BISC 367 Plant Physiology Laboratory SFU

CO_2 exchange in plants

The effect of light intensity and quality on photosynthetic CO_2 fixation and respiration in C3 and C4 plants

Using light energy and chlorophyll plants are able to oxidize water and use these electrons to generate energy and to reduce carbon that is taken up from the atmosphere in the form of CO_2. This is the process of photosynthesis and the end products are energy-rich sugar molecules. The overall stoichiometry of photosynthesis has one mole (or molecule) of oxygen is produced per mole of CO_2 that is fixed or assimilated. This allows us to measure the apparent photosynthetic rate by assessing the amount of O_2 that a leaf produces or the amount of CO_2 that is taken up by a leaf. However, these measurements are complicated by the fact that plants are also respiring as they release the energy stored in sugars and other energy-rich molecules; this process consumes O_2 and releases CO_2 thereby effectively working opposite to what is achieved during photosynthesis.

When we measure net CO_2 uptake by a leaf we do not distinguish between photosynthetic and respiratory gas exchange and simply measure the sum of these two processes. To know the true rate of photosynthesis we need to be able to measure leaf CO_2 output in the light so as to know the rate of respiration. However, simultaneously measuring photosynthetic CO_2 uptake and respiratory CO_2 output is not easy and requires the use of ^{14}C-labeled substrates or tritiated H_2O or $^{18}O_2$. Furthermore, the rate of CO_2 uptake at saturating light intensities may be 30 times the respiratory rate but this situation is complicated by the impact of photorespiration (light-dependent CO_2 output), which varies from plant to plant and is strongly influenced by whether the plant uses C4 or C3 photosynthesis. In C3 plants rates of photorespiration can account for a sizeable fraction of the total gas exchange whereas in C4 plants photorespiration rates are negligible.

A convenient and widely used method to measure the apparent photosynthesis rate (APS) is to use an infrared gas analyzer (IRGA). CO_2 absorbs strongly in certain infrared wavelengths and this absorption is used to measure the CO_2 concentration in a volume of gas. The principle behind the IRGA is to measure the IR absorption and to convert this absorption into an electrical signal and thence to a concentration of CO_2 in parts per million (ppm).

Objectives:

During this exercise you will learn:

- How to use an IRGA to measure changes in CO_2 concentrations
- The effects of light intensity on the rate of CO_2 exchange in two different type of plants (C3 and C4) and how to determine the light compensation point from a light response curve.
- The effects of O_2 concentration on the rate of CO_2 exchange in C3 and C4 plants

- How to use a radiometer and the quantum meter to measure the light intensities and photosynthetically active radiation (PAR) or photosynthetic photon flux density (PPFD), respectively.

Materials

You are provided with:

Plant material (bean and corn)
Calibrated IRGA and light source
Gas cylinder filled with air and/or other gas mixes
Acetate grid to measure leaf area
LabPro connected to a computer

Experiment one: using the IRGA to measure apparent photosynthesis

Procedure:

1) Set up the IRGA apparatus as follows: Connect the pump to the flow meter to the inlet of the leaf chamber. Connect the outlet of the leaf chamber to the humidity sensor to the drying column and finally to the IRGA.

2) Attach a gas bag containing air to the inlet of the pump. Let the system flush for 3 – 5 minutes and record the [CO_2] as reference [CO_2].

3) Set the Logger Pro display so that the y-axis of the graph has a range of approx. 130 ppm CO_2. This range should include values that are up to 30 ppm above the reference CO_2 concentration and up to 100 ppm below the reference value.

4) Seal a leaf inside the leaf chamber and place the LED light source on top of the leaf chamber

5) The leaf chamber has four gas ports that are grouped in pairs. Each pair has a port on the upper surface of the chamber that is located directly above a port on the lower surface of the chamber. These ports distribute the gases to the upper and lower leaf surfaces through manifolds. Each pair of ports is attached to plastic tubing that connects via a Y-connector to a single piece of tubing.

6) Turn on the light adjusting to the maximum output.

7) You will observe a decline in the CO_2 concentration of the gas leaving the leaf chamber as photosynthesis consumes CO_2 delivered in the reference gas. This may take several minutes.

8) When a steady value of CO_2 is reached record the CO_2 value as your "Analysis CO_2" value.

9) Turn the light source off. The CO_2 concentration will increase as photosynthesis stops. You should see a peak in the CO_2 level within the first few minutes. Record the CO_2 concentration attained after the concentration has reached a steady level. This is your "dark analyses CO_2" value.

10) Click the STOP button on the computer screen to stop data collection and save your data ("File", "Save As") giving it a name.

11) Measure the area of your leaf. Note: if the leaf completely filled the chamber the enclosed area is 9 cm^2. If your leaf did not completely fill the chamber you must use the acetate grid to estimate leaf area. Place the grid on the surface of the chamber.

12) Using the acetate grid count the number of interstices that are completely enclosed by the area of the leaf and assign a value of 1 to each. Count the number of interstices that fall on the leaf margin and assign these a value of 0.5. Total the number of interstices and divide that value by 4 to obtain the leaf area in cm^2.

13) Remove the leaf from the leaf chamber. KEEP your leaf we may need it to determine chlorophyll content/ fresh weight/ dry weight if there is enough time.

14) Repeat for the corn plant. In addition, there are other plants available that have been exposed to a variety of environmental stresses. Measure the CO_2 uptake rate for one of these. The plant you have just measured is a control, unstressed plant.

Calculation of the CO_2 exchange rate

Measurements of CO_2 exchange rates in leaves are typically expressed in units of CO_2 exchange per unit time per unit leaf area (μmol $CO_2/m^2/s$). There are other ways that we can express these data such as CO_2 exchange based on leaf weight (dry or fresh) or leaf chlorophyll content. To express your data as μmol $CO_2/m^2/s$ you need to calculate the following:

1) The difference between the CO_2 concentration in the reference and analyses gases. i.e. ΔCO_2 = Reference CO_2 – analysis CO_2
2) Convert the ΔCO_2 value from (ppm) to (μmol CO_2/L) using the following calculation:

$$\Delta CO_2/22.415 \times ([T+C]/T)$$

C = temperature in ^oC
T = absolute temperature (273 K)

3) Multiply the ΔCO_2 by the flow rate (L/s) to get the CO_2 exchange rate per second.
4) Divide the CO_2 exchange rate by the leaf area (m^2) to get μmol $CO_2/m^2/s$.

Experiment 2. Measuring the light dependence of photosynthesis

Procedure:

1) Set up the IRGA and proceed as for experiment one.

2) Seal a leaf from a bean plant inside the leaf chamber.

3) Turn on the light source and adjust to 10% of the maximum output. Record the light output.

4) Observe the decline in CO_2 concentration in the gas leaving the leaf chamber. Record the CO_2 concentration at steady state conditions. This is the "analysis CO_2" at 10% light output.

5) Increase the light output to 20% of the maximum level. Measure the light level.

6) Observe the change in the CO_2 concentration of the gas leaving the leaf chamber. When the CO_2 concentration reaches a new steady state level record the concentration.

7) Increase the light output to 40% and repeat as for steps 5) and 6). Repeat this for a light output of 60% and 80% of the maximum output and for the maximum light level. Remember to measure the light level in each case.

8) Turn off the light and observe the increase in CO_2 concentration. Record the CO_2 concentration when it reaches a steady state level.

9) Stop data collection and follow steps 10) to 13) as for experiment one. Calculate the CO_2 exchange rates.

10) IF there is enough time you can repeat this experiment for the corn plant.

11) Record your results in the table below

Photon flux ($\mu mol/m^2/s$)	ΔCO_2 (ppm)	Photosynthesis rate ($\mu mol\ CO_2/m^2/s$
Bean		
Corn		

Construct a photosynthetic light response curve for the bean and the corn plant.

Determine:

The light compensation point
The rate of dark respiration
The photochemical efficiency
The light saturation point

See the end of the handout for a light response curve and information on these parameters.

Experiment 3. Photosynthetic response to O_2 concentration in a C3 versus a C4 plant

Procedure:

1) Set up the apparatus as in step 1) of experiment one with the exception that you will attach a high O_2 air bag to the flow meter.

2) Allow the system to flush for 3 minutes.

3) **Record the CO_2 concentration as your "reference CO_2 concentration- high O_2"**.

4) Set the Logger Pro display so that the y-axis of the graph has a range of approx. 130 ppm CO_2. This range should include values that are up to 30 ppm above the reference CO_2 concentration and up to 100 ppm below the reference value.

5) Seal a bean leaf inside the leaf chamber as for step 2) of experiment two and proceed to step 8).

6) Stop data collection by clicking on the STOP button on the computer screen and save your data.

7) Detach the high O_2 air bag from the inlet of the pump and seal it. Do not remove the leaf!

8) Attach a low O_2 air bag to the flow meter. Flush the system for 3 minutes and then record the CO_2 concentration as your "reference CO_2 concentration – low O_2". Repeat steps 4) to 6). You will omit step 5) because you have not removed your leaf.

9) Measure the leaf area using the acetate grid and remove the leaf.

10) Repeat for corn.

11) Record your results in the table:

		Bean	Bean	Corn	Corn
O_2 concentration (ppm)	Photon flux ($mmol/m^2/s$)	ΔCO_2 (ppm)	Photosynthetic rate ($\mu molCO_2/m^2/s$)	ΔCO_2 (ppm)	Photosynthetic rate ($\mu molCO_2/m^2/s$)

Construct a light response curve for corn and bean under high and low O_2 conditions. You now have three light response curves:

1) Low O_2
2) High O_2
3) Ambient O_2

Calculate for all three O_2 conditions the:

Light compensation point
Rate of dark respiration
Photochemical efficiency
Light saturation point

Light Compensation point is the light level at which CO2 consumption by photosynthesis exactly matches CO2 production arising from respiration. Extrapolate the linear portion of your light response curve to intercept the *x-axis* at the point where the photosynthesis rate is zero.

Rate of dark respiration can be estimated from you light response curve by extrapolating it to intercept the *y-axis* at the point where the light intensity is zero.

Photochemical efficiency is obtained by calculating the initial slope of the light response curve. This value reflects the efficiency by which light energy is used for photosynthesis.

Light saturation point of photosynthesis is the light intensity at which the light response curve plateaus. Beyond this point light does not limit photosynthesis but other factors become limiting.

A light response curve (taken from "Plant Physiology" Taiz and Zeiger, 3rd edition).