Leaves

Materials

I. Twigs with simple leaves, pinnately compound leaves, and palmately compound leaves (at least one of the types of leaves should have stipules)

2. Fresh grass (or other monocot) leaves
3. A display of various insectivorous and other modified leaves
4. A healthy *Sedum* plant
5. Toothpicks
6. Prepared slides of cross sections of lilac *(Syringa)* and pine *(Pinus)* leaves
7. Prepared slides of hydrophyte versus xerophyte leaves 8. Models and charts of leaves

Some Suggested Learning Goals

1. Understand the difference between *a simple leaf* and a *compound leaf* and know the parts of a *complete leaf.*
2. Know the differences between the *upper epidermis* and *lower epidermis* in a lilac *(Syringa)* leaf, and be able to distinguish *guard cells* from other epidermal cells.
3. Be able, with the aid of a compound microscope, to locate *veins (vascular bundles), palisade mesophyll, spongy mesophyll,* and *stomata* in a cross section of a leaf.
4. Know how a pine leaf differs from a dicot leaf with respect to the form and composition of tissues and cells.
5. Understand the distinctions between dicot and monocot leaves.

Introduction

Leaves are produced in an almost infinite variety of shapes, textures, and sizes. Some of the more common variations are illustrated in the glossary preceding the keys on page 205 and described within the keys. In this exercise, twigs with live leaves are studied first, and common and specialized leaf types are examined with a microscope.

A. Form and Arrangement of Leaves

Examine the provided twigs. Note how the *veins* are arranged in the flattened *blade* of a dicot leaf. Is *a midrib* (larger, central main vein) present? How does the vein arrangement *(venation)* differ from that of a grass (monocot) leaf? Which leaves have *petioles* (stalks)? Are *stipules* (paired, often leaflike or thornlike appendages at the base of the petiole) present on any of the leaves? Do the *margins* of any of the leaves have sawlike *teeth* or *lobes* (larger, usually rounded projections)? Are hairs or wax present on the surfaces of any of the leaves? Is an *axillary bud* present in the *axil* (the *angle-not* structure-formed by the petiole with the blade) of each leaf? Note that a compound leaf, which is divided into *leaflets,* has a single axillary bud in its axil at the base of the petiole, but there never are buds in the axils of the leaflets. The midrib of a compound leaf is called *a rachis.* A compound leaf with pairs of leaflets arranged along the rachis is said to be *pinnately compound;* a compound leaf whose leaflets fan out from a common point is said to be *palmately compound.*

B. Typical Leaf Structure

Select a slide showing a cross section of a lilac leaf (Sy*ringa* xs). Note the upper epidermis, which is one cell thick. Normally it is coated with a fatty or waxy *cuticle,* but the cuticle is usually absent in these slides, having been removed by a solvent during the manufacturing process. Immediately below the upper epidermis are two layers of *palisade mesophyll.* Note that the cells are tightly packed together, and that they contain numerous chloroplasts. Are chloroplasts present in the upper epidermal cells? Below the palisade mesophyll is the *spongy mesophyll.* Note that the cells of the spongy mesophyll are loosely and somewhat haphazardly arranged. Note also that there are numerous air spaces between them, and that spongy mesophyll cells have fewer chloroplasts than palisade mesophyll cells.

Notice that there are *veins (vascular bundles)* scattered throughout the mesophyll. Can you distinguish between thin-walled *phloem* cells in the lower part of a vein, and thicker-walled *xylem* cells in the upper part of a vein? Notice also that the veins are of various sizes, and that some appear in cross section while others appear to have been sliced at an angle or lengthwise. This is because veins run in various directions and at various angles throughout a lilac leaf blade. Examine the *lower epidermis* that covers the lower or undersurface of the leaf. Do you see any differences between the upper epidermis and the lower epidermis? For one thing, there are *stomata* scattered throughout the lower epidermis. The stomata are formed by pairs of *guard cells.* The guard cells are smaller than the other cells of the lower epidermis, and they may appear slightly recessed. Guard cells, unlike the other epidermal cells, contain chloroplasts that play a role in opening and closing the stomatal pores.

C. Pine Leaf Structure

Turn now to a slide of a pine tree leaf *(Pinus* xs). Pine leaves are adapted to areas where little moisture is available to them when the ground is frozen in the winter, and they look quite different from lilac leaves. Note that the xylem and phloem in the center are surrounded by *transfusion tissue* composed of a mixture of parenchyma cells and short tracheids. The outer boundary of the transfusion tissue is marked by a single row of conspicuous cells comprising the *endodermis.* Notice also, depending on the species of pine, that the xylem and phloem may be in two adjacent patches *(vascular bundles),* or there may be a single vascular bundle. Much of the remaining tissue of the leaf is mesophyll, which is not divided into palisade and spongy layers.

Note the two or more large, circular to elliptical *resin canals* in the mesophyll. The cells lining each resin canal secrete resin into the resin canals. The leaf is covered by an *epidermis,* consisting of a single row of cells, but there are recessed pockets scattered throughout the epidermal cells. Within these pockets, locate the pairs of *guard cells* (they look a little like cats' eyes) that form each *sunken stomata.* Sunken stomata are common in desert plants and in other plants, like pine trees, that grow in areas where moisture may be unavailable or in short supply for at least part of the year. Notice that beneath the epidermis there are one or more layers of thick-walled cells constituting the *hypodermis* (not present in lilac and many other leaves). The hypodermis gives support and rigidity to the pine leaf and also affords a measure of protection to the more delicate tissues of the interior.

D. Stripping and Observing a Leaf Epidermis

Your instructor will show you how to strip the epidermis from *a stonecrop* or similar leaf. Have a slide with a drop of water ready, and strip a small piece of epidermis for mounting and microscopic examination. Identify the *epidermal cells, guard cells,* and *stomata.* Besides the obvious difference in shape, how else do guard cells differ from the surrounding epidermal cells? Are the stomata in your epidermis open or closed?

E. Specialized Leaves

Examine a prepared slide that has cross sections of leaves from desert and aquatic plants. What differences can you see in the *mesophyll, epidermis,* and *veins (vascular tissue)* of these plants? Explain how the differences adapt the plants to their respective habitats.

Note the display of insect-trapping and other specialized leaves. How do these leaves differ, at least externally, from typical broad leaves of plants of temperate regions?

Drawings to Be Submitted

1. Draw a COMPOUND LEAF attached to a twig. Label BLADE, and where present, PETIOLE, STIPULES, LEAFLET, AXILLARY BUD, and any other visible parts (e.g., HAIRS, SCALES).
2. Fully label the drawing of the stereoscopic view of a portion of a leaf provided. Labels should include UPPER EPIDERMIS, LOWER EPIDERMIS, PALISADE MESOPHYLL, SPONGY MESOPHYLL, VASCULAR BUNDLE (VEIN), STOMA, and GUARD CELLS.
3. Diagram and label a cross section of a pine leaf. Labels should include EPIDERMIS, HYPODERMIS, ENDODERMIS, TRANSFUSION TISSUE, MESOPHYLL, SUNKEN STOMA, RESIN CANAL, and VASCULAR BUNDLE.
4. Label the portion of the crosssection of a lilac (SYRINGA) leaf provided. Labels should include UPPER EPIDERMIS, LOWER EPIDERMIS, PALISADE MESOPHYLL, SPONGY MESOPHYLL, STOMA, and VEIN.
5. Draw a portion of the epidermis of a stonecrop leaf, showing at least one STOMA, GUARD CELLS, and surrounding EPIDERMAL CELLS.

1. How does *a compound* leaf differ from *a simple* leaf?

2. What fatty or waxy substance present on the outer walls of leaf epidermal cells is

   usually lost in the preparation of slides?

3. When you view a cross section of a leaf with the upper epidermis at the top, where is the *phloem* located in a *vein?*

4. Which of the larger organelles are most abundant in *palisade mesophyll* cells?

5. What specific tissue marks the outer boundary of *transfusion tissue* in a pine leaf?

6. Which tissue lies between the *epidermis* and the *endodermis* in a pine leaf?

7. Where are the *resin canals* located in a pine leaf?

What is their function?

8. What are *sunken stomata?*____

With which types of plants are they associated?______________________________

9. What is the function of a *hypodermis?* ______________________________________

Where is a hypodermis located? ____

10. Apart from size and shape, how do *guard cells* differ from the epidermal cells that surround them? ____

1. What are *stipules?*

2. What is the fatty or waxy substance that coats a leaf epidermis called?

3. What tissue composed of thick-walled cells is found just beneath the epidermis of a pine leaf?

4. In prepared slides of lilac leaves, why are some *veins* visible in cross section while others

are visible in longitudinal section?

5. Which tissue of pine leaves differs from that of lilac leaves in its not being divided into two distinguishable layers?

6. Of which two tissues are leaf *veins* primarily composed?

7. Where are *stomata* generally most abundant in the majority of leaves?

8. Which layer of *mesophyll* is closest to the upper epidermis of a leaf?

9. In what kind of leaf would you expect to find *resin canals?*

10. The two cells that form and surround a stoma are known as

The Leaf

Leaves are the main appendages of the stem, and in most vascular plants, the principal structure for photosynthesis. Although leaves vary tremendously in form and internal structure, most consist of a petiole and a blade. Some of the variation in leaf structure is related to habitat. Aquatic leaves and leaves of dry habitats have special modifications to permit survival in those different habitats. Leaf shapes, margins, tips, and venation patterns are characteristics used to identify different species of flowering plants.

A. Dicot Leaf Structure

Examine a prepared slide of a lilac, Syringa, or similar leaf. Note the large midvein. As you scan your section locate the many branching veins, some of which will be in longitudinal section while others are in cross section.

Observe a portion of the blade to one side of the mid vein. Identify the

- **Upper epidermis**, which has a relatively thin but discernible **cuticle**
- **Palisade mesophyll**
- The **veins**, with their bundle sheaths of sclerenchyma
- **The spongy mesophyll**
- **Lower epidermis**, which also has a cuticle.

The palisade and spongy mesophyll are composed of parenchyma cells, which contain many chloroplasts for photosynthesis. Note the presence of intercellular **air spaces** among the spongy mesophyll cells and the relative distribution of **stomata** and guard cells in the lower epidermis. Most stomata open into an air space within the spongy mesophyll. The mushroom-shaped structures of the epidermis are **trichomes**, or **epidermal hairs**.

Dicot Leaf Cross Section

B. A Monocot Leaf

Observe a corn (Zea mays) leaf section. Note the distribution of veins.

Monocot leaves generally have parallel veins rather than the branching network of veins common to dicot leaves. . Note too that the corn leaf has a uniform mesophyll region rather than distinctive palisade and mesophyll areas.

In the corn leaf the veins are surrounded by a sheath composed of large parenchyma cells. These cells are involved with C-4 photosynthesis. The larger vascular bundles contain extensions of sclerenchyma which connect to the epidermis for support. Identify the xylem and the phloem regions of the veins.

Where are the stomata and guard cells located in the corn leaf?

Monocot Leaf Cross Section

C. Environmental Adaptations of Leaves

Xeromorphic Leaves

Plants which live in arid environments are subject to drought, and often, intense sunlight. Such plants are called xerophytes. These plants are subjected to intense evaporation of water, a resource which is often in short supply. Many such plants have a number of modifications which minimize water loss through transpiration, the
evaporation of water from the plant surfaces. Some plants drop their leaves during periods of drought; cactus plants photosynthesize with modified stem tissue, and lack leaves entirely. Those plants which do produce and retain leaves often have special features which we associate with the xeromorphic leaf. Nerium oleander is a good example of a plant with xeromorphic leaves.

Examine the prepared slide of Nerium oleander leaf, xs. Note the very thick **cuticle** as you focus on the **upper epidermis**. The epidermis is several layers thick, too. The **palisade parenchyma**, beneath the epidermis layers, is in two layers. The **spongy mesophyll** is loosely packed and quite wide. The unusual structures seen in the spongy mesophyll are a type of crystal, called **druses**.

Veins may have bundle sheath extensions in additional to the bundle sheath layer. Look for the mid vein. It has phloem on both sides of the xylem, which is unusual.

As you turn to the **lower epidermis**, note that it, like the upper epidermis, has several layers and a thickened cuticle. As you move your slide along the lower epidermis, note the deep invaginations of the epidermis layer into the lower leaf. These invaginations are called **stomatal crypts**. There are a number of epidermal hairs in the crypts, along with the stomata. All of the stomata are located in the crypts.

Why do you think this is?

Nerium oleander leaf , xs.

Hydromorphic Leaves

The leaves of the water lily float on the surface of ponds and lakes, although the water lily is rooted in the lake bottom. Examine a prepared slide of Nymphaea leaf, xs, to observe modifications water lilies have for flotation. Look first at both **epidermis** layers. Where do you find stomata? Why? Look for small hairs in the lower epidermis layer. Now refocus on the upper epidermis layer. Can you find the **cuticle**? It is very thin. Below the epidermis cells the **palisade mesophyll** consists of three or four overlapping layers of cells, which are fairly loosely packed, allowing for gases to enter from the upper epidermis. Note the huge **intracellular spaces** in the **spongy mesophyll** layer.

The buoyancy of the water lily comes from these large air spaces The spongy mesophyll also contains large, branching, thick-walled **sclerids** for support. There are crystals within the sclerids, too. Note the reduced size of the **veins** in Nymphaea, compared to most leaves. The vascular tissue, especially the xylem, is minimal in most hydromorphic leaves. You should find more phloem than xylem in the vascular tissue as you observe the scattered veins. Nymphaea leaf, xs

Compare the adaptations of the hydromorphic and xeromorphic leaves with the typical mesomorphic dicot leaf, such as Syringa.

Ecological Leaf Type Adaptive Structures Environment

Mesomorphic: Syringa
Xeromorphic: Nerium
Hydromorphic: Nymphaea

D. Stomata Structure in Zebrina leaves

The epidermal surfaces of plants are covered with a protective cuticle.
However, CO_2 must enter the leaf for photosynthesis and the O_2 produced during photosynthesis must be released from the plant. To solve this dilemma plants have specialized cells in the epidermis, called guard cells, which form **stomata** (pores) in the epidermis. Stomata can be open or closed, depending on the turgor of the guard cells. When stomata are open, gas exchange can occur. Unfortunately, large amounts of water are lost from the plant through the open stomata as well. (For example, as much as 90% of the water absorbed by the roots of a corn plant growing in Kansas may be lost through the stomata of its leaves.) To avoid excessive water loss, the guard cells have a mechanism to open the stomata during photosynthetic periods (i.e., daylight hours) and close the stomata when photosynthesis is not occurring.
You will observe guard cells and stomata in the lower epidermis of leaves of Zebrina. Since the regular epidermal cells of Zebrina contain anthocyanin (purple) pigments, the guard cells, which contain chloroplasts, are particularly conspicuous.
Leaf epidermis showing stomata and guard cells

Zebrina Epidermal Peel

• Cut a portion of a leaf from a Zebrina plant.
• With your fingernail or a sharp razor blade, peel a portion of the **lower** epidermis from the leaf, starting at the cut edge. Note: The lower epidermis is purple pigmented.
The upper epidermis is silver and green striped.
• Make a wet mount of the epidermal peel. Try to have the peel flat on the microscope slide; wrinkled portions have too many layers of cells and trap air bubbles.
• Observe your slide with your microscope. After locating guard cells with the lower power magnification, use the 45x objective to observe one of the stomata closely.
Can you see the chloroplasts in the guard cells?
• What is the shape of the guard cells? Note the thickness of the inner walls of the guard cells.
Are any of the stomata open?
Recall from your observation of the prepared slide of a leaf that a stoma opens into an air space of the spongy mesophyll. Of what advantage is this arrangement to the plant for photosynthesis?

E. C4 Photosynthesis and Leaf Structure

Most higher plants use a photosynthetic pathway known as the C3 photosynthetic pathway, where the Calvin cycle of the "dark reactrions" begins with CO_2 (carbon dioxide) combining with ribulose biphosphate (RuBP) to form the 3- carbon compounds, PGA (phosphoglyceric acid) and PGAl, (phosphoglyceraldehyde). Both the light reactions of photosynthesis and the Calvin cycle occur within the same chloroplasts in all of the mesophyll cells. The Ligustrum or Syringa dicot leaf cross section you observed shows the typical leaf structure of a C3 plant. Some plants, known as C4 plants, use a different pathway for carbon fixation, in which CO_2 first combines with PEP (phosphoenolpyruvate) to produce 4-carbon acids, such as oxaloacetic acid or malic

acid. The reaction serves as a CO_2 trap, since the CO_2 taken into the leaf can now be stored in the form of the 4—carbon acids. This is especially beneficial for plants in hot dry areas, which lose lots of water through their open stomata when CO_2 is absorbed. Many C4 plants can "stockpile" CO_2 this way, freeing CO_2 from the acids for the Calvin cycle as needed. Some monocot C4 plants also separate the reactions of photosynthesis into different chloroplasts within different types of cells, another energy conserving
measure. When plants do a lot of photosynthesis, the oxygen produced during the light reactions competes with CO_2 for the ribulose biphosphate (RuBP) enzyme. The light reactions of C4 plants occur in mesophyll cells which surround the veins' enlarged and modified bundle sheath cells. The Calvin cycle occurs in chloroplasts of the enlarged bundle sheath cells. This separation of reactions keeps oxygen away from the cells performing Calvin cycle steps. C4 photosynthesis has several benefits for the plant, resulting in a more efficient rate of photosynthesis. It also results in an interesting modification of the typical leaf anatomy.

Observing a C4 leaf

Corn (Zea mays) is a C4 plant. Observe again the prepared slide of a corn leaf to see the differences in C3 and C4 leaf structure.

• Note especially the layer of round cells which surround the veins in the corn leaf.
This layer is formed by the bundle sheath cells, which contain the chloroplasts in which the Calvin cycle occurs.

• Note, too, that the mesophyll cells are not separated into well-defined palisade and spongy mesophyll layers, such as you observed in the Ligustrum leaf. In the corn leaf, the mesophyll cells surround the bundle sheath cells. Only the light reactions of photosynthesis occur in the chloroplasts of the mesophyll cells. This C4 leaf structure is known as **Kranz anatomy.**

Corn leaf, xs Chloroplasts from bundle sheath cell (left) and mesophyll cell (right) of corn leaf.

• Observe the electron micrographs of the C4 mesophyll and bundle sheath cell chloroplasts shown above. Note the different chloroplast structures in the two cells.
Why does the mesophyll cell have chloroplasts containing lots of grana composed of many thylakoid layers? Why are well-developed grana absent in the chloroplasts of the bundle sheath cell?

• Note the many plasmodesmata which connect the two cells. Why would you expect to see so many plasmodesmata between the mesophyll cells and the bundle sheath cells in the C4 plant?

Part 2

The (Main) PHOTOSYSNTHESIS CENTER
Leaf Observations: Top, Bottom and Cross Section Observation

Objective: To carefully observe several leaves for defining characteristics and match these characteristics (as much as possible) to the traditional labeling identified and shared by botanists.

Materials: Various leaves, tray, dissection kit, compound and dissecting microscope, microscope slide(s)

Procedure: Draw and describe the leaf of 1) a typical maple or oak, 2) a leaf of your choice from three perspectives

a) Top view (the side most facing the sun)
b) Bottom view (the side facing away from the sun)
c) *Cross Section (a cut section of the leaf)

*In order to create a cross section of your leaf to view record the steps in the space below.

Creating a Cross Section of a leaf to view (5 points)

Observations and Results

<table>
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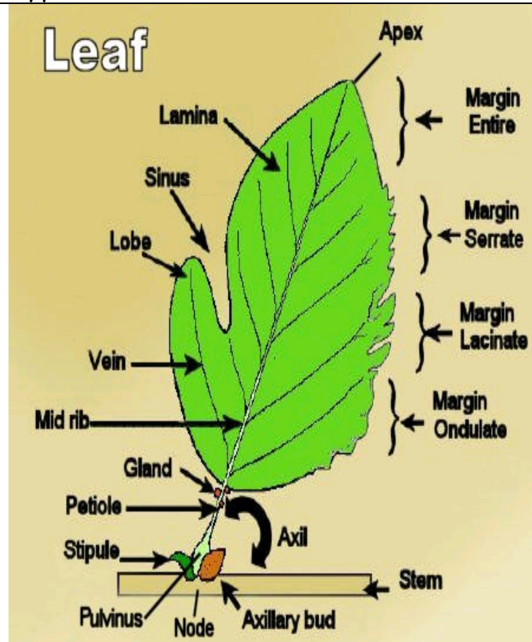
<tr><td>Top</td><td></td><td></td><td>
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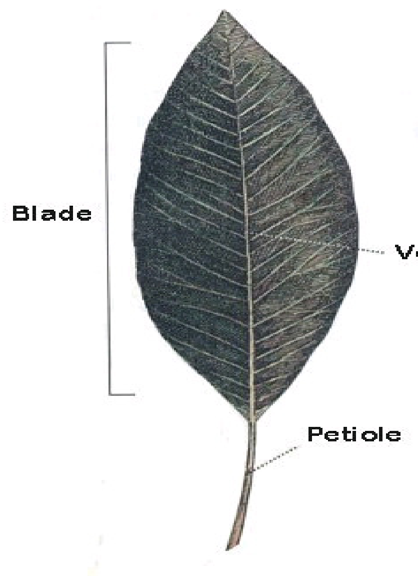
<tr><td>Bottom</td><td></td><td></td><td>
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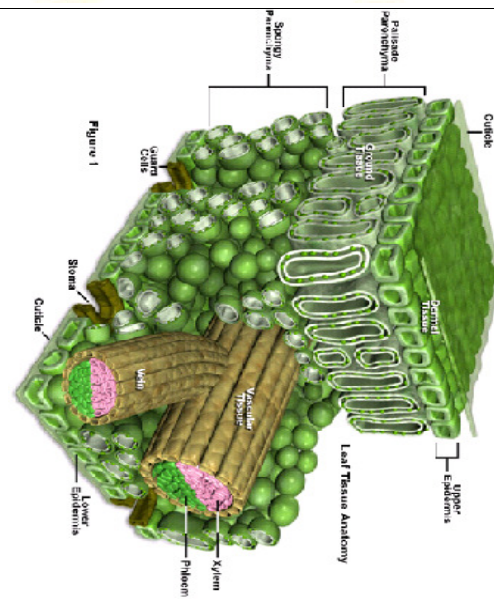
<tr><td>Cross Section</td><td></td><td></td><td>
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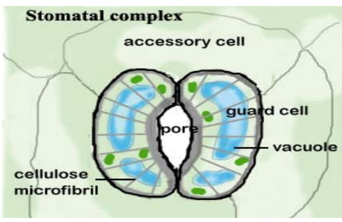
How were the various leaves you observed similar?

How were the various leaves you observed different?

What are the purpose of the veins in leaves?

Do plant cells go through cellular respiration? Explain (including an explanation of where the food used in this process comes from).

Conclusion:
What did you learn?



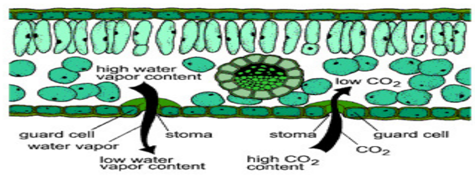


Figure 25. Stomata open to allow carbon dioxide (CO_2) to enter a leaf and water vapor to leave.

Stomata Part 2: Observation of leaf Stomata

1. Choose a leaf from one of the plants. DO NOT REMOVE THE LEAF FROM THE PLANT!!
2. Paint a thick patch (at least one square centimeter) of clear nail polish on the underside of the leaf surface being studied.
3. Allow the nail polish to dry completely for several minutes.
4. Tape a piece of clear cellophane tape to the dried nail polish patch.
5. Gently peel the nail polish patch from the leaf by pulling on a corner of the tape and "peeling" the fingernail polish off the leaf. This is the leaf impression you will examine.
6. Tape your peeled impression to a very clean microscope slide. Use scissors to trim away any excess tape. Label the slide with plant name.
7. Examine the leaf impression under a light microscope at 400X.
8. Search for areas where there are numerous stomata, and where there is no dirt, thumb prints, damaged areas, or large leaf veins. Draw the leaf surface as it appears in your field of view. Then make a detailed drawing of ONE open stomate and one closed.
9. Count all the stomata in one microscopic field. Record the number on your data table.
10. Repeat counts for at least three other distinct microscopic fields. Record all the counts. Determine an average number per microscopic field.

	Leaf 1	Leaf 2	Leaf 3
Stomata in field 1			
Stomata in field 2			
Stomata in field 3			
Average stomata			