

# Mean and Green



## Principle of Plant Biology #9

Plants, like animals, are subject to injury and death due to infectious diseases caused by microorganisms. Plants have unique ways to defend themselves against pests, diseases, and other threats.

Plants encounter many threats in their natural habitats. Herbivores try to eat them, microorganisms infect them, extreme weather stresses and damages them, and other plants compete for resources. Plants are amazing because they must deal with these threats without running away. To defend themselves, plants have many sophisticated adaptations including the production of toxic chemicals.

Many plants make chemicals to inhibit the growth of plants around them. This response is called allelopathy. Allelopathy is difficult to prove in the environment because of the complex interactions found in soil. However, in the laboratory, a bioassay can be used to test for the presence of an allelopathic chemical. A bioassay is a technique to determine the

biological activity of substances. Bioassays can be useful for detecting chemical toxicity.

Germination (when a root comes out of a seed) root length, shoot length, and other characteristics can be measured to understand how allelopathic substances affect growth. Radish seeds are commonly used for these types of bioassays because they are often sensitive to toxic substances. Corn seeds (kernels) are also useful for bioassays. Germinating corn kernels produce a shoot that is easy to measure, and permit experiments that compare a monocot (corn) with a dicot (radish).

In the following experiment, you will test plant tissues to see if they make chemicals that inhibit the growth of radish and corn seedlings.



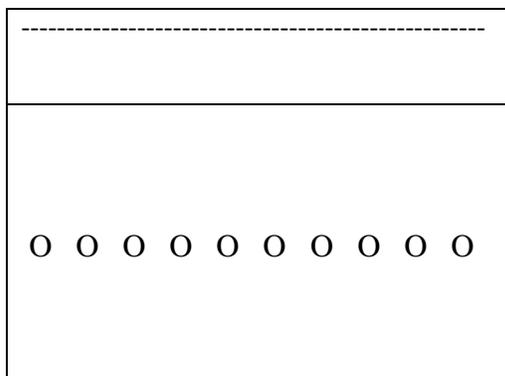
### **Real-world Connection:**

Although we normally think of pesticides and herbicides as chemicals that we spray on plants, plants make many of them on their own. Scientists use bioassays to discover and test chemicals that can improve agriculture, horticulture, and other areas of society.

## Activity: Inhibitory Responses in Plants

### Procedure:

1. Fold two paper towels in half twice and place them into two zip-lock bags. Mark the bags at the bottom corner with the date and your name. Write “Control” on one.
2. Select a plant to test for allelopathic chemicals. Different plant parts can be tested such as leaves, bark, stems, roots, flowers, seeds, and fruit. Tear or cut the plant material into small pieces, and then place it into a mortar with 2 pinches of sand and 20 mL of water. Grind this material for 1 to 2 minutes or until it is pulverized.
3. Filter the solution using filter paper and funnel it into a beaker or cup.
4. Optional step: Test the pH of the plant solution and the water and record.
5. Obtain 20 radish seeds. Place 10 on each paper towel in a line across the middle of each bag. See the diagram below.



Baggie with seeds positioned for bioassay.

6. Add 10 mL of test solution to one bag and spread it over the seeds and towel. Do the

same with 10 mL of water in the “Control” bag. After a few minutes when the solution has thoroughly wet the paper towels, carefully close (zip) the bags and place them in indirect light at room temperature.

7. Optional step: Use the same procedure as you did for radish seeds except use corn seeds. Note that only 5 corn seeds will fit into each bag (so use 10 seeds total). Be sure to position corn kernels shoot side up, with the embryo side facing out. If you intend to measure corn shoots, tape the bags to a vertical surface such as a wall or cabinet. Be careful so that the seeds will stay in position. If some slip, they can be repositioned from the outside of the bag.

### Observations:

Record your data on the next page. Observe and record the number and percentage of germinated radish seeds after 24 hours (Day 1), 48 hours (Day 2), and 72 hours (Day 3). Measure and record root lengths in millimeters at each observation. Finally, determine the average root length for each test solution and the control. If you used corn seeds, measurements should be recorded on a longer timescale (i.e. on days 3, 5, and 7) because the seeds take longer to germinate.

## Student-Designed Experiments

Using the methods you learned in the activity above and the “Guide for Student Experimentation” below, design and carry out your own experiment. Question topics you might consider include the inhibitory effects of other plant tissues collected from a natural setting, garden, or grocery store. You could also try developing your own bioassay using different seeds.

### Data Sheet for Radish Seed Bioassay

Incubation Conditions: Temperature \_\_\_\_\_ Light \_\_\_\_\_  
pH of plant extract \_\_\_\_\_

	Day _____	Day _____	Day _____
<b>Control:</b>			
% Germination			
Average Root Length			
<b>Sample:</b>			
% Germination			
Average Root Length			
<b>Sample:</b>			
% Germination			
Average Root Length			
<b>Sample:</b>			
% Germination			
Average Root Length			

### Data Sheet for Corn Seed Bioassay

Incubation Conditions: Temperature \_\_\_\_\_ Light \_\_\_\_\_  
pH of plant extract \_\_\_\_\_

	Day _____	Day _____	Day _____
<b>Control:</b>			
% Germination			
Average Root Length			
Average Shoot Length			
<b>Sample:</b>			
% Germination			
Average Root Length			
Average Shoot Length			
<b>Sample:</b>			
% Germination			
Average Root Length			
Average Shoot Length			
<b>Sample:</b>			
% Germination			
Average Root Length			
Average Shoot Length			

# Guide for Student Experimentation

## Guidelines for Achieving Great Experiments

1. Ask a very specific, testable question.
2. Test a control for comparison (a group that does not receive the experimental treatment).
3. Use a sample size large enough to allow firm conclusions.
4. To understand a whole population, obtain a random sample of that population to avoid bias.
5. Replicate each part of the experiment (at least 3 times).
6. Hold all variables constant between trials except the variable being tested.
7. Collect quantitative data whenever possible.
8. Measure using metric units.
9. Gather data carefully and accurately.
10. Be objective and honest.

## Introduction

**Question:**

**Hypothesis:**

## Materials and Methods

**Independent variable:**

**Dependent variable:**

**Experimental constants:**

**Control:**

**Protocol:**

**Results**

**Data collected:**

**Other observations:**

**Graph(s):**

**Discussion**

**Interpretation of data:**

**Conclusions:**

# Teacher's Guide to "Mean and Green"

## Links to National Science Education Standards

### Grades 5-8:

- Abilities necessary to do scientific inquiry
- Understandings about scientific inquiry
- Regulation and behavior
- Populations and ecosystems
- Diversity and adaptations of organisms
- Science and technology in society

### Grades 9-12:

- Abilities necessary to do scientific inquiry
- Understandings about scientific inquiry
- Interdependence of organisms
- Behavior of organisms
- Science and technology in local, national, and global challenges

## Materials (per group)

- 20 radish seeds
- 10 corn seeds (optional)
- 2 snack size zip closure bags (usually 16.5 cm to 8.5 cm); 2 more are needed if corn seed is also used
- Paper towels ("Pick a Size" is ideal) (29 x 15 cm a towel)
- 10mL graduated cylinder or another measuring device
- Beakers or plastic cups (to mix solutions)
- Water for solutions and control (tap water is fine)
- Mortar and pestle (or some method to crush plant material such as grinding with stones in a plastic container)
- Several pinches of sand
- Funnel
- Filter paper or coffee filter
- Ruler

- Marking pen
- pH paper (optional)
- Plant material to be tested

Note: The student-designed experiments will require more materials.

## Teaching hints

1. **Preparing corn seed:** Any viable corn seed can be used. Garden seed sold in bulk is often treated with pesticide, indicated by an indelible red powder on the kernel. This seed should be washed with soap and rinsed thoroughly. Liquid dishwashing detergent works well for the wash. Place kernels in a pint jar and fill with water and 4 or 5 drops of detergent. Cover and shake periodically for 3 minutes, drain and rinse 3 times with tap or bottled water. Sweet corn kernels are wrinkled, so starchy corn is preferable. Feed corn works well and is one of the best to demonstrate the pale yellow oval shield (cotyledon) on the embryo side. These seeds will require sorting to remove any cracked or insect damaged. Insects leave a black spot at the small end of the kernel.
2. **Selecting plant material:** Teachers and/or students can collect plant material around the school, from home, or from a grocery store. Common examples of known allelopathic compounds can be found in conifers, hickories, eucalyptus, certain rice species, tree of heaven, kalanchoe, lantana, red maple, sweet bay, box elder, mango, broccoli, and especially walnuts which contain juglone. Many grasses including rye and wheat also inhibit the growth of other grass species. Research has shown that phytotoxic chemicals can be produced from

leaves, roots, shoots, flowers, seeds and fruits and the production of these chemicals can vary with the season of the year and the stage in a plant's life cycle. Therefore, testing different parts of a plant can be an interesting experiment.

3. **Series dilutions:** Another option to testing different plant parts is to have the students do series dilutions of their solution to test. Here the 100% solution is the first one made and then 1mL of this solution is diluted with 9 mL of water. This makes a 10% solution. Using only 5 mL of this solution for the experiment, the student can then take 1 mL of the left over 10% solution and add 9 mL of water to make a 1% solution. Five mL of this solution can be used for another test. This experiment can determine how concentration affects toxicity.
4. **Lack of materials:** Allowing each student group to set up their own demonstration will be more meaningful for students. However, if this is not possible, then you can set up one demonstration at the front of the class with student assistance.
8. **Successful student-designed experiments:**
  - Emphasize the “Guidelines for Achieving Great Experiments.”
  - Have enough plant material available for students to use in their own experiments. Having more allows for better experiments that include replicates, controls, and multiple treatments. Alternatively, you could ask students to bring in what they need.
  - Before students design their experiments, tell them how much time they will have.
  - Allow students to present their experiments and lead a short discussion about each one. Encourage other students to ask questions. Talk about characteristics of good experiments, such as controls, replicates, etc.

## References

- Einheillig F. A. and J. A. Rasmussen. 1978. Synergistic inhibitory effects of vanillic and p-hydroxybenzoic acids on radish and grain sorghum. *Journal of Chemical Ecology* 4:425-436.
- Patterson D. T. 1986. Allelopathy. In: Camper, N. D. (Ed). *Research Methods in Weed Science* pp 125-126.

## Web Resources

- Allelopathy – How plants suppress other plants  
<http://edis.ifas.ufl.edu/HS186>
- Environmental Inquiry –  
How are bioassays used in the real world?  
<http://ei.cornell.edu/toxicology/bioassays/Uses.asp>
- Inhibitory Growth Effects of Conifers  
[www.saps.plantsci.cam.ac.uk/docs/conifer.doc](http://www.saps.plantsci.cam.ac.uk/docs/conifer.doc)

## Acknowledgements

Credits for figures are as follows: Mean cactus, ©iStockphoto.com/Mark Stay; Scientist, ©iStockphoto.com/pidjoe. This work was funded by the Education Foundation of the American Society of Plant Biologists.

