



Hughes
Undergraduate
Biological
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Initiative



THE SECRET LIVES OF PLANTS : EXPLORATIONS IN ALLELOPATHY

Have you ever observed a landscape containing several different plant species? Was the distribution of different species random, or did the plants appear to cluster in a non-random pattern?

Examine the drawings in Figure 1. Assume that each different shape is a different species of plant in a field containing several individuals of three different species. Compare Field 1 and Field 2. Try to think of as many hypotheses as possible to explain why the plants are distributed non-randomly in Field 2. Assume that there has been no intervention by humans. *HINTS: Think about how the seeds might be dispersed. Think about what factors might affect how well seeds germinate and how well adult plants grow.*

Write your hypotheses here.

Many factors influence the distribution of plants in mixed species fields and forests. One potential factor affecting the germination and growth of plants is **allelopathy**. The term allelopathy describes the chemical interactions between plants. Allelopathy may describe interactions in which one plant inhibits (prevents or decreases) the growth or germination of another plant. For example, black walnut trees have long been observed to inhibit the growth of other plants near them. However, the term *allelopathy* may also describe interactions in which one plant stimulates (increases or speeds up) the germination or growth of another plant.

1.) Could allelopathy account for the distribution of plants in Field 2 of Figure 1? Please explain.

2.) How would you test for allelopathy? Think about what kind of experiment you might do to test for inhibitory or stimulatory effects of chemicals from one plant on another plant. Answer the following questions.

a.) How would you measure the amount of inhibition or stimulation?

b.) What conditions would you manipulate or vary in your experiment?


c.) What conditions would you hold constant?

d.) What would your control be?

e.) How many replicates would you do?

Testing for Allelopathy:

The following methods can be used to test for allelopathy. You may be able to think of other ways to test for allelopathy that are different from these methods. If so, you may want to run both types of experiments and compare your results.

 **STEP ONE:** Gather materials. You will need the following materials to complete the experiment:

Distilled or deionized water

Clean glass or plastic containers with lids (one per plant being tested)

Markers for labeling

Chopping knives and cutting board

Cheese cloth or strainer

Petri dishes (1 per replicate. One replicate = one petri dish with 10 seeds)

Filter paper disks, same size as petri dishes (1 per replicate)

Pasteur pipettes or droppers

Seeds {suggestions: **spinach** (Chenopodiaceae), **green beans** (Fabaceae), **lettuce** (Asteraceae), **radish** (Brassicaceae), **broccoli** (Brassicaceae), **cabbage** (Brassicaceae)}
10 per replicate

You may use just one type of seed or several. If using several types of seeds, do at least one replicate per seed type per extract type.

Plant material:

suggestions:

Cabbage (*Brassica* sp.) (Brassicaceae) -- leaves

Parsley (Umbelliferae) -- leaves

Amaryllis (Amaryllidaceae) -- bulb

Walnut (*Juglans* sp.) (Juglandaceae) – hull

Radish (Brassicaceae) – leaves or root

You may test other plant material in addition to these four.

☞ **STEP TWO:** Prepare extracts of the plants you will be testing for their allelopathic effects on other plants. To prepare each extract, chop up the plant and soak pieces overnight in distilled or deionized water. Make sure to wash knives and cutting board between preparation of extracts. After soaking, strain the liquid through cheese cloth or a clean metal strainer to remove solids. Store extracts in sealed, labeled containers in the refrigerator when not in use.

☞ **STEP THREE:** Decide how many replicates will be done using each extract plus the control (plain, distilled or deionized water). One replicate = one petri dish with 10 seeds. For each replicate, complete the following steps.

- 1.) Place a clean filter paper disk in the bottom of a petri dish.
- 2.) Label the bottom of the dish with the type of extract that will be used or "control" if it is a control.
- 3.) Using a pasteur pipette or dropper, wet the filter paper with one of the extracts or with water if this is a control. Make sure the paper is damp but not overwet.
- 4.) Place ten seeds (all the same type) on the surface of the filter paper.
- 5.) Place in a dark warm (25 C) place for 1 week.
- 6.) Continue to wet filter paper as needed. Additional wetting should be done with the same extract or with water for a control replicate.

☞ **STEP FOUR:** Obtain the results.

- 1.) For each replicate, count the number of seeds that germinated and measure the length of the seedling.
- 2.) Calculate the mean length for each replicate by summing the lengths of the seedlings and dividing by the number of seeds that germinated for that replicate.

Example: Six out of ten seeds in one of your replicates germinate. The lengths of these 6 seedlings are 1 mm, 2 mm, 4 mm, 2mm, 3mm, and 3mm. The mean is

$$1 + 2 + 4 + 2 + 3 + 3 = 15, \quad 15/6 = \underline{\underline{2.5 \text{ mm}}}$$

- 3.) Record your data in the tables provided.


Table 1: Average seedling length and # seeds germinated in each replicate of one seed type exposed to one extract type.

(You will need a separate copy of this table for each extract and seed type combination you test.)

Extract type: _____ **Seed type:** _____

replicate number	seedling 1	2	3	4	5	6	7	8	9	10	#seeds germinated	average seedling length

Average # seeds germinated = _____ **Average seedling length =** _____

 **STEP FIVE: Discuss your results and draw conclusions.**

1.) Do your data support that one or more of the plants tested have allelopathic effects on other plants? Which plants?

2.) Explain the evidence from your data that supports this conclusion.

3.) Were there any unexpected results? Can you think of any possible explanations for these unexpected results?

4.) Are there any questions that you might ask if you were able to do additional experiments?

Challenge questions: What factors might select for allelopathy in plants during the course of evolution? Can you think of an experimental design that would test one of your hypothesized factors? Describe your hypothesis and the experiment you would use to test it.