

# Find the Hidden Colors of Fall Leaves

## Introduction

Have you ever wondered why leaves change colors in the fall?

Leaf color is caused by pigments made up of various, color-creating molecules. During warm, sunny months, plants use their leaves to turn sunlight into food—a process called photosynthesis. This primarily uses a pigment that reflects green light.

When days get colder and shorter in the fall, deciduous trees stop making food with their leaves and no longer need the green pigment. Other leaf pigments become visible.

During this experiment, you'll uncover the hidden colors of fall by separating plant pigments with a process called paper chromatography.

## Background

There are many pigments in leaves. Chlorophyll makes them green and performs photosynthesis during warmer months. As the green pigment fades in the fall, other pigments—like yellow, orange, and red—become visible.

Xanthophylls are yellow, and carotenoids are orange. Photosynthesis also uses these pigments during the summer, but chlorophyll is a stronger pigment and overpowers them. Also, these pigments take more time to break down than chlorophyll, so you they become more prominent in fall. There are also anthocyanins, which are an intense red and only appear in the fall.

Using a method called paper chromatography, you can separate a leaf's color pigments. This process dissolves the pigments and allows them to be absorbed by a strip of paper. Larger molecules have a harder time moving in the woven paper and get trapped in the paper first, whereas smaller ones travel farther along the paper. This process separates the mixture of pigments by molecular size and color.

## Needed Materials

- Leaves at five different color stages  
Note: The more the better! About five of each color is best.
- A pair of scissors
- 3–4 drinking glasses
- Rubbing alcohol (isopropyl alcohol)
- A wooden utensil with a blunt end that can crush leaves, like a wooden spoon
- A fork
- 3–4 very small bowls
- Strong, white, heavyweight, ultra-absorbent paper towels
- A ruler
- A pencil
- Toothpicks
- A plate or other surface to protect your working area from stains
- 3–4 tall glass jars, like mason jars
- 9–12 clothespins or large paper clips

## Preparation

- Collect leaves at different stages of color change in the fall, preferably from the same tree.  
Note: Make sure you get leaves that are fresh, not crispy.
- Ask an adult to be your experiment partner!
- Separate your leaves into distinct color groups — green, yellow, and red—with about five large leaves in each group.
- Prepare 15 paper towel strips approximately 1" wide. They should be long enough to touch the bottom of your tall glass jars while still extending over the top.
- With a pencil, gently draw a horizontal line 1" from the bottom of each strip.

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## Procedure

- Use scissors to cut the leaves into small pieces, and then place each group of leaves at the bottom of its own drinking glass.
- Add one tablespoon of rubbing alcohol to each glass.
- Using the blunt end of a wooden utensil, crush the leaves into the rubbing alcohol for about five minutes, until the solution is dark. How has the color of the alcohol changed?
- Let the solution sit indoors, in a dark place, for 30 minutes.
- While leaving the liquid in the glasses, use a fork to remove and throw away all leaf remnants in each solution.
- Pour each solution into its own very small bowl and leave it in a dark place indoors to give the alcohol more time to evaporate. When you stir each solution with a toothpick and it seems thicker, you're ready for the next step.
- Use toothpicks to thoroughly mix the solutions. To make sure you don't mix colors, use a different toothpick for each glass.
- Using a toothpick for each color, smoothly and evenly "paint" some of each solution across a paper towel strip on the pencil line you previously drew. For each color, do this using a total of three or four strips.  
Note: Because some plant pigments can stain, you should do this on a plate or other non-staining surface.
- Allow the strips to dry.
- While the strips are drying, pour enough rubbing alcohol into each tall glass jar to cover just the bottom. Prepare one jar for each color solution.
- With the dry strips, carefully put the pigmented end into the jar until the strip barely touches the alcohol. Drape the top of the strip over the jar's opening and secure it with a clothespin or paper clip. Make sure each strip is only touching the jar where it's secured, not the side.
- Place and secure strips from the same solution

into the same jar, but keep them from touching each other.

- Let the glasses sit for 30 minutes and watch the paper strips. What's happening to the colors?
- When one of the colors reaches the top of a strip, remove all of the strips and let them dry.
- Look at each dried strip. How are the colors different? Do strips from different solutions have unique colors, shared colors, or both?
- Look at the order in which colors appear on different strips. Is the same color at the same place on different strips—or is it at a different place? Do the colors appear in the same separation order or are they separated differently?

## Observations & Results

Using paper chromatography, you were able to separate pigments by the size of their molecules. You should see varying colors at different locations along each strip. Also, the order in which the colors appear should be roughly the same as each different color solution.

Were you able to see multiple bands of color on your test strips? Did you see that some bands differed for different solutions? What are the varying bands of color on the test strips? These are the different pigments in the leaves. The ones you may see on your paper towel strips are green chlorophylls, yellow xanthophylls, orange carotenoids, and red anthocyanins.

Pigments with larger molecules generally stay near the bottom of the strip—where you "painted" on the pencil line—because it's harder for them to travel up the woven fiber of the paper towel. Smaller pigments can more easily climb the paper towel and, consequently, they usually travel farther up the strip.

Because leaf color is dependent on pigments, each displays differently on a paper towel strip. Ex: A strip testing vibrantly green leaves may not show any shades of red (anthocyanins).