Biology 3460 - Lab Exercise 4, Part a
Photosynthetic Gas Exchange Measurements

Objectives:
This lab exercise is intended to:
(1) introduce students to the methods and equipment used in studies of photosynthetic CO$_2$ exchange,
(2) illustrate that net loss of CO$_2$ occurs in a leaf in the dark and that net uptake of CO$_2$ occurs in a leaf at light intensities above the light compensation point,
(3) demonstrate that the initial slope of the light response curve of net CO$_2$ exchange is an indicator of light-use efficiency or apparent quantum yield of photosynthesis,
(4) illustrate that photosynthesis increases with increases in light intensity until a light saturation point is reached
(5) test the effect of different light intensities on photosynthetic capacity and the light response of net CO$_2$ exchange in sunflower (Helianthus annuus) plants.

Introduction
The major objective of this exercise is to demonstrate that plants of the same species have different photosynthetic characteristics depending on the environment in which they were grown. Plants generally have a strong ability to acclimate to specific environmental conditions, often allowing them to optimize the use of a limiting resource. In this experiment you will compare plants that have been grown in growth chambers at different light intensities.

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 eco-physiology of the plant. Important aspects of the light response curve are listed below and shown in figures that are attached.

(i) **The Light Compensation Point.** Extrapolate the linear portion of the light response curve to the point on the $x$ axis where the CO$_2$ exchange rate is zero. The light intensity at this point is called the light compensation point, and it represents the light intensity at which CO$_2$ uptake in photosynthesis is balanced by CO$_2$ loss in respiration.

(ii) **The Rate of Dark Respiration.** If the linear part of the light response curve is extrapolated to intercept the $y$ axis at zero light intensity, the negative rate of photosynthesis at this point gives an estimate of “dark” respiration rate.

* Suggest a way in which it may be measured directly.

(iii) **Light-use Efficiency.** Light-use efficiency or quantum yield 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 incident on the leaf. However, a qualitative measurement of
photochemical efficiency may still be made by calculating the initial slope of the light response curve.

(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$_2$ 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 [ATP-formation due to light reactions]).

(iii) The capacity of the leaf to use energy from photophosphorylation to fix CO$_2$ (dependent on the amount, and turn-over rate, of enzymes involved in the “dark reactions” of photosynthesis).

**Protocol:**

Work as a group of two students to complete this lab exercise. For the net photosynthesis assignment described below, use data collected by your individual group.

Measure rates of net photosynthesis at a range of light intensities from high light to darkness (e.g. 1200, 800, 600, 400, 300, 200, 100, 0 µmol photons m$^{-2}$ s$^{-1}$).

Construct and hand-in a graph that illustrates the light response curve of net photosynthesis for plants grown under high and low light conditions.

Use the excel file (Lab4 Sun&Shade CurveFit.xls) to estimate parameters ($A_{max}$, Dark Respiration Rate, Quantum Yield, Curvature Parameter) for your two light response curve data sets. Use of the excel file for this purpose will be explained during the lab period.

Provide brief answers to the following questions in your report for both light response curves.

1. What is the light intensity at which light saturation of net photosynthesis occurs?

2. What is the rate of dark respiration?

3. What is the light intensity at which net photosynthesis equals zero (or the light compensation point)?

4. What is the apparent quantum yield (or light-use efficiency; initial slope of the curve at light intensities well below light saturation)?
Calculation of CO₂ Exchange Rate

Measurements of photosynthetic and respiratory rates in leaves are usually expressed as rates of CO₂ exchange per unit time per unit leaf area. The units most commonly used are µmoles of CO₂/m²/second (µmol CO₂ m⁻² s⁻¹).

Calculate the difference between the CO₂ concentration in the reference and analysis gases. For example, if an experiment was conducted in air of 350 ppm CO₂, at a flow rate of 0.5 L/min, the depletion of CO₂ due to leaf uptake in photosynthesis at high light may result in an analysis gas CO₂ concentration of 310 ppm. The difference between the reference and sample gas streams (ΔCO₂) in this example would be 40 ppm (or 40 µmol/mol).

\[
\text{CO}_2 \text{ flux rate} = \left[ \Delta \text{CO}_2 \ (\mu\text{mol/mol}) \times \text{Flow rate} \ (\text{mol s}^{-1}) \right] / [\text{Leaf Area} \ (\text{m}^2)]
\]

(1) Convert the Flow Rate from L min⁻¹ into mol s⁻¹:

a) L min⁻¹ into L s⁻¹: divide by 60
b) L s⁻¹ into mol s⁻¹:

\[n = \frac{(PV)}{(R(°C+T))}\]

where \(n\) is mol s⁻¹, \(C\) is the air temperature in °C and \(T\) is the absolute temperature (273 K), \(R\) is the gas constant (0.08314 L bar mol⁻¹ K⁻¹), \(P\) is atmospheric pressure (0.9 bar in Lethbridge), \(V\) is the volume flow rate in L s⁻¹.

At an air temperature of 20°C, and a flow rate of 0.5 L min⁻¹, the molar flow rate is: 0.000307882 mol s⁻¹.

(2) Multiply the ΔCO₂ value by the flow rate (in mol s⁻¹) used in your experiment to obtain a CO₂ exchange rate per second. So the CO₂ exchange rate in our example would be: 40 x 0.000307882 = 0.012315 µmol s⁻¹.

(3) Express your CO₂ exchange rate on a leaf area basis by dividing the CO₂ exchange rate per second by the leaf area in m². If the leaf completely fills the chamber, the area used in the calculation would be 9 cm², equivalent to 0.0009 m². The photosynthetic rate in our example would therefore be 13.7 µmol CO₂ m⁻² s⁻¹, which is a reasonable rate for a C₃ species under ambient conditions.