

**LESSON 21**

# Plant Warfare: Investigating Allelopathy

**OBJECTIVES**

Students will understand the concept of allelopathy and how it affects plant germination and growth. They will understand how allelopathy helps invasive plants outcompete native plants.

**METHOD**

Students experimentally test the effects of plant extracts on seed germination by comparing germination rates of seeds bathed in extract to those in water.

**MATERIALS**

- ✎ Seeds (radish, spinach, and lettuce)
  - ✎ Petri dishes and filter paper
  - ✎ Plastic wrap or parafilm
  - ✎ Disposable pipettes and scissors
  - ✎ Beaker (50 mL or larger)
  - ✎ Distilled water
  - ✎ Grow-lights or south-facing window
  - ✎ Blender (1 for each different type of extract used)
  - ✎ Cheesecloth
  - ✎ Scissors
  - ✎ Graduated cylinder
  - ✎ Balance and weights (grams)
  - ✎ Ruler
  - ✎ Funnel
  - ✎ Flask
  - ✎ Samples of vegetation such as spruce, hemlock, garlic, fir needles, fresh tomato, fresh cucumber, alfalfa sprouts, or knapweed or hawkweed
- If growing alfalfa for extract\*:*
- ✎ Planting flats with inserts
  - ✎ Potting soil
  - ✎ Alfalfa seeds
- \*NOTE: You can grow your own alfalfa sprouts in the classroom or buy them in the grocery store.

**BACKGROUND**

Although plants cannot move around and have no teeth or claws, they still have very effective weapons with which to wage war against their neighbors. Have you noticed that few plants grow underneath pine trees, Taxus bushes (yews) or some other species? These plants compete successfully for resources such as water, sunlight, and soil nutrients by keeping other plants away through the production of chemicals that inhibit the germination and/or growth of other plants around them. This is one adaptation that can give invasive plants, such as knapweed, an advantage over many native and crop plants.

*Your students should have a basic understanding of ecological competition for resources and seed germination before starting this lesson.*

**Grade level:** 9-12

**Subject Areas:** Biology, writing, technology

**Duration:** 1-2 hours, distributed across 2 or 3 class sessions, plus a few minutes daily to collect and record data for 8-10 days, and report preparation.

**Setting:** Classroom

**Season:** Any

**Conceptual Framework Topics:**

Plant competition, allelopathy, invasive species ecology

(Adapted from the *Invasive Plants Taking Root in Alaska Curriculum* and the *Cornell-Boyce Thompson Institute for Plant Research Curriculum Program*)

## Extensions

Have your students research allelopathy in invasive plants listed as Noxious Weeds in Montana.

Some of the students in the class can experiment with the concentration of the plant extract added to the Petri dishes. Have them label their beaker "2X" and increase the amount of plant tissue to 40 grams. Dilute the tissue in 100mL of distilled water. This will give them 2X the ratio of tissue/solvent utilized by the other group to start and enough to make a dilution.

Additional materials:

- Volumetric pipettes for transferring 50mL
- Glass stirring rods

To prepare the dilution:

1. Add 50mL of distilled water to three, clean beakers that are labeled 1X, 0.5X and 0.25X.

2. Use a volumetric pipette to transfer 50mL from the 2X plant tissue preparation to the 1X beaker.

3. Mix with a clean glass stirring rod and transfer 50mL of the 1X to the 0.5X. Mix and repeat to make the other dilutions. There should be 100mL in the 0.25X dilution when complete. **A clean pipette and stir rod should be used for each transfer.**

4. Prepare 5 Petri dishes with either spinach, radish or lettuce seeds as above. Pipette 10mL of each solution to the 4 dishes that are labeled with corresponding concentrations.

One dish should get only clean, distilled water to serve as a control.

## PROCEDURE

Begin by asking your students how animals might compete for and defend resources in short supply, such as food or living space. Responses may include fighting or driving off competitors. Now ask if they think plants do the same things. Brainstorm possible ways plants might outcompete others; discuss adaptive strategies such as fast growth and reproduction, and explain that plants also engage in "chemical warfare." Tell them they are going to investigate whether some plants inhibit seed germination and/or growth in other plants. See if they can come up with some ideas as to how this might be done. You may want to brainstorm some possible plants to use for extract, and/or let them choose from the plants listed in *Materials*.

Give students the student lab sheets and go over the procedures with them.

After students have completed the lab, ask them to summarize their information and address the questions. As a class, discuss *allelopathy* and make sure they can agree on a definition as they compare the group data together as a class.

*To grow your own alfalfa for extract:*

1. Fill one flat of 2x2 cells to the top with potting soil. Plant with approximately 10g of alfalfa seeds that are spread evenly across the surface. Moisten the soil by placing a half gallon (3.7L) of water in the bottom of the planting flat. Water will wick upward into the soil. Sprinkle a small amount of soil over the seeds and mist with water. Keep soil very moist until plants have germinated and are beginning to grow. Place under grow lights or a well-lit area. Let them germinate and grow for 12 days.

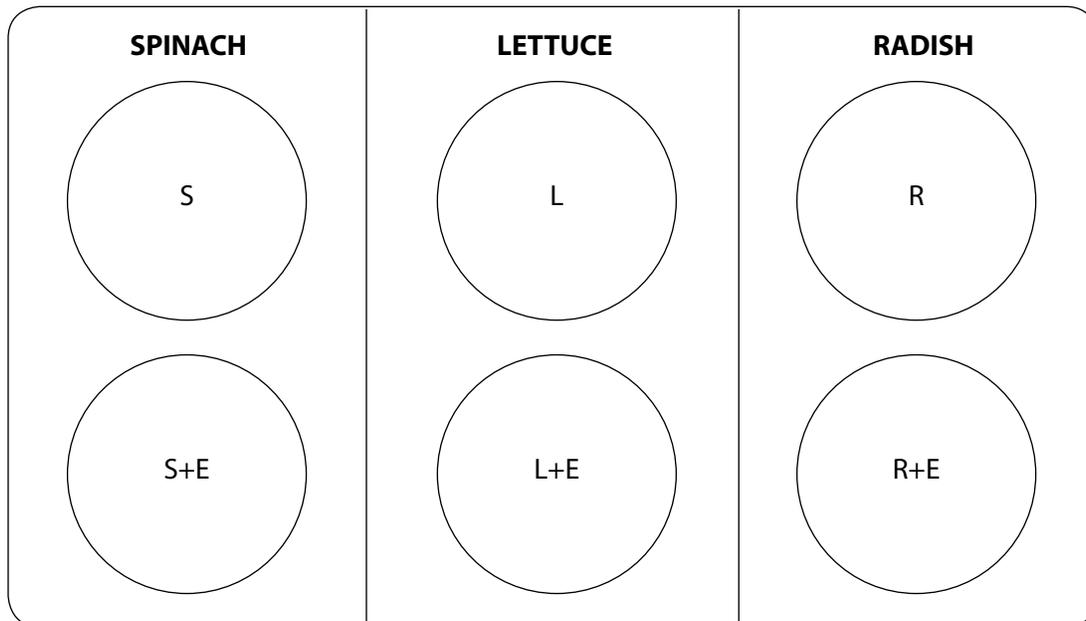
2. Harvest alfalfa sprouts by clipping off stems at the soil surface.

## STUDENT LAB 2

# Plant Warfare: Investigating Allelopathy

In this lab you will prepare an extract from plant tissues and test its ability to affect seed germination. For this exercise, the seeds of three different plants (radish, spinach and lettuce) will represent native plants and you will test the *allelopathic* effect of other plants on their germination.

1. Determine which plant material you will test for allelopathy. If necessary, use scissors or knife to cut one type of vegetation into small pieces before putting it into the blender. Weigh out 25 grams of vegetation.
2. Add 75 mL of distilled H<sub>2</sub>O for every 25 grams of plant material placed in the blender. Blend for 1-2 minutes. Let this sit overnight. This is enough for three different groups.
3. Filter the plant extract with cheesecloth and funnel. Discard the plant material from the funnel.
4. Each group should set out six Petri dishes and label as follows: S, S+E, L, L+E, R and R+E, (E= Extract) Petri dishes should be lined with filter paper or a single sheet of paper towel.



5. Evenly distribute 20 seeds of radish, spinach and lettuce into each corresponding labeled dishes.
6. Use separate pipettes to water each dish labeled (+E) with 10mL of the plant extract and each of the other dishes with distilled water. Be careful to use a different pipette for the extract and the distilled water so as not to contaminate the dishes with the wrong liquid.
7. Cover each with plastic wrap or parafilm and place under grow lights or a well-lit area.

8. Predict which seeds will have the highest rate of germination. What other kinds of observations or measurements could you make to determine whether or not the plant extract has an effect on seed germination? Design a data table to hold your data. The data table should have a space for all the information you'll need to record to answer your question.
9. Make daily observations of the seeds for about 10 days and note the number of seeds germinated (seed coat broken). Also observe and record data on the seedlings' appearance as they emerge (i.e. color, root and shoot lengths, etc).
10. Summarize your data by preparing a graph of the number of seeds germinated over time. Calculate the percent of germination:  
*# seeds germinated per dish / total number of seeds per dish (20)*

Summarize any data you collected on seedling size as well. Write a lab report to share your findings. Address the following:

1. Name the dependent and independent variables in this experiment.
2. How does what you learned relate to plant competition and invasive species?
3. Can you find information on invasive plants in Montana that use allelopathy to outcompete native plants?
4. Can you think of beneficial ways to use this information?
5. How would you improve this experiment if you did it again?
6. Write a conclusion that summarizes what you learned in this activity.