Leaves: Specialized Plant Organs
OBJECTIVES

After completing this lab you will be able to:
1. Describe and name leaf patterns
2. Identify the structural differences between dicot and monocot leaves

LAB PREPARATION

In preparation for this laboratory you should do the following:

1. Read and study this laboratory.
3. Bring your copy of Photo Atlas for Biology to lab.

INTRODUCTION

Leaves, perhaps more than any other plant organ, vary greatly in external form and internal structure. As the main photosynthetic organs of the plant, leaf morphology is largely influenced by the amount of sunlight they receive. Additional environmental factors, such as water availability, wind, temperature, and herbivores, also affect the morphology and arrangement of leaves on a plant.

A. Transpiration and Leaf Anatomy

The carbon a plant uses to make carbohydrates comes from atmospheric carbon dioxide (CO₂). Plants sequester CO₂ through special openings in the leaf surface, called stomata. When the stomata are open, some water vapor is lost from the interior of the leaf. This process of water vapor loss is called transpiration. As water vapor is lost by transpiration in the leaves, more water is pulled into the roots and up the plant body, via the xylem (see Chap. 36 in Campbell for a discussion of transpirational pull).

While transpiration is an integral component of the mechanism responsible for water movement throughout the plant, excessive water loss can threaten the plants continued existence. For this reason, various leaf adaptations have evolved that minimize or control transpirational water loss in most plants. In this laboratory, we will point out some of the more common leaf adaptations.

B. Morphology of Leaves

Leaves generally consist of two parts, an expanded portion called the blade (or lamina), and a stalk-like portion called the petiole. In some leaves, smaller leaf-like structures may exist at the point where the leaf attaches to the stem. These are called stipules. Also, in most
monocots (and some dicots) the base of the leaf is expanded into a sheath which encircles the stem.

1. **Leaf Arrangements (Figure 4-1)**
   a. **Opposite**: two leaves at a node (point of leaf attachment) on opposite sides of the stem
   
   b. **Spiral** (alternate): one leaf per node with the second leaf being above the first but attached on the opposite side of the stem (alternate), or where the successive leaves attach above the first in a spiral.
   
   c. **Whorled**: three or more leaves are attached at one node.

Can you see why an alternate leaf arrangement is just a special case of spiral leaf arrangement?

Dicot leaves can be simple or compound (Figure 4-2). In simple leaves, the blade is not divided into separate parts, although it may have deep lobes. In compound leaves, the leaf is divided into leaflets, with each leaflet usually having its own stalk (called a petiole). There are two types of compound leaves: **pinnate**, and **palmate**. Pinnate leaves have their leaflets attached to both sides of an extension of the petiole, called the rachis, like the pinnae of a feather. Palmate leaves radiate out from the tip of the petiole (like the fingers of your hand), and lack a rachis.

Sometimes it may be unclear whether a leaf is a compound leaf or a cluster of several simple leaves. When in doubt, remember that leaves, simple or compound, have axillary buds in their axils (where they attach to the stem), and leaflets do not.

C. **Internal Anatomy of the Typical Leaf**
   
   The typical components of a leaf are discussed sequentially (as they occur from top to bottom) in the following sections. As you read, refer to Figure 4-3 of the typical dicot leaf. Keep in mind, however, that there are many variations on this basic plan.

1. **Cuticle**
   
   The cuticle is a waxy (hydrophobic), non-cellular layer secreted atop the epidermal cells. Its primary function is to reduce water loss from the leaves.

2. **Upper Epidermis**
   
   The upper epidermis is the outermost cell layer of the leaf, and originates from the protoderm. It is the layer in which the stomata are found. It may also contain any of a variety of specialized cells (reviewed below), depending on the species.
a. **Epidermal cells** are usually a single layer of flattened cells, normally parenchyma, which lack chloroplasts. Their function is to provide mechanical support, regulate water loss, and protect the leaf from environmental intrusion, e.g., wind, excess light, insects.

Some plants have a multiple epidermis that is several cells thick. While some researchers think that a multiple epidermis functions to further decrease water loss from the leaf's interior, others believe that it serves as a water-storage tissue. Both ideas are probably correct.

b. **Stomata** (stomates; Gk. *stoma*, mouth) are the mouths that allow the exchange of gases into and out of the leaves. Technically, the term stomata (plural; singular stoma) refers only to the openings in the leaf. The cells that actually cause the stomata to open and close are called **guard cells**. These look like kidney beans when viewed from the top. It is easy to distinguish these guard cells from the other epidermal cells, because they are the only cells of the epidermis that contain chloroplasts.

c. **Guard cells** often are connected to **subsidiary cells** or **accessory cells** which sometimes look very different from the rest of the epidermal cells. These cells are the only cells that directly communicate with the guard cells.

In terrestrial plants, there are typically fewer stomata on the upper epidermis than there are on the lower epidermis. This is believed to be one of the many adaptations of the leaf structure that serves to minimize loss of water vapor from the interior. Plants of drier environments might be expected to have no stomata on the upper epidermis and very few on the lower epidermis. On the other hand, the density of stomata on the upper and lower epidermis may be rather high in plants adapted to wet areas.

d. **Bulliform cells** are specialized epidermal cells easily distinguished from the other cells of the epidermis. Bulliform cells are conspicuously large cells that are found on the upper epidermis of some monocots leaves. They are believed to be another adaptation that protects the leaf from desiccation. Bulliform cells are particularly sensitive to changes in the water availability of the leaf. If water is not available, the bulliform cells collapse, causing the leaf to roll up, or fold. This reduces the surface area of the leaf exposed to the sun or wind, therefore limiting the amount of water loss from the leaf.

e. **Trichomes** (hairs) are cells which extend out from the surface of the leaf. Some secrete waxes, oils, or sugars. Others are scale-like, or
Laboratory 5: Leaves

3. The Mesophyll

The mesophyll (meso = middle and phyll = leaf) is composed of cells that originate from the leaf’s ground meristem.

The mesophyll is characterized by a fairly large amount of air space and an abundance of chloroplasts. As seen in Figure 3-3, very often it is divided into two fairly distinct layers of parenchyma cells.

a. Palisade Parenchyma (or palisade mesophyll) is the site of the majority of photosynthetic activity. Normally, the palisade mesophyll looks very much like a wall of rectangular bricks, one or more layers deep. (The one shown in Figure 3-3 is two layers deep.) Each brick is a parenchyma cell containing chloroplasts.

b. Spongy parenchyma (or spongy mesophyll) looks very much like a three-dimensional net with a lot of air spaces in it. The parenchyma cells here have conspicuously fewer chloroplasts per
cell than those of the palisade layer. They frequently have rather irregular shapes, with branches (arms) extending from one cell to another. The primary function of the spongy parenchyma appears to be intercellular transport of gasses, water, and nutrients to and from the palisade parenchyma.

Again, you should realize that not all leaves have a mesophyll that is neatly differentiated into two distinct layers. Grasses, for example, have mesophyll tissue that looks the same throughout (no division into palisade and spongy parenchyma). On the other hand, some plants have a palisade layer on both sides of the leaf.

4. Leaf Veins and Associated Cells

The water needed by the mesophyll cells for photosynthesis must be transported from the roots to the leaves, and the photosynthetic products of the leaves (mainly sugars) must somehow get to the rest of the plant. (Remember, like all living cells, plant cells require a food source that, when broken down, will provide them with the energy and raw materials they need for maintenance and growth.) The transport of water and food molecules to and from the leaves is made possible by the xylem and phloem which is continuous with the vascular tissue of the stem and root.

a. Vascular Bundles or veins are little bundles of xylem and phloem which originates from procambium. The xylem functions to deliver water and minerals from the root to the cells, while the phloem transports food molecules from the leaves (the sources) to roots and stem tips (the sinks).

b. Bundle Sheaths are compactly arranged cells, one or more layers thick, that enclose the veins of the leaf. The cells of the bundle sheath often look very much like the parenchyma cells of the spongy mesophyll. The bundle sheath encloses the entire length of the vein, so that the vascular tissue is not directly exposed to the air in the intercellular spaces. This means that all substances entering and leaving the vascular tissues must pass through the bundle sheath cells. Thus the bundle sheath provides a screening service similar to that performed by the endodermis of the root (see Laboratory 3).

c. Bundle Sheath Extensions are columns of cells that connect the bundle sheath cells with the upper and lower epidermis in some leaves. The connections are simply extensions of the sheaths. These extensions provide mechanical support for the leaf and may act to conduct water to the epidermis.

5. Lower Epidermis

The cellular components of upper and lower epidermis of terrestrial plants are very similar, but the lower epidermis frequently has a
higher density of stomata (and trichomes). This adaptation minimizes water loss due to transpiration.

6. Cuticle
The cuticle covering the lower epidermis is this same waxy non-cellular layer that coats the upper epidermis.

D. Which Side Is Up?
The xylem is located more towards the center of a stem in a vascular bundle than is the phloem (see Lab 3). As a result, the xylem is normally located on the top side of a leaf and the phloem on the bottom side. This orientation of xylem and phloem in the leaf vein is the only consistently reliable indicator of which way is up in a leaf. (Remember, not all leaves are neatly differentiated with a palisade layer on top and a spongy mesophyll layer on the bottom, as shown in Figure 4-3.)
LABORATORY EXPERIMENTS

You should work individually in this lab. Be sure to look at the poster of the histology of monocot and dicot leaves.

A. Internal Anatomy of the Leaf

1. Dicot Leaf

   **PROCEDURE**
   
   1. Examine the prepared slide labeled typical dicot leaf.
   2. Identify the cuticle, and upper epidermis.

   Are there any stomata or trichomes visible here?

   3. Below the epidermis is the palisade parenchyma.

   How many cell layers thick is the palisade mesophyll?
   • Note the numerous chloroplasts visible within its cells.

   4. Identify the spongy mesophyll.

   What percentage of the spongy parenchyma is made up of intercellular air spaces?
   
   • The vascular bundles lie on the border between the two mesophyll layers.

   5. Identify their xylem and phloem, and the bundle sheath cells surrounding them.

   What is the function of these three tissues (cells)?
   • Notice that not all the bundles lie in the same plane, i.e., some are in cross-section while others are longitudinal.

   How can you explain this?

   6. Identify the lower epidermis, and locate a stoma.

   Where are the guard cells?
   How many stomata can you count on the lower epidermis? Compare this to the upper epidermis.
2. Monocot Leaf

**PROCEDURE**
1. Now examine a prepared slide of a typical monocot leaf in cross-section.

2. Look first at the upper epidermis. Is there a cuticle present? What about stomata and trichomes?

3. Identify the bulliform cells. What is their function?
   - Notice that the mesophyll here is not differentiated into two distinct layers like that of the dicot.

4. How are the vascular bundles arranged in this leaf, i.e., are they all in the same plane? Explain.

5. Identify the large vessel members that make up the xylem.

6. Find the phloem and bundle sheath cells. How would you tell which way is up on this leaf?

7. Finally, examine the lower epidermis and compare it to the upper. Which has more stomata?

8. Go back and compare the monocot leaf with the dicot leaf you previously examined. How do they differ in thickness, stomata location, mesophyll arrangement, intercellular air spaces, size and shape of the vascular bundles, cell types present?

**B. Stomata**

**PROCEDURE**
1. Obtain a piece of Zebrina leaf and peel off a small strip of the LOWER, purple epidermis. (If you have difficulty obtaining only the epidermis, ask your Teaching Assistant for help.)

2. Quickly mount the epidermis in a drop of water on a microscope slide before it dries out, making sure that the outside of the peel is facing up.

3. Cover the peel with a coverglass and examine it with your compound microscope.
4. Locate and identify the stomata. Are they open or closed?

5. Examine the guard cells surrounding a stoma and the normal epidermal cells that make up most of the peel. How do these two cell types differ? Can you identify the subsidiary cells adjacent to the guard cells?

6. After you have located these cells remove the coverglass and add one or two drops of Lugol's stain to the peel. Cover the peel with the same coverglass and re-examine.

Where is the starch located in the peel?

C. Display: Leaf Modifications

- Although most leaves are specialized for photosynthesis, some plants have leaves that have become adapted by evolution for other functions. Look at some of the examples of modified leaves on display.

- In many plants, brightly colored leaves help attract pollinators to the flower. The purple or pink petals of bougainvillea are actually leaves that surround a small flower. Similarly, the large red petals of poinsettia are modified leaves that surround a cluster of yellowish flowers.

- The spines of cacti are actually modified leaves. The extremely reduced surface area of the leaves minimizes water loss through them. They also protect the plant from herbivores (plant-eaters). At maturity, the spines contain no living tissue. Photosynthesis is carried out mainly by the fleshy, green stems.

- Fleshy, succulent leaves function in water storage and thus help plants to withstand drought. These, and plants such as cacti that have succulent stems, usually have a modified form of photosynthesis called CAM photosynthesis. In such plants, the stomata are closed during the day, thus conserving water; the stomata open at night and allow carbon dioxide to enter. C4 compounds accumulate at night and enter the Calvin cycle during the day. CAM photosynthesis thereby allows the plant to obtain carbon dioxide at night, when transpiration occurs at a slower rate than during the day, and to use the carbon dioxide later. It is a highly advantageous system for succulent plants that grow in hot, arid regions.
• Bulbs are vertical, underground shoots consisting mostly of swollen bases of leaves that store food. You can see the many layers of modified leaves attached to the short stem in an onion bulb. The arrangement of the leaves is concentric so that the older leaves surround the younger ones, with the apical meristem living in the center of the structure.

• Carnivorous (animal-eating) plants obtain some of their nitrogen and minerals by killing and digesting insects. They live in acid bogs and other habitats where soil conditions are poor, especially in nitrogen. Insect traps have evolved by the modification of leaves. The Venus flytrap is a modified leaf with two lobes that close together rapidly enough to capture an insect. Glands in the trap then secrete digestive enzymes, and nutrients are later absorbed by the modified leaf. (Despite its name, the flytrap catches more ants and grasshoppers than it does flies.)

• Trichomes are outgrowths of the epidermis that may occur on stems, leaves, and reproductive organs of plants. A fuzzy or woolly leaf is covered with trichomes. Trichomes play an important role in regulating the heat and water balance of the leaf. A covering of trichomes, such as those on the leaves of African violets, creates a layer of more humid air near the surface of the leaf, enabling the plant to conserve available supplies of water.
LAB SUMMARY

1. Submit copies of drawings you made in your lab notebook for this laboratory. Make certain they are properly labeled with a complete legend, etc.

2. Answer the following discussion questions

a. Describe the three basic patterns of leaf arrangement.

b. Draw a dicot leaf in cross section, name the tissues beginning with the top layer and working down.

c. Give at least two differences between the anatomy of a monocot leaf and a diccot leaf.

d. What three environmental factors which may influence the structure of leaves?

e. What is the usual cycle of stomatal opening and closing in a plant?