How Many Plants Does it Take to Keep You Alive?

Dr. Weise
410 Biochemistry
email: weisesea@msu.edu

At the beginning of class you are expected to turn in a brief summary of the lab and the answers to the 3 questions posed in the introduction (in green).

Introduction:

By now you are very aware that photosynthesis has two major parts. One we call electron transport which occurs on the thylakoid membrane of the chloroplast. The other is called the Calvin cycle and occurs in the stroma of the chloroplast. Chloroplast electron transport splits water at photosystem II and this is where the oxygen that we breathe comes from. The Calvin cycle takes up carbon dioxide via the carboxylation reaction of ribulose-1,5-bisphosphate carboxylase/oxygenase, more commonly called rubisco (we will talk about the oxygenase activity of this enzyme next week). These two processes are very tightly linked and one cannot fully proceed without the other. If we wanted to measure the photosynthetic rate of a plant we could either measure the amount of oxygen produced or the amount of CO_2 taken up. For many reasons it is far easier to measure the amount of CO_2 uptake than it is to measure the amount of O_2 production.

What is the current atmospheric concentration of Oxygen and CO_2 in both % terms and parts per million terms?(This information is easily found in a Google search and a little math).

Given your answer to this question, **why might it be harder to measure the amount of oxygen given off by a plant leaf?**

Both methods are commonly used in plant biology, but measurement of CO_2 uptake using an infrared gas analyzer (IRGA) has become the "gold standard" of photosynthetic measurements.

By now you are also well aware of global warming. Global warming occurs because certain gases (mainly H_2O and CO_2) absorb the infrared radiation in sunlight and prevent its rapid retransmission to space. This is called the greenhouse effect. It is mainly the presence of large amounts of water vapor in earth's atmosphere that results in most of earth being at temperatures hospitable to life as we know it and it is this lack of water vapor, that keep Mars at a chilly -60°C. An infrared gas analyzer uses the greenhouse effect to measure CO_2 and H_2O vapor and thus we can use it to measure the photosynthetic rate and transpiration rate of a plant. In this lab you will quantify the amount of carbon dioxide taken up by a plant and your own respiration rate. Using your knowledge of photosynthetic electron transport, the Calvin cycle and a few assumptions about human physiology you will estimate how many plants it would take to provide enough oxygen production and carbon dioxide uptake to sustain your life.

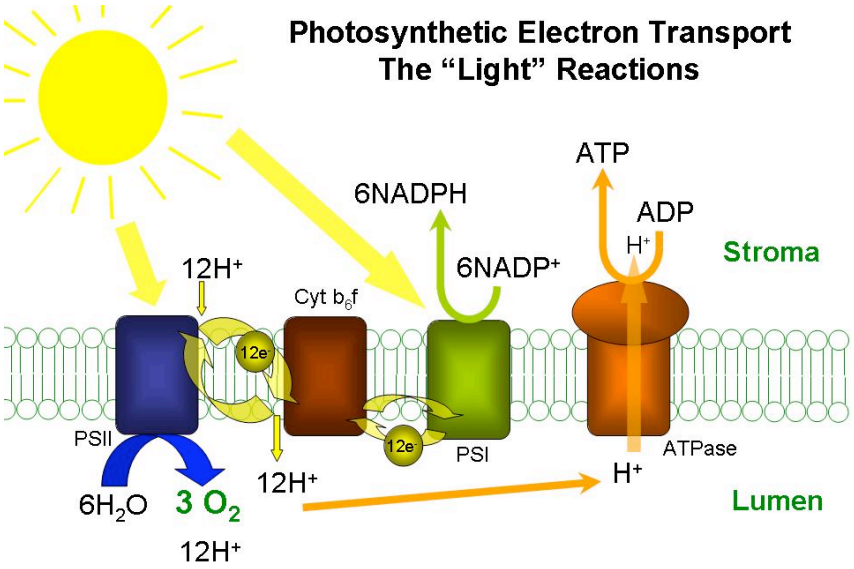

Figure 1. Photosynthetic Electron Transport.
It is on the lumen side of photosystem II that water is split using light energy to yield electrons, protons, and oxygen. PSI is the ultimate destination for the electrons where they are used to reduce $NADP^+$ to make NADPH. The H^+ ions that accumulate on the lumen side of the thylakoid membrane generate a proton motive force which is used by ATP synthase to phosphorylate ADP into ATP.

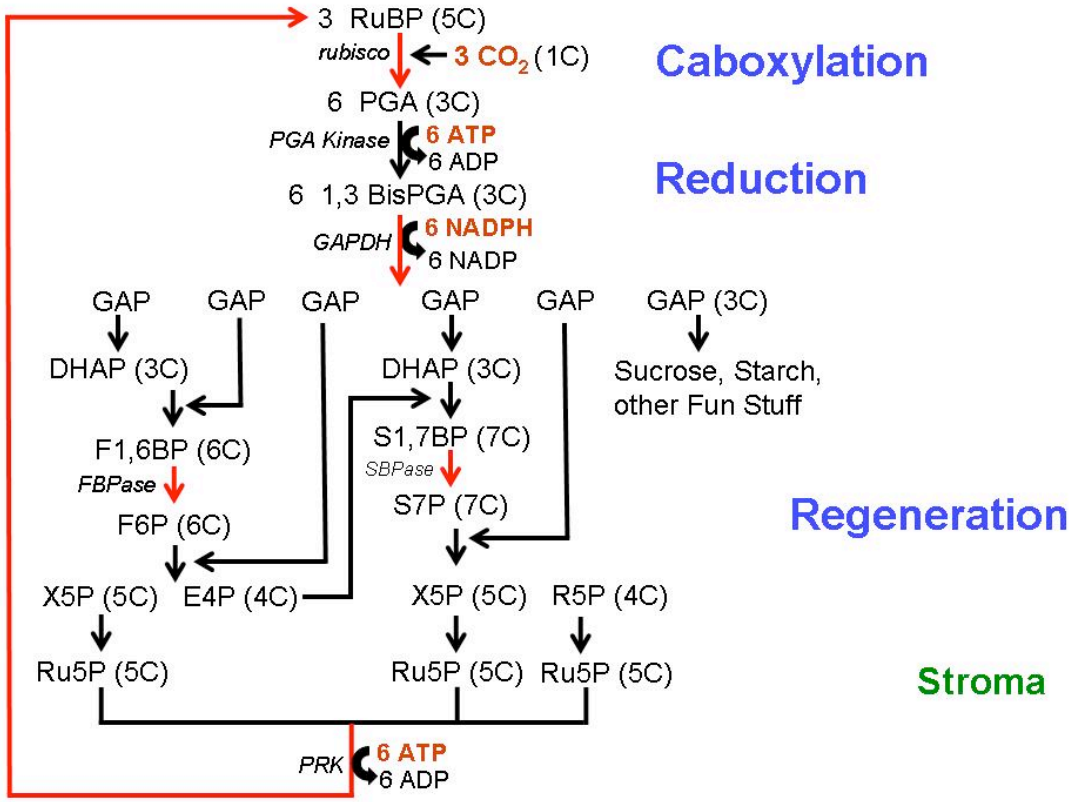

Figure 2. The Calvin Cycle
All the enzymes that make up the Calvin cycle are located on the stromal side of the thylakoid membrane. It is the Calvin cycle which is responsible for the CO_2 uptake in plants. Ribulose-1,5-bisphosphate, a 5 carbon sugar, is carboxylated by rubisco using CO_2 to generate two 3 carbon molecules, 3-

phosphoglycerate (PGA). The Calvin cycle cannot work in the dark due to the need for NADPH and ATP provided by photosynthetic electron transport and because 5 of the enzymatic steps (red arrows) are regulated by pH, Mg^{2+}, or thioredoxin and do not work in the dark.

How do pH, Mg^{2+} change in the chloroplast in the light versus the dark?

Procedure:
You will work in groups of 2 to accomplish all the tasks. However, each student is responsible for all the data in the lab notebook and submitting your own report. Make sure you get all the data. If you don't write it down yourself copy it from your partner later. Because we only have two IRGA's to measure photosynthesis, we can't all use them at the same time so while some groups will start with the Plant Physiology questions others can start with the Human Physiology part.

A. Human Physiology
The amount of carbon dioxide taken up by a single plant is very small (≈ 0.0005% CO_2 in air) compared with the amount of carbon dioxide given off by a human being (5% CO_2 in air). Because of this difference in scale the instruments that we use to measure CO_2 uptake by the plant cannot be used to measure the much larger amount of CO_2 evolved by a human being. The amount of CO_2 we emit per O_2 we consume is called the respiratory quotient (RQ). If we are burning carbohydrates the RQ is 1. So we will use some assumptions and generalizations to estimate our respiration rate.

- How many breaths do you take per minute? sitting still? running?
  Using a stop watch, or any clock that counts seconds, count the number of breaths you take in one minute. Now run up and down the hallway, or go outside, for 5 minutes quickly come back and again measure how many breaths you take in one minute.

- What is the total volume of air you breathe in one minute?
  Hint: The amount of air you breath in and out is called the tidal volume and is ≈ 500 ml

- Of the total volume of air you breathe in one minute how much is oxygen?
  Hint: You breathe out 16% oxygen

B. Plant Physiology
To measure the photosynthetic rate of a plant we will use a commercially available IRGA. The instrument we will use is called a LI-6400 and is the most commonly used IRGA in plant research. In fact the LI-6400 is actually 2 IRGA's. One is called the "Reference" and measures CO_2 and H_2O vapor in the air that has not passed over a leaf. The second one is called the "Sample" and it measures CO_2 and H_2O vapor in the air that is in contact with the leaf. It is the difference between these two IRGAs that gives us the photosynthetic rate of the plant.

The CO_2 concentration is displayed as a mole fraction. In other words you might see on the display 400 μmol/mol which means there are 400 μmol of CO_2 for every mol of air. We can also call this a part per million (ppm) i.e. there are 400 parts of CO_2 for every million parts of air. The H_2O vapor is displayed 2 ways. The first is as mole fraction, in this case it is mmol / mol or parts per thousand. The other way, and far more useful, is as a dew point. A dew point is literally the temperature at which water will form dew, or condense. The higher the dew point the more water vapor is in the air and that water will condense at a higher temperature because there is more of it. You are probably familiar with relative humidity (RH). Relative humidity is not a very useful scientific measurement alone because it is relative. It is relative to temperature. Warm air will hold more water molecules

than cold air. So 90% RH on a summer day is sticky and miserable but 90% RH on a cold winter day still gives you chapped lips and static electric fun.

As you are aware from your previous labs lots of things can influence the photosynthetic rate of a plant. Some of the big ones include light level, temperature, CO_2 concentration, and humidity. We can vary any of these parameters and measure the photosynthetic rate. In fact looking at how photosynthesis changes in response to changes in light, CO_2, temperature etc. can reveal many things about plant metabolism and is a common tool in the plant researcher's tool kit. However, you probably want to get out of lab sometime before the summer so we will pick a fairly standard set of conditions, which we will decide on together in class. If you were asleep during that part of the lecture I'd give your lab partner a quick call because I expect to see these conditions in your write up. The LI-6400 will have been properly calibrated for you, for the convenience of time. So all you need to do is set the conditions, place a leaf in the chamber, often called a cuvette, and make a measurement.

1. Before you clamp the LI-6400 onto your leaf make sure the conditions that set are appropriate for the measurement you wish to make. What is the CO_2 level? Are the lights on and what is the light level? What is the humidity? What is the temperature in the chamber? What is the flow rate?

2. Carefully place a leaf into the chamber, try to fill as much of the chamber as possible to ensure a good signal. Once the leaf is in the chamber gently clamp the leaf in place. Make sure you did not break the petiole.

3. While watching the instrument panel allow the plant to acclimate for 5 – 10 minutes. While you are waiting for the leaf to acclimate keep a careful eye on the leaf temperature, chamber temperature and dew point. We do not want to allow the dew point to climb above the chamber temperature. Why would we not want the dew point to rise above the chamber temperature? If the dew point does start to climb close to the chamber temperature you can make some adjustment to lower the humidity.

a. If you have a good CO_2 signal i.e. the difference between the reference and sample CO_2 is greater than 5 ppm you can raise the flow rate.
b. If the flow rate is already at max and/or the CO_2 signal is low you can scrub the incoming air to remove water vapor and lower the reference dew point.
c. If 1 and 2 fail you can always raise the chamber temperature.

You also will want to "Match" the reference and sample IRGA's to make sure they have not drifted apart. Matching allows the sample air stream that has passed over the leaf to pass through both the sample and the reference IRGAs. When this happens both IRGA's should read the same, if they do not you can "match" them so that they do. This is the equivalent of taring or zeroing a balance before you weigh something.

4. Watch the photosynthetic rate, is it climbing, falling or relatively stable. When you are satisfied that the photosynthetic rate is relatively stable match the IRGA's one last time and make a measurement.

- What is the average photosynthetic rate of the plant?
  Hint: This is given by the LI-COR 6400 in units of μmol CO_2 m^{-2}leaf area s^{-1}
  Now increase the CO_2 level, allow to acclimate for 5 minutes and take another reading. Repeat this until you have measured the photosynthetic rate's at all the CO_2 levels we agreed upon in the lecture

- What is the conductance rate of the plant? How does this compare the conductance you measured using your potometer. Why might these be different? Make sure to note the transpiration rate as well.

- For every μmol of CO_2 taken up by the plant how much O_2 is given off?
  Hint: Use your now extensive knowledge of the stoichiometry of electron transport and the Calvin cycle, think about what links these two processes.

- How much leaf area does a plant have?
  Hint: Take all the leaves off a plant and trace them on a piece of paper. Cut out the pieces of paper and weigh them. Now cut out a piece of paper with a known area and weight that.

C. Putting It All Together

At this point you have collected all the data you need to answer the question, how many plants does it take to keep you alive. You just have put information together, get everything in the same units and remember the ideal gas law from freshman chemistry. There are multiple ways to attack this problem. I will lead you through my way of thinking with the following questions.

- How many moles of O_2 do you use per minute, now per second?
  Hint: This is where you need the ideal gas law PV=nRT,
  R is the ideal gas constant = 8.20973×10^{-2} L atm K^{-1} mol^{-1}, this number is adjusted for temperatures ≈ 25°C

- How many square meters of plant leaf do you need to provide all the O_2 you need?

- How many plants do you need to keep you alive?

D. Other Considerations

- What happens when the lights are off, i.e. at night

- If we keep cutting down the rain forests will we all suffocate?

- How do you think rising CO_2 will affect plant growth?
  Hint: Tell me what you think, but also look up some scientific papers on the subject to expand on your points. Elevated CO_2 studies are a hot topic in botany

Lab Report

Abstract

Even though the abstract comes first a paper, write this last. Keep your abstract short and to the point. The abstract should be 200 words or less. In the abstract you should be able to convey to the reader:

1. Why your experiment is important and where it fits in the larger scheme
2. What the major finding of your experiment were
3. Your conclusions based on those results

Introduction

This doesn't have to be long, 2 paragraphs are more than enough. Be sure to include the background the reader would need to understand the significance of your results. Conclude your introduction by

clearly and concisely stating what you did in this experiment and why you did it. Keep this to 4 to 5 sentences MAX.

You may "borrow" heavily from the introduction I provided in your lab manual but don't copy it verbatim and don't use the same colloquial tone I used in your lab manual.

Materials and Methods
Be thorough here. You want to include all the details necessary so that someone else can duplicate your experiment if they wanted to. The power of science is in its reproducibility. If someone cannot reproduce your results then it is not science. Do not list what you did as discrete steps, it's a science paper not a cook book.

Results
1. A bar graph documenting the average respiration rate (breaths per minute) sitting and running get data from other groups so that you have something to average. Be sure to include standard deviation.
2. A line graph showing how the photosynthetic rate changes with CO_2 concentration. Use only your own data here, photosynthesis can be extremely variable.
3. A line graph showing how the conductance changes with CO_2 concentration, again use only your own data

Please also include text in your results section describing your results. There should be text referring to each of the three figures that I asked you to make above. You can write out how does the respiration rate change from active to inactive. How does the photosynthetic rate change with CO_2 concentration? How does the conductance change with CO_2 concentration?

Discussion
Start your discussion by "discussing" the strongest point of your work first. If the reader could only remember one point from your work what point would you want them to remember.

Some things you will also want to include in your discussion are
1. If the standard deviation is high in your respiration data, why is it high?
2. Why does CO_2 affect the photosynthetic rate?
3. Why doesn't photosynthesis continue to increase with increasing CO_2 concentration?
4. Why does CO_2 affect the conductance rate?
5. How does the conductance you measured compare with the conduce you measured with the potometer?
6. All the questions posed in the "Other considerations" section