Allelopathy

Chapter 1: Introduction

History

Theophrastus (ca. 300 B.C.E.), a student and successor to Aristotle, wrote about allelopathic reactions in his botanical works. He has been called the "father of Botany", and wrote of how chickpea "exhausts" the soil and destroys weeds.

In 1 C.E., Gaius Plinius Secundus, also known as Pliny the Elder, a roman scholar and naturalist, wrote about how chick pea and barley "scorch up" cornland. He also mentioned that Walnut trees are toxic to other plants.

Augustin Pyramus De Candolle, a botanist and naturalist, in 1832, suggested that soil sickness was caused by chemicals released by the crop.

And, in 1907-1909, two researchers, Schreiner and Reed investigated the isolation of a number of phytotoxic chemicals from plants and soils.

What is Allelopathy?

The word allelopathy derives from two separate words. They are *allelon* which means "of each other", and *pathos* which means "to suffer". Allelopathy refers to the chemical inhibition of one species by another. The "inhibitory" chemical is released into the environment where it affects the development and growth of neighboring plants.

Allelopathic chemicals can be present in any part of the plant. They can be found in leaves, flowers, roots, fruits, or stems. They can also be found in the surrounding soil. Target species are affected by these toxins in many different ways. The toxic chemicals may inhibit shoot/root growth, they may inhibit nutrient uptake, or they may attack a naturally occurring symbiotic relationship thereby destroying the plant's usable source of a nutrient.

Are all plants Allelopathic?

Not all plants have allelopathic tendencies. Some, though they exhibit these tendencies, may actually be displaying aggressive competition of a non-chemical form. Much of the controversy surrounding allelopathy is in trying to distinguish the type of competition being displayed. In general, if it is of a chemical nature, then the plant is considered allelopathic. There have been some recent links to plant allelotoxins directed at animals, but data is scarce.

Environmental Impact

Allelopathy is a form of chemical competition. The allelopathic plant is competing through "interference" chemicals. Competition, by definition, takes one of two forms--exploitation or interference.

Competition is used by both plants and animals to assure a place in nature. Plants will compete for sunlight, water and nutrients and, like animals, for territory. Competition, like parasitism, disease, and predation, influences distribution and amount of organisms in an ecosystem. The interactions of ecosystems define an environment.

When organisms compete with one another, they create the potential for resource limitations and possible extinctions. Allelopathic plants prevent other plants from using the available resources and thus influence the evolution and distribution of other species. One might say that allelopathic plants control the environments in which they live.

For Discussion

How can one tell whether a plant is exhibiting allelopathy as opposed to non chemical competition?

Can allelopathic chemicals affect animals, including humans?

How did people first become aware of allelopathy?

Have you noticed allelopathy or allelopathic-like influences in your neighborhood? Elsewhere?

Why is studying allelopathy important?

What are some beneficial implications of allelopathy? How can these be used for the betterment of the planet?

How would you go about testing for allelopathy?

Can you name or identify some allelopathic plants?

Focus: Inquiry based learning

This unit on Allelopathy is inquiry based. The labs and field trips are hands-on and all of the activities require constant student interactions. Because of this, the unit can be modified and adjusted without negative affect.

Chapter 2: Chemical Warfare in the Plant Kingdom

Black Walnut

One of the most famous allelopathic plants is Black Walnut (Juglans nigra). The chemical responsible for the toxicity in Black Walnut is Juglone (5 hydroxy-1,4 napthoquinone) and is a respiration inhibitor. Solanaceous plants, such as tomato, pepper, and eggplant, are especially susceptible to Juglone. These plants, when exposed to the allelotoxin, exhibit symptoms such as wilting, chlorosis (foliar yellowing), and eventually death. Other plants may also exhibit varying degrees of susceptibility and some have no noticeable effects at all. Some plants that have been observed to be tolerant of Juglone include lima bean, beets, carrot, corn, cherry, black raspberry, catalpa, Virginia creeper, violets, and many others.

Juglone is present in all parts of the Black Walnut, but especially concentrated in the buds, nut hulls, and roots. It is not very soluble in water and thus, does not move very rapidly in the soil. Toxicity has been observed in all soil with Black Walnut roots growing in it (roots can grow 3 times the spread of the canopy), but is especially concentrated closest to the tree, under the drip line. This is mainly due to greater root density and the accumulation of decaying leaves and hulls.

Tree-Of-Heaven

The Tree-Of-Heaven, or Ailanthus (Ailanthus altissima) is a recent addition to the list of allelopathic trees. Ailanthone, an allelotoxin extracted from the root bark of Ailanthus, is known for its "potent post-emergence herbicidal activity". Ailanthus poses a serious weed problem in urban areas.

Sorghum

The major constituent of sorghum that causes allelopathic activity is sorgolene (2-hydroxy-5-methoxy-3-{(8'z,11'z)-8',11',14'-pentadecatriene}-p-benzoiquinone). Sorgolene is found in the root exudates of most sorghum species and has been shown to be a very potent allelotoxin that disrupts mitochondrial functions and inhibits photosynthesis. It is being researched extensively as a weed suppressant.

Others

There are many other known allelopathic species, and many that are highly suspected of being allelopathic including various wetland species, grasses, and other woody plants such as Fragrant Sumac (Rhus aromaticus). Tobacco (Nicotiana rustica), Rice (Oryza sativa), Pea (Pisum sativum), and many others, are known to have root allelotoxins.

For Discussion:
Have the students research and discuss other allelopathic plants.

Chapter 3: Procedures and Protocol

Protocol 1. Learning To Identify Signs Of Allelopathy

The best way to study allelopathy is to find signs of it occurring in nature. It is impossible to "see" the toxins at work, but it is possible to see the signs and symptoms caused by the chemicals on surrounding plants. For example, very few plants grow under a Black Walnut and those that do often times look sickly and chlorotic. This is a sign of the allelotoxin, Juglone, at work.

Along with recognizing the signs of allelopathy, one must also be able to identify the plants. Some allelopathic plants, such as Black Walnut, grow in our backyards and on our streets and are easy to identify. Others, like sorghum or chick pea, may be easier to find in rural areas where they are grown as crops or alongside farm land. Some allelopathic plants, especially many of the wetland species, may require special field trips and extra time to find them first and then identify them.

Protocol 2. Harvesting Plants and Plant Parts

Many of the known allelotoxins are very expensive and not easy to come by. Some companies such as Sigma Chemical and Caroline Biological may carry the chemicals, but in solid form that will require extra time and effort to bring to a soluble form that can be used in the lab. However, not every class will have the funds or the access to these chemicals. Thus, it may be that the only way to run the experiments is to have the class harvest their own allelotoxins.

Some research will be required to investigate what plant parts have the highest concentrations of allelopathic species. For instance, the Juglone found in Black Walnut can be found throughout the plant but particularly within the nut hulls, leaves, and roots. Therefore, a class project may be to break into groups and harvest each part and test them accordingly.

It is important when harvesting plants or plant parts to be sure that the plant is not endangered and to be sure that the procedure is carried out in such a way as to bring no harm to the plant or the surrounding area. Of course, in the case of harvesting the entire plant, accommodations must be made.

This field exercise can be done when the class is identifying the allelopathic plants as described in Protocol 1, or can be done as a separate exercise.

Protocol 3. Testing for Allelopathy in the Lab

The effects of allelopathic toxins on sensitive plants can easily be tested in the lab or greenhouse setting. Seeds are the easiest and least expensive to test. Seeds that do not germinate in the presence of allelotoxins are probably displaying toxicity effects.

Plants that become chlorotic and eventually die in the presence of allelotoxins are also showing signs of toxicity to the chemical.

Solanaceous crops, such as tomatoes and peppers, are most susceptible to juglone (the allelotoxin found in Black Walnut trees). The laboratory setting is the perfect place to test the susceptibility of certain plants to various alleltoxins.

Other scientific or research based concepts, such as graphing, dilutions, and general lab protocol will also be covered when certain allelopathy activities are conducted in the lab or classroom setting.

Procedures

1. Familiarize yourself with the allelopathic species in your area. In particular, focus on mature species that are established. These tend to have higher concentrations of the allelotoxin and thus will display better signs and symptoms on any susceptible surrounding plants.

2. If possible, contact a local conservation organization or extension agency, that might have some insight about allelopathy. Your research may be of interest to them and they may offer professional advice or important information.

3. Decide which species and areas should be the focus of your survey.

4. Decide on a survey method. For instance, you may want to conduct the identification field trip one day and then follow up with the harvest field trip another, or you may want to conduct both on the same day.

5. Learn how to identify the species that you will be studying. There are many good Field Guides available, as well as many excellent web sites.

6. Decide how to divide up the area you will be working in.

7. Record what allelopathic signs and symptoms were found, and the species they were found by.

8. Discuss ways to study allelopathy in the laboratory.

9. Gather needed materials. See the materials list at the end.

Lab & Classroom Exercises

A} Identifying Allelopathic Plant Parts: Black Walnut

Research Focus

Have the students discuss why they believe certain plant parts may be more allelopathic than others. Have them conduct some research into this. They can scan the web, be involved in personal communications with professionals in the field, or visit a library.

For Discussion:

*Why are some parts more allelopathic than others?
*How does this affect the organism's ability to out-compete other plants?
*Does this change from season to season? Day to night?
*How can we test these parts and their properties?

Field Harvesting

Conduct a field trip to harvest parts of the Black Walnut tree. These parts will be brought back to the classroom for further investigation and observation.
Students should collect the following:

1. Leaves
   *5-10 leaves per group
   *Place them in paper bags with the group's name or number on them and where on the tree canopy the leaves were collected from.

2. Nut Hulls
   *2-3 nut hulls per group
   *Note: Black Walnut nuts stain skin and clothes.

3. Roots
   *One bunch of roots for the entire class is sufficient
   *Dig a clean hole, CUT a small amount of roots by the drip line
   *Replace the soil
   *Removal of roots is invasive and should ONLY be done if the teacher and students are confident in their techniques and methods.

4. Soil
   *Have students remove soil from a range of areas starting at the trunk of the tree and moving out beyond the drip line.
   *One bucket-full per group will be sufficient

5. Other

Laboratory Procedures

Leaves:

1. Prepare jars with tomato or pepper seeds
    *Place tissue in bottom jar
    *Make moist
    *Place seeds on moist towels (usually ten seeds per treatment)

2. Crumble leaves and place in cheesecloth

3. Rubber band or tie the cheesecloth closed and place into mouth of jar so that the leaf bundle dangles over, but does not touch, the seeds.

4. Place jars in windowsill or under grow light.

5. Observe

Nut Hulls:

1. Using a blender, food processor, or hammer, pulverize the nut

2. Decide on whether to add water or leave as is (a comparison of both ways may be best)

3. Place the nut juice in a petri dish that has been lined with paper towel.

4. Place ten seeds into each prepared petri dish

5. Observe.

Roots:

See Nut Hulls Procedure Above

Soil:

1. Place some potentially allelotoxic soil into containers.
2. Place a known un-contaminated soil into other containers (control)
3. Plant seeds into each
4. Observe

Note: Tomato plants can be used instead of seeds where appropriate.

B} Allelopathy: Tomato Seed Dose/Response Bioassay

Research Focus:

Have students discuss the idea of only certain amounts of allelotoxins being effective against other organisms. Have them conduct some research into this. As with the afore mentioned activity, they can scan the web, visit a library, or be involved in personal communications with a professional in the field.

For Discussion:

*Why would certain amounts of allelotoxins be more effective than others?
*How would this affect susceptible organism's defenses?
*What other factors may be involved?
*How can we test for this?

Doing Dilutions to Test a Chemical’s Toxicity Thresholds

Conduct a laboratory experiment where certain allelotoxins are tested at varying dilutions or concentrations. These dilutions, once observed and analyzed, should give some insight into what amounts of that chemical are most effective against other organisms. This particular experiment uses tomato seeds as a bioassay because tomatoes are a known susceptible species to Juglone. However, the class can decide on other seeds or plants to use (in the case of plants, the dilutions would be poured into the containers or into the solution in the case of hydroponics).

Allelopathy: Tomato seed Dose/Response Bioassay Data Sheet

Name
Date
Chemical tested
100% Concentration
Length of Experiment

Table 1: Seed Germination Data

Concentration (%)	Concentration (mg/L)	# Seeds Germinated/Dish	Average # Seeds Germinated
Control			
0.001%			
0.01%			
0.1%			
1%			
10%			
100%			

Table 2: Radicle Length Data

Concentration (%)	Radical Length (mm)	Average Length (mm)
Control		
0.001%		
0.01%		
0.1%		
1%		
10%		
100%		

What are students looking for?

1. Whether or not seeds germinate.

2. Whether or not plants become affected.

3. For changes in reactions according to treatments.

4. Other: Let them decide what to look for and why.

General Notes on above procedures:

Solanaceous crops, such as tomatoes, peppers, eggplants, etc., are most susceptible to juglone and are thus best to use.

Tomato, eggplant, or pepper plants can be used instead of seeds where appropriate.

Juglone is generally non-toxic to humans but, as with any substance, individuals may be sensitive and might react. It is recommended that pregnant women not come in direct contact with crystallized juglone.