Laboratory
Water Movement through the Xylem

By now you are familiar with the general anatomy of a vascular plant, and know that water is taken up via the roots, travels up the stem, and exits the plant via openings on the leaf known as stomates. This process is known as **transpiration**. Only about 1% of the water taken up by plants is used for metabolic functions such as photosynthesis. The other 99% exits the stomates 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. The movement of water through the biosphere can be traced by studying the **Hydrologic Cycle** (Figure 1)

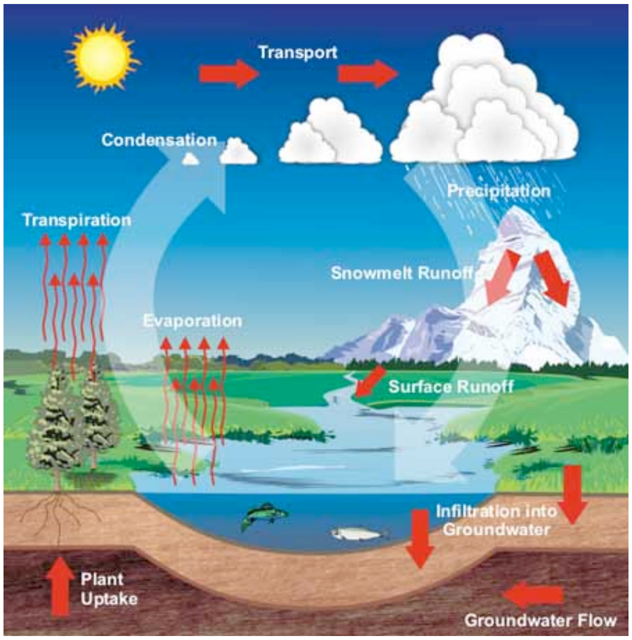

Figure 1. **The Hydrologic Cycle shows the movement of water through many of the biotic and abiotic components of the biosphere.**

Like all living things, plants have the ability to control their internal environment (**homeostasis**). Among their many metabolic talents, vascular plants have the ability to control their osmotic internal environment, despite conditions of extreme heat, drought or wind. This is accomplished both with anatomical and physiological features that make any given species best adapted to survive in its particular habitat and climate.

I. The Forces of Water Movement in Vascular Plants

Vascular plants are characterized by the presence of two types of complex conducting tissue, **xylem,** which transports water and dissolved inorganic substances from the roots to the leaves and **phloem,** which transports dissolved photosynthates (sugars produced by photosynthesis) back and forth between areas of manufacture and storage.

You have no doubt witnessed the fact that 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 might be less familiar with the mechanisms responsible 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?

A. Root pressure

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. (Thus, 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).

In other words, water will always tend to move away from a relatively "wet" area and into an relatively "dry" area. Under normal conditions, the aqueous cytoplasm of root cells contains more dissolved salts and minerals than does the water in the spaces between soil particles (**interstitial** water). Thus, the water potential of the root is normally lower than the water potential of the soil. In the absence of any barriers, water would tend to diffuse from the area of higher water potential (soil) to the area of lower water potential (root tissue).

There is a barrier, however. Just inside the cortex lies the **endodermis** (Figure 2). Endodermis is a specialized layer of cells, each bounded by a strip of suberin to form a Casparian strip. This prevents water from entering the stele between the cells, thus forcing them through the selectively permeable membrane of the endodermal cells.

The osmotic 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

Root pressure is capable of forcing water up from the roots to an altitude of approximately 35 feet. So how does a redwood tree, which might tower hundreds of feet into the air, get water from its roots to its topmost leaves? This requires a separate, but related phenomenon, shoot tension.

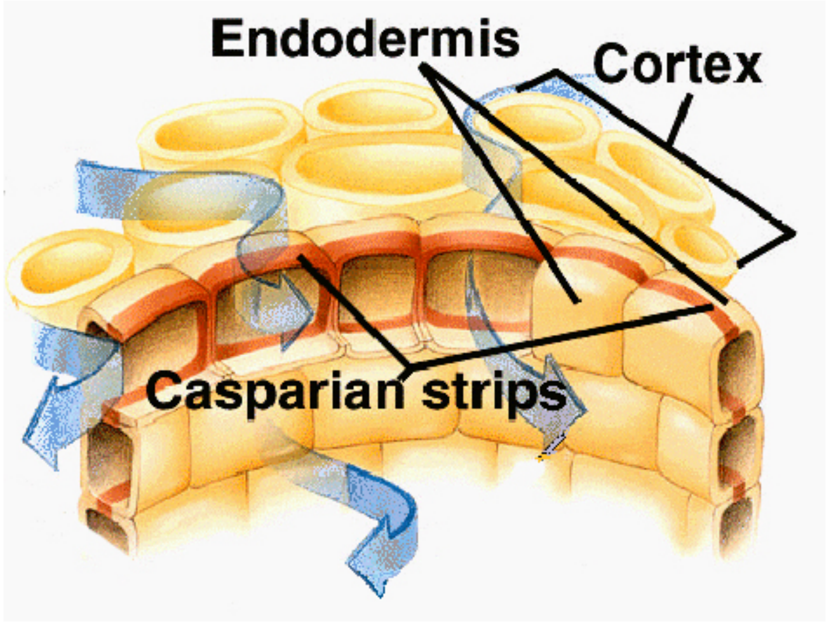

Figure 2. **Endodermis. The blue arrows show the movement of water through the cortex and into the stele via the endodermis plasma membranes.**

B. 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. This process is responsible for the continuous flow of water from the soil to heights as great as 370 feet. 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. This property, which results in water molecules tending to "stick" to one another, is known as **cohesion.** Water also tends to stick to *other* types of polar compounds (**adhesion**), such as carbohydrates, which form the walls of the xylem elements. In small-diameter tubes, such as xylem vessels, cohesion and adhesion combine to form a water column with tensile strength approaching that of a steel wire.

If a thin, unbroken column of water extends from root to leaf in a xylem tube, 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 internal water column. Measured values exerted by the column of water suggest that shoot tension is probably the major force responsible for water movement in vascular plants of any appreciable height.

II. Examining Transpiration

With what you know about plant anatomy and with a general understanding of how environmental changes can affect the way a plant responds to its environment, you and your teammate should be able to come up with some interesting questions about transpiration. Perhaps you would like to test the rate of transpiration between two different species—a hydrophyte and a xeriphyte. Or perhaps you would like to investigate what happens when you manipulate the environmental conditions of a single plant species.

Whatever question you ask, be sure it is relevant and has implications and importance that reach beyond the first year student or "science fair project" level. Why are you using the particular species you chose? Why is the environmental variable you are manipulating interesting? What further implications or larger ideas can be linked to what you are doing here?

Once you and your teammate have decided what experiment to perform, it's time to learn how to use the equipment.

A. Your Experimental Apparatus: 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 3 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.

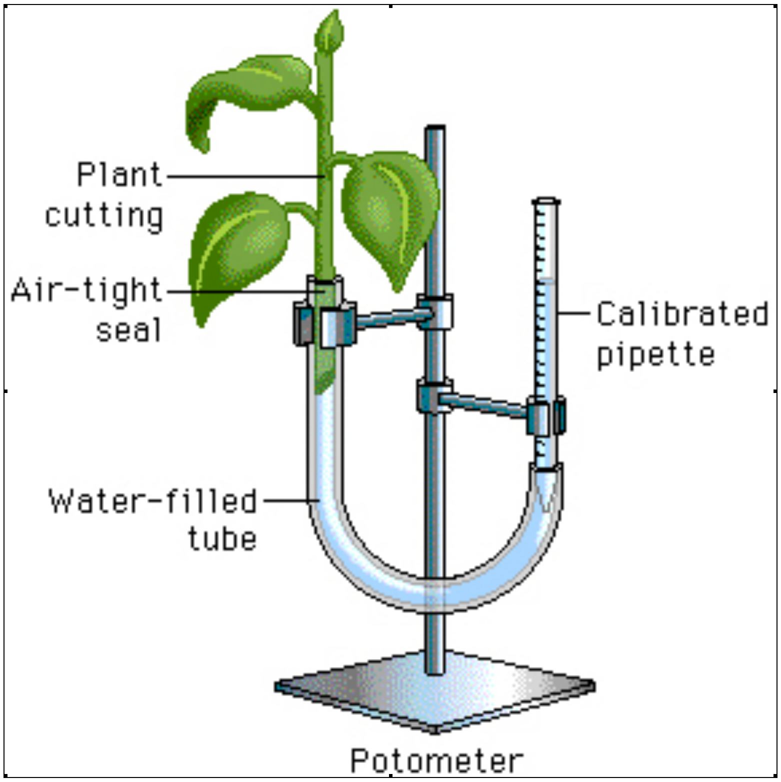

Figure 3. The potometer assembly. Note that you will need to carefully select a pipet and rubber tubing of the appropriate size to fit each leaf or cutting you choose, so that the system will not leak.

B. Formulating a Hypothesis

Before you begin working, your instructor will take you on a brief tour of the native biome in the John C. Gifford Arboretum, where you will see a variety of plant species growing naturally. As you wander the area, be aware that the plants are active, photosynthesizing, respiring and transpiring as you walk by. What questions does this awaken in your mind? Are different plants transpiring at different rates? Are different parts of the same plant doing the same amount of transpirational work? And what about microenvironment? Would a change in light, wind, local humidity, or some other factor change the rate of transpiration? The list of questions is limited only by your imagination and sense of inquiry.

Once back in the lab, you and your teammates should confer and formulate a hypothesis about an observation you made on the tour. Also consider how you will design an experiment to test your hypotheses.

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 examine 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. A table is available on the last page for you 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 **xeriphyte** is a plant evolved to withstand extremely 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 which may not have as obvious water-saving mechanisms, but are xeriphytic nonetheless.

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 ot 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. Most of the ornamental plants around campus are mesophytes.

Devise your hypotheses carefully, noting whether this is a pilot study (which will provide you with observations that you can put to further hypothesis formulation) or whether you have enough knowledge about the transpiration system you are considering to pose multiple, competing hypotheses to explain your observation. List your hypotheses (either multiple, competing or null/alternative) here.

Observation: ____

Hypothesis 1: ____

Hypothesis 2: ____

Hypothesis 3: ____

Hypothesis 4: ____

What is your prediction?

OR:

Null Hypothesis: ____

Alternative Hypothesis: ____

What is your prediction?

Design and perform your experiment. Be sure to record all your methods and data in your lab notebook. Before you begin, you should know what type of statistical test you will use to analyze your data.

2. Climatic effects on 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 (lights, color filters, fans, etc.) which your team can use to modify environmental conditions. Observe them, and then pose hypotheses about transpiration in the plants available to you. Write your hypotheses below.

Observation:

Hypothesis 1:

Hypothesis 2:

Hypothesis 3:

Hypothesis 4:

What is your prediction?

OR:

Ho:

Ha:

What is your prediction?

3. Correlating the physical characteristics of your study organism with its transpiration rate.

Once you have measured the transpiration rates of your subjects, you will need to make some physical measurements to be sure to eliminate as many variables as possible. It is up to you to decide which measurements are most appropriate, but here are a few useful techniques that you may find helpful.

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 the appropriate methods in MS Xcel or similar software, enter the data points for each of your experimental replicates
2. Calculate rates of transpiration by measuring the slope of each run (if you don't remember how to do this, your instructor will be happy to provide you with extra resources). Wherever appropriate (i.e., for multiple runs of a given trial), calculate an average rate of transpiration.
3. Use these transpiration rates in conjuction with the area calculations above when reporting your results.

III. Review of Vascular Plant Anatomy

Once you have set up your experiment, you will have plenty of time between readings to relax and review some plant anatomy. This might also give you some ideas of what to include in the powerpoint presentation you will create for your colleagues (classmates) after you have finished analyzing your data.

Water movement in a 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 general anatomy of the vascular plant.

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).

A. Roots

Figure 4 shows the cross section of a generalized root. Water enters the root across the plasma membrane of the **root hairs** located near the growing tip. Root hairs form as extensions of specialized epidermal cells called **trichomes**. The density and small size of the root hairs results in tremendous surface area for absorption (Figure 5. (Actually, most higher plants absorb the majority of their water through a symbiotic plant/fungus association called **mycorrhizae ("fungus roots).)**

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 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. (Figure 6) Why do you suppose this is advantageous?

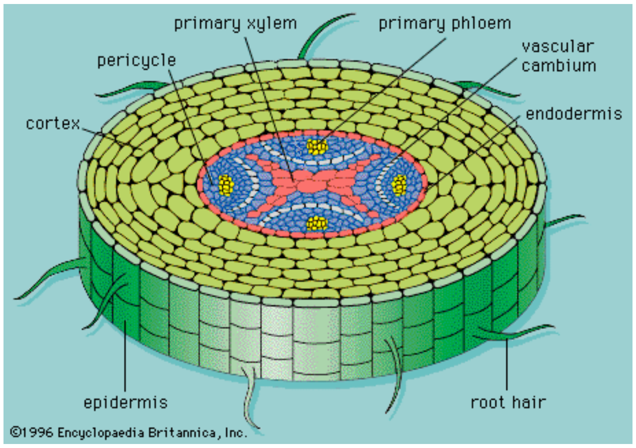

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Figure 4. Cross section through a typical root, showing all major tissue layers. The endodermis is the site of the Casparian strips.

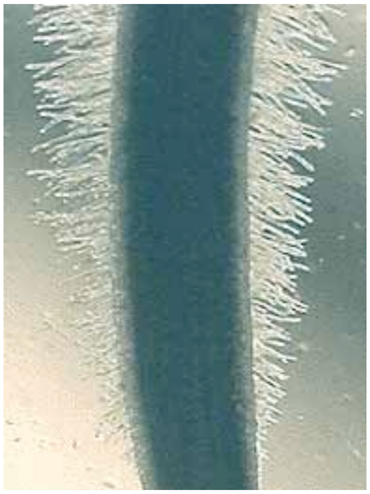

Figure 5. Root hairs

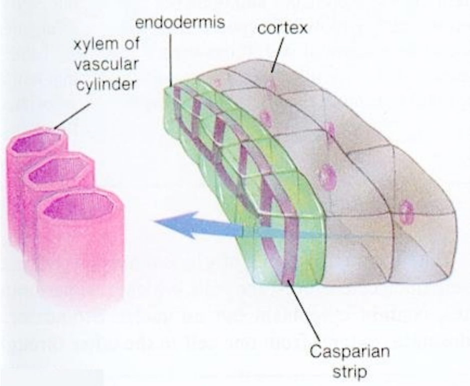

Figure 6. Water traveling from the cortex to the xylem is forced through the endodermal plasma membrane by the waxy Casparian strips, which block water from passing between the cells.

B. Stems

Examine a live piece of the petiole of the leaves of your test subjects. Cut a cross section and observe under a dissection scope. Can you see the xylem and phloem? If you are comparing two species, how does the "tube" arrangment differ between them? For greater resolution, take a look at the typical monocot and dicot stem cross-sections shown in Figure 7. Notice the difference in the arrangement of the xylem elements in these bundles as compared to their arrangement in the elements in these bundles as compared to their arrangement in the stele of the root.

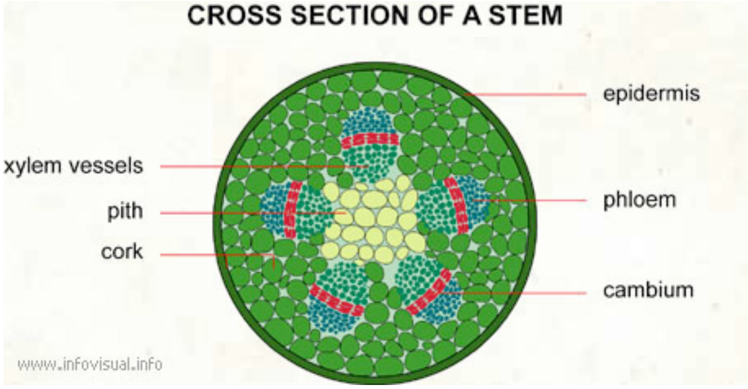

Figure 7. **Cross section through a typical herbaceous dicot stem.**

C. Leaves

Obtain a prepared slide of a *Coleus* leaf in cross-section. The vascular elements leave the stem and enter the leaf, branching into ever-smaller veins, which eventually terminate directly into the **spongy mesophyll**. 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 live leaf, this cavity is coated with mucilage and saturated with water vapor which evaporates from the surface of the spongy mesophyll and diffuses outward through the stomatal opening.

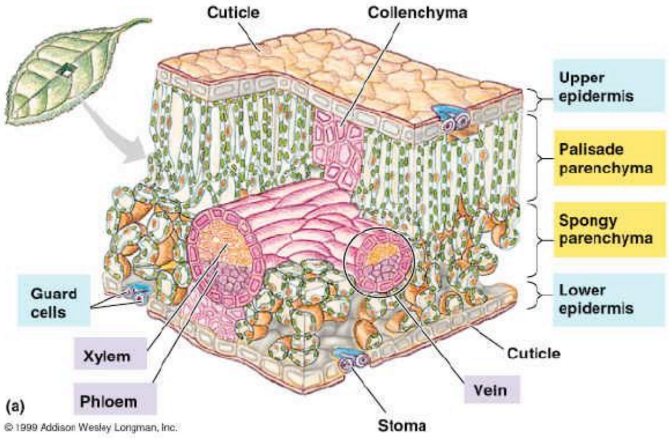

Figure 8. **Cross section of a leaf.**

V. Preparing Your Presentation.

Once you have collected your data, you and your partner/teammates will analyze your results and present your findings in the form of a Power Point presentation. You can find tips for an effective presentation in your online lab manual.

A few questions to consider when preparing your presentation:

Once you have eliminated all but the correct hypothesis, you must explain your results. Observations without explanations are nothing.

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 that might have affected the results of your experiments?

Don't let the above be the only questions you answer. We expect a thorough analysis of every aspect of your results--just as you would give if you were submitting this report for publication to a scientific journal!

Be sure to incorporate what you learn about plant anatomy from Section IV. Refer to your text and lectures, and cite all sources in the literature cited section.

Table ____ .

Trial # :		Trial # :		Trial # :	
time (minutes)	meniscus level (ml)	time (minutes)	meniscus level (ml)	time (minutes)	meniscus level (ml)

Trial # :		Trial # :		Trial # :	
time (minutes)	meniscus level (ml)	time (minutes)	meniscus level (ml)	time (minutes)	meniscus level (ml)

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