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 effect, 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.
4. Learn how to write and interpret scientific papers/reports.
Laboratory Activities:

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

Laboratory Equipment:

1. 40 petri dishes
2. Clean, sterile sand
3. 100 #1 9cm diameter filter paper discs
4. Black walnut plant material
5. Buckwheat plant material
6. Lettuce seeds
7. Barley seeds
8. 20 soaking jars
9. 8 syringes
10. 4 1 L beakers
11. Vacuum filter apparatus
12. 4 plastic tubs
13. Sharpies
14. Forceps
15. Parafilm
16. Refrigerator
17. Ruler
18. Distilled Water
19. Notebook and Pencil/Pen
20. Computer for Analysis and Write Up

Laboratory Procedures:

Pre-Lab Preparation:

Mature, pre-senescent buckwheat leaves and stems and black walnut leaves were collected on September 15, 2011 and October 5, 2011, respectively. The collected plant material was dried in a food dehydrator for 24 hours. 2.5 g and 7.5 g of both black walnut 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 (October 6):

1. Form groups of 2-3 people, for a total of 4 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) = 10 dishes per group.

2. Obtain 50 seeds of both 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% black walnut leachate, and finally the 3.75% black walnut 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. 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 (October 11):

1. Gather your bioassay chambers from the box. For each dish, record the number of seeds that germinated and measure 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.

2. Use the supplied Excel worksheet to compare root and shoot lengths of the different treatments.

3. 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 October 20, 2011. 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. Explain the overall goal of our study.
   3. 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 used (i.e. chi squared, t-tests). You do not have to state that we calculated means and standard errors.

3. **Results** (include the following)
   1. Present the results of the experiment including which varieties and treatments had significantly less germination or growth. **Be sure to reference your figures and provide numerical data** (i.e. lettuce seeds treated with black walnut leachate had significantly less growth than the control with an average of 2 mm and 4 mm growth, respectively (Fig. 2)). **DO NOT JUST STATE WHICH ONE WAS HIGHER or LOWER.**
   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 excel sheet). 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. Include references to at least 2 additional papers—beyond those listed in the lab—on allelopathy and how their results and conclusions match up with ours (one on buckwheat and one on black walnut). **Hint: search for “buckwheat allelopathy” or “black walnut allelopathy” on google scholar (www.scholar.google.com).**

5. **References**
   1. List the papers cited in your introduction and discussion.
   2. Use the laboratory overview as a model for how to cite papers within the text.
   3. Use the following references cited as a model for your references. Use the Entomological Society Journals format with full journal names (not abbreviations) see http://www.entsoc.org/pubs/publish/style/#References_Cited for more details.
References:


