

# Measurement of the Light Dependence of Photosynthesis

## Introduction

In this exercise you will demonstrate that light is required for photosynthesis, and that the rate of photosynthesis increases with light intensity (perhaps until a light saturation point is reached). At that point, the photosynthetic rate is limited either by the ability of the leaf to transduce the light energy it absorbs to chemical energy, or by the supply of some other factor required for photosynthesis.

Although light may seem to be an abundant source of "free" energy in the environment, there are many ecosystems in which the supply of light is the major factor limiting plant growth. In forests, and in densely planted crops, only the upper leaves are exposed to high light levels, and lower leaves, together with those plants that inhabit the understory, intercept only the stray light (sunflecks) that pierces the leaf canopy.

Changes in light level on a global basis may have been responsible for one of the major catastrophic events in the earth's history. It is thought that the impact of an enormous meteorite created an increase in atmospheric particulates that reduced penetration of solar radiation to a level that could not support high rates of photosynthesis. As a result, plant growth was reduced, herbivorous dinosaurs starved, causing starvation of their carnivorous predators.

It has been suggested that should humans be foolish enough to make use of nuclear weapons, this could result in an effect similar to that which exterminated the dinosaurs, causing the onset of a "nuclear winter" which would devastate crop production in regions far removed from those directly devastated by nuclear weapons.

Meanwhile, degeneration of the ozone layer by atmospheric pollutants is allowing a greater flux of solar radiation to penetrate the atmosphere, with adverse effects both on plants and animals. A study of photosynthesis and irradiation, therefore, has great biological, economic and historical relevance, as well as allowing insight into a fundamental process in plant physiology.

## Materials Required

- A higher plant.
- A laboratory stand to which other components are attached.
- A light fitting with a 50W halogen bulb.
- A sliding dimmer control into which the light source is plugged.
- A leaf chamber incorporating an oxygen sensor in its upper lid.
- A light sensor to attach to the chamber base.
- A 200 mL beaker for use as a heat filter (when filled with water).
- An acetate grid for leaf area measurement.
- A nylon-polyethylene gas bag.
- Drinking straws to fill the gas bag.
- A Lab Pro interface (analog to digital converter).
- A power supply for the Lab Pro interface.
- A USB cable to connect the Lab Pro interface to a computer.
- A thermometer measuring in °C.

## Materials and Methods

- (1) Dr. Koning will have arranged the components of the photosynthesis package as described in the General Introduction. But you may need his help in arranging for your particular plant; ensuring that the leaf chamber is at a height to enclose part of or all of a leaf, and that the base of the light fitting is about 11 cm from the surface of the leaf chamber (there should be 6 empty slots on the lab stand between the lamp and leaf chamber brackets). Ensure that the light is off by sliding the dimmer control to its minimum setting before proceeding further. Check that your computer is connected to the USB cable from the Lab Pro interface.

Plant being used: \_\_\_\_\_

- (2) Turn on the computer, and calibrate the O<sub>2</sub> and light sensors...
  - a) Double-click on the Logger Pro icon on the computer's desktop. This will start the Logger Pro software for data collection and analysis.

- b) The software starts with a “dummy” experiment, so choose “Close” from the “File” menubar item. In the dialog box that appears click on the “Don’t Save” button. This should clear out the “dummy” lab.
  - c) Choose “Open” from the “File” menubar item. In the dialog box, select the file: PH1\_SETUP.MBL and click on the “Open” button. In the dialog box click “OK.”
  - d) The computer screen will show two graphs, the upper graph displaying percentage O<sub>2</sub> plotted against time, and the lower graph showing photon flux ( $\mu\text{mol quanta/m}^2/\text{s}$ ) plotted against time. To the right is a table for recording actual data to be plotted on the two graphs, and at the bottom of the screen are the actual readings from the two sensors.
  - e) With the chamber open we will now calibrate the Oxygen Sensor. Click on the “Collect” button near the top in the center of the screen. The oxygen sensor reading at the bottom of the screen and appearing in the data table should be at or near 20.7%. If it does not, Dr. Koning will help you calibrate this to read 20.7% with a small screwdriver, or perhaps some software tweaks!
  - f) Now we need to calibrate the light sensor. It is assumed that your leaf is covering the sensor such that only green light is reaching it. For typical leaves we will want to calibrate this to a “medium” setting (for 0-100 PFD) using the toggle switch on the amplifier for the light sensor. In the software choose “Calibrate” under the “Experiment” menubar item.
  - g) In the dialog box select the “Sensor Setup” tab. Click on the “CH2” button and select the calibration for 100qu. NOTE: if you have an exceedingly thick leaf, we may use the low (0-60 PFD) switch position on the amplifier and the 60qu calibration. Put the dialog box away.
  - h) Click on the “Stop” button that replaced the “Collect” button.
- (3) Ensure that the leaf chamber gaskets have a very thin coating of vacuum grease. If any grease is visible, you do not need to apply more. With the light off, seal the leaf inside the leaf chamber so that no part of the leaf is shaded by the O<sub>2</sub> sensor or the gas inlet and outlet ports. It does not matter if the leaf is too large to be fully sealed within the chamber, and any "excess" may protrude out of the chamber without influencing your results. When closing the chamber turn the thumb-screws finger tight only.
  - (4) Fill the beaker to the top graduation with water. Place the beaker on top of the chamber so that it covers the major part of the leaf area.
  - (5) Click on the "Collect" button on the bottom left hand side of the computer screen. The button will change to a "Stop" button, and data will begin to appear on the two graphs on the screen, and as numerals on the bottom of the screen. The initial O<sub>2</sub> concentration should be close to 20.7% O<sub>2</sub>, and the initial photon flux should be close to zero.
  - (6) Using a drinking straw, inflate the plastic gas bag with your breath being careful not to put pressure on the seams by over-inflating the bag. Seal the bag with the luer lok plug provided. Depending on your metabolic condition, your exhaled breath should contain between 16 and 18% O<sub>2</sub>, and 3 to 5% CO<sub>2</sub>. Under normal conditions in humans, one molecule of CO<sub>2</sub> is produced in respiration for every molecule of O<sub>2</sub> consumed, so if your breath contains 18% O<sub>2</sub> it should also contain 2.75% CO<sub>2</sub> i.e. atmospheric O<sub>2</sub> concentration (20.7%) minus breath O<sub>2</sub> concentration (18%) plus the CO<sub>2</sub> concentration in the laboratory (typically 0.05%).
  - (7) Remove the luer lok plug from the tubing on the bag, and attach this tubing to one of the gas ports on the upper surface of the leaf chamber. Remove the luer lok plug from the opposing corner of the chamber. Press the bag gently so that your breath is flushed through the chamber. After approximately 10 seconds of flushing, remove the bag from the inlet port, seal the bag and seal

both ports of the leaf chamber with the luer lok plugs provided. Observe the decline in the O<sub>2</sub> reading on the computer screen until this reaches a stable value (this could take 4 minutes).

Breath oxygen concentration: \_\_\_\_\_%O<sub>2</sub>

- (8) When the O<sub>2</sub> reading on the screen has reached a steady value, switch on the light by sliding the dimmer control to its maximum setting. Make a note of the irradiance reading at the bottom of the screen and then **turn off the light immediately** by sliding the dimmer control to its minimum setting.

Maximum irradiance: \_\_\_\_\_ $\mu\text{E}/\text{m}^2/\text{sec}$

- (9) Stop data collection by clicking on the "Stop" button on the computer screen. You must now adjust the range of values displayed on the axes of the upper O<sub>2</sub> graph so that the lowest value is close to that of your exhaled breath, and the highest value is slightly above that of atmospheric pO<sub>2</sub>. Using the mouse, double-click on vertical (%O<sub>2</sub>) axis of the upper graph. In the dialog box, click the manual scaling radio button. Then enter a minimum value for the axis that is 0.5% units lower than the O<sub>2</sub> concentration of your breath (e.g. if your breath contains 18.5% O<sub>2</sub>, type 18). Next, enter a maximum value for the axis of 22% O<sub>2</sub>. Repeat for the lower graph. Setting the minimum to a value 10 units above the maximum irradiance value you recorded above.
- (10) Your experiment should take approximately 60 minutes to complete. If necessary, adjust the time axis on both graphs to a maximum of 60 minutes by using the mouse to double-click on the horizontal axis and change the maximum to 60 minutes.

Your software is now ready to do your project! We will do the entire rest of the lab exercise in one data run...so here goes!

- (11) Click on the "Collect" button on the computer screen. Remove the plugs from the leaf chamber and reflush the

chamber with breath from the gas bag. Reseal the gas bag and the chamber and let the O<sub>2</sub> concentration in the chamber stabilize at a low value.

- (12) Observe the changes in the O<sub>2</sub> concentration graph for 5 to 10 minutes which will later give us a starting rate.
- (13) After observing and recording in the computer these changes for 5 - 10 min, **leave the data collection running**, but remove the luer lok plugs from the chamber. Attach the gas bag to a chamber inlet port and flush the chamber for 10 seconds by pressing gently on the bag, and then re-seal the chamber and the gas bag with the luer lok plugs.
- (14) Switch on the light again, and set its output with the dimmer switch to a PFD value of approximately 20% of the maximum irradiance noted in step 8 above.
- (15) After switching on the light, photosynthesis should begin. Measure the increase in pO<sub>2</sub> of the chamber for 5 - 10 min, and then repeat step 13.
- (16) Increase the output of the lamp to 40% of maximum irradiance, and repeat steps 15 and 16 until you have measured photosynthetic rate at a number of light intensities equal to 0, 20, 40, 60, 80, and 100% of the initial light output.
- (17) After you have made all your measurements, stop the experiment by clicking on the "Stop" button.
- (18) Save your data by selecting the "Save as..." item under the "File" menu item. In the dialog box, give your data an appropriate group file name, and save it to the default folder.
- (19) You now quit Logger Pro by selecting the "Quit" option under the "File" menu item. Be sure you have indeed quit all the way. You can then close the cover of the computer, and disconnect it from the USB cable. The next group will bring their own computer to attach.

(20) Remove the beaker from the chamber and detach the leaf from the plant. Then using the setscrew on the bottom of the chamber, remove the light sensor gently. Finally detach the leaf chamber from its mounting bracket with the leaf enclosed. There are two wing nuts that accomplish this. Be careful not to touch any hot surface of the lamp or its fitting while doing this. You cannot go far with the chamber because of the still-connected oxygen sensor. Place the chamber with its contained leaf over a sheet of white paper. Place the acetate grid on the surface of the chamber so that it covers the leaf. Count the number of interstices completely enclosed by the area of the leaf. Any interstices falling exactly on the leaf margin should be given a value of 0.5. Sum the results, and divide the total by 4. The value you obtain is equal to the area of the leaf in  $\text{cm}^2$ .

Surrounded interstices = \_\_\_\_\_

Edge interstices x 0.5 = \_\_\_\_\_

Sum = \_\_\_\_\_

Leaf Area: Sum/4 = \_\_\_\_\_  $\text{cm}^2$

(21) Remove the leaf from the chamber and leave everything in good condition for the next group.

(22) Record the ambient temperature of the laboratory from the thermometer. \_\_\_\_\_  $^{\circ}\text{C}$

## Data Analysis

The  $\text{O}_2$  sensor measures only the partial pressure of  $\text{O}_2$  present in the leaf chamber, it does not measure the rate at which this  $\text{O}_2$  is produced. The rate of a process, such as photosynthesis, is expressed as the rate of increase in a product of that process (or a decrease in the substrate for the process) per unit time. To measure the rate of photosynthesis in your experiment, you will need to measure the increase in  $\text{pO}_2$  within the cuvette as a function of time. This is achieved by measuring the gradient of the  $\text{O}_2$  response which, when the x axis of your graphs is presented in min, will give a rate in  $\% \text{O}_2$  per min. The procedure for analyzing your data is as follows:

- (1) Reopen the computer cover. Double-click on the Logger Pro icon on the computer's desktop. You will likely get a dialog box telling you that the port was not found (no, duh!), so just click the "OK" button. This will bring up another dialog box; just click the "Cancel" button. Then, again, close out the dummy lab. Open the file containing your data. Your data run and graphs should reappear just as you saved them.
- (2) Select the "Examine" option under the "Analyze" menu item. This sets up a behavior for the mouse pointer. When you point to a place on the upper graph, a vertical line extends from top to bottom of the screen showing you where the examining function is looking. It also scrolls the data table at right and highlights a particular row of data corresponding to the point displayed at the pointer on the screen.
- (3) Find the beginning of your 0% maximum irradiance run. Drag from that point to the end of that particular run. Now the range of data are highlighted in the table. Select the "Linear Fit" option under the "Analyze" menu item. The computer will do a linear regression fit for these data and display the line on the graph and show you the  $y=mx+b$  formula for this fitted line. An  $r^2$  value is also shown. Double-clicking on the equation box will allow you to select a color for this line to help it stand out from others you are going to produce. Finally, you should drag the equation box by its title bar close to the portion of the graph that it describes. Record the slope value (m) in the table below. Also record below the PFD value from the highlighted row in the data table.
- (4) Repeat step 3 above for each of the runs at different PFD values. Changing the color of the lines and moving the equation box each time will help you keep track of your progress. Recording the values in the table below as you go will also help.

## Calculations

Each slope (m) value from each regression that you performed represents the rate of increase of O<sub>2</sub> concentration in the chamber with time. As such, each of these values are rates of photosynthesis expressed as %O<sub>2</sub> per min. However, photosynthesis is usually expressed in terms of μmoles of O<sub>2</sub> evolved per unit leaf area per unit time i.e. in units of μmol O<sub>2</sub>/m<sup>2</sup>/min. To make this conversion, the following procedure is required.

The m value is the O<sub>2</sub> concentration of the chamber increased by m %O<sub>2</sub>/min.

m %O<sub>2</sub> is equivalent to 10 000 m part per million (ppm) O<sub>2</sub> which, in turn, is equivalent to 10,000m μL of O<sub>2</sub> per L of gas in the chamber.

At STP 1 μmole of any gas occupies 22.413 μL, so at the temperature T of the laboratory, 10,000m μL of O<sub>2</sub> contains:

$$10,000 \text{ m} / [(273+T)/273] \cdot 22.413 \text{ } \mu\text{moles of O}_2.$$

Let this number = Y μmoles of O<sub>2</sub> (i.e. Y is the number of μmoles of O<sub>2</sub> that were produced per liter of gas in the chamber per minute).

To obtain a photosynthetic rate we must now multiply Y by the volume of the chamber expressed in liters. The chamber is designed so that when closed it has a fixed internal volume of 0.047L. Therefore, in our example, photosynthetic rate would be 0.047 Y μmol O<sub>2</sub>/min/leaf. To express this rate on a leaf area specific basis (e.g. μmol O<sub>2</sub>/cm<sup>2</sup>/min) it is necessary to divide the value by the area of the leaf (in cm<sup>2</sup>) that you obtained using the acetate grid.

## Results

m (%O <sub>2</sub> /min)	Photon Flux (μmol quanta/m <sup>2</sup> /s)	Photosynthetic Rate (μmol O <sub>2</sub> /min/cm <sup>2</sup> )

When you have calculated rates of photosynthesis at each light intensity used in your experiment, present your data as a graph with photosynthesis rate plotted on the y axis and light intensity on the x axis. Using Excel, do a linear curve-fit (regression) for at least the first few points. What does the y-intercept tell you? What does the x-intercept tell you?

A photosynthetic light response curve for a generalized leaf might show an initial rise followed by a leveling off phase. At low light intensities, photosynthesis increases linearly as light is increased. This is because at these intensities the rate of photosynthesis is limited by the rate of the light reactions. Insufficient photons are being supplied to the leaf to produce the ATP and reductant required to sustain maximum photosynthetic rates. At higher light intensities there is less of an increase in photosynthetic rate per unit increase in light intensity, and eventually photosynthesis reaches light saturation at the highest light intensities used in the experiment. Under these conditions, the light reactions of photosynthesis are maximized, and the photosynthetic rate is limited either by the supply of CO<sub>2</sub> to supply the photosynthetic dark reactions, or by the turnover rate of the photosynthetic enzymes. Or, in the case of shade-tolerant plants, outright phototoxicity!

The photosynthetic light response curve of a particular plant is influenced by many factors, and a study of the components of the curve can tell us a great deal about the physiology and ecophysiology of the plant. Important aspects of the light response curve include:

- (i) **The Light Compensation Point.** Find the x-intercept in the light response curve (at the point where photosynthetic rate is zero). The light intensity at this point is called the light compensation point, and it represents the light intensity at which O<sub>2</sub> production in photosynthesis is balanced by O<sub>2</sub> consumption in respiration. You probably recall the significance of this intensity in the care and maintenance of plants.
- (ii) **The Rate of Dark Respiration.** The y-intercept in the light response curve (at the point where the photon flux is zero) is likely a negative value. This point gives an estimate of "dark" respiration rate. Why is the value negative?
- (iii) **Photochemical Efficiency.** Photochemical efficiency may be defined as the increase in photosynthetic rate achieved per unit increase in light absorbed by the leaf. In your experiment, you did not measure light absorbance by the leaf, but only the amount of light transmitted through the leaf. However, a qualitative measurement of photochemical efficiency may still be made by calculating the initial slope of the light response curve. You might think about how sun/shade leaves might differ in this aspect.
- (iv) **The Light Saturation Point of Photosynthesis.** The light intensity beyond which the light response curve plateaus is called the light saturation point of photosynthesis. At this point increases in light intensity do not cause increases in photosynthetic rate, so other factors apart from the supply of light must be limiting the photosynthetic process. These factors include:
  - (i) The supply of CO<sub>2</sub> to the leaf.
  - (ii) The ability of the leaf to transduce the light energy supplied into chemical energy for photosynthesis (dependent on the photophosphorylation capacity of the leaf).
  - (iii) The capacity of the leaf to use energy from photophosphorylation to fix CO<sub>2</sub> (dependent on the amount, and turn-over rate, of enzymes involved in the Calvin cycle).

Did you measure the light saturation point in your experiment? If not, why do you think the light saturation point was not reached? If the light saturation point was reached, do you think that CO<sub>2</sub> supply was the major factor limiting photosynthesis at this point? How would you test this? Remember that your breath contains approximately 100 times the concentration of CO<sub>2</sub> in the atmosphere.