Looking Closely at Leaves

At Mount Holyoke, plants are ubiquitous and you walk by them (or on them) every day without giving them a second thought. You might even mentally classify them as "boring" because they don't move around or seem particularly active – if so, you're mistaken. Plants are as complex as you are and face as many functional problems and tradeoffs in their lives as any animal. They're just less obvious about it..

Today, we will examine one of the major functional problems in a plant's life: striking a balance between photosynthesis and water loss. Plants are autotrophs – they make their own sugars to use either in cellular respiration or as structural elements of their tissues. The sugar-manufacturing process is called photosynthesis, and uses carbon dioxide (CO$_2$) extracted from the atmosphere, water (H$_2$O) brought up from the roots, and light energy from the sun. In most plants, the bulk of photosynthesis takes place in specialized organs called leaves.

But leaves have a major dilemma. Photosynthesis requires light and CO$_2$, both of which are collected through the surface of the leaf. Therefore, if a leaf increases its surface area, it could collect even more light and CO$_2$, and make more sugar for the plant. But water is also required for photosynthesis, and as it is brought up from the root system it tends to escape from the leaf through its surface, particularly as light warms the leaf. So, larger leaf surfaces will also increase the amount of water that evaporates from the leaf, increasing the plant's vulnerability to desiccation. It is this tradeoff between making food and preventing desiccation in a plant’s particular habitat that shapes leaf anatomy, both on the gross and microscopic levels.

Most plants share a few basic features that defend against desiccation while still allowing CO$_2$ to enter the leaf. Leaf surfaces are covered in a transparent waxy layer that can let light through but keeps water from escaping. But the waxy coating also prevents CO$_2$ from entering, so plants make tiny breaches in the layer called stomata to let the CO$_2$ in. Stomata are holes that lead to the interior of the leaf, and any one leaf is covered with many stomata. Unfortunately, water can still escape from the interior of the leaf through the stomata. Plants attempt to control their water loss by closing their stomata when water is less available; this control comes from pairs of guard cells which flank each opening and respond to the water content of the plant; when its water content is high – the cells open, when it is low, they close the opening.

As you proceed through today’s lab, you should draw the leaves you see, both with your eyes and under the microscope. Your drawings, along with written descriptions and interpretations of your observations, will be due in lab next week.
PART 1: An Experiment in Transpiration

Chlorophyll does a better job absorbing some wavelengths (colors) of visible light than others. The more efficiently chlorophyll absorbs light, the more photosynthesis is performed. We also know that plants have an optimal light level for photosynthesis, and if they receive less light, there will be less photosynthesis. What relation, if any, is there between the wavelength or the amount of light and the amount of transpiration (water lost through leaves)? You will design a simple experiment, looking either at different light levels or different wavelengths of light on the rate of transpiration.

You have available: Small bean plants growing in pots, plastic bags that can be sealed around the plants, packets of desiccant that can pick up water transpired by the plants if they're sealed in bags with the plants, and an electronic balance that can measure the amount of water the desiccant packets pick up (As long as you weigh them before you seal them up with the plants).

Design a simple experiment, including a control, and predict what you will find in a couple of hours. We will check on the results at the end of this lab.

PART 2: Leaves to the naked eye.

You will begin the next part of today’s lab by taking a trip to the Mount Holyoke College Greenhouse, where you can see plants that have evolved strategies to living in several very different environments, each with different amounts of available sunlight and water availability.

Enter the greenhouse complex at the main entrance, then turn left and walk through the Show House to the Succulent House. The Succulent House contains many plants that have evolved in habitats with low moisture conditions, called xeric environments. The classic xeric environment is a desert; in addition to little available water, these habitats also have high light levels and high temperatures that accelerate water loss. The succulent house contains (among others) two important types of xerically adapted plants: the Cactacea and the Euphorbiaceae. These groups are not closely related: the Cactacea are primarily New World plants, the Euphorbiaceae are Old World, and their xeric adaptations were evolved independently.

Find examples of both the Cactacea (cactuses) and the Euphorbiaceae (euphorbs). Can you tell them apart without looking at the labels? What similarities do they share?
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Where are the leaves on these plants? How large are they compared to the rest of the plant? How does their surface area compare to their volume?

If the plant is leafless, where is photosynthesis occurring? Where are the stomata located?

Why are the stems so thick and fleshy? Many of these plants look pleated – what function do you think the pleats might have?

Next, go back to the Main entrance, then turn left and walk all the way back to the Conservatory. This room contains plant species native to tropical and subtropical habitats generally characterized by high humidity and good water availability. But many of these plants also grow under poor light, because they live in the shade of larger trees.

Find the Elephant ear and the Banana tree. These plants are (respectively) understory and midstory plants, which are often shaded by other, larger trees. How does the shape of their leaves reflect this environment?
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Do these plants seem to be storing water in their leaves? How does their leaf shape compare to the plants in the Succulent house?

Why might high humidity reduce the amount of water loss these leaves experience?

Although you might not expect it, tropical rain forests also contain plants with xeric adaptations. These plants, which include the bromeliads and epiphytes like orchids, live either in the crooks of branches in large tropical trees or attached to the tree bark. They have very small root systems which limits the amount of water they can absorb, even though it rains every day.

Find a bromeliad in the Conservatory. How do its leaves resemble a classical xeric desert plant?

Can you identify another adaptation that lets the plant collect water and minerals?

Walk next door to the Orchid House to see the orchids. How do their leaves resemble a classical xeric desert plant?
While in the Orchid house, take a look at the plants living in the pool. Are these plants likely to get high or low levels of light? Do they have to control water loss? How do their leaves reflect this?

Consider the water fern (*Salvinia*) and the water lily (*genus*). Where do you predict these plants keep their stomata? Why? (You’ll get a chance to test your hypothesis back in lab).

**PART 3: Leaves at the microscopic level.**

Back in lab, your task is to look for stomata on leaves. Again, stomata are the pores through which CO$_2$ enters the leaf and water is lost, and each one is surrounded by a pair of guard cells. Guard cells can only expand lengthwise when they fill with water, but because they are attached at the ends they get forced apart in the middle, opening the stoma between them. The plant controls stomatal opening by varying the concentration of K$^+$ ions in the guard cells. As long as a high K$^+$ concentration is maintained, the guard cells remain turgid and the stomata are open. But this requires continuous active transport and a constant supply of ATP. Which means that these cells have to make and metabolize a lot of sugar.

Observe the stomata on some of the bean leaves available. In each case, bend the leaf until it cracks, and with forceps peel off a couple of pieces from the epidermis. The pieces need only be a couple of millimeters long, and it is OK if they remain attached to bits of more intact leaf. Mount a piece in a drop of distilled water, and observe it through a compound microscope.

Draw and label a stoma. What are the tiny green objects inside the guard cells? How many stomata are in one field of view?
In addition to the bean leaves, we have an assortment of leaves from other plants for you to examine. Prepare leaf peels from some of these plants, then draw and count the stomata in a field of view. How many stomata are present in a xerically adapted leaf compared to a leaf from a more humid environment?

Which side of the leaf has the most stomata? Is it different in a bean? A blade of grass? A water lily? Why might there be differences in stomata location?

Use the microscope to look at a transverse section of leaf from *Peperomia* - a xerically adapted tropical plant. What features do you see that might improve the plant's ability to retain water?

Look at some of the photographs around the room that were taken with the scanning electron microscope. Do the stomata look very different at this level of magnification? Are there differences in stomata morphology in different species?

**PART 4: The Transpiration Experiment**

Weigh the desiccant bags near the end of the lab period. How long has the experiment been running? Do the plants look stressed (wilted, cooked)? Do you notice anything qualitative that might affect your experiment? Where has more water been transpired? What does the control show? Is the result what you expected?

Some thought experiments – not to be turned in, but they might turn up on an exam…
You measured transpiration. The original question asked whether transpiration varied with the amount of photosynthesis. How might you measure photosynthesis directly (in a 150 bio lab setting).

It is possible for light intensities that differ by a factor of two to yield the same amount of photosynthesis. Explain.

Imagine setting a bright light much closer to the plants than in your experiment, and then shading one plant so it received exactly as much light as one of your plants received. If the plant showed much more transpiration in this treatment, what might be the reason?

What might happen if you sealed 4 bags of desiccant with each plant rather than one? What are the variables that matter in problems involving diffusion, and how would they change if there was more desiccant?