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# Water Transport in Plants: Anatomy and Physiology

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**Abstract:** This investigation presents a condensation of two, 3-hour laboratory sessions. In one phase, we adopt a classic dye uptake experiment to determine the path of upward water transport in a detached shoot. Photography of stem thin sections taken with a digital video camera aided by compound and stereomicroscopes and a computer significantly enhance the presentation of observations and their interpretation. In a second phase, we determine the quantitative role of leaves in determining the rate of transpiration using a moderately priced, very sensitive and stable electronic potometer.

**Keywords:** anatomy, dye, physiology, plant, potometer, sap, transpiration, xylem

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## Introduction

This document is a condensation of two sequential 2- to 3-hour laboratory experiences intended for first-year college majors in biology. The introductory biology course for which this investigation was designed has sections of plant structure and function, animal structure and function, and ecology. The laboratory component has the following elements.

- Emphasis on the process of science rather than specific topics.
- Hands-on, inquiry-based activities.
- Collaboration within and between 3-member student teams.
- Collection of both quantitative and qualitative data using high quality instrumentation.
- Computers to aid data collection and analysis.
- Computer networking to aid collaboration and communication of scientific findings.
- Opportunities for student projects which extend investigations to asking new questions and testing new hypotheses.

For investigations relating to plant biology, we study variables that affect the path and rate of water uptake by plant shoots. Students first expose halves of an herbaceous stem partially split vertically to different aqueous dye solutions and observe with the unaided eye and microscope tissues in the path taken by upward travel of the solutions. In a second phase students determine with an electronic potometer the effect of relative leaf area on the rate of transpiration. Both investigations offer several opportunities for modification or expansion into projects with a significant inquiry-based learning component, and either may be used in a semester without the other.

These investigations have been in use for about five years. Modifications have been made to a commercial potometer that improve the frequency of successful first-time setup and virtually eliminate a major hazard, contamination of the electronic pressure sensor with water. It takes one staff person with undergraduate helpers a full day to reset four rooms for each of the two combined investigations, primarily because the electronic probes are stored away when not in use. Students typically complete each investigation (dye uptake, transpiration) in 2 hours and no one takes longer than 3 hours.

## Student Outline

### Background

#### *Dye Uptake*

Flowering plants (angiosperms) have a well-developed structure in their roots, stems, and leaves that is responsible for the transport of liquid water carrying dissolved minerals upward from the soil and some organic substances of tissue origin as well. A continuous supply of water is needed to replace water lost from aerial parts of the plant by transpiration and to deliver solutes to growing and developing tissues. The water moves in specialized plant tissue adapted for rapid, long distance transport.

Biological dyes (also called stains) can be used to enhance observation of plant tissues and specifically those transporting water. (1) If the dye is applied to a fresh thin cross-section of stem or other organ, it may adhere to walls of most or all cells revealing general tissue organization (anatomy) and cell structure. Some dyes even produce different colors revealing different polymers in the cell walls and this is helpful for tissue/cell identification. (2) If, on the other hand, the end of a cut shoot is placed into a solution of dye and solution is drawn into the stem by normal physiological processes, only those cells being used for water transport will pick up the dye providing it is not able to spread

horizontally to neighboring tissue. A thin cross section of the stem, viewed under a compound microscope, will reveal dye attached to the walls of certain cells thus marking the path taken by the solution. Both approaches (1) and (2) are used in this investigation.

### *Transpiration*

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, 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 one or more possible 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 with a potometer. The potometer is a device designed for measuring rates of water uptake by a shoot. In the type you will use the cut end of the shoot's stem is inserted into a flexible tube containing water and connected to a pressure sensor. Uptake of water by the plant from the tube 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.

It is important to realize that the cut shoot may take up water faster than the intact plant under the same environmental 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 biological factors on uptake, but not the true rate of uptake for an intact plant.

## **Learning Goals**

### *Dye Uptake*

When you have completed this investigation, you should be able to...

1. Adjust a student compound microscope for optimal viewing.
2. Prepare a stained plant stem cross section suitable for viewing and identifying tissues.
3. Identify major plant stem tissues.
4. Identify the plant species used in this investigation.
5. Discuss the anatomical arrangement of transport tissues in a plant stem.
6. If available, use the instructor workstation to take and save digital images of tissues viewed with a research-grade compound microscope.

### *Transpiration*

When you have completed this investigation, you should be able to...

1. Assemble and trouble-shoot the plant potometer apparatus and attach a plant with a leak-free connection.
2. Describe how the potometer works, its principle of operation.
3. Describe environmental factors that affect the rate of transpiration.
4. Describe the role of stomata in transpiration.
5. Use *Logger Pro* to collect and analyze data.
6. Convert changes in pressure to volume of water lost by transpiration.
7. Interpret graphical results, i.e. understand what the line slope indicates about transpiration rate.

## Activities

### *Activity 1 – Food color uptake by a carnation stem*

Start with this activity. The scientific question being asked is this: what will be the distribution of several different food colors taken up by separate portions of a carnation stem split lengthwise? What would blending or color separation in the petals imply about vascular tissue arrangement in the stem?

1. Set up an experiment of food coloring uptake by carnation (Methods, Part A).
2. Be sure you take the advice of trimming the stem to ensure color uptake into the flower petals in about an hour or so.
3. Allow food color uptake until the petals are well colored. This will take about an hour. Start Activity 2 while this treatment is occurring.
4. Document the outcome with a digital still camera if available.

Use this demonstration to predict the outcome of the next investigation with a whole plant which will take longer to develop.

### *Activity 2 – Dye uptake by a split stem*

The question being asked is this: what will be the distribution in stem and petiole tissue of two different dyes taken up by a split plant stem? The results of this activity and Activity 1 should be compared.

1. Choose a dicot plant, either sunflower or tomato. Half should use sunflower and half should use tomato.
2. Prepare the shoot (Methods, Part B) for the split stem dye uptake experiment. Reserve the stem segment you cut off for use in tissue identification (Activity 3).
3. Start the dye uptake (Methods, Part B). Observe the shoot from time to time by unaided eye for evidence of upward movement of dye. It is probably not possible to detect the exact dye front as it advances up the stem or leaf unless the plant is especially transparent such as jewel weed (*Impatiens*), but you should be able to determine if dye is present or absent in a particular stem and leaf region.
4. Switch to Activity 3 or 4 while this experiment is progressing. Allow as much time as you can for dye uptake, minimally one hour.
5. Return to this activity. Examine the shoot by unaided eye and note the pattern of dye travel in the veins of the stem and leaves. Is it confined to certain regions of these organs? Does it move out into some leaves or portions of leaves and not others?
6. If possible take digital still camera photos of your experiment clearly showing the experimental setup and the outcome visible to the unaided eye.
7. Make thin cross sections at places along the stem appropriate for determining the outcome of the treatment at the microscopic level. See Methods, Part C for sectioning technique. We suggest you examine the midpoint of each internode and each leaf petiole near its attachment to the stem. Keep track of stem and petiole cross-section orientation on your slides so you can relate your observations back to the intact plant.

8. Prepare fresh mounts of these sections. Remember that "fresh mounts" are prepared by placing tissue in a small drop of water on a glass slide and covering with a glass cover slip. Do not use dye solution for these fresh mounts; the tissue is already stained.
9. Observe sections for location of dye adhering to cell walls in certain tissues and for location of active tissue. We recommend using a stereomicroscope with video camera for capturing a view of the entire stem, and a compound microscope with video camera for capturing views of individual vascular bundles and cells. Identify the cells and tissue(s), referring back to your knowledge obtained in Methods, Part C for identification.
10. Document your cross section observations with digital pictures.
11. Interpret your observations and share these ideas with your colleagues.

### *Activity 3 – Identifying stem tissues in the plant species chosen for Activity 2*

The primary question is: what tissues are present in the stem of the plant used in Activity 2 and what is their arrangement? A secondary question is: what is the fundamental difference in the arrangement of vascular tissues in a monocot compared to your dicot?

1. Follow Methods, Part C. Use the segment of stem cut off in Activity 2.
2. Identify all of the stem's major tissues. Choices are: epidermis, cortex (collenchyma, parenchyma, sclerenchyma), vascular tissue (xylem, phloem), and pith. To identify tissues and cells consult any resources available to you such as your textbook, a Photoatlas, prepared slides, or images provided by the instructor.
3. Repeat with a piece of stem tissue from the monocot sorghum. Be careful – the stem may be wrapped with several layers of leaves which you might think are part of the stem.
4. If possible take digital pictures of your best cross sections for presentation and discussion at the end of the lab period and your lab report.

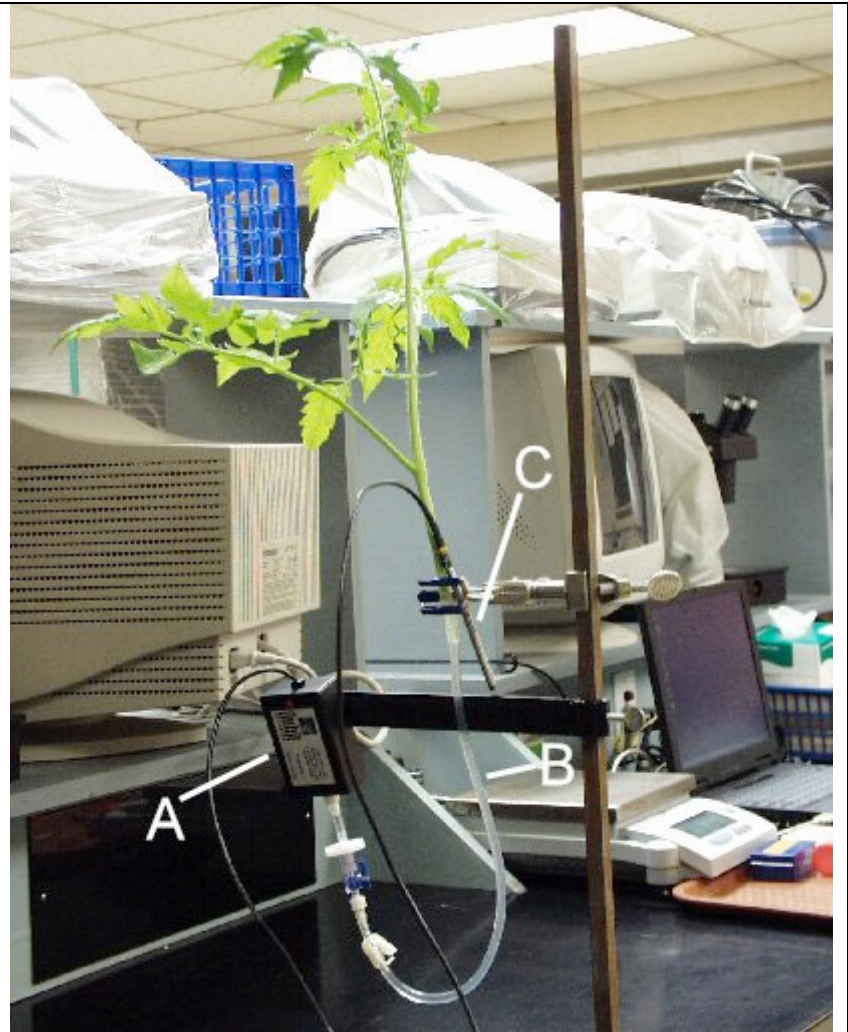
### *Activity 4 – Transpiration*

The question is this: is the rate of transpiration related to the total leaf area of a plant?

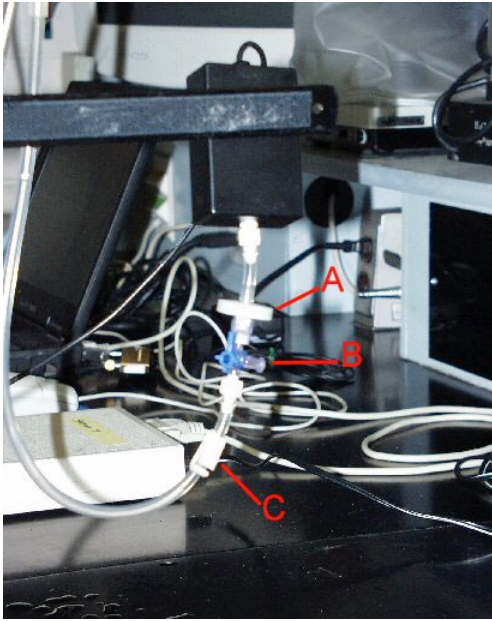
1. Follow Methods, Part D for setting up the electronic potometer (Figs. 1 and 2) and using the *Logger Pro* software. The starting point for this investigation is with *Logger Pro* data collection stopped (the default condition) and the blue handle on the 3-way valve at position 1 (Fig. 3) which closes the branch to the pressure sensor.
2. When you are ready, click on the "Collect" button to start collecting data (the button label changes to "Stop") and turn the blue handle on the 3-way valve to position 2 (Fig. 3) which closes the branch to room air. Leave *Logger Pro* in the collecting data state for the rest of the experiment.

**Figure 1.** Electronic potometer setup with plant shoot.

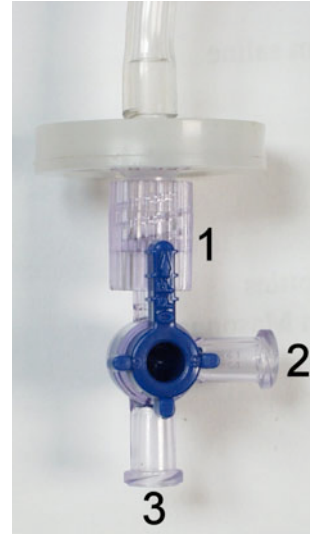
A = Electronic pressure sensor.  
B = Water-filled connecting tube in which pressure changes are generated by the plant (A and B combined represent the potometer).  
C = Electronic temperature sensor.



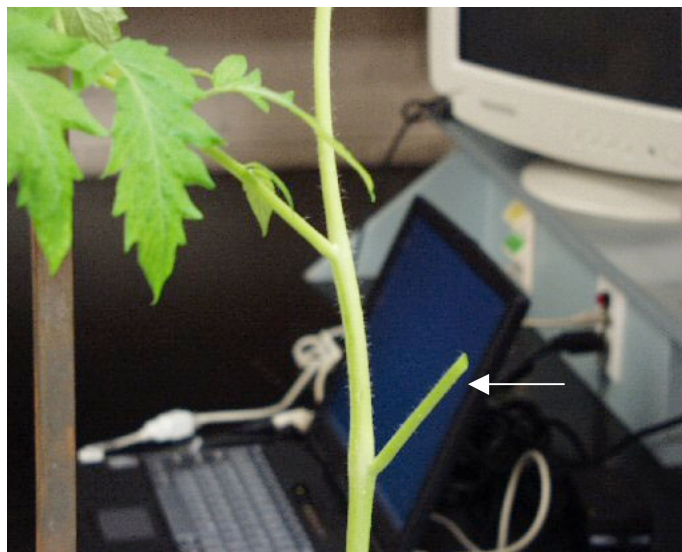
3. Collect data for 30 seconds. Move the blue handle on the 3-way valve quickly to positions 1 and 3 and then back to 2. This resets the potometer tube and the pressure sensor to room air pressure so there is not a buildup of negative pressure.
4. Continue collecting data for another 30 seconds. Repeat the pressure reset and collect data for a 3<sup>rd</sup> run of 30 seconds. More replicates can be obtained but three is usually enough to get a useful average response. At the end of the series, and indeed, whenever you are not collecting data, set the blue valve handle to position 1.
5. Sever a leaf petiole about midway between stem and leaf blade (Fig. 4). This helps prevent air intrusion when the potometer is under negative pressure. Collect three more sets of 30-second data.



**Figure 2.** Close-up of potometer.  
 A = Membrane to prevent water backing up into pressure sensor. B = 3-way valve. C = Pinch clamp.



**Figure 3.** 3-way valve. The blue “arm” is positioned upward blocking air flow to that branch. Use this setting before data collection.



**Figure 4.** Tomato plant with one leaf removed. Note complex leaf shape so each small blade is only part of the leaf, and that detachment has left a stub (arrow) of petiole to minimize air being drawn into the stem.



6. Repeat leaf detachment and data collection until the shoot has only a cluster of very small leaves at its apex. These can be removed as if they are one leaf so all that remains is the stem. Collect one last set of 3 runs and stop *Logger Pro* data collection.
7. Save the file (.MBL extension) to the computer desktop as a hedge against data loss. Then determine the slope of each run using the appropriate *Logger Pro* function (see manual). Slopes should be determined over the first few seconds because the rate of water uptake can decrease as negative pressure builds up in the potometer.
8. Convert rates in Kpa/min to  $\mu\text{L}/\text{min}$  using the conversion factor given in Methods, Part D.
9. Construct a graph with “Leaf Number Remaining” as the independent variable on the X axis and “Transpiration Rate ( $\mu\text{L}/\text{min}$ )” as the dependent variable on the Y axis. For a more meaningful graph, weigh each leaf as it is removed and use “Leaf Mass Remaining (% of total)” as the independent variable, or estimate leaf area and use “Leaf Area Remaining (% of total)”.

The potometer can be used for measuring positive pressure changes with a decapitated stem and attached root caused by root pressure. See Methods, Part D.

#### *Challenge Questions*

1. What percentage of transpiration occurs through the leaves; through the stem?
2. How high could the plant pull a column of water in five minutes?

### **Methods**

#### *Part A – Food Color Uptake by Carnation*

The following instructions are for a demonstration in which food color is taken up by a fresh cut stem of carnation with white flower.

1. Arrange four small tubes (1.5-ml microcentrifuge tubes suggested) in a suitable rack into a tight square pattern. Put 0.5 to 1 ml of each unique food color into separate tubes – use red, yellow, green, and blue.
2. Trim a cut carnation stem with flower under water to about 25 cm long. This length will include more than one internode.
3. Quickly split the stem at the cut end into 4 sections along a length of about five cm.
4. Support the stem with a ring stand and clamp and put each section into one of the color solutions.
5. Allow solution uptake to progress for about an hour or so. The time will depend on length of stem and possibly other factors such as relative humidity in the room, air movement around the flower, and condition of the stem.
6. Note the pattern of color in the white flower petals. Is it sectorial or blended? Attempt to explain the result. Photograph the flower with a digital camera if available and include the image in your report.

#### *Part B – Dye Uptake into a Split Stem of a Plant Shoot*

1. Sever the stem of an intact herbaceous plant with a razor blade close to the soil surface.

2. Immediately (within 2 seconds) submerge the cut stem end (not the whole shoot) in water and cut off an additional 5-10 centimeters of stem below the water surface to eliminate air that may have entered into the vascular tissue. The shoot may be kept with its cut stem end in water for as long as needed before treatment with dye.
3. Fill two small tubes separately with full strength solutions of Toluidine Blue O and Amaranth Red. Place them close together in a tube rack.
4. Split the stem internode (avoid a node) toward the leaves far enough to be able to place each portion a little way into one of the dye solutions. Note orientation of the split with respect to the leaf arrangement at the lowermost node. Check with your instructor about orientation of the split because it will have a major influence on the dye distribution.

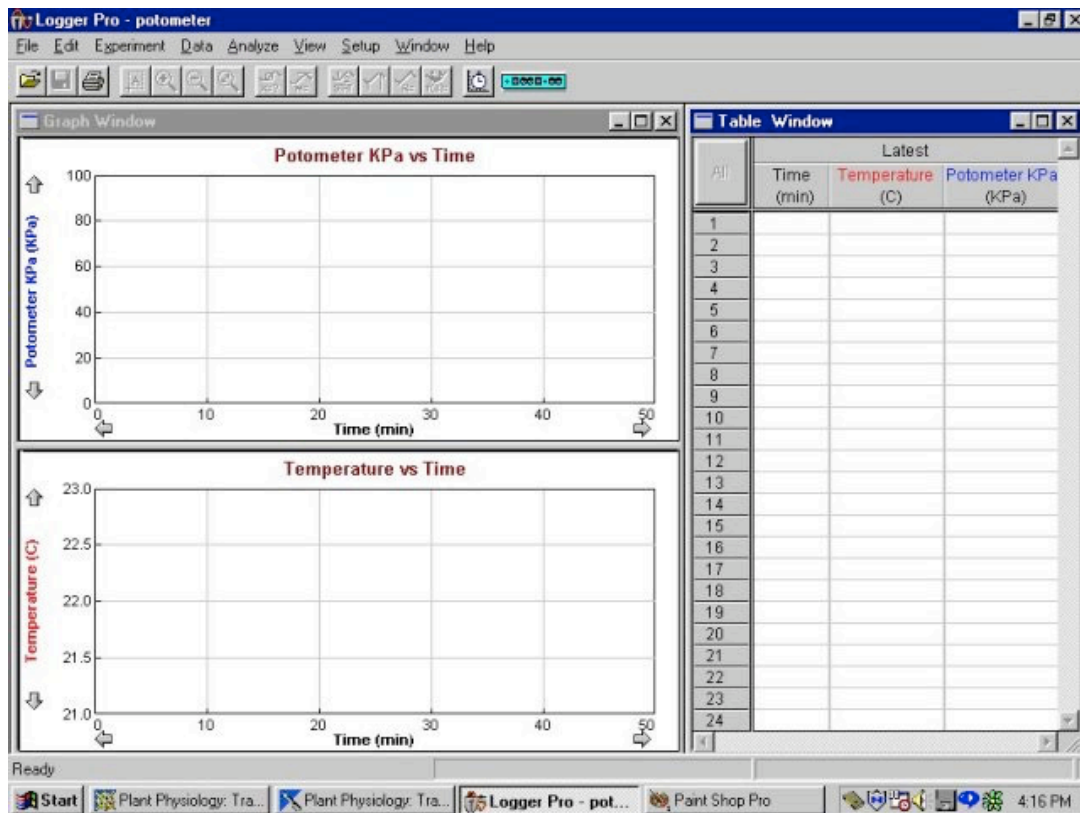
#### *Part C – Staining All Tissues of a Stem*

1. Prepare a thin section of stem tissue using a fresh razor blade. You can do this free hand but you will have better results if you use a hand microtome if available. This device consists of a brass cylinder with threaded plunger. A small length of stem is placed between halves of a cylinder of some soft material such as foam insulation which is inserted into the barrel of the microtome giving a snug fit. The threaded plunger is advanced in small increments, about half a turn, and a fresh razor blade is sawed across the protruding tissue/foam to produce a very thin slice. A drop of water on the tissue/foam adds lubricant which aids cutting without tearing. This same technique is used for sectioning stem after dye uptake.
2. Quickly place the tissue in a drop of dye on a glass slide. Toluidine Blue O (TBO) is particularly good for this. It is a metachromatic stain that produces different colors depending on the polymer to which it adheres. Primary walls (parenchyma, collenchyma, and phloem) are purple and lignified secondary walls of xylem tracheids and vessels (a subtype of vascular tissue) and sclerenchyma are blue, while some other cells may take on a greenish color. Full strength (1% w/v) is much too strong for this; all cell walls will be dark purple. Try a 10-fold dilution of the stock solution (0.1% v/v) and if this is too strong dilute it even more. You should see some tissues with bright blue and others with light purple walls and perhaps other colors as well.
3. Let the section sit in the dye for a minute or two. Blot away excess dye and rinse section with a drop of water. Place a drop of water on the section, apply a cover slip, and observe. If under or over stained adjust the dye concentration and repeat with a fresh section.

#### *Part D – Using Logger Pro and Setting Up the Potometer*

Logger Pro is best learned by referring to instruction manuals from Vernier and Qubit Systems. Listed here are some of the custom settings that can be changed to suit the situation.

- The number and type of window displayed can be changed. The standard configuration for the potometer apparatus is a pressure sensor window, a temperature sensor window, and a table (Fig. 5)
- The automatic timeout for a data collection session can be changed. For the potometer it is typically set to 60 min.
- The graph axes can be adjusted to show any range of values. For the potometer pressure is set to 0-100 KPa and temperature is set to 21-23°C (Fig. 5).



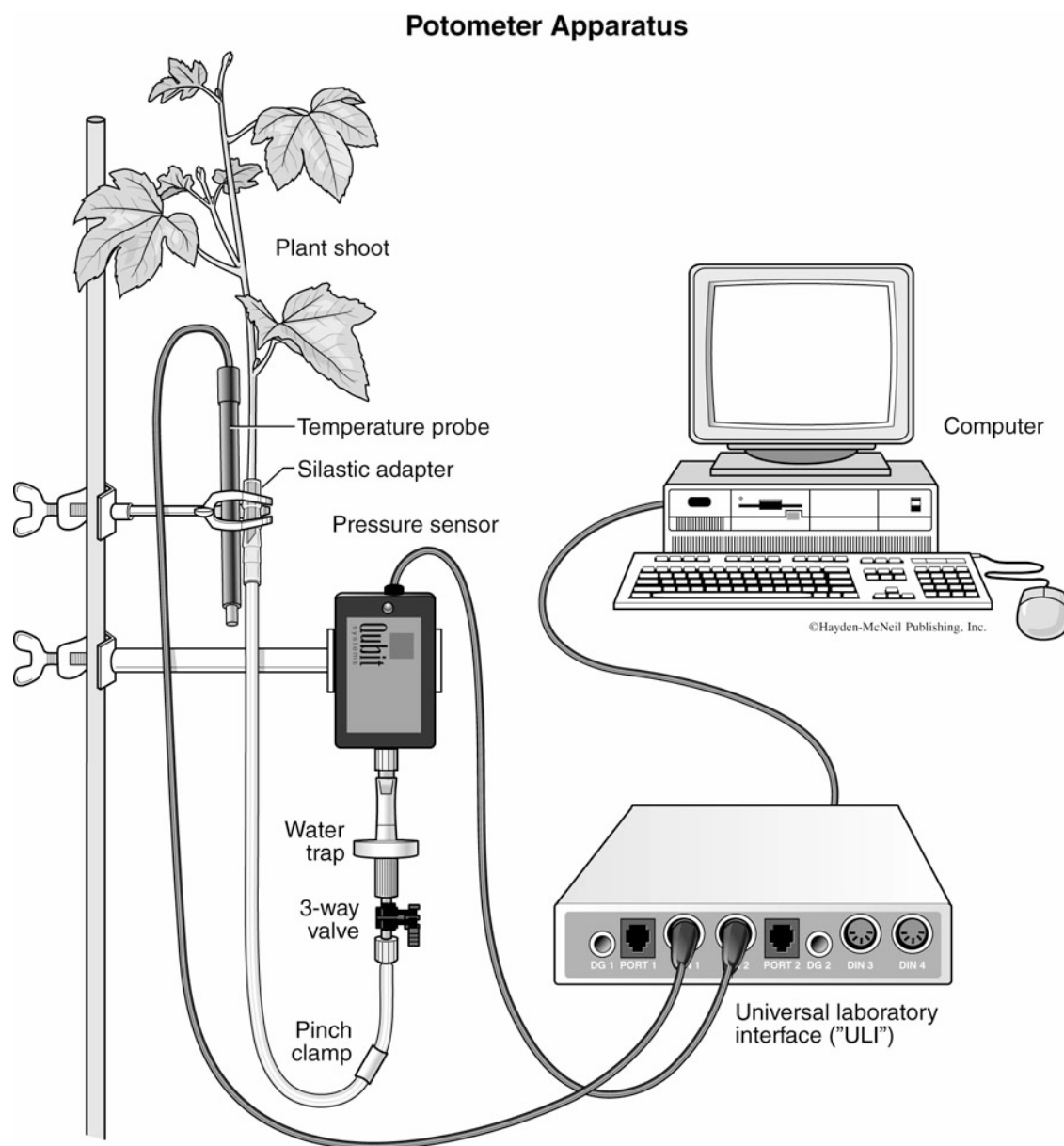
**Figure 5.** Sample screen from *Logger Pro* for potometer data collection. Pressure and temperature are recorded graphically in the left-hand window and in a table in the right-hand window.

It is important when saving files that they not overwrite the “template” file with which you started. Therefore you must use the “Save As” procedure and give the file a unique name. Also if you exit the application without storing data in a file with a new name and you made one of the changes given above, you may get a dialog box asking if you want to save the setting. Always say no to prevent overwriting the calibration file.

### *Potometer*

Setting up the potometer with attached plant shoot is a challenge but with some patience and attention to detail it can be successful on first try. Figures 6-17 show various stages of the process which is also described here. There are two main precautions. One is to prevent air bubbles in the potometer. The second is to keep water out of the pressure sensor, 3-way valve, and filter.

1. First check to make sure all of the electrical connections are set properly (Fig. 6).
2. Next check to make sure the pressure sensor is working. Fit an empty plastic syringe tightly into the open end of the flexible potometer tubing and create a gentle suction. Observe the pressure sensor reading in a little box at the bottom of the *Logger Pro* window. A decrease in pressure indicates a functional sensor.

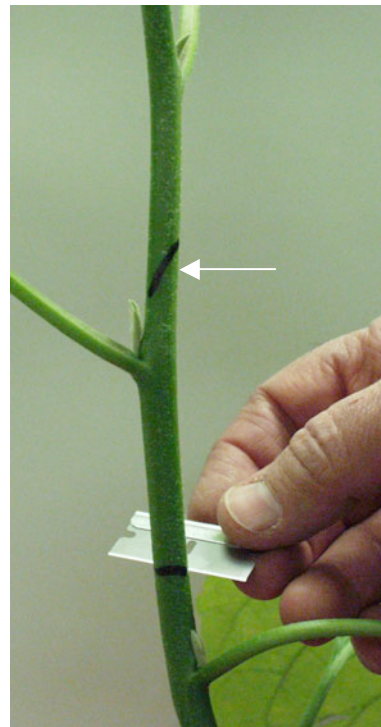


**Figure 6.** Schematic diagram of potometer setup. Reprinted with permission from Hayden-McNeil Publishing, Inc.

3. Sever the stem of an intact herbaceous plant with a razor blade so the stem is about two inches longer than you will use in the potometer and a bit wider than the Silastic adapter tube (Figs. 7, 8). Immediately (within 2 seconds) submerge the lower portion of stem (not the entire shoot – keep leaves dry) in a shallow pan of water and cut off about two inches of stem using an angle cut to remove any vascular tissue containing intruded air (Fig. 9). Leave the cut end under water until the potometer tube is ready.



**Figure 7.** Eggplant. Arrow shows position of initial cut. Plant (>8 weeks) is older than necessary.



**Figure 8.** First cut to sever shoot from root. Note angled mark (arrow) on internode where stem will be recut under water.



**Figure 9.** Stem recut under water.



**Figure 10.** Choosing Silastic adapter size to fit stem snugly. Note 2 nested sizes are needed.

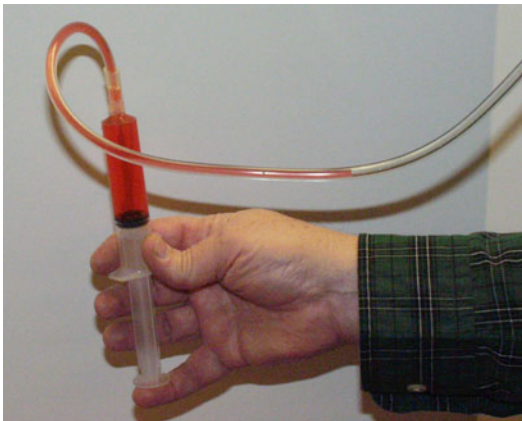
4. Detach the potometer tube from the pressure sensor and take it to the work area set up for working with plants and water. Select the short Silastic adapter tube/s needed for a tight fit with the stem (Fig. 10). Fill a syringe with water (Fig. 11), attach it to a Silastic adapter (Fig. 12), open the pinch clamp and fill the potometer tube (Fig. 13) to within a cm or two of the Luer fitting (Fig. 14). Close the pinch clamp, remove the syringe, and add water to fill the tube all the way to the end of the Silastic adapter.



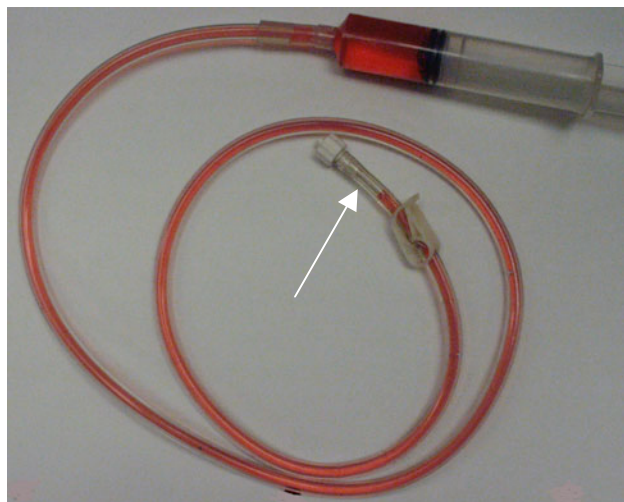
**Figure 11.** Filling the syringe.



**Figure 12.** Syringe attached to Silastic adapter of potometer tube.



**Figure 13.** Filling the potometer tube. Red food color was added to the water to improve contrast for the picture. Pinch clamp is open (not shown).



**Figure 14.** Filled potometer tube. Note air space (arrow) deliberately left between Luer connector, and closed pinch clamp.

5. Insert the cut stem end snugly into the Silastic adapter tube (Fig. 15) making a tight fit (Fig. 16). There must not be any air trapped in the water column all the way from the cut stem to close to the other end of the tube. Open the white pinch clamp and leave it open from now on. From this point on you do not have to hurry because the plant can draw water into the stem from the potometer tube.



**Figure 15.** Inserting stem into water-filled Silastic adapter.



**Figure 16.** Stem tightly seated in Silastic adapter. Pinch clamp should now be opened.

6. Set the 3-way valve to position 1 (Fig. 3) and attach the potometer tube to the valve. The filter above the valve prevents water getting into the pressure sensor that would stop it from working.

Here are some helpful hints for a successful potometer setup.

- If air is going to leak into the system, it can occur as early as one minute after setup, and it will be revealed by no change in the potometer pressure reading. Sometimes the same plant can be used if you act quickly and recut the stem a ways away from its end. However it is better to be safe and use a fresh plant if available.
- 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. Often selection of smaller diameter flexible Silastic tubing (the short tubing attached to the longer, less flexible tubing) will do the trick. Put a little water around the stem where it enters the Silastic tube as a sealant.
- Look for damage to the cut stem end which might allow air to leak into the potometer tube, or contours on the stem surface that may prevent a tight seal.
- Be aware that a particular plant may be unresponsive for reasons that are not obvious. Try a different one if available.

Root pressure can also be measured with the same potometer apparatus arranged in “reverse”.

1. Fill the potometer tube with water leaving airspace of two to three centimeters where the tube attaches via Luer lock to the 3-way valve. This is a much larger air space than used for measuring transpiration, and it is necessary to prevent water from getting into the valve. Note that root pressure pushes water towards the pressure sensor (positive change in pressure), whereas transpiration pulls water away from the pressure sensor (negative change in pressure).
2. Attach the flexible Silastic tube connector to the cut stump of a plant stem with intact roots (leave cut plant in its pot). Use a plant that appears to be oozing fluid at the cut stem surface. The soil should be wet (soil moisture content can be a variable for study).
3. Open the tubing clamp and record pressure changes for several minutes. You can fiddle with the elevation of the pressure sensor above the plant and see if this has any influence on the rate of sap flow.

### Scientific Questions for Projects

These are only some of the possible scientific questions you could ask. You, in consultation with the instructor, are encouraged to suggest and pursue others. Limit your investigation to only one question and do it well in the time available, repeating it as necessary to obtain usable and consistent data.

#### *Dye Uptake*

1. Is the pattern of transporting tissue the same in every internode of a plant?
2. Is some vascular tissue or its individual cells more active in upward transport compared to other tissue or cells?
3. Is there a unique set of vascular tissues that supply water to a particular leaf or do these same tissues continue upward and supply water to additional leaves?
4. Does a cut in transporting tissue prevent upward movement of water beyond the cut?
5. Does removal of a leaf affect the path taken by water in its upward travel in the internodes below and above the node where the leaf was removed?
6. Is living tissue necessary for water transport?

#### *Transpiration*

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 on a per leaf area basis?
4. Is there a quantitative relationship between transpiration rate and number or size of leaves on the stem?
5. Does exposing a stem's cut surface to air for a while affect its ability to subsequently supply water for transpiration?
6. Is root pressure real?
7. What is the maximum force that root pressure can generate?
8. Does root pressure depend on soil moisture level?
9. Does the viscosity of water influence transpiration rate?
10. Is the rate of transpiration affected by substances that are potentially harmful to leaf tissue, such as dilute alcohol or weak acids (e.g. acetic acid)?



## Notes for the Instructor

### Timing

These investigations are condensed from two, 3-hour laboratories scheduled in succession. There is too much for students to accomplish in one 3-hour meeting. A better plan would be to expand back into two sessions of 2-3 hours each. In the original format the dye uptake part, i.e., “functional anatomy” is placed first. This provides an introduction to plant stem anatomy and a visualization of functional water conducting tissue. The transpiration phase follows in a second week and yields quantitative information about water transport and factors affecting it. The electronic potometer can also be “reversed” and used to determine root pressure. Both investigations are “cook book” in the sense that the scientific questions and methods to answer them are given. However both investigations offer ways for extension into open-ended projects.

### Student Preparation

It is necessary that students have studied prior to the lab basics of plant anatomy, particularly of the stem, and the process of transpiration and the path of sap on its way from root to leaf. The authors have a set of digital images available on request (or possibly included on the *Proceedings* CD with this document) that can form the basis for a tour of stem anatomy in dicot and monocot species. Sunflower and corn seem to be the standards in textbooks these days but we also have some images of modest quality from other species used in these investigations.

A typical method for inducing students to read and think about a lab is to use a pre-lab quiz. We have found that better results are obtained if they are required to arrive at lab with a well thought out and written Work Plan. This conclusion is based subjectively on relative levels of procedure understanding and numbers of mistakes made during experimentation. A sample Work Plan for this workshop is given in the Appendix.

### Choice of Dye

TBO has proven to be the best general purpose dye because of its metachromatic behavior as a histochemical stain. It is also very mobile in most stems (not carnation however) and irreversibly stains cell walls as it passes by. The literature advises that acid stains should be preferred over neutral and alkaline. We have had some success with aqueous solutions of Alcian Blue and Amaranth Red, and the latter is used along with TBO in the dye uptake investigation here. Food colors are useful for uptake into sectors of split white carnation stem where it will travel only to a region of flower that is serviced by the stem sector below. However they are not suitable for anatomical study because they do not bind to cell polymers.

### Choice of Plant Species

For dye uptake any herbaceous dicot is potentially suitable. Our favorite is tomato, but we have had success with green bean, sunflower including dwarf cultivar, pepper, squash, egg plant, and soybean. Sorghum is a better choice for monocot than corn in the young stage because the stem is enough elongated to extend far enough above soil level and provide material for anatomical study. The corn stem at the same age is shorter and very hard to find in cross section; students think they are seeing stem when it is just several wrapped around leaves.

For transpiration only dicots with a smooth and nearly circular stem give a tight enough seal in the potometer to be useful. Favorites are tomato, sunflower, and egg plant. For monocots sorghum can be used after cutting and peeling some of the lower leaf tissue exposing the stem.

### Equipment and Methods

Software and electronic equipment are subject to change and consequently so are directions for their use. Therefore this document does not have detailed instructions for our current version of *Logger Pro* software (v. 2.2.1) which is already outdated. Instead we indicate some of the customizations one can make to the graphic interface and advise that students refer to instruction manuals for details. Manuals are available from Vernier Software and Technology ([www.vernier.com](http://www.vernier.com)), and Qubit Systems, Inc. ([www.qubitsystems.com](http://www.qubitsystems.com)).

### Laboratory Safety

Students should have eye protection when handling the dye solutions TBO and Amaranth Red.

### Calculations

For the transpiration investigation as given to students leaf area is disregarded. When students extend the experimental question into a project, estimating leaf area or measuring leaf mass should be considered. The factor for converting 1 unit of pressure change (kilopascal) into water volume was obtained by attaching an empty 1 ml syringe to the potometer and withdrawing 1 ml of water. This should be confirmed before acceptance.

### Projects

Our students are given one or two weeks to design a laboratory project which has a basis in one of the preceding developed investigations (dye uptake or transpiration). A project starting point can be to state a scientific question and then continue with a hypothesis and experimental design. However we prefer to start at the second step, give questions to students we know they can explore with the materials and equipment available and within the available time. Suggestions for projects are given in the Student Outline.

### Sample Data

Appendix B shows sample data for dye uptake (Fig. 17) and transpiration (Figs. 18, 19) experiments. Additional sample photomicrographs of stem cross sections taken by instructors and students can be requested from the authors.

### Student Evaluation

Our students are evaluated from laboratory reports modeled on the primary science literature and a practical final lab examination. Except for a team project based on a one or two-week investigation, these reports are abbreviated in one way or another. The Results section is always written out in full. This is sometimes accompanied by one other full section chosen from Introduction, Methods, or Discussion or by a content outline of all three other sections.

## Materials

### Dye Uptake

- Six-week old monocot (sorghum) and herbaceous dicots (sunflower, tomato) – A team of three students works with one species; plan on one plant per team and a few extra for the lab. Plants are grown in a glasshouse in standard potting mixture.
- Carnation flowers with long stems – from local florist; one per team and a few extra for the lab
- Prepared slides of monocot (corn) and dicot (sunflower) stems
- 1% and 0.1% (w/v) aqueous Toluidine Blue O (TBO) – stored at room temperature and filtered each year before use. Prepare from powder and distilled water.
- 1% (w/v) Amaranth Red – stored at room temperature. Prepare from powder and distilled water
- Food colors – red, yellow, green, blue
- Razor blades
- Glass slides and cover slips
- Hand microtome – ours are antiques; can be made from metal pipe, nut, washer, bolt. Instructions are available from authors.
- Foam board
- Cork borer
- Containers for treatments with dyes (1.5 ml microcentrifuge tubes, small glass test tubes)
- Racks to hold tubes – plastic, or drilled wooden block
- Ring stand and clamp
- Photoatlas or other printed source of stem anatomy images
- (optional) Compound and stereo microscopes (Meiji) with digital video camera (Hitachi) and video capture board; one per lab. Total cost approximately \$5,000 at year 2001 prices from Martin Microscope Company; <http://www.martinmicroscope.com>.

### Transpiration

The total cost for equipment minus computer at year 2004 prices was about \$710; Qubit Systems, Inc., <http://www.qubitsystems.com>. Teams of three students works with one potometer.

- Six-week old monocot (sorghum) and herbaceous dicots (eggplant, sunflower, tomato) – We plan on teams working with one species of 2-3 plants to be prepared for mishaps
- Electronic potometer (Qubit Systems, Inc. Model S191, \$145)
- Computer (PC, but devices and software for a Mac are also available)
- Lab Pro Interface, power supply, and computer cable (Qubit Systems, Inc. Model C410, \$275) – This replaces the Universal Laboratory Interface (ULI) used in the present investigation.
- Lab Pro software (Qubit Systems, Inc. Model C901, \$150) – This has replaced the *Logger Pro* software from Vernier (resold by Qubit)
- Temperature sensor (Qubit Systems, Inc. Model S131, \$40)
- Laboratory stand (Qubit Systems, Inc. Model 100, \$100)
- Tygon and Silastic tubing with Luer connector (initially supplied with Qubit Systems order; additional sizes and replacements available from Fisher as Tygon and Dow Corning Silastic tubing)
- Three-way valve (Value Plastics, Inc; 888-404-5837; [www.valueplastics.com](http://www.valueplastics.com); # VPB 1000079N three-way stopcock)
- Water trap filter (Nalgene 25mm syringe filter; PTFE 0.02 um; Nalgene # 199-2020 or Fisher # 09-740-38A; \$10.64 per 50)
- Razor blades
- A very large, shallow pan for recutting stem under water

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## About the Authors

**Robert Hodson** is a faculty member in the Department of Biological Sciences at the University of Delaware since 1969. He received a bachelor's degree from the University of Minnesota and MS and PhD degrees from Cornell University. He did postdoctoral work at Brandeis University in the Department of Biology. At the University of Delaware he has taught plant physiology, cell and molecular biology, and introductory biology for both conventional and honors science majors. His research interests have been physiology and genetics of nitrogen assimilation by *Chlamydomonas* and comparative structure and function of apolipoproteins in humans and turtles. He received a university-level Outstanding Teaching Award in 2000.

**John Acuff** is a staff member in the Department of Biological Sciences at the University of Delaware. He received a bachelor's degree from the University of Delaware in Entomology and Applied Ecology, and holds a degree in Mechanical Engineering and Design from Penn State University. He has served as a research field supervisor for the State of Maryland and the USDA, and as a task force design engineer for the DuPont Company. His major responsibility at UD is for the operation of the second semester introductory biology course which covers plant and animal biology and ecology, and he has also taught both first and second semester laboratories. He is self-taught in computer assembly and networking and has designed and has built networking systems in five introductory biology laboratory rooms.

## Appendix A – Sample Work Plan

- A. Do the carnation food color uptake experiment.
1. Recut stem to suitable length (25 cm).
  2. Dispense food color (4 colors) into microcentrifuge tubes (half full).
  3. Split stem at cut end into quarters about 3 cm long.
  4. Insert each section into a different food color solution.
  5. Support stem.
  6. Put aside and start next activity.
  7. (later) Finish by photographing flower.
- B. Start dye uptake experiment (dicot only).
1. Choose species and obtain one plant.
  2. Prepare stem for exposure to dyes. Reserve small stem piece.
  3. Insert stem into dye(s) and support stem.
  4. Put aside and start next activity.
- C. Observe stem anatomy.
1. Prepare thin cross sections from reserved stem piece.
  2. Immerse in drop of diluted TBO and apply cover slip.
  3. Observe under compound and stereo microscopes and capture images.
  4. Identify tissues.
  5. Save image files on computer desktop in folder I create and name.
- D. Start transpiration experiment.
1. Start *Logger Pro* application.
  2. Become familiar with *Logger Pro*.
  3. Obtain plant (same species used for dye uptake).
  4. Prepare shoot and attach to potometer.
  5. Collect water uptake (pressure change) data with intact shoot.
  6. Sequentially remove leaves and collect water uptake (pressure change) data after each removal. Consider the shoot apex and its cluster of tiny leaves as one leaf. Consider weighing leaves removed.
  7. Save data file to computer desktop in folder I create and name.

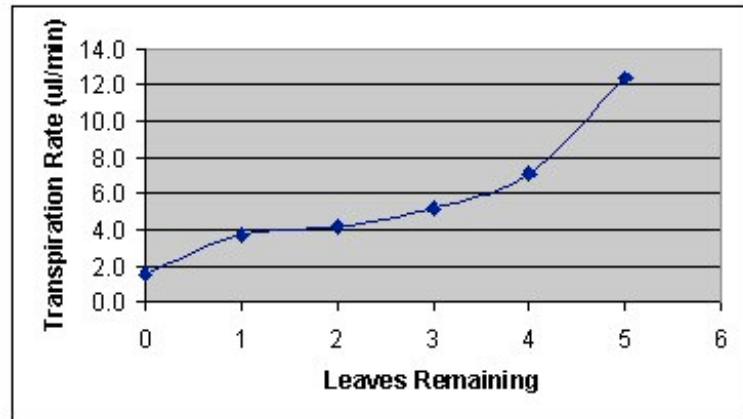
### TAKE A WELL EARNED BREAK

- E. Finish transpiration experiment.
1. Determine average transpiration rates (microliters per minute) versus number of leaves on shoot and enter into spreadsheet.
  2. Create appropriate chart (X-Y scatter recommended)
  3. Save to computer desktop in folder.
- F. Finish dye uptake experiment.
1. Prepare appropriate stem cross sections and make wet mounts (in water only).
  2. Observe sections with microscope (stereo preferred to capture whole stem).
  3. Capture images and save to desktop folder.
  4. Interpret results.
- G. Present results and discuss.

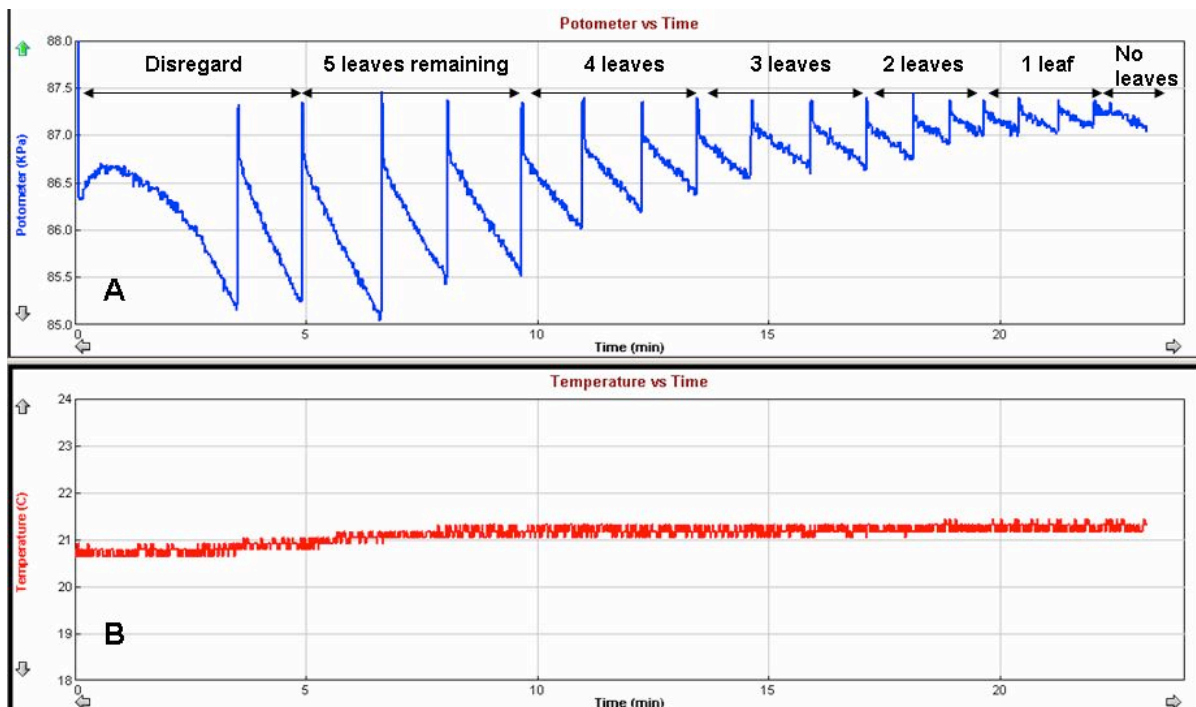
## Appendix B – Sample Student Data



**Figure 17.** Stereomicroscope photomicrograph of dye uptake pattern in split stem of sunflower; first internode above split. Toluidine Blue O taken up by the left half and Amaranth Red taken up by the right half.



**Figure 18.** Transpiration rate of tomato shoot as a function of leaf number. Data obtained from experiment shown in Fig. 19.



**Figure 19.** Logger Pro graphs of (A) pressure and (B) temperature for a tomato plant mounted in a potometer and subjected to sequential leaf removal. The intact plant had five larger leaves. Leaf area was not determined.