ivy) using various positions on a light table (gel reader) as the light source; it also explains how to graph the data using Excel and Paint, and how to carry out the calculations to determine the rates of photosynthesis and cell respiration.

This exercise is appropriate for use by first-year undergraduate students. Because the protocol can also be used as the basis for independent student experimentation, it could also be used by upper-level students in a botany or ecology course. Set-up for the exercise requires only the advance procurement of the leaf material and the preparation of two solutions. The actual assay, as described here, requires about 2 hours for completion; an additional 1.5 hours are needed to graph the data and perform the calculations. Because the data analysis requires mathematical manipulation with which students may not have experience (finding the reciprocal of a value), it is necessary to allocate sufficient time for this step.

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Student Outline

Background

The floating leaf disk assay (FLDA) utilizes the rate at which oxygen is produced or consumed as a measure of the processes of photosynthesis and respiration, respectively. Disks are punched from leaf tissue and a vacuum is used to replace air in the spongy mesophyll with liquid, making the disks sink. As photosynthesis takes place in light, oxygen is produced. The accumulating gas makes the disks buoyant and they float. The rate of photosynthesis in the leaf disks is calculated after determining the time required for submerged leaf disks to float. When the leaf disks are put in the dark, oxygen is consumed in cell respiration and the floating leaf disks sink. The rate of respiration in the tissue is related to the time required for the floating disks to sink.

Procedure

Infiltrating leaf disks

1. Crease the piece of weighing paper down the middle and flatten it out. Using the single-hole punch, cut 15 leaf disks onto the weighing paper. Avoid areas of the leaf that include veins. Make sure each disk is a complete circle and has no "tails" of epidermal tissue.
2. Remove the plunger from the syringe by pulling it straight out of the barrel.
3. Pour the leaf disks from the creased weighing paper directly into the barrel of the syringe. Make sure they are all at the bottom of the syringe barrel. Re-insert the syringe plunger.
4. Push the plunger as far down the barrel as possible without crushing the leaf disks.
5. Label a 50-mL beaker *infiltration solution*. Pour about 50 mL of infiltration solution into the beaker. Then draw about 5 mL of infiltration solution into the syringe from the beaker.
6. Invert the syringe so the tip points upward and swirl the contents briefly. Holding the syringe in this inverted position, press on the plunger until one or two drops of solution emerge from the tip of the syringe.
7. Turn the syringe so the tip points downward and expel excess infiltration solution back into the beaker so that only about 3 mL remain in the syringe.
8. Place the #10 rubber stopper flat onto the lab bench in front of you. Firmly place the tip of the syringe directly onto the rubber stopper, so that the syringe tip is sealed air-tight on the surface of the stopper.
9. Slowly pull back on the plunger, up to the 10 mL mark, creating a vacuum within the syringe barrel. Hold the vacuum for 15-20 seconds and then slowly release the vacuum by returning the plunger to its original position.
10. Hold the syringe up in front of you and swirl the contents. The leaf disks should all sink to the bottom of the solution in the syringe. If they do not, repeat Steps 8 and 9 until all disks have sunk in the infiltration solution.
11. The plunger needs to be removed from the syringe barrel, but without losing any liquid in the process. To do this, pull the plunger up to the end of the barrel and then place your finger over the tip of the syringe to seal in the liquid as you retract the plunger completely.

Conducting the assay for photosynthesis

1. Label a clean, empty 100-mL beaker as *Trial 1*. This will be the reaction beaker.
2. Measure out 50 mL of bicarbonate buffer in a graduated cylinder and **leave it in the cylinder**.
3. Empty the infiltration solution and the 15 leaf disks from the syringe into the reaction beaker. If some of the disks cling to the inner wall of the syringe, use small amounts of the 50 mL of bicarbonate buffer to rinse the remaining leaf disks into the reaction beaker. Then pour the rest of the bicarbonate buffer from the graduated cylinder into the reaction beaker.
4. Immediately swirl the reaction beaker and place it on a designated spot on the light table. Start the timer.
5. As the leaf disks settle out in the beaker, use the stirring rod to gently separate any that overlap. The goal is to have each leaf disk sitting flat on the bottom of the beaker and separate from each other, to maximize exposure to the light coming from below.

6. Each minute, record the number of disks that have completely floated to the surface of the solution in the beaker. Gently stir the solution above the level of the disks.
7. Stop when 10 disks have reached the surface.

Conducting the assay for respiration

1. Move the beaker off the light table to the bench. Remove the disks that never floated to the surface so that the only disks left in the beaker are the 10 disks that floated to the surface.
2. Place an opaque cover over the beaker of disks from the photosynthesis assay. Set the timer. Each minute, remove the opaque cover and observe the number of disks that have sunk to the bottom of the beaker. Stir the contents of the beaker gently with the stirring rod for a brief time and replace the cover. Stop when all of the floating disks have sunk. Repeat the entire assay (for photosynthesis and cell respiration) for a total of three times with different disks but at the same designated spot on the light table.

Analyzing the data

1. Construct a scatter graph of the results from the two parts of your experiment, following the directions below. Do this for each of the three trials. Follow the directions to determine the ET_{50L} and ET_{50D} from the figure.
2. From the graphs, you determined values for ET_{50L} and ET_{50D}. Keep in mind that these values represent the **E**ffective **T**ime needed for **50%** of the disks to either rise in the **L**ight or to sink in the **D**ark. These values can't be used in this form, however, but must be mathematically transformed. Here's why: Suppose you determined two different ET_{50L} values, 10 minutes and 20 minutes. The shorter value—10 minutes—would indicate the faster rate and the longer value—20 minutes— would indicate the slower rate. In other words, the faster rate is the smaller number and the slower rate is the larger number. That doesn't make sense.
3. Therefore, we use the reciprocal of ET_{50L} instead of the actual value. The faster rate would now be 1/10 min = 0.10 min-1 and the slower rate would be 1/20 min = 0.05 min-1. The faster rate is now greater than the slower rate. That makes sense.
4. The same would be true of ET_{50D}, so we use the reciprocal, $1/ET_{50D}$ instead.
5. Calculate the rate of photosynthesis (PS) and the rate of respiration (RS) for your designated spot on the light table. Use the average ET_{50L} and ET_{50D} values from your three trials.
   - In the dark, disks carry out respiration only. Therefore, the rate of respiration (RS) can be determined by noting the effective time for half of the floating disks placed in the dark to sink (ET_{50D}). The equation for RS, then, is: $RS = 1/ET_{50D}$.
   - In the light, plants carry out **both** photosynthesis and respiration. Therefore, this calculation is a little more complicated. The rate of photosynthesis (PS) can be determined by noting the effective time for half of the submerged disks placed in light to float (ET_{50L}. The actual rate of photosynthesis is proportional to ET_{50L}, thus:
     $PS = 1/ET_{50L}$
   - But since both photosynthesis and respiration occur in the light, $1/ET_{50L}$ is equal to the rate of photosynthesis minus the rate of respiration: $1/ ET_{50L} = PS – RS$. And, re-writing the equation for PS gives us:
     $PS = 1/ ET_{50L} + RS$
6. NOTE TO INSTRUCTOR: Omit this step from the directions if you don't want students to know from the outset about the different light intensities on the light table. [Incorporating the data from the entire class, construct a scatter graph with no line to show the relationship between PS and light intensity; insert the line of best fit. Construct a second scatter graph with no line to show the relationship between RS and light intensity; insert the line of best fit.]

Table 1. Data for FLDA at position ______; in the light (NDF = number of disks **floating**)

Time (min)	Trial 1		Trial 2		Trial 3	
	NDF	%	NDF	%	NDF	%
0	0	0	0	0	0	0

Table 2. Data for FLDA at position ______; in the light (NDF = number of disks **floating**)

Time (min)	Trial 1		Trial 2		Trial 3	
	NDF	%	NDF	%	NDF	%
0	10	100	10	100	10	100

Constructing the graphs

1. In Excel, enter the times in column A and the % disks floating in column B. Keep in mind that the data from each trial consist of the values when the disks were in the light and the values when the disks were in the dark. Therefore, enter the “Light” values first and then the “Dark” values directly after, with the times running uninterrupted from “Light” values to “Dark” values. In other words, if the last “Light” reading was at 16 minutes, your first “Dark” reading would be entered as 17 minutes. Use XY scatter > Straight lines and markers.
2. In Layout, select Gridlines > Primary vertical > Minor.
3. Add the axis titles with units of measure.
4. Click on one data point > Format data series > Marker fill > No fill > Close.
5. Cut (control + C) and paste (Control + V) the figure into Paint.
6. In Paint, select the Line drawing tool> Third line option. Draw a vertical line on the gridline that marks where the light was turned off.
7. In Paint, select the text box tool. Insert the terms “Light” and “Dark” on the two sides of the vertical line. Select the Paintbrush > Large dot option. Fill in the data points for the values that represent times in the dark.
8. In Paint, select the Line drawing tool > First line option. On the “Light” side, draw a horizontal line from the 50% point on the y axis to the graph line. Then draw a vertical line down to the x axis. Use a text box to label this ET_{50L}. Do the same thing on the “Dark” side, going first from the Light/Dark vertical line to the graph line, and then from the graph line to the x axis. Use a text box to label this ET_{50D}.
9. Your resulting graph should look like Figure 1. Cut and paste the graph into Word. Include all three graphs in the same figure. Add the legend in Word to the entire figure (with the three graphs).

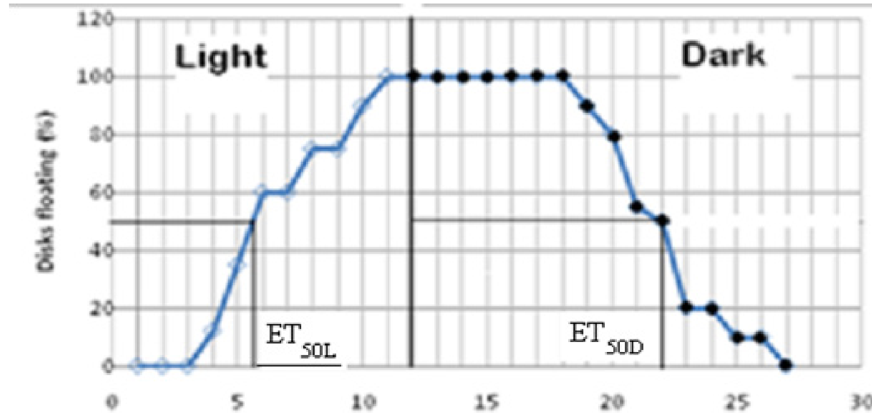

Figure 1. Sample data from a trial of FLDA.

Materials

Per class

(2) Light tables (gel readers)
(2) Light meters
~1.6 L bicarbonate buffer, room temperature
~350 mL infiltration solution, room temperature

Per group of three students

10-mL plastic syringe
#10 rubber stopper
Fresh leaves of English Ivy, *Hedera helix*
(3) 100-mL glass beakers
50-mL graduated cylinder
Stirring rod
Hand-held, single-hole punch
Featherweight forceps
Stopwatch timer
50-mL graduated cylinder
Piece of 4” x 4” weighing paper
Opaque beaker cover (plastic soda cup is OK)
~50 mL of infiltration solution
~200 mL of bicarbonate buffer
Marker

Notes for the Instructor

From a conceptual view, students should have already learned about photosynthesis prior to conducting the lab.

During the laboratory exercise

It helps if the students do a practice assay to work out the kinks in the “choreography” before they actually begin the experiment. In the assay for photosynthesis, the students work initially with 15 leaf disks. That way, if one or two disks are stubborn and don’t float, it’s not a problem. When students determine the % disks floating for their data table, however, they should base the % value on only 10 disks, not the entire group of 15. Make sure the students remove the “non-floaters” before they begin their assay in the dark.

Pay particular attention to students in these parts of the protocol:

- Steps 8-9 of **Infiltrating leaf disks**: Make sure students push down on the stopper with the syringe and pull up on the plunger at the same time. It may help to have one student carry out each task.
- Step 6 of **Conducting the assay for photosynthesis**: The point is not to create a vortex but to break surface tension that may be holding the disks down.
- Step 1 of **Constructing the graphs**: Make sure students enter the data correctly, with “Light” % values first, followed by “Dark” % values in the same column (Column A) of the Excel spreadsheet.

I don’t tell students that the six spots at the light tables are areas of different light intensities. We address that issue when the six groups arrive at different values for PS.

In calculating PS and RS, the student groups work on large dry-erase boards, writing out the calculations for each of their three trials. That way, I can keep track of their thinking more easily.

Make sure the students understand why the ET_{50} values have to be transformed to reciprocals. This is crucial to an understanding of the lab results.

Each group records their values for PS and RS at the lab blackboard and we discuss reasons for the differences. This may take some time, especially since the PS values vary and the RS values don’t. When a student suggests light intensity, we measure the intensity at the six positions on the light table. Then students graph the relationship between PS and RS and light intensity. The idea of light as a limiting value can be discussed at this point. If the results do not suggest a saturation point, the following websites address saturation point and the discussion question I give the students if their data doesn’t include the saturation point.

- **http://www.marietta.edu/~spilatrs/biol103/photolab/saturati.html**
- **http://www.marietta.edu/~spilatrs/biol103/photolab/photosyn.html**

Follow-up to the laboratory exercise

Once students understand the basic design of the FLDA, they can develop their own adaptations of the assay and carry out additional experiments. They could, for example, study PS in shade plants and sun-loving plants, PS in young leaves and older leaves, or the effect of wavelength of light on PS.

Literature Cited

Armstrong J.E. 1995. Investigation of photosynthesis using the floating leaf disk assay. **http://www.bio.ilstu.edu/armstrong/biolab/cellbio/phynex1.htm**.

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About the Author

Janice Bonner has been teaching introductory and general education biology courses at Notre Dame of Maryland University for 24 years. Before that, she taught high school and junior high science. She received her degree in Curriculum and Instruction from University of Maryland.

Appendix

Solutions for a class of ~18 students working in groups of three

The solutions described in this exercise were prepared with specific components. Substitutions (e.g., citric acid monohydrate for anhydrous citric acid) may not produce the same results.

Bicarbonate buffer solution
Dissolve together in ~250 mL dH_2O: 6.98 g citric acid* and 35.10 g Na_2HPO_4** (pH = 6.8)
Then add 4.00 g $NaHCO_3$
Bring volume to 2000 mL
* anhydrous free acid (FW 192.130)
**dibasic anhydrous (FW 141.96)

Infiltration solution
350 mL bicarbonate buffer solution (above)
7 drops Tween (added with transfer pipet)

Other required material

Plant Material

Some directions for FLDA recommend using spinach leaves from the grocery store. We did not have consistent success with this plant material. After experimenting with numerous types of plant tissue, we found that English ivy (*Hedera helix*) produces the most consistent and rapid results. Ivy can be obtained in flats from a home improvement center, in pots from nurseries, or by taking cuttings. We take clippings of the English ivy that grows outside our campus building just before lab begins and place them in a plastic bag with a damp paper towel. There are several things to keep in mind if you use this source of plant material, however. First, the leaves must be large enough to enable students to punch the disks without encountering large veins; therefore, larger leaves work better than smaller ones. Second, if cuttings are used, keep in mind that plant material picked for use in a morning lab will not necessarily be viable for use in an afternoon lab on the same day. Third, if cuttings are used, keep in mind that environmental conditions (temperature/ humidity) may adversely affect the quality of the plant material.

Light table (gel reader)

Prior to the beginning of lab (and before the students arrive) I use the light meter to locate 6 spots on the two light tables (gel readers) that represent a broad range of light intensity, three on one light table and three on the other. I draw three circles the size of the beakers that students will be using and label them A-F. Each group of students works with one circle of light throughout the experiment.

Hole punches

Make sure that the hole punches produce disks with sharply defined edges and no trailing strings of epidermis. It works best to keep a set of hole punches “dedicated” to this lab.

Light meters

These should be designed for use in a biology laboratory (capable of reading in lux) not for photography.

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Citing This Article

Bonner, J.M. 2012. Studying Photosynthesis and Respiration in *Hedera helix*. *Tested Studies for Laboratory Teaching,* Volume 33 (K. McMahon, Editor). Proceedings of the 33rd Conference of the Association for Biology Laboratory Education (ABLE), 390 pages. http://www.ableweb.org/volumes/vol-33/?art=1

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