PLANT PHYSIOLOGY: TRANSPIRATION

PLEASE…
When you arrive in the lab, do not engage in unstructured exploration of the computer and electronic apparatus. Leave the computer cover closed and wait for instructions from the TA.   As you work with the computer, do not change desktop and sound settings. We need to have the computers look and operate exactly as they did when we set them up. Thank you.

INTRODUCTION

Flowering plants (angiosperms) transport water in the form of a continuous column of liquid inward from the root surface to the xylem and upward in the stem from root to shoot and out into the leaves. In the leaves and other aerial organs possessing stomata (flower petals and sepals, some fruits, stems) liquid water turns to vapor and diffuses out into the atmosphere by the process of transpiration. A continuous supply of water is needed to replace water lost from aerial parts of the plant by transpiration. This replacement water moves upward in plant tissue, xylem, that is adapted for rapid, long distance transport.

The rate of transpiration, and hence of upward movement of water in the plant, depends on several factors. Read about this topic in your text and be prepared to suggest in a lab discussion several factors and how their relative contribution to water movement may be assessed.

The rate of water movement up a cut stem of a shoot (the stem is part of a shoot bearing leaves) caused by transpiration can be quantitatively estimated by a potometer. The potometer is a device designed for measuring rates of water uptake by a stem. The cut end of a stem with leaves is inserted into a chamber containing water. The chamber is a sealed, flexible, uncalibrated tube connected to a pressure sensor. Uptake of water by the plant from the chamber causes a decrease in pressure in the water column (a negative pressure) and this decrease is detected and quantified by the pressure sensor. The advantage of using an electronic pressure sensor is that it is very sensitive and stable. When connected to a computer, the observer can obtain useable results in only a few minutes (or just as quickly learn that there is an air leak and the potometer-plant must be set up again!).

It is important to realize that the cut shoot takes up water faster than the intact plant under the same conditions. This is because a major resistance to water uptake located at membranes of root cells (endodermis) has been removed. Thus a potometer connected to a cut shoot can estimate the influence of environmental and internal factors on uptake but not the true value of uptake for an intact plant.

SYNOPSIS

Herbaceous (nonwoody) angiosperms, both monocots and dicots, provide the experimental material. The stem of a cut shoot is inserted a little way into a flexible plastic tube nearly filled with water. The other end of the tube is attached to a sensor that can detect very small changes in pressure (pressure change caused by as little as one microliter of water removed by the plant from the tube). As water is drawn out of the plastic tube by the plant a partial vacuum is produced, and this is detected and transduced to an analog signal (voltage change) by the sensor, converted to a digital signal by an analog-to-digital converter box (ULI), and recorded by a laptop computer running LoggerPro software. Various factors that may affect transpiration, which in turn affect rate of water uptake by the stem, are available for study.

CONCEPTUAL OBJECTIVES

- Learn about plant stem structure and function.
- Learn about the energy source for upward movement of water in plants and how the energy is used.
- Learn about environmental factors that affect transpiration.
- Gain more experience in designing experiments, analyzing data, and interpreting results

PROCEDURAL OBJECTIVES

- Learn how to use and care for delicate electronic equipment.
- Learn how to collect and analyze data with assistance from a computer.

PREPARATION

- Read chapters 35 and 36 in the Campbell et.al. 5/e text.
- Observe the instructional potometer video @ http://www.udel.edu/introbio/208/potometer.mov

HYPOTHESIS OR SCIENTIFIC QUESTION

ACTIVITIES

Before the experimental phase of the investigation begins, engage in a class discussion of methods you will use today and of precautions you should take.

A. <u>Basic Procedure</u>

If you are not familiar with the *Logger Pro* computer application, printed tutorials are on reserve in each laboratory room.

a. Running *Logger Pro* with the Potometer

1. Make sure the ULI (Universal Laboratory Interface) and computer are turned on. If the ULI is not turned on, the computer will not know it is attached to a COM port when you execute the next step.

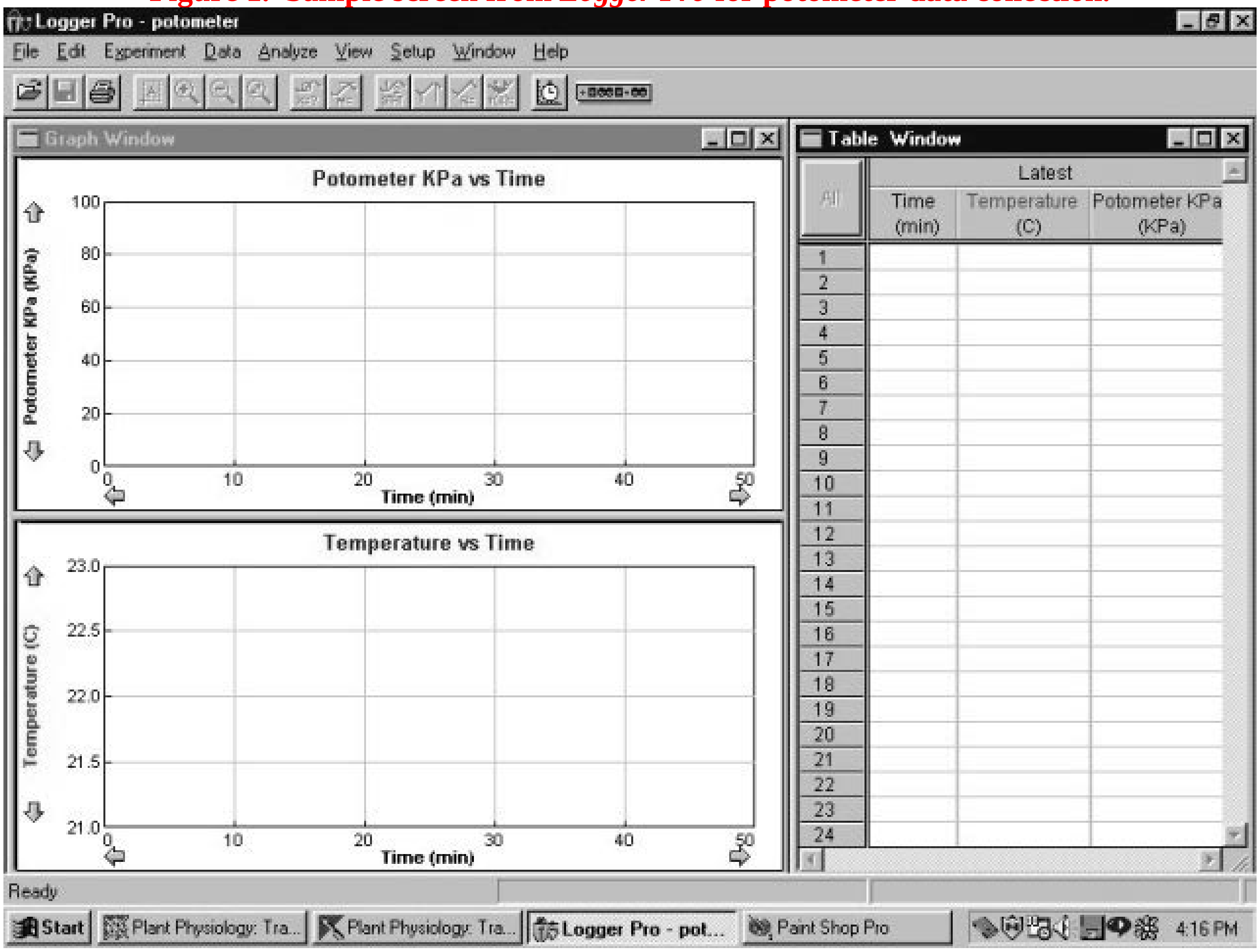
2. Double click on the 'My Documents' icon. A window will appear showing files in the 'My Documents' folder.

3. Double click on the file 'potometer.MBL' (the file name may be just 'potometer' without the .MBL extension showing). The computer screen will show two graph windows and one table window (Fig. 1). At the bottom of the screen there are two little windows showing probe outputs in real time. When collecting data, the upper graph window displays the potometer readings in KPa (Kilo Pascals) and the lower graph window displays the temperature probe readings. The table window displays numerical values from both probes and is used when analyzing the data or saving data to your floppy disk.

4. You are now ready to collect data. To do so, click on the 'Collect' button. Click on this button again (its name will have changed to 'Stop') to stop data collection.

1. **IMPORTANT: If you exit the application without storing data in a file with a new name, you may get a dialog box asking if you want to save the setting. Always say no to prevent overwriting the calibration file.**

Figure 1. Sample screen from *Logger Pro* for potometer data collection.



b. Learning to Use Logger Pro

Logger Pro can be intuitive for some students and intimidating for others. If you are comfortable with computers and not hesitant to learn by trial and error, then take a tour of the software on your own. Find out how to do the following:

- change the time interval set for collecting data (data collection stops at the end of the set time) and the scales for the X and Y axes,
- save your data to a floppy disk as an *.MBL file (replace the * with a name) or a plain text file readable by Microsoft Excel,
- calculate an average of several data points and a slope and Y intercept.

If you prefer a tutorial approach, then follow the directions in "*Tutorial: Temperature Measurement with Logger Pro*" available in the laboratory room. There are three additional tutorials that may be taken as needed.

c. Set-up File Characteristics
The sensors are already connected to the proper DIN's on the ULI, and each DIN is already assigned the correct calibration file. You can change various aspects of the graphs such as ranges on the Y-axis and data collection time on the X-axis. **Always save data to your floppy disk, never to the hard drive.**

Helpful hint: To modify the ranges of the X- and Y- axes click on the maximum and/or minimum values and type in the values you desire. Note that the maximum time value that you select for the X-axis limits the time over which you can collect data for a particular run. Data collection will stop automatically at the end of the time period specified. If your experiment is still running, select 'Data' from the main menu and then 'Store Latest Run'. You may then restart data collection. (Click on 'Collect'). Data from the first part of your experiment will remain on the screen as a faint trace and new data will be plotted in bold. You may collect numerous runs in this way. Each run will be collected to a separate data table. When you save your data, all the runs will be saved under the same file name. You can customize most of the options of any graph by double clicking on appropriate portion of the graph (such as the graph title, data line, etc.) and selecting options from the dialogue box that is displayed.

d. Experimental Procedures (see Figure 2 below)

Some words of caution
The procedure is deceptively easy but requires careful attention. Don't walk around in the room with the cut stem end exposed to air. Don't blindly ram the stem into the potometer tube and assume it will work. Look for air leaking into the potometer tube and do what is necessary to prevent it. Look for damage to the stem cut end which might allow air to leak into the potometer tube.

1. Sever the stem of an intact herbaceous plant with a razor blade. Choose a plant stem that is enough wider than the internal diameter of the potometer connecting tube to make a tight fit.

2. **Immediately** immerse the cut stem end (not the whole shoot) in water and cut off an additional centimeter or two of stem below the water surface to eliminate air that may have entered a little way into the vascular tissue. [Note: one variable you could test is not to recut the stem end and see if this has an effect on transport.] Keep the cut end under water until you are ready to connect it to the potometer.

3. Fill the potometer connecting tube (2, Fig. 2) with water leaving a 1-cm air bubble at the Luer lock end which will be connected to the pressure sensor (1, Fig. 2), and clamp the tube shut with the white clamp provided. Filling technique will be demonstrated.

4. **Quickly** remove the cut stem from the water and insert the cut end snugly into the connecting tube making a tight connection. There must not be any air trapped in the water column all the way from the cut stem end to about 1 cm from the Luer lock end of the tube. It is absolutely essential that no air be drawn into the stem either during transfer to the potometer or during the experiment.

5. Release the white clamp on the connecting tube to equalize pressure in the water column and then reclamp.

6. Attach the Luer lock to the pressure sensor, and open the white clamp. **Make sure no water gets into the electronic pressure sensor; if it does, stop working and consult with the TA.** The pressure sensor chamber may have to dry for a few days before it can be used again.

7. Click on the "collect" button on the computer screen to start collecting data. Either let the preset time expire, or click on 'Stop' to end data collection. If there is no change in pressure, or if there is some change at first which then stops, ALWAYS suspect that air has been drawn into the stem.

8. To save data, click on 'File' and 'Save As', change the drive letter to your floppy disk in the a: drive, give the file a unique name, and click 'Save'. **Do not use the 'Save' icon at the top of the screen because this may overwrite the calibration file.**

   The file(s) you save following this procedure are in .MBL format and can be opened and read only with the Logger Pro program. If you want to work on data with another computer, you need to create a plain text file (.txt) or import the data into an Excel spreadsheet (.xls).
   You can export numerical data to other applications such as a spreadsheet or graphing application easily using the Windows clipboard. The following instructions are from the Help file of Logger Pro.

   Export Data command (File menu):
   This option exports data to a tab-delimited text file. Only raw data including time and manually-entered data from the Latest data run are exported to the file. Calculated columns or curve fit columns are not saved. A time stamp, column names, short names, and units are included the file. After choosing this option, enter the name of the file you wish to create in the save file dialog.

   Note: Do not confuse this option with the Save or Save As options which save all the details of the current experiment. Use the Export Data option only if you want to create a text file that can be read by other applications such as spreadsheets or word processors.

   Hint: Instead of exporting data using text files, it is often easier to copy the values from a Data Table window and paste them into another application.

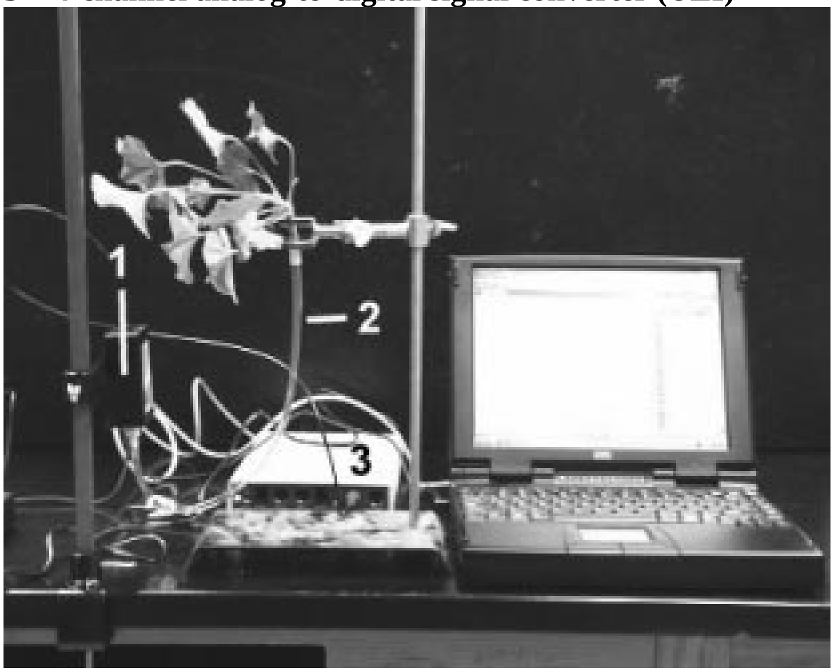
   Copy Data to Other Applications (such as Excel):
   (a) Select the desired data columns in the Data Table. You can select all columns and runs by clicking the All button in the Table window. You can also select a region by dragging across the graph window, which selects the corresponding data rows.
   (b) Choose Copy from the Edit menu.
   (c) Switch to the application where you want to paste the data. You don’t need to quit Logger Pro.
   (d) Click to indicate where the data are to be pasted.
   (e) Choose Paste from the Edit menu.

9. Repeat data collection according to your experimental design and objectives, obtaining repetitions or changing the variable.

10. Calculate rates of water uptake (transpiration) in which units of KPa are converted to volume and the rate is calculated on a per minute basis. Conversion: 72 KPa = 1 ml.

Figure 2. Electronic Potometer Setup

1 = Electronic pressure sensor
2 = Water-filled connecting tube in which pressure changes are generated by the plant (1 and 2 combined represent the potometer)
3 = 4-channel analog-to-digital signal converter (ULI)



B. <u>Some Variables for Investigation</u>

1. Does air movement have any effect on transpiration rate?

2. Does relative humidity of the air surrounding a shoot have any effect on transpiration rate?

3. Do different plant species have the same rate of transpiration?

4. Are leave or stems more important as evaporative surfaces for transpiration?

5. Is there a quantitative relationship between transpiration rate and number or size of leaves on the shoot?

6. Does exposing a cut stem to air affect its ability to supply water for transpiration?

7. Is root pressure real or only theoretical?

Note: you may want to wonder if a dicot has the same rate of transpiration as a monocot. Unfortunately the readily available monocots such as corn don't make tight connections in the potometer tube and are thus not suitable.

MATERIALS PROVIDED

Potometer Apparatus (6 per lab) - from Qubit Systems Inc (www.qubitsystems.com)

- Laptop computer with *Logger Pro* software
- Universal Lab Interface (ULI)
- ULI cable
- ULI power supply (AC adapter)
- S191 Potometer connected to DIN 2
- Tygon and Silastic tubing with Luer connector
- Temperature probe connected to DIN 1

Other

- An assortment of 6-week old herbaceous dicots (tobacco, zinnia, sunflower, tomato, soybean) --- basically anything with a round, smooth stem that is about 4-6 mm in diameter.
- Razor blades
- Fans
- Plastic bags
- Vaseline
- A plastic pan for recutting stem under water