Plant Pigments

During winter, there is not enough light or water for photosynthesis. The trees will rest, and live off the food they stored during the summer. They begin to shut down their food-making factories. The green chlorophyll disappears from the leaves. As the bright green fades away, we begin to see yellow and orange colors. Small amounts of these colors have been in the leaves all along. We just can't see them in the summer, because they are covered up by the green chlorophyll.

The bright reds and purples we see in leaves are made mostly in the fall. In some trees, like maples, glucose is trapped in the leaves after photosynthesis stops. Sunlight and the cool nights of autumn cause the leaves turn this glucose into a red color. The brown color of trees like oaks is made from wastes left in the leaves.

It is the combination of all these things that make the beautiful colors we enjoy in the fall.

All summer, with the long hours of sunlight and a good supply of liquid water, plants are busy making and storing food, and growing. What about wintertime? The days are much shorter, and water is hard to get. Plants have found many different ways to get through the harsh days of winter.

Some plants, including many garden flowers, are called "annuals," which means they complete their life cycle in one growing season. They die when winter comes, but their seeds remain, ready to sprout again in the spring. "Perennials" live for more than two years. This category includes trees and shrubs, as well as herbaceous plants with soft, fleshy stems. When winter comes, the woody parts of trees and shrubs can survive the cold. The above ground parts of herbaceous plants (leaves, stalks) will die off, but underground parts (roots, bulbs) will remain alive. In the winter, plants rest and live off stored food until spring.

As plants grow, they shed older leaves and grow new ones. This is important because the leaves become damaged over time by insects, disease and weather. The shedding and replacement continues all the time. In addition, deciduous trees, like maples, oaks and elms, shed all their leaves in the fall in preparation for winter. "Evergreens" keep most of their leaves during the winter. They have special leaves, resistant to cold and moisture loss. Some, like pine and fir trees, have long thin needles. Others, like holly, have broad leaves with tough, waxy surfaces. On very cold, dry days, these leaves sometimes curl up to reduce their exposed surface. Evergreens may continue to photosynthesize during the winter as long as they get enough water, but the reactions occur more slowly at colder temperatures.

During summer days, leaves make more glucose than the plant needs for energy and growth. The excess is turned into starch and stored until needed. As the daylight gets shorter in the autumn, plants begin to shut down their food production.

Many changes occur in the leaves of deciduous trees before they finally fall from the branch. The leaf has actually been preparing for autumn since it started to grow in the spring. At the base of each leaf is a special layer of cells called the "abscission" or separation layer. All summer, small tubes which pass through this layer carry water into the leaf, and food back to the tree. In the fall, the cells of the abscission layer begin to swell and form a cork-like material, reducing and finally cutting off flow between leaf and tree. Glucose and waste products are trapped in the leaf. Without fresh water to renew it, chlorophyll begins to disappear.

The bright red and purple colors come from anthocyanin (an-thuh-'si-uh-nuhn) pigments. These are potent antioxidents common in many plants; for example, beets, red apples, purple grapes (and red wine), and flowers like violets and hyacinths. In some leaves, like maple leaves, these pigments are formed in the autumn from trapped glucose. Why would a plant use energy to make these red pigments, when the leaves will soon fall off? Some scientists think that the anthocyanins help the trees keep their leaves a bit longer. The pigments protect the leaves from the sun, and lower their freezing point, giving some frost protection. The leaves remain on the tree longer, and more of the sugars, nitrogen and other valuable substances can be removed before the leaves fall. Another possible reason has been proposed: when the leaves decay, the anthocyanins seep into the ground and prevent other plant species from growing in the spring.

Brown colors come from tannin, a bitter waste product. Other colors, which have been there all along, become visible when the chlorophyll disappears. The orange colors come from carotene ('kar-uh-teen) and the yellows from xanthophyll ('zan-thuh-fil). They are common pigments, also found in flowers, and foods like carrots, bananas and egg yolks. We do not know their exact role in leaves, but scientists think they may be involved somehow in photosynthesis. Different combinations of these pigments give us a wide range of colors each fall.

As the bottom cells in the separation layer form a seal between leaf and tree, the cells in the top of the separation layer begin to disintegrate. They form a tear-line, and eventually the leaf is blown away or simply falls from the tree.

One more important question remains. What causes the most spectacular display? The best place in the world for viewing fall colors is probably the Eastern United States. This is because of the climate there, and the wide variety of deciduous trees. The brightest colors are seen when late summer is dry, and autumn has bright sunny days and cool (low 40's Fahrenheit) nights. Then trees make a lot of anthocyanin pigments. A fall with cloudy days and warm nights brings drab colors. And an early frost quickly ends the colorful display.

It is time to discover, first hand, these pigments found hiding out in a typical green deciduous tree leaf. Use the following pages to investigate some of these plant pigments.

Paper Chromatography Lab

Introduction:

This experiment is conducted to investigate the components of Plant Pigments while visibly separating them. There are a couple of different types of components in plant pigments, and they became clearly visible during this lab. The most important and abundant chemical pigment found in plants is chlorophyll. This pigment exists in two forms; ***chlorophyll a*** and ***chlorophyll b***. Chlorophyll absorbs two main colors from light quite well. These are blue, and red. The chlorophyll reflects green light very well, however, the two different types of chlorophyll have their maximum absorption at different wavelengths of light. Chlorophyll a, being the main photosynthetic pigment, has a primary purpose to convert light energy to chemical energy used by the plant itself. Chlorophyll b absorbs light in a region of the spectrum apart from the dominant chlorophyll, and transfers the energy it produces to chlorophyll a. Along with chlorophyll b in transferring their energy produced to the dominant chlorophyll, other pigments are found in plants. Carotenes, xanthophylls, and anthocyanins, which are orange , yellow, and red/purple respectively. Since chlorophyll is such a dominant pigment in green plants, this domination hides the color of the other pigments in the leaves. This causes most plant leaves to appear green most of the time. During the autumn, however, the chlorophyll starts to break down, causing the carotenes and xanthophylls to show their bright red, orange and yellow colors.

These brilliant colors can be separated another way. This different technique, known as paper chromatography, separates mixtures in a liquid into individual components. The technique is based on the fact that each substance in a mixture has a specific affinity for a solid surface and a specific **solubility** in different **solvents**. By this method, the solid surface is the cellulose fibers in the chromatography paper, and the solvent is the solution that is placed in the bottom of the developing chamber (beaker).

This separation takes place through a process of **absorption** and **capillary action**. A small amount of the leaf pigment mixture is placed at the bottom of the strip of chromatography paper. The chromatography paper is then placed in the developing chamber with a solvent, which wicks up the paper, pulling the solvent up the paper by capillary action, and the mixture of pigments is dissolved as the solvent passes over it. The different components of the mixture move upward at different rates. A compound with greater solubility will travel farther than one with less solubility. The pigments then show up as color streaks on the chromatography paper. These substances have formed a pattern called a chromatogram on the chromatography paper.

The R_f values for each pigment is calculated to establish the relative rate of migration for each pigment. This value represents the ratio of the distance a pigment traveled on the chromatogram relative to the distance the solvent front moved. Scientists use the R_f value of a sample to identify the molecule. Any molecule in a given solvent matrix system has a uniquely consistent R_f value. The formula for this value is as follows:

$$R_f = \frac{\text{Distance each pigment traveled}}{\text{Distance solvent front traveled}}$$

Purpose:

The purpose of this lab is to introduce students to paper chromatography and to extract the different pigments found in **deciduous** leaves.

Hypothesis:

Using paper chromatography, the pigments that give a leaf its color can be separated and observed to determine the R_f value of each pigment.

Materials:

Leaves of deciduous trees with any of the following colors: (green , red, orange, purple, yellow, mixed, etc.)
Chromatography solution (2-ethyl alcohol:1-acetone) (stoppered 250 ml Erlenmeyer flask)
Chromatography paper (10 cm X 0.8 cm wide)
Metal coin (a penny works well)
Small test tube (1cm X 10cm)
Test tube rack
Disposable plastic pipette
Scotch tape
Metric ruler
Scissors
Pencil

Procedures:

1. Create **two** strips of chromatography paper with the following dimensions:

   Tape → 10 cm long X 0.8 cm wide ← Tape

2. Using a graphite pencil to draw a faint horizontal line 1.5 cm from one end of both strips.

3. Choose a leaf from the stock pile.
   Using the edge of a coin, apply pigment from a leaf onto the penciled horizontal line of the strip of chromatography paper. This is best achieved by placing the leaf over the horizontal line, while pressing the coin downward, use a rolling motion to trace the line with the coin. Apply a generous amount of pigment from the leaf. Make your pigment cover the entire horizontal line. This is the initial pigment line and must remain above the top level of chromatography solution.

4. Use the disposable plastic pipette to pipette exactly 1 ml of chromatography solution into the bottom of the small test tube.

5. Place the strip of paper chromatography paper into the test tube. After the pigments have completely separated and the solvent front has reached the top of the chamber, remove the strip, mark each pigment front and solvent front with a pencil line before it evaporates. Measure and record the distance the solvent and each pigment traveled. Use a calculator to determine the R_f values for each pigment.

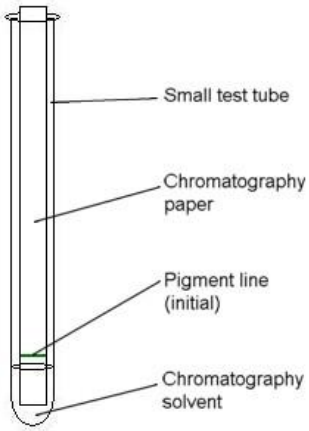

Figure 1

Table 1

Band #	Pigment	Color	Migration distance (mm)	R_f value
1	Carotene	Orange		
2	Xanthophyll	Yellow		
3	Chlorophyll a	Light green		
4	Chlorophyll b	Dark green		
5	Anthocyanin	Red, Purple		
Solvent				