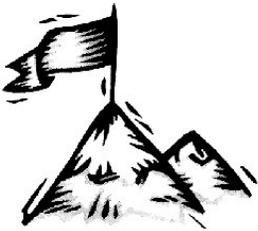


Lab 4 – The Scientific Method and Allelopathy



I. Central questions

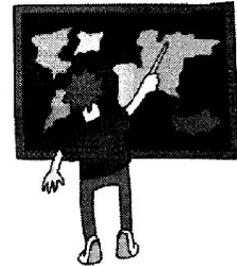
- How does the scientific method work?
- How do plants affect each other?



II. Learning Objectives

At the end of this lab you will be able to

- Understand the process of the scientific method
- Design and set up a controlled experiment to test a hypothesis
- Understand the concept of allelopathy and its role in plant interactions



III. Introduction and Background

In the past two weeks you have learned how biologists classify and identify organisms and also how scientific observations can lead to questions and hypotheses. This week we will focus on the “heart” of the scientific method: hypothesis testing.

All science depends initially on **observation** to identify a problem or pattern of interest. What scientific research does is seek to answer “how” or “why” that pattern or problem exists. The first step of the scientific method is to develop a **hypothesis** based on your observations and relevant background information to explain the problem. Often there are multiple hypotheses that could potentially be correct. The job of the scientific method is to investigate these hypotheses to eliminate or reject those that are incorrect. This is done by formulating **predictions** that follow from each hypothesis, and then **testing** those predictions either by rigorous further observations or by experiment. Note that an experiment is not always necessary to falsify a hypothesis---in fact, some branches of science such as astronomy rarely use experimentation---but where possible, a manipulative experiment is usually the most effective way to establish cause and effect. If the test results do NOT support the predictions of a particular hypothesis,

then this hypothesis can be rejected. If the results DO support the predictions of only one hypothesis, then the hypothesis can be tentatively accepted.

Now we are ready to apply the scientific method to a real question about plant competition. There are many ways that organisms of different species compete with one another for resources. This is known as **interspecific** (between-species) competition. When you think of the word 'competition', you might imagine individuals moving around, aggressively interfering with each other's access to resources. This may sometimes be the case for highly mobile animals, but what about less mobile organisms like plants?

You might have observed that under different tree species there are different amounts of plant growth. What causes this variability? One possibility is **allelopathy**, by which some plants chemically inhibit the germination, or growth of competitors. The word comes from the Greek words *allelon*, meaning "of each other", and *pathos*, meaning "to suffer". The chemicals may be released from plant tissues directly into the air (volatile chemicals), or released into the soil from the roots or from decomposing leaves.

The phenomenon of allelopathy has been known for over 2000 years, since it was noticed that certain plants including chick peas (*Cicer arietinum*) inhibit weeds and some crop plants from growing around them. Other examples of plants with allelopathic effects include the black walnut tree (*Juglans nigra*), as well as black mustard (*Brassica nigra*), an introduced Mediterranean plant that is an invasive species in California grasslands (see <http://www.calflora.org>).

This 'chemical warfare' between plants may have potentially important applications for agriculture. For example, when planning a crop rotation schedule, it is important to know if certain crops leave chemicals in the soil that may inhibit growth of future crops. Asparagus is known to have such effects, especially on future asparagus growth. This **intraspecific** (within-species) allelopathy is known as **autotoxicity**. Allelopathy is also of interest as an alternative method of weed control. Instead of traditional herbicides, plants such as rye that produce allelopathic chemicals could be used as cover crops to control weed growth. Alternatively, allelopathic chemicals could potentially be identified, isolated and synthesized to be used as 'natural herbicides'.



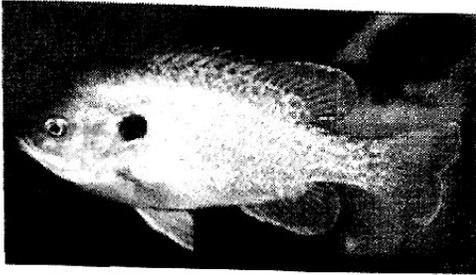
IV. Lab instructions

Work in a group of

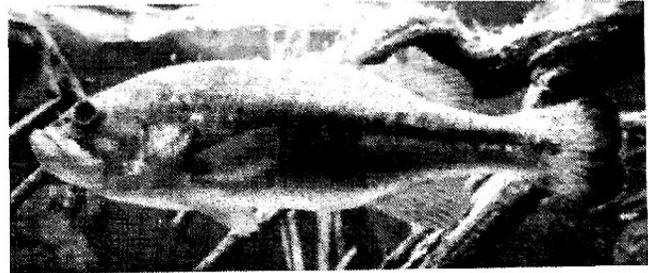
Exercise 1. Designing an Experiment (this part of the laboratory was designed by Aviva Liebert)

You should already have some background in the scientific method, so we will review the process briefly. We'll start with a discussion of a "virtual" experiment so that we can review the important steps of the scientific method before you dive in and design a real one yourselves.

Imagine that you have gone fishing in several ponds containing two types of fish, bluegill sunfish (*Lepomis macrochirus*) and largemouth bass (*Micropterus salmoides*).



Bluegill Sunfish



Largemouth Bass

Photos by Jeff Grabarkiewicz, Wayne Davis, and Todd Crail
http://www.epa.gov/bioiweb1/html/photos_fish_freshwater_sunfishes.html

You have noticed that in ponds where you find more bass, there are fewer bluegill. After seeing this pattern several times, you develop the hypothesis that the bass are predators of the bluegill and are therefore driving down their numbers.

Working together in groups of 3-4, answer the following questions and prepare to share your answers with the rest of the class.

1. What alternative hypothesis might explain the observed pattern?

2. What specific predictions follow from the different hypotheses?

3. What simple experiment could you do to test these predictions?

4a. In your experiment, what is the independent variable? _____

4b. What is the dependent variable? _____

5. What variables need to be controlled? _____

6. How often should you replicate your experiment to gain sufficient confidence in your results?

7. Let's say you replicated your tests an appropriate number of times and your results were always supporting one of your hypotheses. Have you proven that this hypothesis is correct? Why (not)?



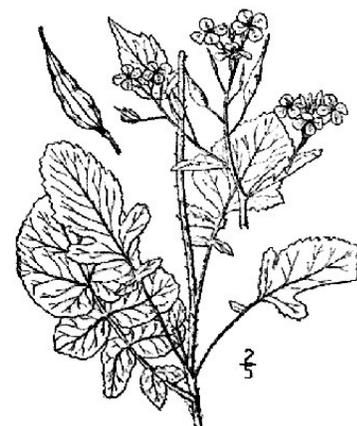
Where we are headed

- In this lab, we are practicing the scientific method and experimental design. This will help us in our final comparison between the values of the native plant garden and the more “exotic” area on campus. It will also help you understand and evaluate experiments done by other people.
- Allelopathy is but one way that plants can negatively affect each other. Keep this in mind when thinking what allows certain plant species to become invasive.

Exercise 2. Allelopathy

Let's investigate the effect of ground-up leaf litter from two different tree species on the germination rate of a fast growing plant: radish (*Raphanus sativus*).

1. In a group, go out on campus and observe growth patterns of plants under different plant species. As a class, agree on two plant species to test, preferably those with different growth patterns beneath.



Radish (*Raphanus sativus*); USDA-NRCS PLANTS Database / Britton, N.L., and A. Brown. 1913. Illustrated flora of the northern

2. Per group of four, collect a small handful of pure leaf litter of those two tree species. You will work in groups of four; however, the whole class should work on the same tree species – *why?*
3. Come back to class.
4. Formulate the research question.

5. Formulate multiple (2) hypotheses.

6. For each hypothesis, formulate a prediction.

These are the materials each group has available to them:

six 5-inch pots
potting soil
radish seeds

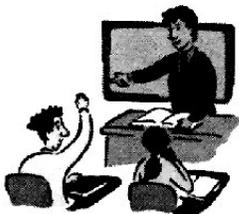
blender
measuring cup

7. Describe the test you are planning to conduct. Be as specific as possible. What are your independent, dependent, and control variables?

8. Are there any other variables that might influence your results that you cannot control?

9. As a class, discuss the experimental designs of each group. Agree on one procedure, to ensure that you have **replicates**.
10. Set up your experiments. In your lab notebook, make note of the procedure used. Be sure to write down specific details including numbers of seeds, amounts of ground litter, volume of water to be added, etc.
11. Before you leave class, devise a plan to water your plants between this class and the next class meeting, and turn in this handout.

Next week we will collect data and work on analyzing and interpreting the results of this experiment.



V. Review questions

1. What are the steps of the scientific method?
2. Define the terms control variable, independent and dependent variable.
3. Why is it beneficial to have multiple hypotheses?
4. Define allelopathy, give an example.
5. Why should everyone in the class follow the same experimental set-up?
6. Critique your original experimental set-up in light of the set-up the class is doing now.



VI. Homework assignment

- Answer the review questions above and turn them into your instructor at the beginning of next week's lab.