LABORATORY OVERVIEW:

**Allelopathy** is an interaction between plants where one species releases biologically active compounds into the surrounding environment that affect other plants, most often in an inhibitory way (Golisz, 2008). Common effects of allelopathy include reduced seed germination, reduced seedling growth, and reduced survival rates. Allelopathy can be considered a form of **antibiosis** and when used to suppress weeds can be considered a biological control. It can also be considered a form of cultural control, especially if the crop itself is allelopathic.

Allelopathy may play an important role in the success of invasive species, and could be used to suppress weeds through cover crops (Ridenour, 2001, Bais, 2003, Bertholdsson, 2005). Both weed and crop plant species have the potential to be allelopathic. Determining if allelopathic inhibition is actually occurring in a system is a multistep process. First, an investigator will determine if an extract of a plant affects the growth of other plants in the lab through a bioassay. If the extract has an affect, then the investigator will attempt to demonstrate allelopathic inhibition in the field, as well as isolate the suspected allelopathic compound.

LABORATORY OBJECTIVES:

1. Investigate the phenomenon of allelopathy.
2. Determine the allelopathic potential of two plant species.
3. Discuss the implications of allelopathy for the management of agroecosystems.
Laboratory Activities:

1. Setup seed germination arenas
2. Hypothesize results
3. Break down arenas
4. Data analysis
5. Lab report

Laboratory Equipment:

1. 50 petri dishes
2. Clean, sterile sand
3. 100 #1 9cm diameter filter paper discs
4. Wheeler Rye plant material
5. Buckwheat plant material
6. Lettuce Seeds
7. Giant Foxtail Seeds
8. 25 soaking jars
9. 25 syringes
10. 4 1 L beakers
11. Vacuum filter apparatus
12. 4 plastic tubs
13. Lab Tape
14. Black Sharpie
15. Forceps
16. Parafilm
17. Incubator
18. Refrigerator
19. Ruler
20. Distilled Water
21. Notebook and Pencil/Pen
22. Computer for Analysis and Write Up

Laboratory Procedures:

Pre-Lab Preparation:

Five Wheeler Rye seeds were each planted in 10 pots on 10/25/10 in the greenhouse. The pots were watered every 2-3 days. Five buckwheat seeds were each planted in 10 pots on 10/25/10 in the greenhouse. The pots were watered every 2-3 days. Plant material was harvested on 11/08/09. The collected plant material was dried in a food dehydrator for 24 hours. 2.5 g and 7.5 g of both Wheeler Rye and Buckwheat plant material were weighed out and placed in a 1L beaker. 200mL of distilled water was added to each 1L beaker, and the plant material soaked in it for 2 hours at room temperature. The resulting solutions were filtered under vacuum to obtain a 1.25% and 3.75% leachate of each plant. Each leachate was placed in an appropriately labeled tub for common use by the investigation teams.
Day 1 (November 9):

1. Form groups of 4-5 people, for a total of 5 teams. Each team will prepare a complete replicate (2 plant species: foxtail and lettuce) x 5 treatments (4 leachates plus a control treatment of distilled water).

2. Obtain 50 seeds of all three plant species. Distribute the seeds evenly among five soaking jars (4 leachates + distilled water). Label each jar with one of the five leachate/treatments, and then add just enough of the leachate or distilled water to the appropriate soaking jar to cover the seeds. Leave the seeds to soak for 1 hour.

3. Next, prepare the bioassay chambers. Obtain 15 petri dishes. Weigh out 36g of sand into each petri dish, and level the sand. Place a filter paper disk on the sand surface in each dish. Use lab tape to label the lid of each dish with the date, team (use last name initials), seed species, and treatment type.

4. Once the bioassay chambers have been prepared, you will irrigate each chamber with the appropriate treatment. Using clean syringes each time, add 8ml of each treatment to the correct dishes. Each treatment should only have to be added to 3 dishes.

5. (If there is a shortage of syringes, you can get by with two per team. Use one syringe to add the distilled water first, then the 1.25% Wheeler Rye leachate, and finally the 3.75% Wheeler Rye leachate. Use the second syringe to add the 1.25% Buckwheat leachate, and then the 3.75% Buckwheat leachate. (In that order.)

6. Develop a hypothesis and predictions.

7. Once the seeds have soaked for an hour, you may add them to the chambers. Using forceps, place 10 seeds into each dish and arrange them in a circle.

8. Take 15 filter paper discs and soak 3 in each treatment. Place the soaked filter paper discs in the appropriate dishes to cover the seeds.

9. Place the correctly labeled petri dish lids on the bioassay chambers, and seal them with parafilm.

10. Finally, carefully place all of your bioassay chambers in the box. They will be allowed to sit at room temperature for 72 hours, long enough for the seeds to germinate. Once they have germinated, the instructor will transfer the bioassay chambers into the refrigerator to arrest growth until the next lab session.

Day 2 (November 16):

1. Gather your bioassay chambers from the refrigerator or box. For each dish, measure with a ruler the length of the hypocotyl (root) and the epicotyl (shoot) of each seed that germinated to the nearest millimeter. Record the data on your datasheet, and enter a dash if there is not a measurable root or shoot. Also, record the number of seeds that germinated and the number of seeds that did not germinate.

2. Sum up the total root length and total shoot length for each chamber. Calculate a combined total for roots and a combined total for shoots for each treatment. Determine the number of observations for roots and shoots in each treatment. Then calculate the mean root length and the mean shoot length for each treatment.
3. Use the supplied Excel worksheet to compare root and shoot lengths of the different treatments.

4. Analyze your results and write up a lab report.

**Laboratory Report:**

You will be required to write a laboratory report for this lab. It will be due on November 25, 2009. The lab will need to include figures showing results as well as the statistical analysis (you will be provided with the latter). Please write the report as if you were writing a journal article (i.e., do not merely answer the questions posed in the discussion). Your lab report should include the following sections:

1. **Introduction** (include the following)
   1. Introduce the topic using the provided information.
   2. Pose the hypotheses that were tested.

2. **Materials and Methods** (include the following)
   1. List the pre, during, and post lab methods as well as the data analysis steps taken.

3. **Results** (include the following)
   1. Present the results of the experiment including which varieties and treatments had significantly more infection.
   2. Create numbered figures showing mean percentage ± Standard Error of The Mean (SEM) root growth and seeds germinated (see the example in the provided analysis pack). Refer to these figures (by number) in the appropriate places in the results and discussion.

4. **Discussion** (include discussion of the following)
   1. Which plants appeared most prone to allelopathic effects? How might this influence organic planting and weed management strategies?
   2. How reliable do you think the test used in this lab is to predict allelopathic potential in the field?
   3. Is allelopathy an example of biological control or cultural control?
   4. How might things be done differently in future experiments?

5. **References**

**References:**


