Herbivory and Cyanide Production

Lab Activity Extension

Background

This activity is designed to be an extension activity to the White Clover Cyanogenesis Lab. It is best used ***after*** performing the Cyanogenesis Lab Assay provided with this website. However, it can also be used separately in conjunction with a lesson on natural selection and adaptation.

Plants are ***autotrophs,*** producing their own food supply through photosynthesis. Plants are ***sessile***, or literally, rooted to the ground, so they cannot move away from predators trying to eat them. Instead of moving, many plant species have developed other defenses. Spines, thorns, a nasty taste, and toxins are some defenses against herbivory. Others could include waxy cuticles, hairy leaf surfaces, or dying quickly after reproduction to avoid predators finding them.

Studies have revealed that two key selective factors in the evolution of the cyanogenesis polymorphism in white clover are herbivore defense and energetic costs associated with producing the cyanide toxin. Experiments have shown that cyanide-producing clover plants are typically protected from small herbivores such as slugs, snails, voles and insects, which are a major source of predation. In regions with many herbivores, such as warm climates, cyanogenic plants are often more fit than acyanogenic (non-cyanide-producing) plants. However, due to the energy necessary to produce cyanide, these plants also show lower growth and reproduction rates than acyanogenic clover; acyanogenic plants seem to flower earlier and more abundantly than their cyanogenic counterparts. It is hypothesized that this is due to the fact that they are not expending energy on producing toxins to ward off herbivores. Thus, although acyanogenic plants may show lower fitness if herbivores are abundant, they may be at a competitive advantage in cooler climates where herbivore defense is less critical for survival

Past experiments have shown that in higher elevations, where there would be less herbivore presence due to cooler climates, a higher proportion of plants are typically acyanogenic, and that in warmer climates and at lower elevations, where plants are exposed to a greater degree of herbivores, populations show an increase in the frequency of cyanogenic plants.

In this activity, students will gather data on the amount of herbivory affecting the plants that were gathered for the Cyanogenesis Lab, then compare this to the phenotypes indicating cyanogenic vs. acyanogenic plants in their classroom populations. They will then be able to use the website database to compare their results to data in other regions of the United States to analyze any relationship between herbivory, and location of various populations of clover.

Materials:

- Magnifying lenses/loupes
- Writing/drawing material
- Graph paper

Observation/Exploration

1. Examine the plants that were brought in for the Cyanogenesis Assay portion of the lab. Do any of the plants show signs of herbivory? (This includes partially or totally eaten leaves and stems)
   ____
   ____

2. Are there parts of the plant that seem to have been eaten more than others? Why do you think this occurs?
   ____
   ____

3. What characteristics might a plant have that may attract or deter more insects or animals than others?
   ____
   ____
   ____
   ____

4. How do you think your climate or location will affect the amount of natural predators to the white clover population in your area?
   ____
   ____
   ____
   ____

Instructions:

After observing your plants for signs of herbivory, choose 10 leaves from the plant samples brought in by your group. Remember to include all three leaflets of the clover leaf as one leaf sample.

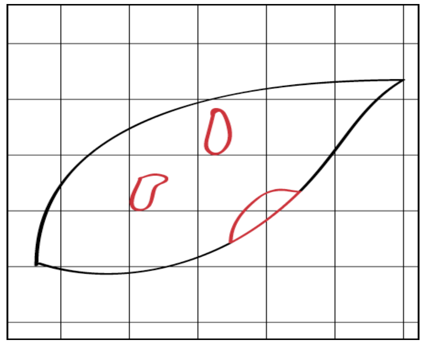
Calculating single group data:

1. In the lab, we are going to calculate the approximate area of each leaf that has been removed by herbivory.

2. Take one leaf from your plant, place the leaf on a piece of graph paper, and trace the outline of the entire leaf.

3. Next, trace the outline of any portions of the leaf that were removed by herbivory

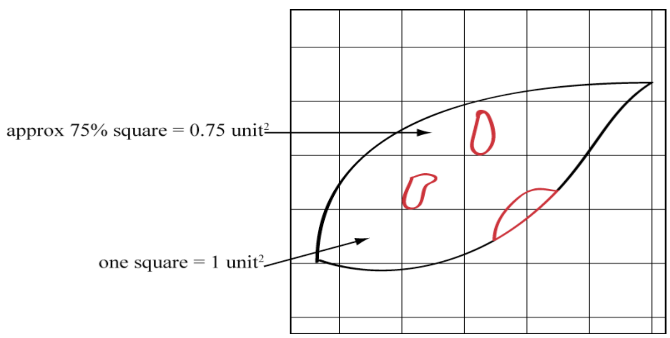
4. You should end up with a drawing that looks like this:



5. Next you will calculate the total area of the leaf (outlined in black) and the area that has been removed by herbivory (outlined in red).

6. The total area will be calculated by first counting the approximate number of squares on the graph paper that are included in your drawing. If only part of a square is included within the drawing, then record the approximate proportion of the square.

7. Each square is equal to 1 mm^2, therefore by adding the total number of squares, you can estimate the total area in mm^2.



8. The above example shows a total of:
0.1 + 0.2 + 0.2 + 0.2 + 0.75 + 0.95 + 0.95 + 0.25 + 0.1 + 0.8 + 1 + 1 + 0.5 + 0.3 + 1 +0.95 + 0.5 + 0.05 + 0.02 + 0.1 + 0.05 = 9.97 $units^2$

Record the total area of each leaf in your individual group data chart

9. The total area that has been removed by herbivory will be calculated in a similar manner by simply counting the number of squares included in the damaged portion of the leaf.

The above example shows a total removed area of:
0.25 + 0.25 + 0.1 + 0.15 + 0.2 + 0.05 = 1.00 $units^2$

Record the total area of herbivory present on each leaf in your individual group data chart

10. Add the column for Total Area to get a unit of Total Leaf Area surveyed
    Record this total in your Individual Group Data chart. This is the number that Will be used in the Class Data Chart

11. Add the column for Total Area of Herbivory. Record this total in your Individual Group Data chart. This is the number that will be used in the Class Data Chart.

Calculating the Class Data: Percentage of Herbivory

1. Each group will record their Column Totals for Leaf Area, and Herbivory Area on a Transparency of the Class Data Chart provided by the teacher.
2. Fill in your own Class Data chart using the data provided by other groups.
3. Calculate the percentage of herbivory present in your clover population by:
   a. Adding together all of the Leaf Areas: Record the total in your class data chart.
   b. Adding together all of the Herbivory areas: Record the total in your class data chart
   c. Divide the Total area of Herbivory / The Total area of Leaf then multiply the answer by 100.

   $$\left[\frac{\text{The area removed by herbivory}}{\text{The total area of the leaf}}\right] \times 100$$

   d. This number is the % of Herbivory affecting your location of clover.

Individual Group Data

Leaf #	Area of Leaf In mm^2	Area of Herbivory In mm^2
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		
Total		

Class Data

Group #	Total Area of Leafs Surveyed in mm^2	Total Area of Herbivory Surveyed in mm^2
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		
Total		

Herbivory Analysis

Summary:

1. From your class data, what % of herbivory is affecting your school's local White Clover population?

   ____________________.(*You will input this percentage in the "submit your data" page of the website.)*

2. Why might you expect more herbivory in warmer climates than in cooler climates?____

   ____

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3. Now compare your data to the website database. Choose any climate other than your own to look at the number of cyanogenic plants they had vs. herbivory, and acyanogenic plants vs. herbivory. Using the Clover Project website, find four other locations on the map. Choose two climate zones warmer than yours, and two climate zones that are cooler than yours. Record your findings here:

Climate Zone	Class Data for % Cyanogenic	Class Data for % Acyanogenic	Class Data for % Herbivory

   A. Does this comparison support the given hypothesis? Why or Why not?

4. Based on your climate zone, did your results prove or disprove that the frequency of cyanogenic vs. acyanogenic plants appears relative to the % of herbivory found in that area? ______________________________________

5. Looking back at the class data for the gene frequencies of cyanide producing plants in this population, compare that to the herbivory data you've just collected. What do you find?

   ____
   ____
   ____
   ____
   ____

6. The current hypothesis is that warmer climates in lower elevations and latitudes would have a higher frequency of cyanogenic plants than cooler, higher elevations. Does your data support this hypothesis? Why or Why not?

   ____
   ____
   ____
   ____