

RESPONSES TO ABIOTIC FACTORS.

INTRODUCTION

Climate and the environment often have drastic effects on the behavior and physiology of an organism. Increases in temperature, for example, can raise metabolic rates and affect the course of development. Humidity levels and the availability of water often disturb reproduction and/or the distribution of organisms. In a similar vein, the chemical composition water (pH, salinity, etc) affect development and various aspects of the animal's physiology (look at the effects of acid rain on wildlife, for example). Sometimes two or more environmental properties work together to challenge an animal (as wind speed increases, so do water losses in most terrestrial animals).

Each animal has an ecological minimum and maximum tolerance for temperature, humidity, salinity, and other environmental factors between which they can survive (albeit under stress and for only a short period of time). Thus, although you could tolerate being locked naked in a 0° walk-in freezer for several minutes, it's unlikely you'd survive for very long. Likewise, an animal may get by for short periods near their tolerance limits, but the stress can affect feeding, resistance to disease, and reproduction. Any condition that approaches the limits of an organism's **tolerance range** is said to be a **limiting factor**. Over a more narrow range animals have preferred or **optimal** ranges for temperature, humidity, and so forth. In this laboratory you will explore factors related to humidity and water balance in a variety of animals.

Transpiration by Plants.

MATERIALS:

- Potometer (or 1.0 ml pipette with rubber tubing), razor blades, pans of water, incandescent lamps (40-60 Watt bulbs), small fans, petroleum jelly, applicator sticks, paper towels or cotton balls.
- Living *Coleus*, pea, bean, or sunflowers (Optional: evergreen leaves).

Plants living in aqueous habitats have little or no trouble exchanging gasses with the environment because their cuticle is thin. This allows both oxygen and carbon dioxide to diffuse between the plant and the surrounding medium. Terrestrial plants, however, have evolved a thick cuticle (mainly to help cut down on water losses). By solving one problem, however, the land-dwelling plants have created another: how to allow free passage of O₂ and CO₂ into the leaf (needed for respiration and photosynthesis).

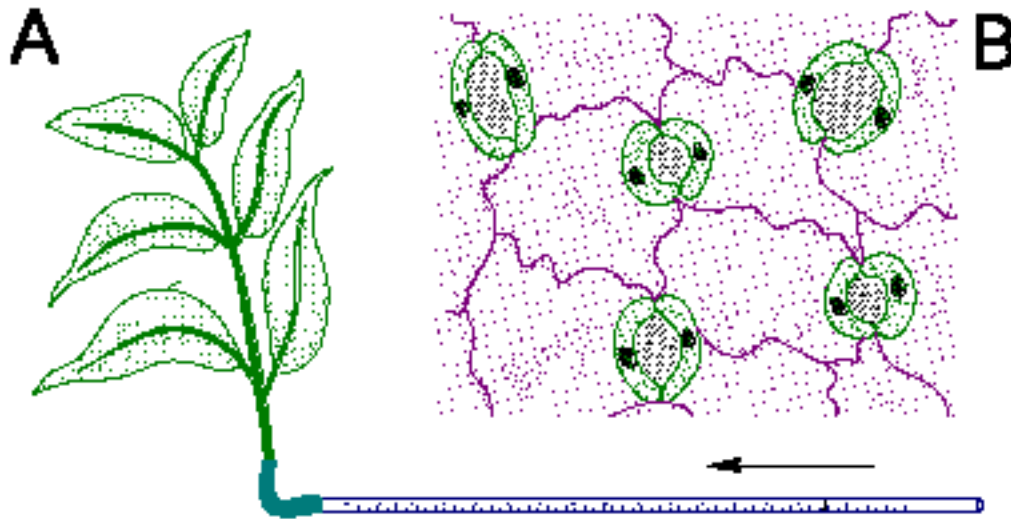
The problem was solved through the evolution of leaf stomata (which allow exchange of gasses with the atmosphere). In this exercise you will demonstrate the functioning of leaf stomata by observing a process called "transpiration"; the loss of water vapor from a plant's leaves. As water evaporates from the internal surfaces of the mesophyll layer, it diffuses through stomata to the surrounding air. Transpiration is the main force by which sap is drawn up from the roots of the plant (much as a drink is sucked through a straw). It also helps to cool the plant (plant perspiration?).

PROCEDURE:

CAUTION: Work quickly during the following procedure. Make sure to maintain the column of water by cutting and working with the stem under water whenever possible. To make it even more challenging, try not to get the leaves wet while working on the stem.

1. **Preparation of Plant Leaves.** Work in groups of three or four. Select a long branch with healthy leaves (choose one that is about the same diameter as the hole in the stopper or rubber tubing you will use). Make a clean cut at the base and **immediately** transfer the cut end to a pan of water (try to keep the leaves dry). Make a second oblique cut a cm or so above the first. From now on, do not expose the cut end to the air since an air bubble will form in the vascular tissue of the plant. If the column of water in the plant is not continuous, the experiment will not work.

2. **Building a Pipette Potometer.** Attach one end of the rubber tubing to the pipette and fill with water by immersing in a pan of water (Fig 1). Insert a pencil in the free end of the rubber tube and roll the rubber back on itself to form a cuff. Work under water and gently insert the cut end of the stem in the end of the rubber tube. Roll the cuff back over the stem and apply a seal of petroleum jelly with an applicator stick. You can now remove the stem with the attached pipette from the pan of water. If the water begins to drain out of the tube, immediately immerse the apparatus so you won't get an air bubble in the vascular tissue (check your seals before continuing; do not pass go, do not collect \$200.00). If all goes well, support the leaf in an upright position by leaning it against your comatose lab partner or laboratory apparatus (a beaker or test tube rack, for example)



Potometer setup (A) and stomata (B)

3. **Recording Rates of Transpiration: Effect of Light.** If your leaves are wet gently pat them dry with a paper towel or ball of cotton. Tap the open end of the potometer or pipette to move an air bubble into the scaled portion of the tube (opposite the plant). Move a lamp near the plant and turn it on (don't place the lamp so close that it will overheat or burn the leaves). Allow the leaves to bathe in the eerie glow of Edison's infernal light for two minutes, then note the bubble's start position. Record the movement of the air bubble at one-minute intervals for 5 min (enter these data in the results section). If the bubble just sits there or moves away from the plant, check for leaks. You may have to cut another plant (be more careful this time and keep the cut end under water). Turn off the lamp but do not move it out of position. After allowing two minutes for equilibration, record the initial position of the bubble and then its progress up the tube at one-minute intervals for five minutes. Record these data in the results section under "Reduced Illumination".
4. **Recording Rates of Transpiration: Effect of Wind.** Move a small fan near the plant and, with the lamp still off, record the movement of the bubble as before (don't forget the start position). Enter these data in the table column marked "Dark with Breeze". Turn the lamp back on, allow 2 minutes for equilibration, and record the effects of light with a breeze on the rate of transpiration.
5. **Recording Rates of Transpiration: Sealing the Stomata.** Turn the fan off and coat the upper surface of the leaves with petroleum jelly. Be careful not to get any petroleum jelly on the bottom surface since you only want to seal the stomata on the upper surface at this time. Illuminate the plant and record the transpiration rate as before. Now cover the bottom surface of the leaf and make your recordings. What effect does light, wind, and sealing of stomata have on the transpiration rate of your plant. From these data can you determine which surface of the plant has the most stomata? Speculate why this distribution of stomata has evolved.
6. **A Model of Transpiration.** Download and run the transpiration model. Vary temperature, stomata density, and other factors to determine their effects on plant transpiration.

RESULTS AND DISCUSSION

Transpiration by Plants. Enter the results of your transpiration experiment in the following table, then compute the mean. Make sure you enter the volume moved, not the actual reading. If, for example, the bubble started at 0.5 ml and moved to 1.5 ml during the first minute, you would enter "1.0 ml" ($1.5 - 0.5 = 1.0$). The value for the second minute would be the difference between the second and first minute readings (and so on).

Minutes	Full Light	Reduced Light	Dark with Breeze	Light with Breeze	Lower Leaf Coated	Top & Bottom Coated
Start Vol						
1						
2						
3						
4						
5						
Mean						

Briefly summarize the effects of light, dark, and the presence of wind on transpiration in your plant. Which treatments opened the stomata and which closed the stomata?

From these data, is the distribution of stomata the same on the top and bottom surfaces of the leaf (Explain). Also, speculate why this distribution of stomata evolved.

Compare and contrast your data to that of the transpiration model.
