

# A Study of Allelopathy in Plants

## SYNOPSIS FOR CORE EXPERIMENT

Students will test the effects of the water-soluble extract or leachate of alfalfa leaves on radish, white clover, and rye grass seeds. They will determine whether the leachate from the alfalfa leaves contains allelopathic agents that affect the germination or growth of the target seed species.

## APPROPRIATE BIOLOGY LEVEL

Introductory and advanced

## TEACHER PARTNER

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## Directions for Teachers

*Note to Teachers: Information below is given for the Core Experiment. Additional information needed for each variation of the Core Experiment may be found beginning on page 31. For the page number of a specific variation, check the At-A-Glance Map.*

### GETTING READY

See sidebars for additional information regarding preparation of the lab.

### OBJECTIVES FOR CORE EXPERIMENT

*At the end of this lab, students will be able to:*

- Identify the parts of a plant that may produce allelopathic substances.
- Describe the observable effects of allelopathy on seed germination, seedling appearance, and growth of roots and leaves.
- Discuss the ecological implications of allelopathy.

### MATERIALS NEEDED

*For the teacher preparation, you will need the following for a class of 24:*

- ♥ 2 shallow 30 x 60-cm plastic planting trays or 18 10-cm planting pots
- ♥ 8 kg (16 lbs) topsoil or potting soil
- ♥ 100 g alfalfa seeds
- ♥ 1 balance
- ♥ 1 weigh boat
- ♥ 1 window with southern exposure or 1 fluorescent Gro-Lite™ bank with automatic timer
- ♥ 18 disposable 10 x 150-mm petri dishes
- ♥ 1 refrigerator

*You will need the following for each group of four students in a class of 24:*

- ♥ 5 g freshly cut alfalfa shoots
- ♥ 1 pair of scissors
- ♥ 1 150-mL glass beaker
- ♥ 50 mL distilled water
- ♥ 1 permanent marking pen
- ♥ 18 9-cm disposable, plastic petri dishes
- ♥ 6 40 x 40-cm layers of cheesecloth
- ♥ 1 50-mL flask
- ♥ 54 sheets of 8-cm filter paper, coffee filters, or white paper towel sheets
- ♥ 2 5-mL disposable, plastic pipettes or syringes without needles
- ♥ 60 seeds each of radish, white clover, and rye grass
- ♥ 18 80 x 10-mm strips of Parafilm™ (optional)
- ♥ 1 metric ruler
- ♥ 1 data journal

### LENGTH OF LAB

A suggested time allotment follows:

*Day 1* (30 to 45 minutes)

- Prepare the alfalfa extract and petri dishes.

*Day 2* (5 minutes)

- Examine the extract for color change and evidence of chemicals leaching into the water.

*Day 3* (30 to 45 minutes)

- Remove alfalfa leaves from the leaf extract. Set up the control and experimental petri dishes with target seed species and either the leaf extract or distilled water.

*Day 4* (10 minutes)

- Check for seed germination.

*Days 5 and 7* (10 to 20 minutes)

- Observe and record the number of germinated seeds and their appearance.

*Day 9* (45 minutes)

- Make final observations of seed appearance and the number of seeds that have germinated. Count the number of leaves on each seedling and measure the root and shoot lengths.

## TEACHER'S NOTES

### PREPARATION TIME REQUIRED




*15 days before lab* (1 hour)

- Plant seeds.

*1 day before lab* (30 minutes)

- Locate and set out student supplies.

### SAFETY PROCEDURES

-  Avoid plants that elicit allergies.
-  Wash hands after touching plants.
-  Students with allergies should wear nonallergenic gloves.

### DIRECTIONS FOR SETTING UP THE LAB

#### Planting the Alfalfa

- Weigh approximately 50 g of alfalfa seeds for each plastic planting flat. Spread the seeds evenly across the soil surface and cover them with approximately 1 cm of soil.
- Water the seeds thoroughly and place them in a well-lit area. The plants should have 16 hours of daylight for optimal growth. If using natural light, a southern exposure is preferable. If artificial light is used, place the plants approximately 0.5 m (20 inches) away from the light. It is suggested that 6 120-cm (48-inch) fluorescent light bulbs be used.
- Continue to water as needed to keep the soil moist.
- Expect sprouting in 3 to 5 days. The alfalfa will be ready to harvest within 10 to 15 days.

### TEACHER BACKGROUND

#### Content Information

Ecology is the study of interactions between organisms and their environment. One type of interaction frequently studied by ecologists is the competition between two or more species for limited resources. When a resource such as water is in limited supply, those species that need it will compete for it.

Competition may be defined as one organism having a negative effect on another by restricting its access to a resource that is in limited supply. Competition may be intraspecific, occurring between individuals of the same species, or interspecific, occurring among different species. One possible result of competition is the extinction of a species. The Russian biologist G.F. Gause demonstrated this possibility with two species of *Paramecium* (Towle, 1989). When grown together in a laboratory culture, one species always became extinct, even though each species grew well alone.

Another possible outcome of competition is specialization of each species in their use of resources. As this happens, their resource requirements no longer overlap entirely and they are able to coexist in the same environment. Competition between species can be an important factor influencing the distribution and evolution of species.

There are two general ways an organism can limit the resources available to another organism—exploitation or interference competition. In exploitation competition, one species is better adapted for using the limited resource. This indirectly deprives the other species of the resource. An example is a desert plant that has an extensive root system which allows it to use water that would otherwise be available to another plant species. The second type of competition, interference, occurs when one species directly prevents the other from using the resource by either physical or chemical means. Allelopathy is a form of chemical interference competition utilized by plants.

The word allelopathy is derived from the root words *allelon*, “of each other,” and *pathos*, “to suffer.” Allelopathy involves a chemical inhibition of one species by an-

other. Molecules produced by one plant, mostly secondary metabolites, are released into the environment and then affect the growth and development of neighboring plants. In California's Mojave Desert, for example, creosote shrubs are thought to exclude burro-weed by exuding a chemical from their roots. This chemical may inhibit the root growth of the burro-weed (Dutton, 1993).

Allelopathic chemicals may be present in any part of a plant including the leaves, flowers, fruits, stems, roots, rhizomes, and seeds. These toxins can affect a target species in a number of ways. For example, they may inhibit the target species' nutrient uptake, or they may inhibit shoot or root growth. Some plants, such as clover, have a symbiotic or mutually beneficial relationship with nitrogen-fixing bacteria that provides nitrogen for the plant's growth. An allelopathic agent against clover might directly attack the symbiotic bacteria and destroy the plant's source of usable nitrogen.

Allelopathy is not a recent discovery. In 300 BC, Theophrastus realized that planting chick peas made the soil unsuitable for many other plants. Earlier records of allelopathy date back before 300 BC when Democritus reported that weeds could be controlled by using naturally occurring plant products and trees could be killed by treating their roots with a mixture of lupine flowers soaked in hemlock juice. In 1 AD, Pliny, the Roman naturalist, reported that barley and bitter vetch prevented the growth of other plants in the same soil. In more recent history, A.B. Massey (1925) determined that toxic substances released into the soil by walnut trees inhibited the growth of almost all other plants. Some researchers, however, contend that other factors, such as competition for light or nutrients, are the reason why certain plants are unable to grow together and that allelopathy is not a factor.

### Pedagogical Information

The following is a chart of some concepts related to this lab and some student misconceptions of these concepts.

Correct Concept	Misconception
<ul style="list-style-type: none"> <li>Plants can interact with other plants and their environment.</li> <li>Plants compete for limited resources.</li> <li>Plants form allelopathic substances that can inhibit the growth of other plants.</li> </ul>	<ul style="list-style-type: none"> <li>Plants have no effect on each other.</li> <li>Only animals compete for resources.</li> <li>Plants form chemical substances only against insects.</li> </ul>

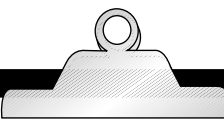
### INSTRUCTIONAL PROCEDURES FOR THE CORE EXPERIMENT

#### Introduction

Start by brewing a pot of tea to help introduce allelopathy. Ask the students what is creating the smell and color in the tea. Relate the smell to the chemicals in the tea plant, such as tannin.

Have some potted herbs, such as mint or pennyroyal, for students to rub between their fingers. Discuss the presence of chemicals that are responsible for the odors that they emit. Freshly cut pine boughs also work well to illustrate the point that plants contain many chemicals.

Discuss the botanical origin of many of our modern medicines. Some of these medicinal substances are derived from common plants and trees that students may have growing in their own backyards. An example is foxglove (*Digitalis purpurea*), the source of a drug used to treat some heart conditions. One of the reasons for the



### TEACHING TIPS

- Store the alfalfa-leaf extract in the refrigerator to control contamination.
- If student lab time is limited, the teacher may prepare the alfalfa-leaf extract in advance. Prepare 300 mL of extract per class.
- During field testing, contamination by bacteria was sometimes a concern. The concern lies in whether the effect on the target species was truly allelopathic, therefore plant-plant interaction, or an action of bacterial contamination.
- To manage time and the large number of petri dishes, do not have each student group set up each target species. Rather, have each student group be responsible for setting up one target species group and providing their data for a class set of data.
- If time is limited, fresh alfalfa sprouts can be purchased in the produce section of many grocery stores. Be aware that ungerminated seeds and seed coats are often mixed in with the alfalfa sprouts. One container of alfalfa sprouts may not provide sufficient plant material.
- Instead of petri dishes, students may use large culture dishes with larger-sized filter paper or resealable plastic bags and paper towels. The amount of alfalfa tea and distilled water will need adjustment so that the filter paper is moist, but not oversaturated with water.
- Agricultural and garden supply stores have a good supply of a variety of seeds. Some seeds are also available through biological supply companies.
- Seeds are available from the beginning of February or from some garden supply stores throughout the year. If you anticipate doing the lab in the fall, purchase seeds during the prior spring or early summer.
- Check with students as to the types of trees and plants they may have in their yards; many, such as black walnut, mint, and ragweed, are good sources of allelopathic substances.  
Weed seeds can be purchased from:  
Valley Seed Service  
P. O. Box 9335  
Fresno, CA 93791  
559.435.2163



## TEACHING TIPS

(continued from page 23)

- Avoid overwetting the paper lining the petri dish. Paper should be damp throughout the lab with no standing water. Excess water can be poured off provided it is done for all petri dishes.

preservation of the tropical rain forests and other areas is the potential for discovery of valuable medicines.

Not all substances that plants produce are useful as medicines; many are toxins. Some chemicals produced by plants serve as defenses against predators by making the plant distasteful or toxic. The oils produced by poison ivy are a good example. Even the medicinal chemical extracted from foxglove is toxic in larger doses. Remind the students that allelopathic substances are not just found in the leaves, but also may be present in other parts of the plant, like the roots, stems, and flowers.

Discuss allelopathy as a form of interference competition and relate it to broader ecological and evolutionary principles. You could also mention the practical human applications related to agriculture.

### HYPOTHESIS GENERATION

The following discussion and activities are designed to elicit questions that students can develop into hypotheses.

Students should generate a hypothesis for the Core Experiment based on what they know about the production of chemicals by plants as a form of competition. They should consider how these chemicals affect other plants.

Tell the students that alfalfa sprouts and some common seeds are available for the experiment. Alfalfa sprouts are believed to have an allelopathic effect on some plants. The students need to develop a technique to remove the allelopathic chemicals from the alfalfa leaves. They probably will come up with some great ideas for drying the leaves or using chemical extraction. Remind them that water is a universal solvent and is probably the solvent most likely to occur in nature.

### Sample Hypotheses

- Alfalfa-leaf extract will prevent the seeds from germinating.
- Alfalfa-leaf extract will affect the geotropic response of the germinating seeds.
- After germinating, seeds treated with alfalfa-leaf extract will not continue to grow.
- Leaf extract from alfalfa will have a negative effect on the germination and seedling height of radish, white clover, and rye grass seeds.

*On the following pages are a sample hypothesis, procedure, and data analysis set with interpretation that students might develop for the Core Experiment. It is followed by a related test question and answer for teacher evaluation. This example has been included as a potential outcome of the activity and should not be given to the students. Students should develop their own hypotheses and procedures. Make sure they understand that there is not just one correct hypothesis, procedure, or data set. The Variations of the Core Experiment will give each group of students the opportunity to expand on the Core Hypothesis. Additional test questions are found on page 30.*

### Question

Will alfalfa-leaf extract affect seed germination or growth?

### Hypothesis

Leaf extract from alfalfa will have a negative effect on the germination and seedling height of radish, white clover, and rye grass seeds.

### Rationale

Alfalfa sprouts are believed to have allelopathic effects that may affect common seeds, such as radish, clover, and rye.

Procedure  
Alfalfa-Leaf Extract Preparation

1. Harvest 5 g of alfalfa shoots by cutting them close to the soil surface. See Figure 1.

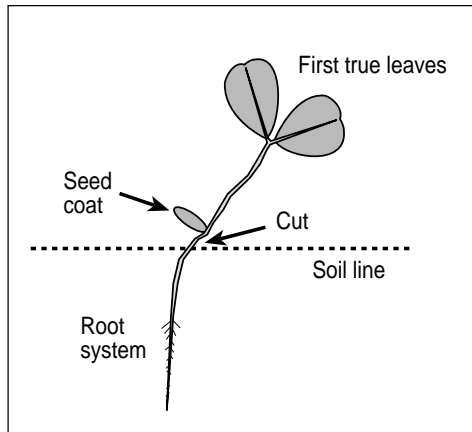


Figure 1. Alfalfa shoot.

2. Remove any loose soil from the shoots.
3. Place the alfalfa shoots in a 150-mL beaker and add 25 mL of distilled water. See Figure 2.

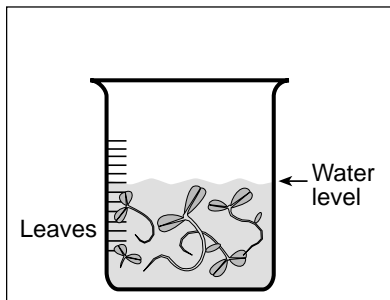


Figure 2. Beaker with submerged shoots.

4. Press the shoots down so they are submerged totally and cover the beaker with Parafilm™ or plastic wrap to prevent evaporation.
5. Let the alfalfa shoot "tea" steep for 2 days in a cool place. If the room temperature is above 22°C, place the "tea" in a refrigerator.
6. On the second day of steeping, examine the "tea" for color, odor, and consistency.
7. Label the "tea" *alfalfa-leaf extract*. If you are unable to continue with the experimental design setup at this time, store the extract at 4°C in a refrigerator. It should remain stable for a few days.
8. You will need 6 petri dishes for each target seed species—3 controls and 3 treatments. Label each petri dish with petri dish number, treatment or control, target species, date, and group initials as shown in Figure 3.

TEACHER'S NOTES

## TEACHER'S NOTES

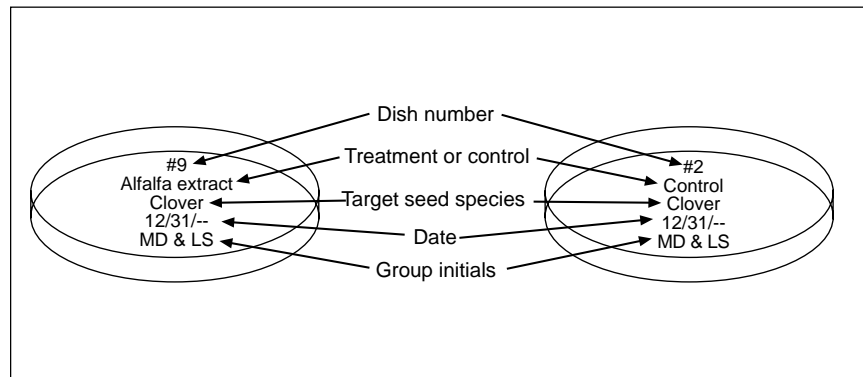


Figure 3. Labeled petri dishes.

### Target Seed Setup

1. Pour the alfalfa-leaf extract through several layers of cheesecloth to remove any suspended matter and collect the liquid in a 50-mL flask. This extract will be used to treat the seeds.
2. Line each petri dish with 3 sheets of filter paper.
3. Add 5 mL of distilled water to each of the control dishes with a 5-mL disposable pipette or syringe.
4. Add 5 mL of leaf extract to each of the treatment dishes with a 5-mL disposable pipette or syringe.
5. Place 10 radish seeds in each of the 3 control petri dishes and each of the 3 treatment petri dishes. Spread the seeds evenly in the dish. Cover with the lid. If necessary, seal with Parafilm™ or tape to prevent evaporation.
6. Repeat Step 5 using the white clover and the rye grass seeds.
7. Place all the petri dishes in a warm place with filtered light. Direct sunlight may alter the chemical compounds in the extract.

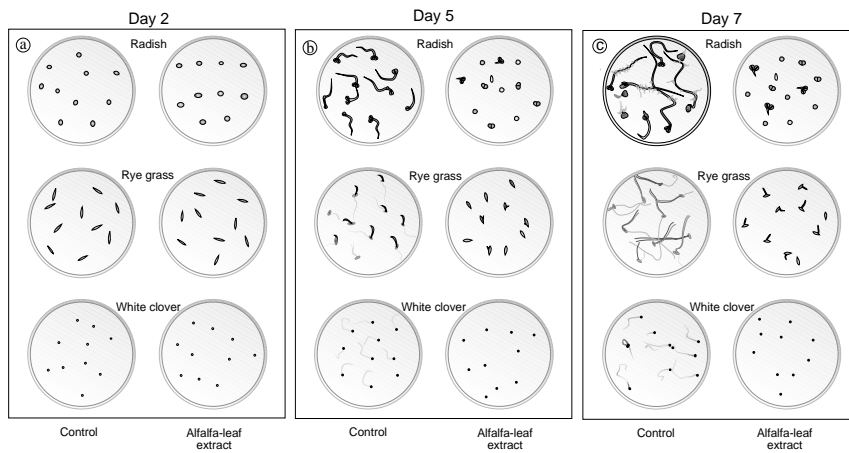
### Seed Observation and Data Collection

1. Check the petri dishes the following day for evidence of germination or sprouting. Record these observations as Day 1.
2. On Days 2, 5, and 7, record the number of germinated seeds and descriptions of each seedling's appearance in each dish.
3. On Day 7, also measure the shoot and root length of each seedling.

### DATA ANALYSIS AND INTERPRETATION

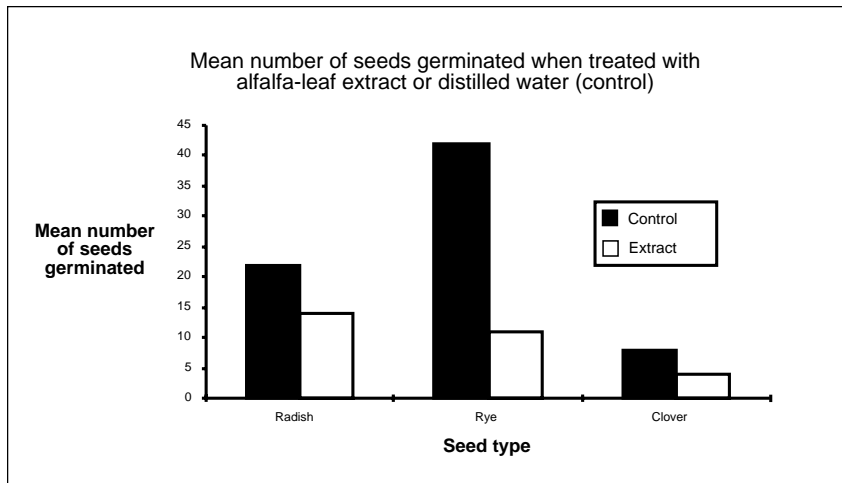
#### Sample Data 1

For each of the 3 target species of radish, white clover, and rye grass, compare the experimental and control groups, with respect to the mean number of seeds germinated, the mean number of leaves produced, the mean shoot length, the mean root length, and other responses of the different target species, such as changes in color or number of leaves. Consider whether all the target species responded equally to the alfalfa-leaf extract.



**Figure 4.** Results of alfalfa-leaf extract treatment on rye, clover, and radish seed germination at Days 2, 5, and 7.

**Sample Data 2**



**Graph A.** Effects of alfalfa-leaf extract on seed germination.

**TEACHER'S NOTES**

**Interpretation**

The results support the hypothesis. For all 3 target species — radish, white clover, and rye grass — the alfalfa-leaf extract had a negative effect on the germination of the seeds.

### Interpretation

The results do not support the hypothesis. There is no difference between the experimental and the control groups of any of the target species. This suggests the alfalfa-leaf extract had no allelopathic effect.

### Alternative Interpretation

The lack of negative effects does not rule out necessarily the possibility of allelopathy; it only indicates that there is no evidence of it. The allelopathic agent may not be water soluble or the extract may not have been strong enough to cause an effect.

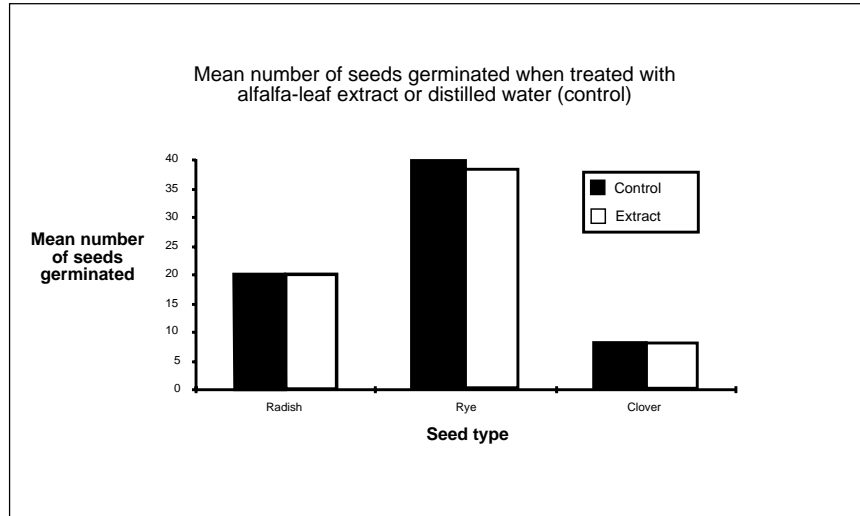
### Interpretation

Radish and clover both showed reduced germination in the presence of alfalfa-leaf extract; clover showed a greater reduction in germination than did radish. Rye grass germination was not affected by alfalfa-leaf extract.

### Answer to Test Question

Some plants may be more resistant to the allelopathic substances produced by alfalfa. Others may not be sensitive to the substances at all.

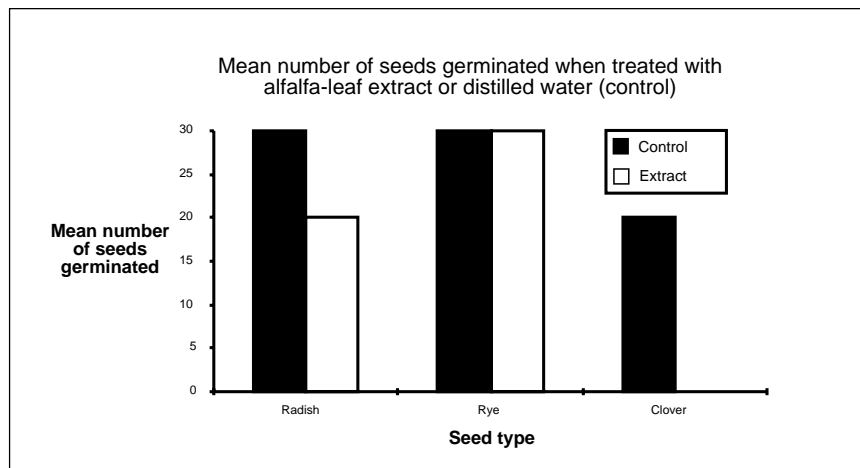
### Sample Data 3



Graph B. Effects of alfalfa-leaf extract on seed germination.

### Sample Data 4

The 3 target species may not respond equally to the alfalfa extract. Different species may have different sensitivities or different types of responses to the allelopathic substance. The germination of one species may be inhibited, but that of another may not be. See Graph C. Nitrogen-fixers such as clover may be affected indirectly through inhibition of the nitrogen-fixing bacteria in their roots.



Graph C. Effect of alfalfa-leaf extract on seed germination.

### TEST QUESTION

Why might the alfalfa-leaf extract have different effects on the different species of plants?

### STUDENT DESIGN OF THE NEXT EXPERIMENT

After students have collected and analyzed the data from their experiments and shared results and conclusions with the class, encourage them to brainstorm ideas for experiments they could do next. They should think of questions that occurred to them as they conducted their first experiment. Ask them what quantifiable experiments could be done based on observations they have made.



Have students return to their experimental lab groups to share ideas before writing their proposals. Questions students may suggest include the following:

- Does heat destroy the allelopathic agent?
- Could direct exposure to sunlight or ultraviolet light alter chemicals in plants that are known to produce allelopathic effects?
- Are there important allelochemicals in familiar fruits or vegetables?
- If bacteria grow in the leachate, what inhibits germination — the bacteria or the extract?

#### SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

These are possible ways to modify this specific activity for students who have special needs, if they have not already developed their own adaptations. General suggestions for modification of activities for students with disabilities are found in the AAAS *Barrier-Free in Brief* publications. Refer to p. 15 of the introduction of this book for information on ordering FREE copies of these publications. Some of these booklets have addresses of agencies that can provide information about obtaining assistive technology, such as Assistive Listening Devices (ALDs); light probes; and talking thermometers, calculators, and clocks.

#### Blind or Visually Impaired

- Allow students to bend a leaf of each plant to smell it for identification during the introduction to potted herbs.
- Use larger seeds. Radish, white clover, and rye grass seeds are too small to be handled effectively by most students who are visually impaired. Garden peas and some varieties of beans are adequate. Also, seeds that are used for edible sprouts, such as lentils, mung beans, and sunflower seeds, are good choices. (Park Seed, 1 Parkton Avenue, Greenwood, SC 29647 or Reay's Ranch Market, 3360 East Speedway, Tucson, AZ 85716).
- Do not use a magnifying glass. It is not an option for the blind and not satisfactory for students who are visually impaired.
- Alfalfa seeds present no problems as they are used in large quantities and not counted manually. Separate the alfalfa leaves from the roots by touch.
- Label beakers with braille. Labels can be made by inserting a sheet of self-adhesive labels into a braille slate.
- Reserve a section of a refrigerator shelf for students who are disabled. They should not handle other items to find their material labeled with braille.
- Use plastic margarine or cheese spread containers with lids, or resealable plastic bags in place of petri dishes. These containers measure about 5-cm high and make suitable sprouting chambers. Line them with filter paper or paper toweling cut to size. Petri dishes are too shallow to accommodate the larger seeds with their sprouts.
- Use a 10-mL disposable syringe to add water to the seeds. The barrel of the syringe can be notched to show the position at 10 mL. When shoots and roots appear, they should be measured with a braille ruler.

#### Deaf or Hard-of-Hearing

- Practice good communication skills. They are important not only for the student who is deaf or hard-of-hearing, but also for the teacher and classmates. The student who is deaf may be able to perform every portion of a biology experiment, but performance is no longer a solo act.
- Make lab partners aware that they must be alert to relay impromptu comments by the instructor during the investigation.
- Alert the lab group that they should be willing to inform their non-hearing



## TEACHER'S NOTES

## TEACHER'S NOTES

partner about dangers in the classroom unless the biology room is equipped with flashing lights, such as a fire alarm alert.

- Assign partners who have good note taking skills to deaf students who speak. Deaf students who neither read lips nor speak need skill with a note pad and friends who will assist them.

### Gifted

- Explore variables that may affect allelochemicals such as concentration, time of year, or growing conditions of allelopathic plants or target species.

### Manually Impaired

- Allow students with manual impairment to work with materials that they can handle most easily. For some it will mean using larger seeds such as sunflower seeds, peas, lentils, mung beans, squash, and green beans for the reaction with alfalfa solution. For others, it may mean taking notes with a tape recorder while the lab partners plant and evaluate seeds.
- Measure water in a short, wide graduated cylinder or a measuring cup if handling droppers requires finer motor skills than some students possess.

### Mobility Impaired

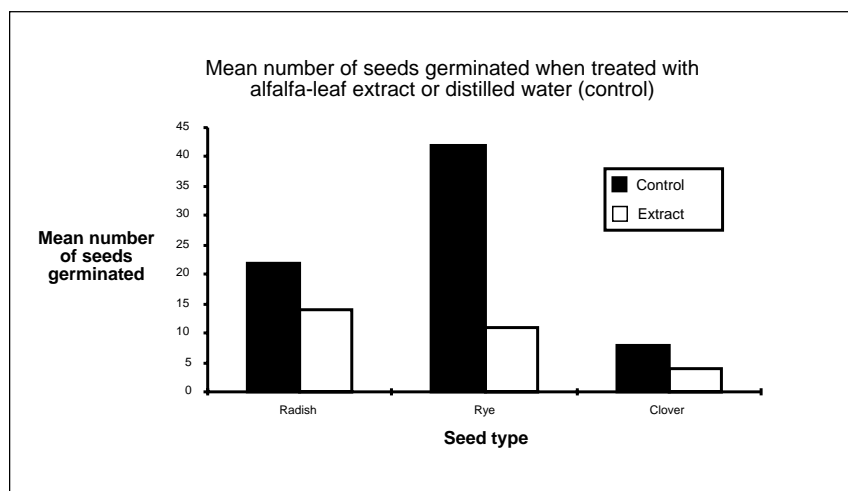
Mobility impaired students should have no problems with the experiments in this unit.

- Provide a table of suitable height, preferably one large enough for the lab group. Students in wheelchairs need a comfortable place to work. Where counter tops in the biology lab are too high to be reached from a seated position, or the student is unable to transfer to a chair, a portion of the apron of the table may need to be altered to allow the arms of the wheelchair to fit beneath it.
- Provide adequate space for wheelchairs to access supply areas within the classroom. If the room does not have adequate space, lab partners can provide these items.

## ADDITIONAL TEST QUESTIONS

Test questions for the Core Experiment may also include the following:

1. The seeds of three plant species were exposed during germination to either distilled water or alfalfa-leaf extract that has allelopathic effects on certain plant species. See Graph D.



Graph D. Effects of alfalfa-leaf extract on seed germination of three plant species.

When treated with alfalfa-leaf extract, the clover seeds showed a lower germination rate than the radish and rye grass seeds. This was due to:

- A. The effects of alfalfa-leaf extract.
  - B. An intrinsic characteristic of the clover.
  - C. The allelopathic effects of the alfalfa-leaf extract and factors that could be intrinsic to the clover species.
  - D. The radish and rye grass seeds not being sensitive to the allelopathic chemicals produced by the alfalfa.
2. An equal number of seeds were watered with distilled water and alfalfa-leaf extract to:
- A. Show that the added nutrients in the leaf extract would help germination.
  - B. Compare the numbers of seeds that germinated when treated with distilled water versus those treated with alfalfa-leaf extract.
  - C. Test the distilled water for allelopathic chemicals.
  - D. Provide the amount of water the seeds needed to germinate.

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## POSSIBLE SOURCES OF MENTORS

Check with biology and agriculture departments at local universities. Much research on allelopathy is done by agricultural researchers. A state, local, or federal agency might be able to refer you to possible mentors.

## VARIATIONS ON THE CORE EXPERIMENT

After completing the Core Experiment, students should use the results to develop a variation on that experiment. The following directions are meant only as a guide for the teacher. They suggest possible hypotheses students may develop and data that may result.

**Note to Teachers:** Only information that is unique to each Variation of the Core Experiment is found in this section. Teacher information for each variation is the same as that found in the Core Experiment, except as noted. Materials listed in this section are needed in addition to the materials listed for the Core Experiment.

### Answers to Additional Test Questions

1. C
2. B

### Answers to Questions and Analysis on Student Page

1. Answers will vary depending on the outcome of the experiment. However, previous experiments indicate that alfalfa-leaf extract appears to reduce germination in white clover and radish, but not in rye grass. Leaf growth is inhibited in all 3 species. Sensitivity varies among the species; white clover is the most sensitive.
2. Examples include inaccurate measurement of extract, distilled water, or seedling length; spacing of seeds; and poor germination conditions, such as an environment that is too cold.
3. Answers will vary.
4. See variations for suggestions.
5. Allelopathy is a type of interference competition. Competition between two species occurs when a resource, such as nutrients, is in limited supply. Interference competition occurs when one species directly affects the other, for example, by attacking it. Allelopathy is a form of interference competition that occurs among plants. Molecules produced by one plant, mostly secondary metabolites, escape into the environment and then affect the growth and development of neighboring plants.
6. Allelopathic plants may explain why some crops cannot be grown together and why a border separation between fields or crop rotation may be necessary. Allelochemicals have potential applications as "natural herbicides."

## TEACHER'S NOTES

## VARIATION 1

## The Effect of Different Concentrations of an Allelopathic Agent on Seed Germination and Growth

## SYNOPSIS

Students will test the effects of different concentrations of the allelopathic agent produced by alfalfa leaves. They will determine if the concentration affects the germination and growth of seeds of the target species.

## ADDITIONAL MATERIALS NEEDED

*You will need the following for each group of four students in a class of 24:*

- 🌿 15 g freshly cut alfalfa leaves
- 🌿 7 10-mL flasks
- 🌿 1 10-mL graduated cylinder
- 🌿 12 disposable 10 x 150-mm petri dishes
- 🌿 36 8-cm pieces of Whatman filter paper
- 🌿 7 disposable 5-mL plastic pipettes or syringes without needles
- 🌿 120 target plant seeds of the same species

## HYPOTHESIS GENERATION

## Question

Will the concentration of an allelopathic chemical influence its effects on other plants?

## Sample Hypothesis

An increase in the concentration of the allelopathic agent will cause a decrease in the number of germinated seeds and in seedling growth.

## Rationale

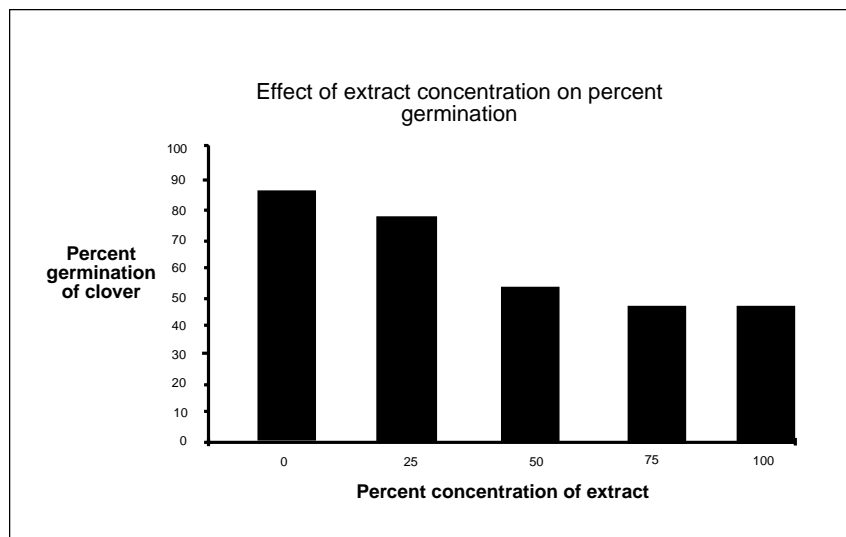
The effect of the allelopathic agent may depend not just on its presence, but on the quantity to which the target species is exposed.

## Sample Experimental Procedure

1. Prepare 200 mL of highly concentrated alfalfa-leaf extract solution according to the procedure in the Core Experiment. To concentrate the solution, increase the amount of alfalfa leaves used to prepare the extract.
2. Prepare dilutions of 100%, 50%, 25%, and 0% using the concentrated alfalfa-leaf extract and distilled water. For the 100% solution, use the undiluted extract only. For the 75% solution, mix 7.5 mL extract with 2.5 mL distilled water; for the 50% solution, mix 5.0 mL of the extract with 5.0 mL of distilled water; for the 25% solution, mix 2.5 mL of the extract with 7.5 mL of distilled water; and for the 0% solution, use distilled water only.
3. Line each petri dish with 3 sheets of filter paper and label each dish with the appropriate concentration of the alfalfa-leaf extract solution. There should be 3 treatment dishes for each concentration.
4. Add 5.0 mL of distilled water to each control dish.
5. Add 5.0 mL of the appropriate concentration of leaf extract to each treatment dish.
6. Continue the experiment according to the sample procedure for the Core Experiment.

## SAMPLE DATA ANALYSIS AND INTERPRETATION

### Sample Data



**Graph E.** Germination of seeds treated with increasing concentrations of alfalfa-leaf extract.

### TEST QUESTION

Based on the results of your experiment, what happened to the number of germinated seeds as the concentration of alfalfa-leaf extract increased? Why do you think this occurred?

### SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

#### Blind or Visually Impaired

- Use different sizes of syringes to measure the liquid to make the serial dilutions of the concentrated alfalfa extract. The barrels of the syringes should be marked appropriately. With many measurements to make in this experiment, the student may wish to use other methods.
- Weigh liquids on a balance in a container of known mass. With 1 mL weighed at 1 gram, serial dilutions can be made readily. Students who are blind readily use the double and triple beam balances. The weights on the beams slide into notches readily counted by touch to find the value. With a piece of tape placed at the zero point, the student is able to tell when the pointer is centered by touch. A quadruple beam is available in which the notch slide is calibrated with the braille ruler to show the number of grams per centimeter.
- Expect all students to present laboratory reports containing the same items. Students who use braille should write their report in braille for their records. For the benefit of the sighted teacher, a typewritten report should be presented.
- Use raised-line drawings if drawings are included. Labels should be in print and in braille. Braille graph paper is available or it can be made by the students on their brailier. When several bars are on the same graph (see Allelopathy Graph A), different tools and techniques are used to raise the area in each bar. A drawing such as Figure 2, which shows a beaker with leaves, stem, and roots in water, can be made as a raised-line drawing. A pointed tool sketches the drawing on braille paper that has been placed on a heavy rubber mat. (A clipboard with several layers of heavy cloth will serve also.) The size and type of lines are made by using

### Interpretation

Accept the hypothesis if a decrease in the number of germinated seeds and the amount of growth of the seedlings was observed when there was an increase in the concentration of the allelopathic agent.

Reject the hypothesis if the number of germinated seeds and the amount of seedling growth was approximately the same or greater for all the concentrations of the alfalfa-leaf extract. The hypothesis also should be rejected if all the seeds exposed to the alfalfa extract showed less growth than the control seeds or if the degree of response is uncorrelated with concentration. This suggests that any amount of the allelopathic agent is harmful to seed germination and growth.

### Answer to Test Question

Answers may vary depending on the outcome of the experiments. Generally, as concentration increases, the number of germinated seeds will decrease. This suggests that effects of allelopathic chemicals increase as the concentration increases.

**TEACHING TIPS**

- To facilitate the separation of the alfalfa roots from the soil, grow plants in a mixture of 4 parts sand to 1 part topsoil. Sift the topsoil before mixing to prevent large organic particles from adhering to the roots, or use potting soil.
- Students should keep the roots alive while collecting the extract. They can do this by gently washing off the sand and placing the plants in a small jar with enough distilled water to cover the roots. See Figure 5. Allow the plants to remain in the water for 2 to 4 days.

a stylus, a dull pencil, a tracing wheel, a stick from an ice cream bar, or a special tool that leaves a dappled effect. Teachers and laboratory partners can likewise use this technique to make a quick drawing to explain some item in the laboratory.

- Provide students who are visually impaired the opportunity to examine models of plant parts and seeds if they are available in the science department.

**VARIATION 2**

**The Allelopathic Effects of Alfalfa Roots on Seed Germination and Growth**

*Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 2.*

**SYNOPSIS**

Students will test the effects of the extract of alfalfa roots on the germination and growth of radish, white clover, and rye grass seeds. They will determine whether the alfalfa roots produce allelopathic substances.

**ADDITIONAL MATERIALS NEEDED**

*You will need the following for each group of four students in a class of 24:*

- ☛ 1 tray of alfalfa, planted in sandy soil
- ☛ 1 50-mL beaker or baby food jar
- ☛ 4 kg (8 lbs) sand
- ☛ 1 kg (2 lbs) topsoil or potting soil

**HYPOTHESIS GENERATION**

**Question**

Do roots contain allelopathic substances?

**Sample Hypothesis**

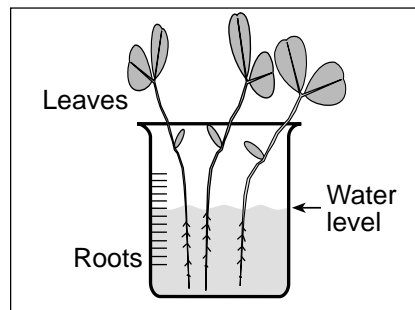
Alfalfa roots produce allelopathic agents that reduce the number of germinated seeds and the amount of seedling growth.

**Rationale**

Students should provide their own rationale.

**Sample Experimental Procedure**

1. Prepare the alfalfa extract by allowing 5 g of alfalfa roots to steep in 25 mL of distilled water for 2 days. See Figure 5. Refrigerate after 1 day.

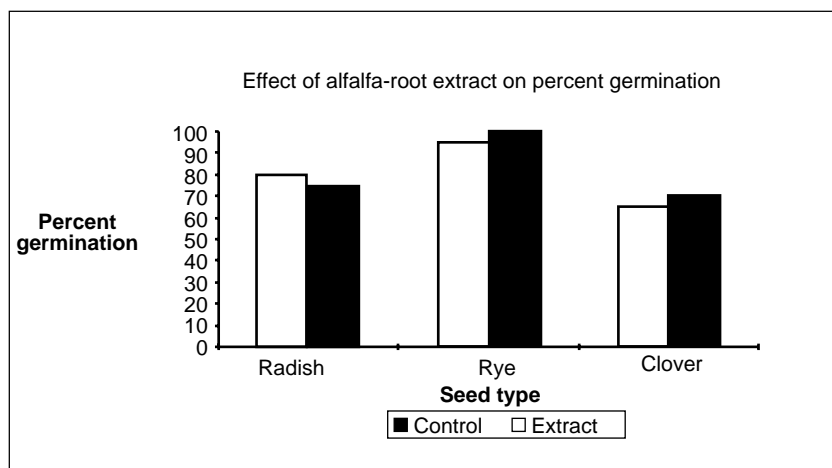


**Figure 5.** Roots of alfalfa sprouts submerged in water.

2. Continue by following the sample procedure for the Core Experiment using the alfalfa-root extract instead of the alfalfa-leaf extract.

### SAMPLE DATA ANALYSIS AND INTERPRETATION

#### Sample Data



**Graph F.** The effect of alfalfa-root extract on seed germination.

#### TEST QUESTION

Did the alfalfa-root extract affect the seed germination or growth of any of the 3 target species?

### VARIATION 3

#### The Allelopathic Effects of Alfalfa Seeds on the Germination and Growth of the Seeds of Other Plants

*Note to Teachers:* In addition to the information found in the Core Experiment, the following material has been provided for Variation 3.

#### SYNOPSIS

Students will test the effects of the extract of alfalfa seeds on the germination and growth of radish, white clover, and rye grass seeds. They will determine whether the alfalfa seeds produce allelopathic substances.

#### ADDITIONAL MATERIALS NEEDED

*You will need the following for each group of four students in a class of 24:*

- 20 g alfalfa seeds
- 100 mL distilled water

#### HYPOTHESIS GENERATION

##### Question

Do alfalfa seeds produce allelopathic agents that reduce the germination and growth of seeds?

##### Sample Hypothesis

Alfalfa seeds produce allelopathic agents that reduce the germination and growth of radish, white clover, and rye grass seeds.

#### Interpretation

Accept the hypothesis that the roots of alfalfa plants produce an allelopathic substance if there is a decrease in the germination and growth of the seeds of radish, white clover, and rye grass.

Reject the hypothesis if the germination and growth of the seeds exposed to the alfalfa-root extract is the same as or greater than the control seeds.

#### Answer to Test Question

Answers will vary depending on students' results. However, tests generally show that alfalfa-root extract has no allelopathic effects on the germination of seeds.

## TEACHER'S NOTES

### Interpretation

The hypothesis should be accepted if a decrease in the germination and growth of the seeds of radish, white clover, and rye grass was observed in the seeds treated with alfalfa-seed extract.

The hypothesis should be rejected if the radish, white clover, and rye grass seeds exposed to the alfalfa-seed extract showed the same amount or more germination and growth as the seeds grown with distilled water.

### Answer to Test Question

Answers will vary depending on students' results. However, tests generally show that alfalfa-seed extracts have no allelopathic effects on the germination of seeds.

### Rationale

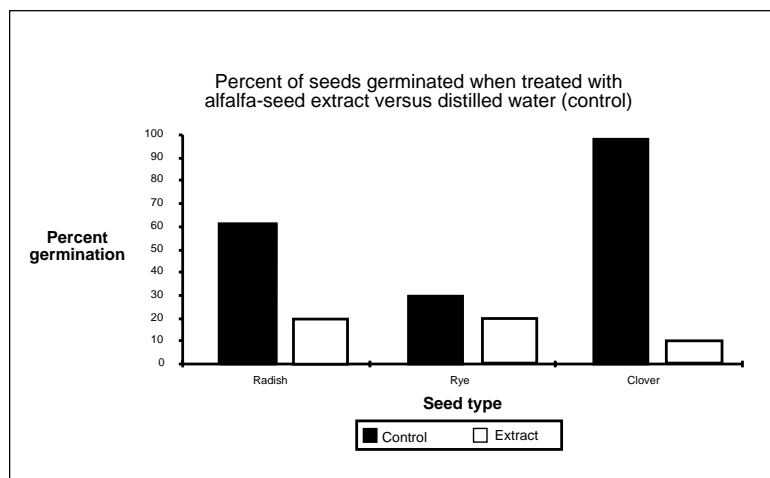
Students should provide their own rationale.

### Sample Experimental Procedure

1. Make a seed extract using 20 g of alfalfa seeds soaked in 100 mL of distilled water for 2 days. Refrigerate after 1 day.
2. Continue by following the sample procedure for the Core Experiment using the seed extract instead of the leaf extract.

### SAMPLE DATA ANALYSIS AND INTERPRETATION

#### Sample Data



Graph G. Effects of alfalfa-seed extract on germination.

### TEST QUESTION

Did the alfalfa-seed extract affect the germination or growth of any of the 3 target species' seeds?

## VARIATION 4

A Comparison of the Allelopathic Effect of Leaf Extract from Different Plant Species on Radish, White Clover, and Rye Grass

*Note to Teachers:* In addition to the information found in the Core Experiment, the following material has been provided for Variation 4.

### SYNOPSIS

Students will test the allelopathic effects of various plants on the germination and growth of radish, white clover, and rye grass seeds. They will compare the allelopathic effects of these plants to the effect of alfalfa-leaf extract.

### ADDITIONAL MATERIALS NEEDED

You will need the following for each group of four students in a class of 24:

- 250 g fresh cut leaves from celery, ragweed, mint, chickpea, walnut, or oak
- 250 g fresh alfalfa leaves
- 250 mL distilled water



## SAFETY PROCEDURES



Ask students about allergies. Avoid plants that elicit student allergies.

## HYPOTHESIS GENERATION

### Question

Do the leaf extracts of any other plants affect the germination and growth of the target species?

### Sample Hypothesis

Greater inhibition of germination and growth of seeds is obtained using black walnut-leaf extract as compared to alfalfa-leaf extract.

### Rationale

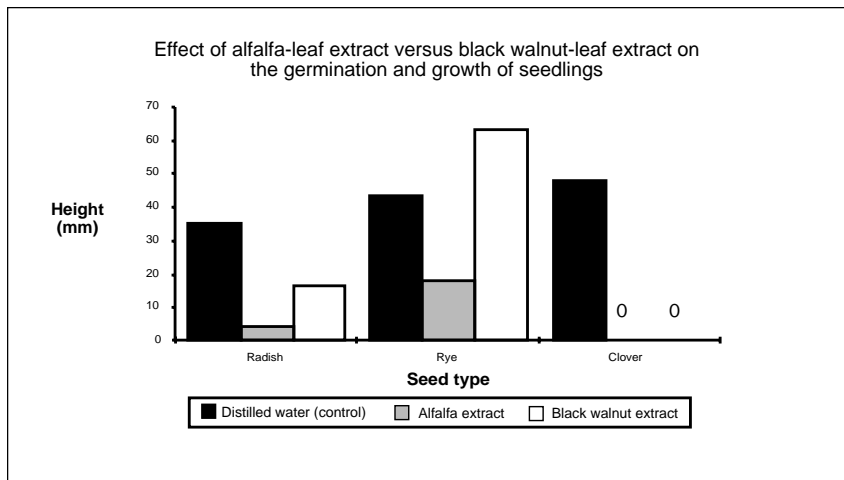
Black walnut is known to inhibit the growth of most plants. The black walnut extract differs from alfalfa extract and may affect more species or may affect some species more severely.

### Sample Experimental Procedure

Duplicate the procedure for the Core Experiment, replacing the alfalfa leaves with leaves from the black walnut tree for the second treatment group. When preparing the extract, use 250 g of leaves in 250 mL of distilled water.

## SAMPLE DATA ANALYSIS AND INTERPRETATION

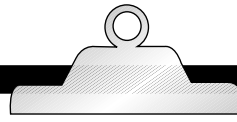
### Sample Data



**Graph H.** Comparison of the effects of black walnut and alfalfa-leaf extracts on seeds.

## TEST QUESTIONS

1. Compare the germination of clover seeds exposed to alfalfa-leaf extract to the germination of clover seeds exposed to black walnut-leaf extract. If there is a difference, what factors might explain it?
2. Why would different plants show varying degrees of allelopathy?



## TEACHING TIPS

- If black walnut trees are not available in your community, test plants such as celery, ragweed, mint, chick-peas, walnut, and oak for allelopathy. Students may want to compare several species from the same genus.
- Results from the Core Experiment may be used while testing the other plant, but only if the tests are run concurrently.
- Black walnut is not common in New England.

### Interpretation

Accept the hypothesis if the seeds showed less germination and growth when exposed to the black walnut extract than when exposed to the alfalfa extract or to distilled water. This suggests that black walnut leaves show a stronger allelopathic effect than alfalfa.

Reject the hypothesis if the same number or more seeds germinated and there was equal or greater growth when the seeds were exposed to the black walnut extract than when exposed to the alfalfa extract.

### Answers to Test Questions

1. Answers will vary depending on student results. Differences may be due to different chemicals acting as the allelopathic agents.
2. Allelopathy helps plants compete with other plants for limited resources. Varying degrees of allelopathy in plants may suggest different levels of competitive stress or reflect intrinsic metabolic differences among plants.

**TEACHING TIP**

Students can test a variety of suspected allelopathic plants for their effects on several different common weeds and agricultural plants. They should try to find an allelopathic plant that prevents the growth of the weeds but does not harm the crop plants.

**VARIATION 5**

## The Use of Allelopathic Plants as “Natural” Herbicides




*Note to Teachers:* In addition to the information found in the Core Experiment, the following material has been provided for Variation 5.

**SYNOPSIS**

Some researchers are interested in whether allelochemicals can be used as natural herbicides against weeds, those plant species that are considered noncultivated or undesirable. Students will test the extract from black walnut leaves for allelopathic effects on seeds of a weed plant and a crop plant, such as sunflower.

**ADDITIONAL MATERIALS NEEDED**

*You will need the following for each group of four students in a class of 24:*

-  50 g black walnut leaves
-  60 weed plant seeds
-  60 sunflower seeds

**HYPOTHESIS GENERATION****Question**

Can black walnut-leaf extract be used as an herbicide?

**Sample Hypothesis**

An extract of black walnut leaves will prevent weed plant seeds from germinating but will not affect the germination of sunflowers.

**Rationale**

Black walnut is known to have an allelopathic effect on most plants, but is known to stimulate the growth of sunflower seeds. Therefore, it is reasonable to consider it as an herbicide for the weedy plants that may grow with sunflower crops.

**Sample Experimental Procedure**

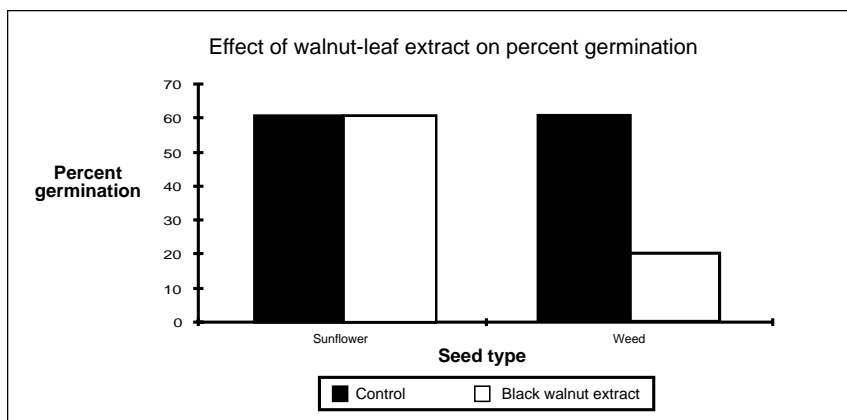
Repeat the sample procedure for the Core Hypothesis. Prepare an extract from the leaves of a black walnut tree. Test for the effects of this extract on the germination of weed plant seeds and sunflower seeds.

**SAMPLE DATA ANALYSIS AND INTERPRETATION****Sample Data**

Possible results for this variation include:

- Germination rate varies.
- Germination rate is reduced.
- Neither the sunflower nor the weed plant seeds germinate.
- The sunflower seeds do not germinate, but the weed plant seeds do germinate.
- The sunflower seeds germinate, but the weed plant seeds do not germinate.
- Both the sunflower and weed plant seeds germinate.

The walnut-leaf extract can be considered an effective herbicide only if the sunflower seeds germinate, but the weed plant seeds do not.



**Graph I.** The effects of walnut-leaf extract on seed germination.

### TEST QUESTION

Did you see any effect of the allelopathic plant against the weed seeds or the crop plant seeds? Based on these findings, would you recommend the allelopathic plant leaves as a natural herbicide?

## VARIATION 6

### The Effects of Light on the Production of Allelopathic Substances

*Note to Teachers:* In addition to the information found in the Core Experiment, the following material has been provided for Variation 6.

### SYNOPSIS

Students will test the allelopathic effect of the extract from alfalfa plants grown in different amounts of light. They will determine if the amount of light has an effect on the amount of allelopathic substance that the plant produces.

### ADDITIONAL MATERIALS NEEDED

*You will need the following for each group of four students in a class of 24:*

- 🌿 2 4-L (1-gallon) pots
- 🌿 8 kg (16 lbs) topsoil or potting soil
- 🌿 50 g alfalfa seeds
- 🌿 3 30-cm<sup>2</sup> layers of 12-gauge cheesecloth
- 🌿 1 light meter

### HYPOTHESIS GENERATION

#### Question

Will the amount of light that alfalfa seeds are grown in affect their allelochemical activity?

#### Sample Hypothesis

The leaf extract from alfalfa plants grown in less light will have diminished allelopathic effect on the germination and growth of radish, white clover, and rye grass seeds.

### Interpretation

Accept the hypothesis if there is no difference in the germination rate of the sunflower seeds in the treatment group and the control group, but the weed plant seeds treated with the extract show less germination than those watered with distilled water.

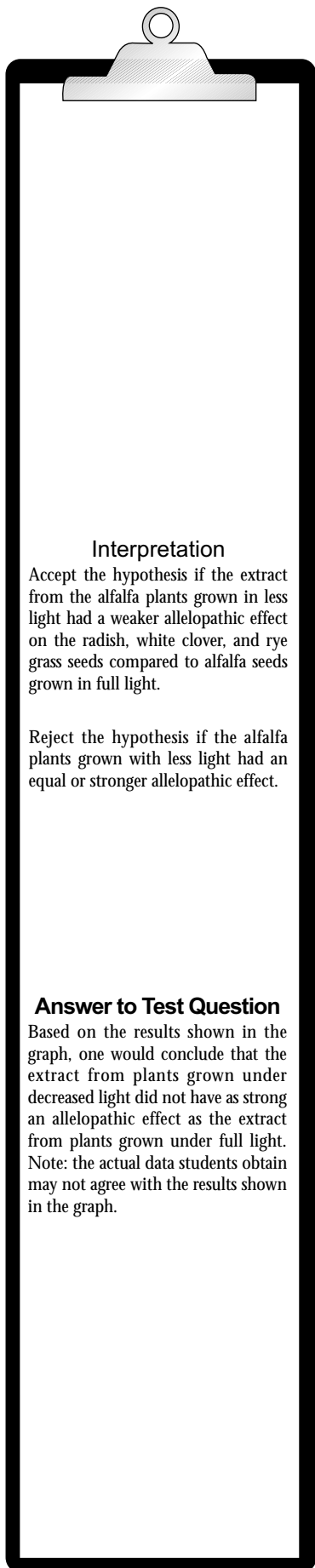
Reject the hypothesis if the extract has an allelopathic effect on the sunflower seeds or if it did not have an allelopathic effect on the weed plant seeds.

### Answer to Test Question

Answers will vary depending on the students' results.

### TEACHING TIPS

- If a light meter from a camera is available, use it to determine the number of layers of cheesecloth needed to achieve the different levels of shading.
- Gauge of cheesecloth varies.
- Three layers of 12-gauge cheesecloth will reduce the light level by approximately 50 percent.



### Interpretation

Accept the hypothesis if the extract from the alfalfa plants grown in less light had a weaker allelopathic effect on the radish, white clover, and rye grass seeds compared to alfalfa seeds grown in full light.

Reject the hypothesis if the alfalfa plants grown with less light had an equal or stronger allelopathic effect.

### Answer to Test Question

Based on the results shown in the graph, one would conclude that the extract from plants grown under decreased light did not have as strong an allelopathic effect as the extract from plants grown under full light. Note: the actual data students obtain may not agree with the results shown in the graph.

### Rationale

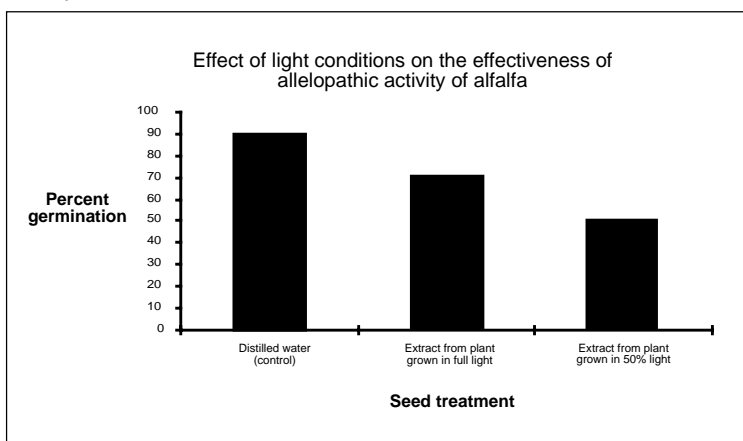
The amount of light from photosynthesis is known to have an effect on plant growth. Therefore, it may affect the production of allelopathic chemicals in alfalfa.

### Sample Experimental Procedure

1. Grow 1 pot of alfalfa under 3 layers of cheesecloth or enough to reduce the light level by approximately 50 percent.
2. Grow the control alfalfa in full sunlight.
3. Prepare extracts from each set of alfalfa plants and treat the radish, white clover, and rye grass seeds as in the Core Experiment. Include a control of distilled water.
4. Compare the effects of the extract from plants grown in low light and plants grown in full light.

### SAMPLE DATA ANALYSIS AND INTERPRETATION

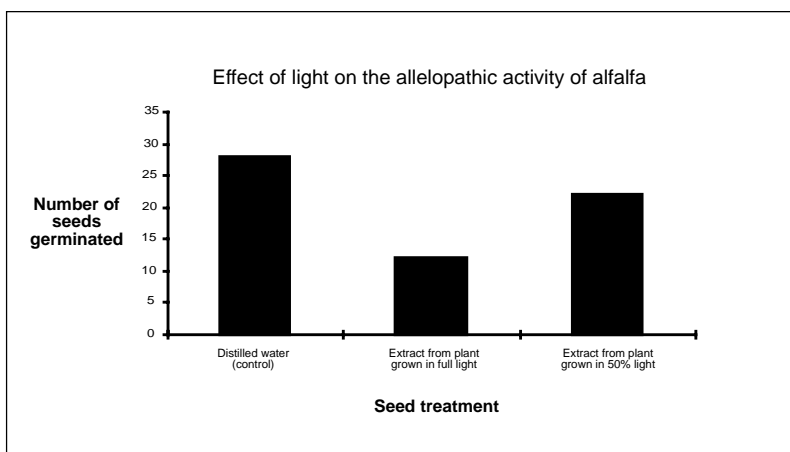
#### Sample Data



Graph J. Effects of the amount of growing light on the allelopathic activity of alfalfa.

### TEST QUESTION

Based on the following graph, what would you conclude about the effects of light level on the production of allelopathic chemicals?



Graph K. The effect of light level on production of allelopathic chemicals.

## SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

### Blind or Visually Impaired

- Use a light probe to determine the number of layers of cheesecloth needed to achieve the different levels of shading. The light probe has the same purpose and basic structure as the camera's light meter, but the light probe uses sound to register the amount of light. The higher the pitch, the greater the amount of light. Earphones are available to use with the light probe when the tone is disturbing to classmates.

## VARIATION 7

### The Effects of Nutrients on the Production of Allelopathic Substances

*Note to Teachers:* In addition to the information found in the Core Experiment, the following material has been provided for Variation 7.

#### SYNOPSIS

Students will test the allelopathic effects of the extract from fertilized and unfertilized alfalfa plants to determine if treatment with fertilizer has an effect on the production of allelopathic substances.

#### ADDITIONAL MATERIALS NEEDED

*You will need the following for each group of four students in a class of 24:*

- 🌱 2 4-L (1-gallon) pots
- 🌱 2 kg (4 lbs) topsoil
- 🌱 8 kg (16 lbs) sand
- 🌱 50 g alfalfa seeds
- 🌱 all-purpose 20-20-20 liquid plant fertilizer
- 🌱 target species seeds, such as white clover

#### SAFETY PROCEDURES

- 👤 Follow the instructions carefully when preparing the fertilizer solution.
- 🧼 Wash hands before and after lab.
- 🚫 Do not eat or drink fertilizer solution.

#### HYPOTHESIS GENERATION

##### Question

Will fertilizer affect the production of allelopathic chemicals in alfalfa?

##### Sample Hypothesis

Treatment with a fertilizer will increase the allelopathic effect of alfalfa on white clover or another target species' seeds.

##### Rationale

As the availability of nutrients increases, more nutrients will be available for the production of allelochemical compounds.

##### Sample Experimental Procedure

1. Grow 2 pots of alfalfa in sandy soil. See Teaching Tips for sandy soil composition.
2. Treat the plants in 1 pot with fertilizer that can be applied as a liquid. Treat the control plants in the other pot with an equal amount of tap water, but no fertilizer.

## TEACHER'S NOTES

#### TEACHING TIPS

- Use a mixture of 4 parts sand to 1 part topsoil.
- Sandy soil is lower in nutrients.
- 20-20-20 refers, respectively, to the nitrogen, phosphorous, and potassium levels in the fertilizer.

## TEACHER'S NOTES

### Interpretation

Accept the hypothesis if the alfalfa plants that were treated with fertilizer had a greater allelopathic effect on the seeds. This suggests that the nutrient level of the growing medium affects the production of allelopathic substances.

Reject the hypothesis if the alfalfa plants grown with fertilizer did not have a greater allelopathic effect on the seeds.

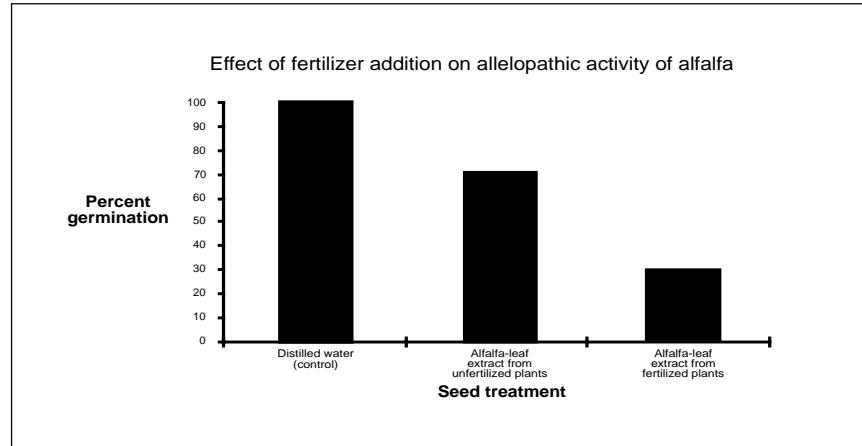
### Answer to Test Question

Based on the results shown in the graph, the plants grown with low nutrient levels did not show as great an allelopathic effect as the plants grown with high nutrient levels. Note: the actual data students obtained may not agree with the results shown in the graph.

2. Continue the experiment by growing the seeds as in the Core Experiment. Germinate 1 set of seeds using extract from the fertilized alfalfa plants and 1 set using extract from the unfertilized alfalfa plants. Include a control germinated with distilled water.

## SAMPLE DATA ANALYSIS AND INTERPRETATION

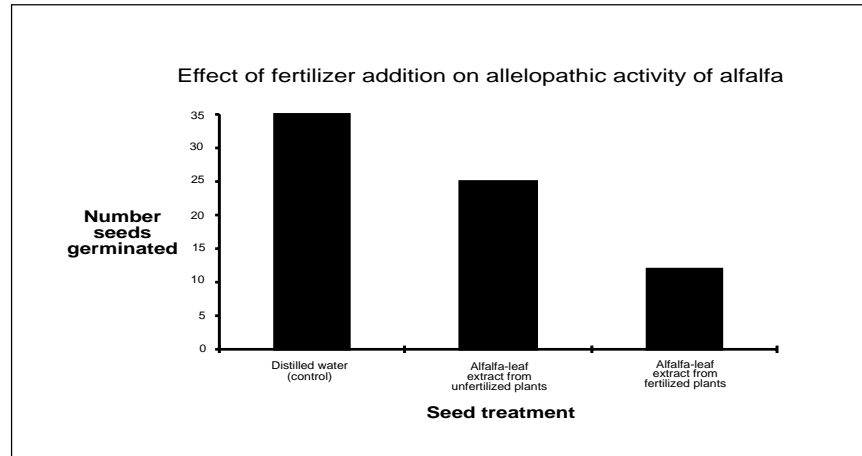
### Sample Data



Graph L. Effect of fertilizer on alfalfa's allelopathic activity on a target species.

### TEST QUESTION

According to the following graph, what would you conclude about the effects of nutrient level on the production of allelopathic substances?



Graph M. Effect of fertilizer on alfalfa's allelopathic activity on a target species.

## SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

### Blind or Visually Impaired

- Use 20 drops/mL of liquid fertilizer to provide a specific number of drops per liter. A bottle of diluted fertilizer should be provided for students who are blind.
- Use graduated cylinders that have raised lines on the outside that can be counted by running a fingernail or a plastic card down the side of the cylinder.
- Detect the meniscus in clear water with a light sensor. Liquid with a slight coloring is easier to read.

## VARIATION 8

### The Effect of Herbivory on the Production of Allelopathic Agents

*Note to Teachers:* In addition to the information found in the Core Experiment, the following material has been provided for Variation 8.

#### SYNOPSIS

Students will test the allelopathic effect of alfalfa plants that have been subjected to herbivory, or the grazing by insects or other organisms, as compared to the effect of plants that have been protected from herbivory. Students will determine whether herbivory affects the production of allelopathic agents.

#### ADDITIONAL MATERIALS NEEDED

*You will need the following for each group of four students in a class of 24:*

- 🌿 2 4-L (1-gallon) pots
- 🌿 8 kg (16 lbs) topsoil or potting soil
- ✂️ 1 pair of scissors

#### HYPOTHESIS GENERATION

##### Question

Will the allelopathic activity of alfalfa be affected by herbivory or grazing?

##### Sample Hypothesis

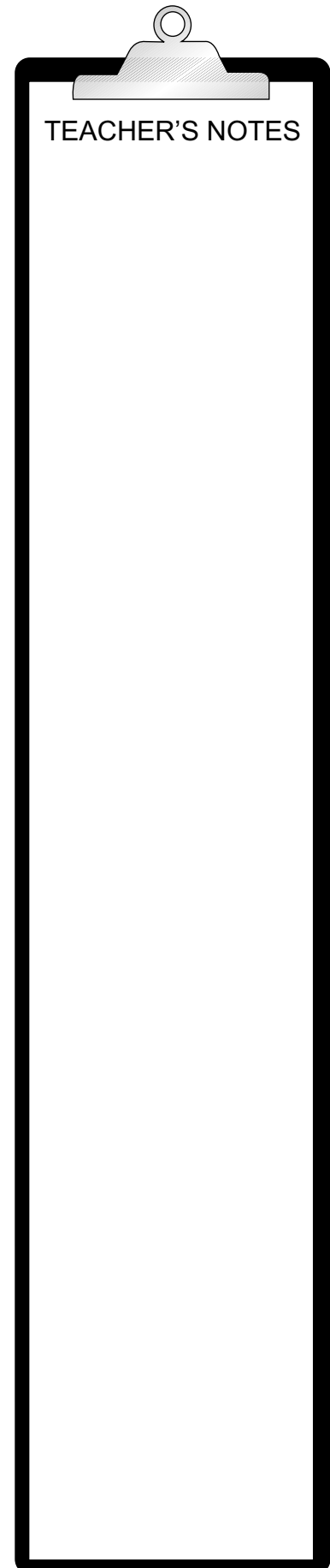
The extract from alfalfa plants that have been exposed to herbivory will have a greater allelopathic effect on seed germination.

##### Rationale

It is known that plants can produce protective chemical substances in response to herbivory. These substances also may have observable allelopathic effects.

##### Sample Experimental Procedure

1. Plant 2 pots of alfalfa.
2. After about 7 days, trim approximately 1 cm off the tops of the alfalfa plants in 1 of the 2 pots to simulate the effects of herbivory.
3. Grow the control pot of plants alongside the treatment pot, but do not trim the control plants.
4. Continue to grow the plants for an additional 7 days.
5. Harvest the alfalfa plants from each pot after 14 days of growth and repeat the procedure used in the Core Experiment with each pot of alfalfa.



### Interpretation

The hypothesis should be accepted if the extract from the plants that were subjected to herbivory had a greater allelopathic effect on the seeds than extract from ungrazed plants. This would suggest that herbivory influences the production of allelopathic substances.

The hypothesis should be rejected if the extract from the plant exposed to herbivory does not have a greater allelopathic effect on the seeds than extract from the ungrazed plant. This would suggest that herbivory does not influence the production of allelopathic substances.

### Answer to Test Question

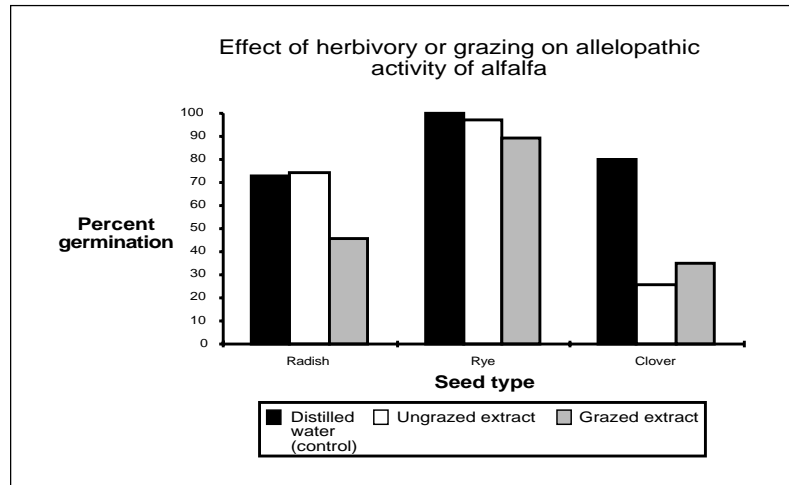
Answers will vary depending on experimental results.

### TEACHING TIPS

- Factors, such as day length, may vary during the 8-week growing period. So, it is best to grow the alfalfa under artificial lights with timers. Plants should receive 16 hours of light and 8 hours of dark.
- A modification of this variation is to examine the effects of plant age on the allelopathic agents produced by trees. Local park authorities may be able to tell you the age of the trees. If you cannot determine the exact age of the tree, you can estimate a range of “younger” to “older” trees based on trunk diameter.

## SAMPLE DATA ANALYSIS AND INTERPRETATION

### Sample Data



**Graph N.** Comparison of the effects of alfalfa extract obtained from plants that have been subjected to herbivory.

### TEST QUESTION

Based on your experiments, does herbivory affect the production of allelopathic agents in alfalfa? How do your data support your answer?

### SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

#### Blind or Visually Impaired

- Students who are visually impaired should have no difficulty in performing all parts of this experiment on the effect of herbivory on the production of allelopathic agents. Provide an assigned area to locate plants labeled in braille.

## VARIATION 9

### The Influence of Plant Age on the Allelopathic Effect of Alfalfa

*Note to Teachers:* In addition to the information found in the Core Experiment, the following material has been provided for Variation 9.

### SYNOPSIS

Students will determine whether the allelopathic effect of alfalfa plants varies with the age of the plant.

### ADDITIONAL MATERIALS NEEDED

*You will need the following for each group of four students in a class of 24:*

- 🌿 4 4-L (1-gallon) pots
- 🌿 8 kg (16 lbs) topsoil or potting soil
- 🌿 100 g alfalfa seeds
- 🌿 1 artificial light bank
- 🌿 1 24-hour timer



## HYPOTHESIS GENERATION

### Question

How does the age of a plant affect the potency of allelopathic chemicals?

### Sample Hypothesis

The leaf extract from older alfalfa plants will have a greater effect on the germination and growth of seeds.

### Rationale

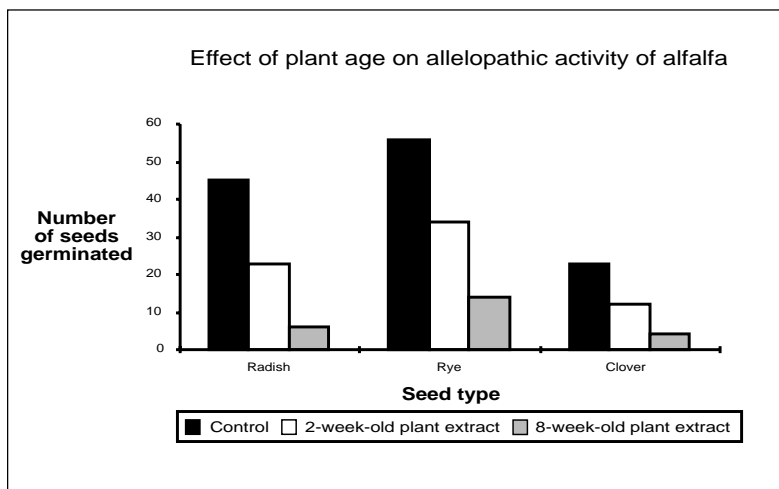
Concentration of allelochemicals can vary with the age of the plant. Therefore, the intensity of the allelopathic effect may vary with the age of the plant.

### Sample Experimental Procedure

1. Plant 1 pot of alfalfa.
2. Six weeks later, plant the second pot of alfalfa.
3. Grow plants 2 more weeks.
4. Repeat the Core Experiment to test the allelopathic effects of plant age.

## SAMPLE DATA ANALYSIS AND INTERPRETATION

### Sample Data



Graph O. Effects of plant age on the allelopathic potency of alfalfa-leaf extract.

### TEST QUESTION

Did the effects of the allelopathic substance on seed germination and growth vary with the age of the alfalfa plant?

### Interpretation

Accept the hypothesis if the older alfalfa plants had a greater allelopathic effect on the germination and growth of the seeds.

Reject the hypothesis if there was no difference in the allelopathic effect of the alfalfa extract from plants of different ages or if older plants had a lesser effect than younger plants.

### Answer to Test Question

Answers will vary depending on students' experimental results.

**TEACHING TIPS**

- Agricultural and garden supply houses usually have a good supply of inoculum for nitrogen fixation.
- To facilitate the separation of the alfalfa roots from the soil, prepare a mixture of 4 parts sand to 1 part topsoil to grow the alfalfa. Sift the topsoil before mixing or use potting soil to prevent large organic particles from adhering to the roots.
- Sterilize the soil in an autoclave or pressure cooker at 15 pounds per square inch (psi) for at least 15 minutes. This will kill all soil bacteria. Keep the soil wrapped in several layers of heavy duty aluminum foil to prevent contamination until it is needed.
- Plants can be dried in envelopes or in any type of folded paper.
- Plants also can be dried in a conventional oven set at 200°F or air dried.

**VARIATION 10****The Effect of Allelopathic Substances on the Formation of Root Nodules**

*Note to Teachers:* In addition to the information found in the Core Experiment, the following material has been provided for Variation 10.

**SYNOPSIS**

Students will determine if the extract from alfalfa leaves will affect the formation of root nodules in white clover by symbiotic, nitrogen-fixing bacteria.

**ADDITIONAL MATERIALS NEEDED**

*You will need the following for each group of four students in a class of 24:*

- 🌿 120 white clover seeds, or another plant known to nodulate
- 🌿 2 kg (4 lbs) sterile, sandy soil
- 🌿 1 autoclave or pressure cooker
- 🌿 0.3 g inoculum
- 🌿 12 10-cm diameter plastic pots
- 🌿 1 microscope
- 🌿 1 drying oven or conventional oven (*optional*)

**SAFETY PROCEDURES**

Students should wash hands well after handling the inoculum.



The teacher should supervise all autoclaving of the soil. The steam is extremely hot, and sandy soil takes 30 to 60 minutes to cool for handling.

**HYPOTHESIS GENERATION****Question**

Is the formation of root nodules of legumes affected by alfalfa-leaf extract?

**Sample Hypothesis**

The extract from alfalfa leaves will inhibit the formation of root nodules by symbiotic, nitrogen-fixing bacteria.

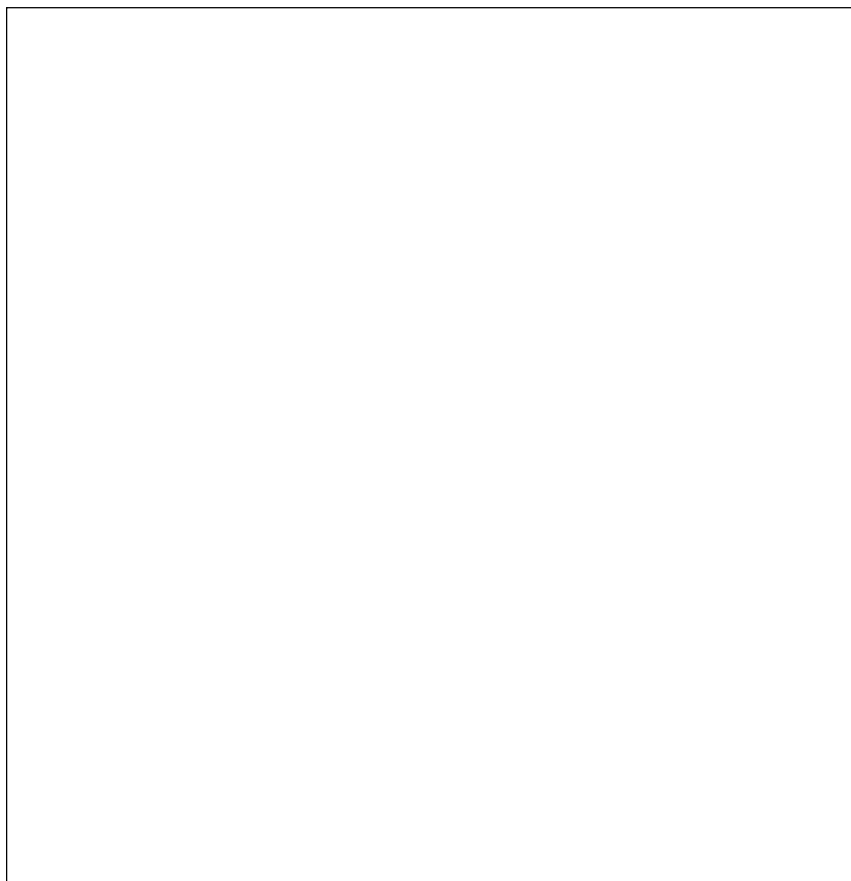
**Rationale**

The chemical interaction between the legume and the nitrogen-fixing bacteria is affected negatively by allelochemicals.

**Sample Experimental Procedure**

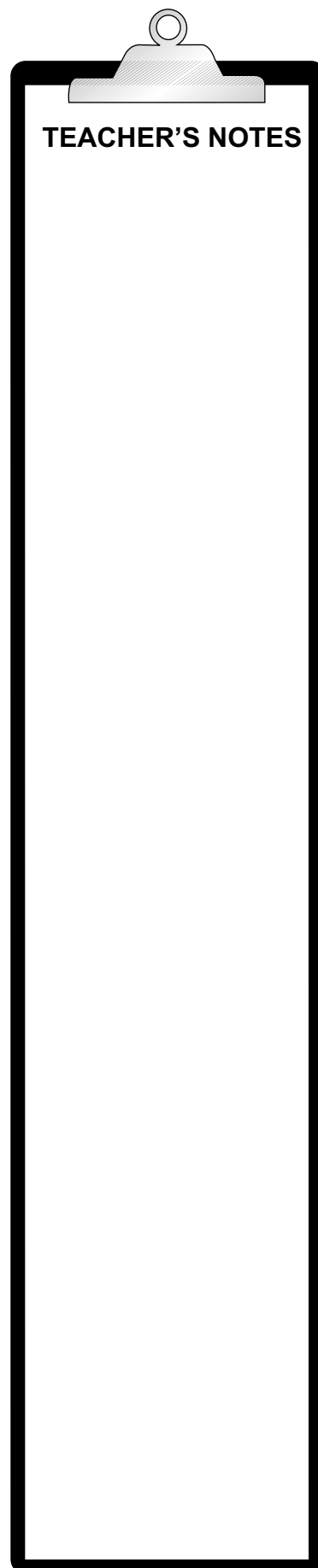
1. Prepare alfalfa-leaf extract as described in the Core Experiment.
2. Fill 12 pots with sterilized sandy soil 1 cm from the top.
3. Label 3 pots with each of the following treatments:
  - no inoculum/treated with distilled water
  - no inoculum/treated with alfalfa-leaf extract
  - inoculum/treated with distilled water
  - inoculum/treated with alfalfa-leaf extract

4. Plant 10 clover seeds in each pot. Water each pot with either distilled water or the alfalfa-leaf extract as the pot's label indicates.
5. Separate the pots to be inoculated from those that will not be inoculated.
6. Inoculate the appropriate pots by sprinkling 0.1 g of inoculum onto the soil. Be sure to inoculate well away from the uninoculated soil so it does not become contaminated.
7. Grow the clover for 10 to 14 days.
8. Gently remove the plants from their pots with roots and soil intact.
9. Carefully separate 5 plants from the soil and rinse them to remove any residual soil.
10. Use a microscope to count the number of nodules on each plant's roots. See Figure 6.



**Figure 6.** Results of alfalfa-extract on white clover nodule development.

11. Harvest the remaining plants and rinse off the soil.
12. Dry the plants in a drying oven at 80°C. You may wish to separate roots from stems and leaves before drying in order to analyze them separately.
13. Once plants are dry, determine the biomass (dry mass).



### Interpretation

Accept the hypothesis if fewer nodules were formed by the clover plants that were treated with the alfalfa-leaf extract.

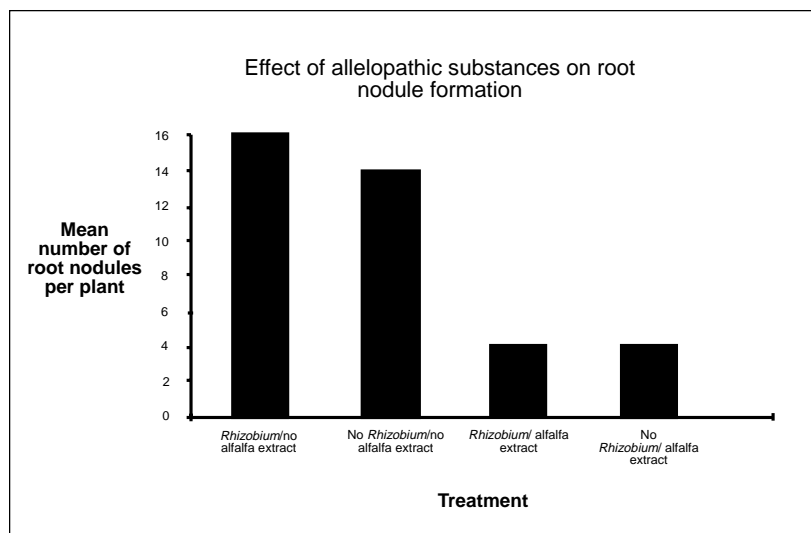
Reject the hypothesis if there was no difference in the number of nodules formed by the clover plants that were treated with alfalfa extract and the plants treated with distilled water.

### Answer to Test Question

The alfalfa and clover will grow well the first 2 or 3 years. Eventually, the alfalfa may become chlorotic and will not thrive. If fertilizers containing nitrogen are added to the soil, the alfalfa crops will thrive. After this time, the clover will fail to thrive.

## SAMPLE DATA ANALYSIS AND INTERPRETATION

### Sample Data



Graph P. Effect of alfalfa-leaf extract on the number of root nodules.

### TEST QUESTION

Based on the results above, describe the effects of cultivating alfalfa and clover in the same section of land for a period of 10 years.

### SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

#### Blind or Visually Impaired

- Have students assist in planting and caring for the alfalfa plants. They should not handle the inoculum unless they are wearing rubber gloves.
- Wash the root nodules free of the inoculum for student examination.
- Have laboratory partners inform visually impaired students of the results of the microscopic work.

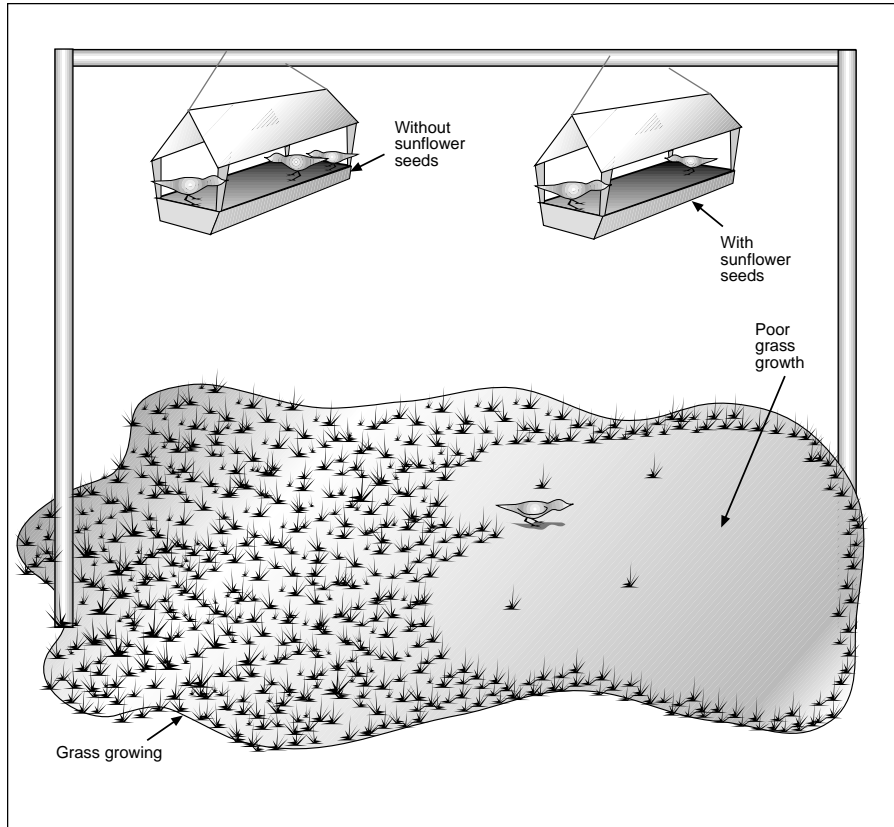
#### Manually Impaired

- Where there are investigations such as Variation 10 that use nitrogenous inoculum, it is best that students with manual impairments watch their lab partners. Some might be able to put seeds in the flower pots but would need friends to carry large items to the growing area. They need lab partners who not only will aid them but who will see that they also share the work when possible.

# A Study of Allelopathy in Plants

## Directions for Students

### INTRODUCTION



**Figure 1.** Two bird feeders located in the same area. One has sunflower seeds; the other does not.

Have you ever noticed bare spots under certain trees where flowers, even weeds (uncultivated plants), will not grow? Look at Figure 1. What observations can you make? Have you observed this in your community? In 300 BC, Theophrastus realized that planting chick peas made the soil unsuitable for many other plants. Earlier records of allelopathy date back before 300 BC when Democritus reported that weeds could be controlled by using naturally occurring plant products and that trees could be killed by treating their roots with a mixture of lupine flowers soaked in hemlock juice. In 1 AD, Pliny, the Roman naturalist, reported that barley and bitter vetch prevented the growth of other plants in the same soil. Later reports showed that even the rain or dew washing from pine needles onto crop plants caused damage.

In more recent history, A. B. Massey (1925) determined that toxic substances released into the soil by walnut trees inhibited the growth of almost all other plants. It eventually was suggested that some plants exude chemical substances that are injurious to other plants. This phenomenon has been observed in nature, most notably in California's Mojave Desert where creosote shrubs exclude burro-weed by exuding a chemical from their roots that may inhibit the root growth of the burro-weed (Dutton, 1993). Some researchers, however, contend that other factors, such as competition for light or nutrients, are the reason why certain plants are unable to grow together and that allelopathy is not a factor. How might you test whether a plant has allelopathic effects on another plant? With your class, you will develop a hypothesis to answer a question about allelopathy and design an experiment to test this hypothesis. This experiment will serve as the basis of other experiments.

## OBJECTIVES

*At the end of this lab, you should be able to:*

- Identify the parts of a plant that may produce allelopathic substances.
- Describe the observable effects of allelopathy on seed germination, seedling appearance, and growth of roots and leaves.
- Discuss the ecological implications of allelopathy.

### SAFETY NOTES



Let your teacher know if you are allergic to any of the plants used in this experiment.



Wash your hands after touching plants.



Students with allergies should wear nonallergenic gloves.

## MATERIALS NEEDED

*For each group of four students, you will need the following:*

- ♥ 5 g freshly cut alfalfa leaves
- ♥ 1 pair of scissors
- ♥ 1 150-mL glass beaker
- ♥ 18 disposable 10 x 150-mm petri dishes
- ♥ 1 permanent marking pen
- ♥ 54 sheets of 8-cm Whatman filter paper
- ♥ 60 seeds each of radish, white clover, and rye grass
- ♥ 1 50-mL flask
- ♥ 2 5-mL disposable plastic pipettes or syringes without needles

## STUDENT LITERATURE SEARCH SUMMARY WITH REFERENCES

Do a literature search on the topic of allelopathy. Summarize your findings and cite your references. Your teacher may be able to suggest some references.

## HYPOTHESIS GENERATION

Think about what effects an allelopathic plant might have on the germinating seedlings of another species. From the information you have on this topic, develop a hypothesis that could be tested in a controlled experiment that gathers quantitative data. Explain the reasoning behind your hypothesis.

Answer the following questions:

1. What is the question you are investigating?
2. Why is it important to control variables other than the one you are investigating?

## PLAN OF INVESTIGATION

Make a numbered list of the steps you will use to investigate your topic. Answer the following questions:

1. How many samples have you included?
2. What will you measure?
3. How can you show your results in a graph?

Design an experiment to test your hypothesis. Be sure that you include an experimental control and enough replicates to provide reliable data. Consider how you will analyze and present your results. Write the procedures you will follow.

**You must have your teacher approve this protocol before you begin the experiment.**

## QUESTIONS AND ANALYSIS

Once you have collected and analyzed your data and graphed your results, answer the following questions:

1. Do these data support your hypothesis? Discuss the evidence you used to come to this conclusion.
2. What were some of the major sources of error in your experiment?
3. What changes, if any, would you make in your procedures?
4. What other experiments would you do as an extension of this study?
5. How does this lab relate to ecological principles in nature?
6. How might some of your findings be applied, for example in agriculture?

## DESIGN OF VARIATIONS OF CORE EXPERIMENT

After you have discussed the results of the Core Experiment with your classmates, write down questions that occurred to you as you tested the allelopathic effects of alfalfa on seed germination. Design an experiment that is quantifiable and write your procedure in a numbered list of steps. Questions other students have studied include the following:

- Will the concentration of an allelopathic chemical influence its effects?
- Do roots contain allelopathic substances?
- Do alfalfa seeds produce allelopathic agents that reduce the germination and growth of seeds?
- Do the leaf extracts of any other plants affect the germination and growth of the target species?
- Can black walnut be used as an herbicide?
- Will the amount of light that alfalfa seeds are grown in affect their allelochemical activity?
- Will fertilizer affect the production of allelopathic chemicals in alfalfa?
- Will the allelopathic activity of alfalfa be affected by herbivory or grazing?
- How does the age of a plant affect the potency of allelopathic chemicals?
- Is the formation of root nodules of legumes affected by alfalfa-leaf extract?