Laboratory: Water Movement in Vascular Plants

This week, your objective is to learn about the function of one aspect of plant physiology: the movement of water through the vascular system. You will work in teams of four to design and perform an experiment that will help you understand the general workings of one of the two types of vascular tissue in plants, the xylem. This system of microscopic tubes, extending from the roots, through the stems, and finally to the leaves, is responsible for moving water from the soil, through the plant, and out the leaf openings known as stomates.

I. Transpiration and the Vascular System

About 1% of the water taken up by plants is used for metabolic functions such as photosynthesis. The other 119% exits without ever being used by the plant. This process, known as transpiration, is ecologically vital, however. It is one of the most important ways that water travels from the soil and back into the atmosphere, where it can then return to earth as life-giving precipitation.

Before you begin today’s exercise, be sure to review the [Hydrologic Cycle](#) and understand the role played by plants in this important biogeochemical cycle.

![Figure TR-1. The Hydrologic (Water) Cycle.](image)

Water Movement-1
II. Vascular plant anatomy

Like all living things, plants have the ability to control their internal environment. Land plants (embryophytes) produce hormones that allow them to bend towards light, manufacture toxins at the site of physical injury to prevent herbivory, and may even be able to intimately control their osmotic internal environment, despite conditions of extreme heat, drought or wind. The non-vascular plants (bryophytes: mosses and liverworts) lack a complex system for water transport, and hence remain small; most can survive only in relatively moist environments. But vascular plants (tracheophytes) have conducting tissue that confers some independence from a wet habitat.

Tracheophytes have a complex system of “tubes” (the vascular system) to transport water and solutes. Phloem travels throughout the plant, and transports organic solutes in an aqueous solution in every direction. Depending on the season and the plant's needs, phloem can even change its flow direction. Xylem starts at the roots, runs through the stems and ends at small pores (stomates) in the leaves. Stomates can open and close, depending not only on time of day, but also upon the osmotic condition of the plant. Xylem, which transports water and dissolved minerals, runs from the roots up through the stems and up to the stomates. Refer to your text to familiarize yourself with diagrams of xylem and phloem in typical plant roots and stems.

The process of water movement through xylem and out the stomates is known as transpiration. Although about 99% of the water taken up by the roots is transpired directly into the atmosphere without taking part in a plant's metabolism, that 1% is all that's needed for the plant to maintain its primary metabolic functions, including photosynthesis and respiration.

As you probably have noticed, plants wilt if the soil is too dry, and if the plants are watered in time, they will quickly regain their normal appearance. You might also know that certain species of plants are better able to withstand drought than others. You are probably less familiar with the mechanisms that cause these phenomena. Before we consider comparative plant homeostasis, let's get to the basics: How does water get from the soil into the roots and from the roots into the stem and leaves?

Water movement in the living plant is the result of the integrated activity of all of the plant organs—root, stem and leaf. While your potometer is quietly yielding data for you, review the anatomy of the vascular plant in your textbook (pay special attention to the figures, and know where the various types of conducting tissues are located, relative to one another, in root, stem and leaf. Be sure you are able to incorporate this information into your presentation.

Try to imagine the path of water transport through the plant as consisting of three regions: an absorbing surface (root), a conducting region (stem) and an evaporative surface (leaf) from which water is ultimately lost as vapor via the stomates.

A. Roots

A plant’s root hairs are located near the growing tip of the root. Root hairs form as extensions of specialized epidermal cells called trichomes, and are the site of water absorption. The density and small size of the root hairs provides the plant with a large surface area for absorption.

Water absorbed by root hairs is transferred by osmosis and diffusion through the root cortex to the stele of the root, where the xylem is located. Bordering the stele is a
Water Movement

A single layer of cells known as the endodermis. Each of these cells is banded vertically by a Casparian strip made of waxy substance called suberin. The Casparian strips effectively prevent water from entering the stele interstitially (i.e. between the cells), forcing water to travel through the selectively permeable cell membrane and into the cytoplasm. Why do you suppose this is advantageous?

B. Stems

Examine a live piece of the petiole of your experimental species. Cut a cross section and observe with a dissection scope. Can you see the xylem and phloem? If you are comparing two different species, how does the “tube” arrangement differ between them? Use your text and the illustrations therein to identify the structures.

In a woody dicot stem, the individual vascular bundles are eventually replaced by concentric rings of inner xylem and outer phloem. Separating the rings of xylem from the phloem is a thin layer of embryonic tissue. This cylindrical layer, which gives rise to both types of vascular tissue, is known as the vascular cambium.

C. Leaves

Study your text diagrams of leaf anatomy. The vascular elements leave the stem and enter the leaf, branching into ever-smaller veins, which eventually terminate directly into the spongy mesophyll of the leaf. The mesophyll region is exposed to the atmosphere through openings on the epidermis of the leaf called stomates. Each stomate is bordered by two guard cells, which open or close the stomate, depending upon environmental and internal plant conditions. Locate a stomate on your slide and observe the "empty" region inside. In a transpiring leaf, this small cavity is coated with mucilage and saturated with water vapor which evaporates directly from the surface of the spongy mesophyll and diffuses outward through the stomatal opening.

III. Root pressure and Guttation

To understand the phenomenon known as root pressure, we must understand the nature of water potential. Water potential is a measure of the free energy of water. This cannot be measured directly, but the water potential of different systems can be compared. By convention, 100% pure water containing no dissolved substances is said to have water potential equal to 0.0 bars (a bar is a metric unit of pressure).

When substances are dissolved in pure water, there are relatively fewer water molecules per unit volume than in pure water, so the free energy of the water (water potential) decreases, becoming a negative number. (Note that water potential can never be greater than zero!). Water always moves from an area of high water potential (high relative water molecule concentration) to an area of low water potential (low relative water molecule concentration). You can think of it as water always tending to move from a relatively "wet" area into a relatively "dry" area.

Under normal conditions, the aqueous cytoplasm of root cells contains more dissolved salts and minerals than does the water contained in soil. Thus, the water potential of the root is lower than the water potential of the soil (there are relatively fewer water molecules in the root, compared to the soil), and by osmosis, water tends to move from the soil into the root tissue through the semi-permeable root cell membranes in a special layer of the root known as the endodermis (Figure TR-2). The osmotic pressure...
pressure generated by uptake of water by the roots is known as root pressure. Water potential in stem tissue is even lower than that in the root, and still lower in the leaves. Hence, water absorbed by the roots tends to travel away from the roots towards the stem, and away from the stem towards the leaves.

The stump of a recently felled tree or a topped herbaceous plant often will give visual evidence of root pressure by exuding of water out of the cut end of the stump. Root pressure is caused by a combination of osmotic pressure and active absorption of water which forces water into the xylem of the root so that the water column is pushed upward, somewhat like water through a hose.

As root pressure forces water from the roots through the leaves, water exits through the hydathodes, specialized structures located along the leaf margins at the tips of the veins. Under conditions of high humidity, this water does not evaporate, and tiny droplets of water form at the edges of the leaves where the hydathodes are located. This droplet formation, called guttation (Figure TR-3) offers clear evidence of root pressure, but it is observed only in relatively small, herbaceous plants. Root pressure is capable of forcing water up from the roots to an altitude of approximately 35 feet.

So wait a minute....how does a redwood tree, which might tower hundreds of feet into the air, get water from its roots to its topmost leaves?
IV. Shoot tension and Transpiration.

The most important force driving water through vascular plants is **shoot tension**, the negative pressure generated as water evaporates from the stomates. It is this process which causes the continuous flow of water from the soil to heights as great as 370 feet, the heights of some of the tallest redwoods. Shoot tension is generated primarily by **transpiration**, the evaporation of water molecules from the stomates.

Shoot tension depends upon the peculiar property of water molecules to form hydrogen bonds with each other (**cohesion**) and with other polar substances (**adhesion**), such as the walls of xylem elements. These properties, which result in water molecules tending to "stick" to one another and to the xylem walls, create a tiny column of water, with the tensile strength approaching that of a steel wire, in each xylem tube. If a thin, unbroken column of water extends from root to leaf in the xylem cells, water evaporating through the stomates will generate a negative pressure on the entire column of water right down to the root. At the root, water will be absorbed in response to the stress on the column, following the potential gradient of water molecules. Measured values exerted by the column of water suggest that shoot tension is probably the major force responsible for water movement in vascular plants.

V. What Factors Affect Transpiration?

Now that you are familiar with the anatomy of a typical plant and with the processes by which water moves through such a plant, you should be able to consider some of the variability in environmental conditions and plant anatomy to ask a question about what might affect transpiration rate. Work in teams of four.

A. Your Equipment: The Potometer

The device used to measure the transpiration rate of a leaf is called a **potometer** (from the Greek word roots *pot*, meaning “to drink” and *meter*, meaning “measure”). The type of potometer you will use consists of a ringstand with a burette clamp which supports a leaf connected to a pipet by a piece of rubber tubing. See Figure TR-4 to understand the construction of the potometer.

Properly set up, the potometer can be used to measure the rate of transpiration of various species of plants, or of the same species of plant under various environmental conditions. It is up to the investigator to use this tool wisely to answer interesting questions about plant transpiration.

B. Asking a Question and Designing an Experiment

Two of the many factors affecting plant transpiration are described below. Your group should read both parts 1 and 2, then choose one of them, ask questions and design an experiment to test the ideas inspired by the descriptions. You should be quite familiar with how to do this by now!

Your TA will guide you on a brief tour of the native biome and the Gifford Arboretum behind the Cox Building to search for plants that interest you and may inspire an experiment. You can find an illustrated guide to some of the species in the area at [www.bio.miami.edu/dana/161/leafguide.html](http://www.bio.miami.edu/dana/161/leafguide.html). Any leaf that has a petiole able to fit into the tubing we have available can be used for this experiment.
With so much variety at your disposal, it is easy to become distracted and fail to perform an experiment with only one variable. Consider carefully whether you will test only one species under different climatic conditions, different species under different climatic conditions, or something else. Whichever you choose to do, remember that when you are attempting to test an hypothesis, you may manipulate only one variable at a time! Be sure that once you have designed your experiment that you have varied only ONE aspect of the system, or you will not be able to say anything meaningful about your results.

**Figure TR-4.** The potometer assembly. Note that you will need to carefully select a pipet and rubber tubing of the appropriate size to fit each leaf you choose, so that the system will not leak.

Consider some of the following factors to perhaps gain inspiration for a relevant experiment to design and conduct in the next few hours. Use Table TR-1 to record your raw data.

1. **Species differences in transpiration rate**
   As an observant biologist, you have undoubtedly noticed the immense physical variety of plants, even in your own back yard. They have evolved different morphologies and features due to selective pressures exerted by pollinators, herbivores, climate and other environmental factors. The climate in which a plant evolves has a profound effect on the characteristics which affect water transport.

   A **xerophyte** (or xerophyte) is a plant evolved to withstand very dry environmental conditions. Adaptive features that help conserve water include a thicker waxy cuticle, stomates recessed into crevices, stomates that open only at night, reduced leaf surface
(or no leaves at all!), hairy leaves (to reduce wind evaporation) and many other features. A cactus is one of the most familiar examples. Many other species, such as oleander, creosote, sage, and even some of our local pineland species are other xeriphytes may not be as conspicuously "designed" to conserve water, but are still considered xeriphytic.

A **hydrophyte** is a plant evolved to live in very wet conditions. Such plants often have a reduced waxy cuticle, rapid transpiration rate, lots of air spaces in the leaves and other features designed to promote flotation, prevent "water logging" and conserve energy that would otherwise be used to construct the water-saving features of terrestrial plants (from which hydrophytes all evolved). An example would be a water lily, pickerel weed or any number of aquatic or semi-aquatic plants.

A **mesophyte** is a plant evolved to survive best in moderate conditions—neither extremely dry nor extremely wet.

Formulate some questions about transpiration rate. Now take one of those questions and formulate your null and alternative hypotheses.

**Ho:**

**Ha:**

What is your prediction?

And now the fun part! Design and perform your experiment. Be sure to record all your methods and data in your lab notebook. Before you leave lab, you should know what type of statistical test—if any—you will run on your data to test your hypotheses.

### 2. Climatic effects on water transpiration rate

Under natural conditions, plants are subject to many factors which may increase metabolic and/or transpiration rate. These include wind (which increases evaporation from the stomates), high light levels and temperature extremes, to name only a few.

Available in lab are several tools which your team can use to modify environmental conditions. Examine them, and then ask some questions about the plants available in lab. Now design a null and alternative hypothesis which you will test in lab today.

**Ho:**

**Ha:**

What is your prediction?
3. How does plant morphology affect transpiration rate?
Once you have measured the transpiration rates of your subjects, you will probably want to make some physical measurements that will help you explain your results. It is up to you to decide which measurements are most appropriate, but here we provide you with a few useful techniques.

a. Visualizing stomates (density, shape, state of closure, etc.)
Apply a very thin layer of clear nail polish to the leaf surface, letting it dry and then carefully peeling off the dried polish and transferring it to a dry slide. If your first preparation is not good, try again. Using the compound microscope, examine the impressions of the stomates.
Do stomates occur on both the upper and lower epidermis? If so, on which surface are they more abundant? How might this have affected your experiments? What is the density per unit area of stomates in your study organisms? Does it vary among species? Do stomate shape and state of closure vary among species and depending upon recent climatic conditions?

b. Calculating leaf surface area
If you plan to examine your data with a statistical test, it’s important to keep everything except your manipulated variable as constant and quantified as possible. To this end, you should calculate the functional evaporative surface area of each of the species you have used in your experiment (e.g., volume of water transpired per unit area per unit time) so that you can adjust for differences among your subjects. Why is this important?

1. Weigh your leaf (Do you think you should include the petiole, or weigh only the evaporative surface? Justify your answer.)
2. Use a pair of scissors or single-edged razor blade to cut three or four squares of known area (you may pick the area yourself) out of the leaf. Weigh them. You now have enough information to calculate the mass of your leaf per unit area.
3. You now know the mass of your entire leaf, as well as the mass of your leaf per unit area. We think you can do the rest.

c. Calculating transpiration rate
1. Using appropriate computer software, create a figure showing the rate of transpiration for your experimental runs.
2. Calculate rates of transpiration by measuring the slope of each run (if you don’t remember how to do this, refer to Appendix #4 of the online lab manual). Wherever appropriate (i.e., for multiple runs of a given trial), calculate an average rate of transpiration.
3. Use these transpiration rates in conjunction with the other measurements and calculations above when reporting and explaining your results.
Table TR-1. Raw data for transpiration trial runs

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VI. Analyzing and Reporting Your Results

Once your data have been compiled, use the statistical test you chose in advance to analyze your results. Do your results indicate that you should reject or accept your null hypothesis? Once you have made this decision, you must explain your results.

If you compared transpiration rates among different species of plants, you might wish to consider...

1. What physical adaptations might the different species have that would affect their transpiration rates?
2. What physiological adaptations might be present to account for transpiration differences? (Be as specific as possible.)
3. What confounding factors might cause you to misinterpret your results?

If you compared transpiration rates of leaves under different environmental conditions, you may wish to consider...

1. How might your results differ if you direct the air flow onto the upper surface of the leaf versus the lower surface? Why?
2. How might your results differ if you direct the light onto the underside versus the upper surface the leaf? Why?
3. Can you think of any confounding factors which might have affected the results of your experiments?

Don’t limit yourself to the questions above. Give a thorough analysis of every aspect of your results—just as you would for publication to a scientific journal. Refer to your text and lectures, and cite all sources in your presentation.