

FRST 200 -- PHYSIOLOGY LAB

November 5/7, 2019

WHEN DO LEAVES CEASE PHOTOSYNTHESIS IN THE AUTUMN?

The purpose of this lab is to introduce you to some techniques used to assess the physiological status of forest plants, and to illustrate some principles from lecture. We will use foliage from *Weigela florida* (Bunge.) A. DC., a member of the *Caprifoliaceae* native to China and Korea. The day before the lab, branches in various stages of fall senescence were taken from trees outside the building. The branches were placed with their cut ends in tap water to wait overnight near a window. We will test the hypothesis that senescing leaves continue to be capable of photosynthesis until all their chlorophyll has disappeared. In other words, do they stop photosynthesis and then turn yellow? Or do they start to turn yellow before they stop photosynthesizing? Naturally we expect that fully yellow leaves will no longer be able to photosynthesize, but they may still have a high respiration rate. To find out, you will test leaves in three colour classes: 1) fully green; 2) yellowish-green; 3) fully yellow

Please work in groups of three or four (depending on lab section). Data will be pooled to enable you to write your individual reports. With the help of the instructor and the TAs you will measure dark respiration rate, photosynthetic rate, chlorophyll content, leaf area used and leaf fresh mass used. We will return dry mass data to you later.

A. Getting Started

Each group will work with one leaf from each of the three categories identified above. Use scissors to cut a strip of leaf tissue, ~1 cm wide, on either the left or right side of the mid-rib. For each leaf strip you can start at either step **B** or **C**, but make sure both are completed before going to **D**. Please stagger your measurements.

B. CO₂ Exchange

1. Place the tissue into a test tube (use 3 test tubes per group, one for each color class; stagger these). Seal the tube by placing a rubber serum cap over the end. Try not to breathe near the test tube while you're filling it. Wrap the tube in foil to exclude all light, but permitting access to the serum cap. Make sure the tube is labeled so that you can identify it.
2. Through the serum cap, remove a 1 mL sample of air from the test tube using a syringe. Plug the needle by stabbing it into a rubber bung. **Immediately** place the tube in the water bath for dark incubation. Record the time!
3. Take your syringe to the Infrared Gas Analyzer (IRGA). There may be a line-up of students waiting. When it's your turn, quickly inject the sample of air into the IRGA. Record the peak height from the IRGA as shown on the chart recorder in chart units (CU). Full scale is 100 CU. The initial or starting CO₂ concentration can then be calculated from:

$$\text{CO}_{2\text{INIT}} = \text{CU} \times \text{slope}$$

where "slope" is the slope of a predetermined standard curve in units of $\mu\text{L CO}_2 \text{ L}^{-1} \text{ air CU}^{-1}$ (i.e., the same as parts per million, ppm). This number will be provided.

4. Wait ~30 minutes. While you're waiting, start the next tube. Still waiting? Record the temperature of the water bath. Why is temperature important?
 5. Remove the tube from the bath and immediately take a second 1 mL air sample. Record the time. Determine the CO₂ concentration at the end of the dark period (CO_{2DARK}) as above.
 6. Remove the foil wrapping from your tube and place it back in the bath under the light. Make sure the leaf tissue is facing upwards and is not shaded by anything (e.g., your labels). Check the temperature as before. A light meter is available to measure the photosynthetic photon flux density (PPFD; units = $\mu\text{mol quanta m}^{-2} \text{ s}^{-1}$). It's best to do this at the end of the experiment. Why does PPFD matter? How does this value compare to full sunlight?
 7. After ~15 minutes, remove the tube from the light and immediately take a third 1 mL air sample. Record the time. Determine the final CO₂ concentration (CO_{2LIGHT}) as above.
- Proceed to **C** if not yet completed for this sample.

C. Leaf Area, Fresh Mass and Chlorophyll Content

To convert your data to rates (either respiration or photosynthesis) you will need to know how much leaf area was in each test tube. Determine the “projected” leaf area in cm² by putting the tissue strips through the leaf area meter (consult TA – take a mean of three measurements; you may need to correct these measurements for the sensitivity of the meter). After measuring the leaf area, retrieve the tissue and determine the fresh mass using the balance (if available). Finally, use the chlorophyll meter to measure chlorophyll content index (CCI) in the middle on the upper side of your leaf strips (consult TA).

Go back to **B** if not yet completed for this sample.

D. Dry Mass

If steps B & C have both been completed, fold each leaf strip into a small foil packet, label with a felt pen (names and colour class please) and deposit at the front bench. Later, a TA will dry these packets to get the dry mass.

F. Finishing Up

Report the colour class type, CO_{2INIT}, CO_{2DARK}, CO_{2LIGHT}, leaf area, fresh mass and chlorophyll content index on the sheet provided. Have these numbers checked before you leave and please turn the sheet in at the front.

Further calculations can be left for later, but you may want help or advice now. First, calculate the difference between CO_{2INIT} and CO_{2DARK}. Then, calculate the difference between CO_{2DARK} and CO_{2LIGHT}. Knowing that the volume of the test tube was 18 mL, calculate how many μL of CO₂ each strip produced in the dark and how many μL of CO₂ it consumed in the light by multiplying these differences by 0.018 L. Note that CO_{2DARK} is your starting CO₂ concentration for the photosynthesis measurement. Finally, calculate the CO₂ exchange rates in units of $\mu\text{L CO}_2 \text{ cm}^{-2} \text{ leaf area h}^{-1}$ as follows:

$$\text{respiration rate} = (\mu\text{L CO}_2 \text{ produced}) \div (\text{leaf area}) \div (0.5 \text{ h})$$

$$\text{photosynthetic rate} = (\mu\text{L CO}_2 \text{ consumed}) \div (\text{leaf area}) \div (0.25 \text{ h})$$

[Note: you may need to adjust the times if the dark or light incubation periods differed from 30 and 15 minutes, respectively]

You can also calculate “leaf mass per area” (abbreviated to LMA) which is a commonly used metric in ecology to determine a plant’s investment (in tissue and nutrients) in a leaf given its spread (i.e., area). Some portion of the weight can also come from starch if the leaf is actively photosynthesizing. Leaves may lose weight if continuing to respire or actively exporting remaining carbohydrate. LMA is calculated by dividing the dry mass weight (in g) by the leaf area (in m²). To change mg weights to g divide by 1000. To change cm² to m² divide by 10,000. Why wouldn’t we just use the fresh weight to determine LMA?

PHYSIOLOGY LAB REPORT:

Your report will be your own (it is not a group report). This should be in 1.5 line spacing and written in 12 point font. You will hand in your report via Turnitin.com again.

Introduction – this should be maximum one page. Start with a broad view and become more focused to outline the point of the experiment, plus introduce one or two testable questions (testable means you can answer the question from the results of this experiment). You should then give a hypothesis (prediction) to your testable question. The null hypothesis is always “no difference between groups”. You should present your hypothesis of the experiment’s outcome and briefly explain why you expect that hypothesis to hold.

Materials & Methods – this should be maximum two pages. Order the methods as you conducted the experiment. Briefly explain the plant materials used, followed by the testing methodologies. (If you did section C first, present this part *before* section B or vice versa depending on what you did). You should also present the equations/calculations that were done as part of this experiment and explain what each equation means for the experiment (in other words, why did you have to use that equation as part of the experiment?). If you added any additional calculations, please include them in your results.

Results – this should be maximum two pages of text. (Graphs and tables do not count towards this limit). Like the last report, you should report on the different trends you observe. All numerical results and calculations should be reported in one or two tables. Don’t forget to include units! (how might you format a table so that it is as easy as possible for your reader to compare the averages from the different colour classes?). You should also graph the following against colour class: a) mean CCI, b) mean photosynthetic rate, c) mean respiration rate, and d) mean LMA. These are bar graphs.

Discussion – this should be a minimum of one page to a maximum two pages of text. Your discussion must deal with expected vs. observed differences (do you accept or reject your hypothesis? Did the leaves behave as expected?). It may turn out that other things are important to discuss and therefore worthy of analysis, such as the dry mass to fresh mass ratio. You should discuss sources of error (e.g., variation in CO₂ concentration from tube to tube). In doing your analysis and write-up, make sure to think carefully about what the gas exchange rates (that are

standardized for leaf area) tell you that the unprocessed CO₂ concentrations do not. Also, you can note how this experiment might be improved or made more realistic.

This report requires that you cite references. If it is not your idea and isn't common knowledge, you must include a citation.

You should aim to cite information from a book on the subject or primary literature (scientific journal articles, technical reports). Plant and tree physiology textbooks are good sources for background information. Blogs, Youtube videos, lecture notes, and websites are not good sources (**don't cite them**). This is because they are not refereed (reviewed by experts and editors).

Wikipedia, like any encyclopedia, is not really an acceptable reference, but it's a reasonable place to get some ideas.

Formatting for citations – because there are many information sources you may end up using, the following website gives guidance on how to use and cite these different sources. It uses the standard APA style:

<http://www.lib.sfu.ca/help/cite-write/citation-style-guides/apa>

The report is due in 2 weeks (Nov 19th or 21st depending on the lab section you attended to do the experiment).

We will send data and Turnitin details following the Thursday section.