Lab 9: Competition in Plants: Allelopathy

Please Read and Bring With You to Lab

What you should bring:

Notebook (for recording data; you will not turn it in) & pencil or pen.
This handout

What you will be provided:

Per group:

3 Petri dishes (both top and bottom), 10 cm diameter
6 sheets of round filter paper, 6-9 cm diameter
Lab marker
Lettuce seeds (at least 150)
≈10g of Brittlebush leaves
≈10g of Lettuce leaves
Mortar and pestle
Distilled water
Cheesecloth
10 ml pipettes

Objectives:

- To determine if extracts from brittlebush leaves contain allelopathic compounds.
- To test deviations from a random outcome with goodness of fit tests.

Preparation:

Students should read this handout and Chapter 13 "Competition" in Molles or similar ecology text.

Introduction

Competition in plant communities can be quite intense. Plants are known to produce a wide variety of chemicals, called secondary metabolites. Plants use these compounds for a variety of functions, including defense against herbivory and in interspecific competition. Allelopathy is the production of specific biomolecules by plants that negatively influences the growth and development of neighboring plants. How might this structure the pattern of plants in natural ecosystems? Competition in deserts may be especially intense due to the scarcity of both water and soil nutrients.

Plants may release allelopathic compounds through a variety of mechanisms, including via the roots, secreted and washed off leaves, or released as fallen leaves decompose. Several of the common local desert plants release allelopathic compounds. Creosote bush (*Larrea tridentata*), for example, releases allelopathic compounds via its roots that suppress root development by other plants. Brittlebush (*Encelia farinosa*) is a common perennial plant present in the Arizona Uplands division of the Sonoran Desert. It is a small shrub that plays an important role in creating microclimates that may facili-

tate the establishement of other plants (i.e., they act as nurse plants). We might therefore expect that, like creosote, it will will try to protect its resources with allelopathy.

In this laboratory you will determine the effect of the secondary metabolites of brittlebush. We will test the effect on lettuce (*Lactuca sativa*) seeds, as these germinate easily and rapidly given the laboratory conditions.

Procedure

1. You will work in groups of 3 for this lab.

2. Synthesizing the extracts.

A. Take a handful of lettuce leaves and grind them in a mortar for about 2 minutes.

B. Weigh out 5 g of ground lettuce and place it in a small beaker. Add 50 ml of water to the beaker, and press down the lettuce to make sure it is submerged.

C. Let the lettuce leaves steep for 30-60 minutes.

D. While the lettuce is steeping, thoroughly clean the mortar and pestle.

E. Repeat the above steps with the brittlebush leaves and let these steep for at 30-60 minutes as well. Note: *always prepare the lettuce first, before the brittlebush*.

F. Prepare two funnels by taking a piece of cheese cloth and folding it several times and placing it in the funnel

G. After the lettuce and brittlebush have steeped, pour the contents of the beaker through the funnel into another beaker. The filtered solution is the extract you will be using. Be sure to use a separate funnel and cheesecloth for the lettuce and brittlebush.

H. Once finished, clean the mortar, pestles, beakers and funnels thoroughly with soap and water. Throw the cheesecloth filter in the trash.

3. Germination setup.

A. While you are waiting for lettuce and brittlebush extracts to steep, prepare your Petri dishes by placing a piece of filter paper in the bottom of each dish.

B. Count out exactly 50 lettuce seeds and spread them out on the filter paper of one of the dishes (make sure they are all on the filter paper). Repeat for the other two dishes.

C. Cover your seeds with another piece of filter paper.

B. Once your extracts have sufficiently steeped, add 10 ml of distilled water to the top filter paper in the first dish. To the second dish add 10ml of lettuce extract, and to the third add 10ml of brittlebush extract.

D. Label the dishes. The dishes will be allowed to germinate for 3-4 days. At this point they may be frozen to disrupt any delayed germination and growth.

4. Checking Dishes

A. These steps will be done in lab next week.

B. Determine the number of seeds that have germinated in each Petri dish.

C. Verify that you did indeed add 50 total seeds to each dish.

Analysis

To test for the effect of treatment on germination rate, we need to use a **Chi-square goodness of fit test**. In a goodness of fit test, the frequencies of predicted outcomes are compared to the observed results. To generate the expected frequencies based on our null hypothesis of no difference in germination frequency, we add up the total number of germinated seeds and divide by three (since there were three treatments).

The **Chi-square (χ^2) test** calculates a test statistic derived from how much each category deviates from its expected outcome using the formula:

$$\chi^2 = \sum[(\text{observed-expected})^2/\text{expected}]$$

Note that χ^2 is the name of the test statistic. Do not square it or take the square root.

You then compare this to the Chi-square distribution at (number of categories)-1 degrees of freedom. For 2 degree of freedom, the critical value ($\alpha = 0.05$) is 5.991. Thus, if $\chi^2 > 5.991$ you can reject the null hypothesis. You can also use *Excel* to calculate an exact *p*-value. This analysis is available in the "Statistical Analysis" *Excel* file.