

PLANT ANATOMY AND TRANSPORT

Robert C. Hodson
University of Delaware

keywords: plant, stem, transport, xylem, microscopy, transpiration

This document was originally addressed to students. It has been modified to include material intended for instructors only.

INTRODUCTION

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. A continuous supply of water is needed to replace water lost from aerial parts of the plant by transpiration and to deliver minerals to growing and developing tissues. The water moves in specialized plant tissue adapted for rapid, long distance transport.

Biological stains can be used to enhance observation of plant tissues and more specifically those transporting water. If the stain is applied to a fresh thin 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 stains even produce different colors revealing different polymers in the cell walls and this is helpful to tissue/cell identification. If, on the other hand, the end of a cut shoot is placed into a solution of stain and solution is drawn into the stem by normal physiological processes, only those cells being used for water transport will pick up the stain, providing the stain is not able to spread to neighboring tissue. A thin section of the stem, viewed under a compound microscope, will reveal stain attached to the walls of certain cells thus marking the path taken by the solution. Both approaches will be used in this investigation.

SYNOPSIS

Herbaceous (nonwoody) angiosperms, both monocots and dicots, provide the biological material. Free-hand stem sections are stained and observed to determine their anatomy. Dicot stems are compared to monocot stems. In the experimental phase of this investigation, upward movement of colored water in cut shoots (stem plus attached leaves) is detected by eye and cellular path determined by microscopic observation of stem sections. Variables which might affect water transport are selected and applied to the shoots. Variables may alter the rate of upward travel or the path taken, and different plant species may be chosen for study.

CONCEPTUAL OBJECTIVES

- Learn about plant stem structure and function.
- Learn about environmental factors that affect transpiration.
- Learn some differences between monocots and dicots.
- Gain more experience in designing experiments and interpreting results

PROCEDURAL OBJECTIVES

- Learn how to prepare and observe plant tissue using thin sections in wet mounts and a compound microscope.

PREPARATION

- Read Chapters 35 and 36 in Campbell et.al. *Biology*, fifth ed.
- Examine Van De Graaff and Crawley, *A Photographic Atlas for the Biology Laboratory*, Morton Publishing Company, 3/e pp82-98.
- Develop an hypothesis or pose a scientific question which you can test using the methods described below.

ACTIVITIES

Part A - Staining All Tissues

Plan ahead and choose the plant species you will be using for the investigation in Part B.

The purpose of this activity is for you to discover the anatomical arrangement of the three fundamental tissue types (dermal, vascular, ground) in the specific plant you will use in Part B. Then, the Part B activity will reveal which of these tissues is involved in long distance transport of water upward in the shoot, and the effect of various treatments on the path taken by the water.

- Prepare a thin section of stem tissue using a fresh razor blade. You can try to do this unaided but you will have better results if you use a hand microtome (demonstrated by TA).

- Quickly place the section in a drop of stain on a glass slide. Toluidine Blue O (TBO) is particularly good for this. It is a metachromatic stain, that is it produces different colors depending on the polymer to which it adheres. Primary walls (ground tissue, phloem subtype of vascular tissue) are purple and lignified secondary walls of xylem tracheids and vessels (a subtype of vascular tissue) are blue, while some other cells may take on a greenish color.
- Let the section sit in the stain for a minute or two. Blot away excess stain and rinse section with a drop of water. Place a drop of water on the section, apply a cover slip, and observe. If overstained dilute the stain with water and repeat with a fresh section. How much dilution of stain? Depends on how overstained the section is. Try diluting with 2 volumes of water and adjust to more or less dilute as needed.
- Using the prepared slides, the photoatlas, and your textbook identify all of the tissues present in the stem section.
- Tempted to try this with roots or leaves? We have had difficulty doing this. Roots are either too thin to handle or too fibrous to cut smoothly. Leaf sections have to be thinner than the leaf thickness to lie with the cut surface up on the slide, and that is too thin to prepare by hand.

Part B - Staining Tissues Involved in Upward Water Transport in the Stem

1. Basic Procedure

The basic procedure requires a steady hand and close attention to detail.

- Sever the stem of an intact herbaceous plant with a razor blade close to the soil surface.
- **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.]
- **Quickly** transfer the cut end of the stem to an aqueous solution of dye. Don't walk around the room with the cut stem end exposed to air --- have the dye solution right next to where you will cut and recut the stem. [The dye is very dark. Use a clean pipet to put dye into the tube so that portion of the stem in the tube but not in dye solution may be observed.] Record the time of immersion if this is important to your investigation. It is important that the chosen dye adheres to the cells along the water conduction path if you want to determine its cellular path. If it does not, when you mount a thin section of the stem tissue in water for observation with the compound microscope, the dye will diffuse away and disappear. For this purpose, toluidine blue O and amaranth red work especially well. However, if all you want to do is observe the migration of dye in the intact shoot, say into the petals of a white flower, then food coloring is suitable.
- 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 tissue. Usually about a half hour is adequate for exposure to dye solution unless you need shorter or longer times for a particular predetermined reason.
- Note by unaided eye the pattern of dye travel in the veins of the shoot. Is it confined to certain areas of tissue in the stem, leaves, or flowers? Does it move out into some leaves and not others? You think of other questions like this that you could ask and collect data to answer them.
- At the conclusion of treatment cut the stem into sections, e.g. in the middle of each internode and at a node. Examine with unaided eye the arrangement of stained tissues .
- Now, prepare thin sections [demonstrated by instructor] of one or more ends of the large stem sections obtained in the previous step to determine the cellular path of the dye solution. 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). Observe sections for location of dye adhering to cell walls in certain tissues. Identify the cells and tissue(s), referring back to your knowledge obtained in Part A for identification.

While you are waiting for the dye front to advance up the stem, try your hand at preparing a "peel" of leaf lower epidermis or making a replica of the leaf epidermis with finger nail polish. This will allow you to assess the state of the stomata --- whether they are relatively open or relatively closed. This state might affect the path of dye travel and be worth knowing. The technique of peeling epidermis or making a replica will be demonstrated, and you will have help interpreting your observations of stomata.

2. Some Variations for Today's Investigation

These are only some of the possible questions you could ask experimentally. You, in consultation with the TA, are welcome to suggest and pursue others. Limit your investigation to only one or two variations and do these well in the time available, repeating as necessary to obtain usable and consistent data. Before proceeding, create an hypothesis and predict the outcome.

It is suggested that you focus your scientific questions or hypotheses on the path of water travel rather than on its rate of travel.

1. Are there any differences in the upward transport paths in the stem of a dicot and a monocot?
2. Does leaving a cut stem exposed to air have any effect on the subsequent ability of the stem to transport water?
3. Is there a unique set of vascular tissues that supply water to a particular leaf?
4. Are leaves necessary for movement of water up the stem?
5. Does the vascular system run unbranched along the entire length of a stem?
6. Does a cut in a vascular bundle prevent movement of water beyond the cut along the entire length of the stem?
7. Is the pattern of water movement in a dicot leaf different compared to a monocot leaf?

We could go on with more questions, but you get the idea. We will provide you with a variety of plant species. You take it from there!

DATA ANALYSIS

This is a qualitative study, so data analysis should consist of visual observations. At the conclusion of the investigative phase, each team should give a brief oral presentation of findings. If compound and stereo microscopes with digital video camera and a digital still camera are available, use them to illustrate your presentation.

MATERIALS PROVIDED

- A selection of monots (corn) and herbaceous dicots [several from the following list: soybean, sunflower, tomato, tobacco, zinnia, egg plant, okra, green pepper, marigold)
- White carnation flowers with stems
- Prepared slides of monocot and dicot stems and leaf epidermis
- 1% w/v aqueous Eosin Y (Brilliant Red) - store at room temperature and filter each year before use
- 1% w/v aqueous Acid Blue 25 - store and room temperature
- 1% w/v aqueous Toluidine Blue O - store at room temperature and filter each year before use
- 1% w/v aqueous Amaranth Red - store at room temperature
- Food coloring - from grocery store, full strength
- Razor blades
- Hand microtomes
- Cork borers
- Foam board
- Fans
- Vaseline
- Containers for treatments with stains (beakers, flasks, 1.5 ml microcentrifuge tubes, 12 and 50 ml plastic tubes)
- Racks to hold containers
- Leaf peel materials (clear fingernail polish, forceps, clear tape)
- Compound microscope

NOTES TO INSTRUCTORS

1. Dyes (stains) are obtained from Fisher, Sigma, or similar source of chemicals. Seeds for plants are obtained from Agway or other seed store. Six-week old plants are about optimal but they may be younger or older. A standard potting soil and common growth conditions (glasshouse, fluorescent lights in lab, etc.) are used. The hand microtome is a brass cylinder with threaded screw insert. Stem tissue is wedged between chunks of foam board, moistened, and sliced as thinly as possible. Usually a half-turn of the threaded screw is about right.
2. White carnation is particularly useful for studying linear movement of water. One can split the stem into 2, 3, or 4 longitudinal sections (1-2 cm long splits) and immerse the cut end of each section in a different color solution. Food coloring works well. Toluidine blue O for some reason does not move past the first node.
3. Corn leaves are very useful for studying the movement of water in major veins. Cutting a vein in the leaf blade can lead to some unexpected results. Comparison to similarly treated dicot stem is instructive.

Copyright: R. Hodson, University of Delaware, 2000