Plant Chemical Defenses & Bioprospecting: A Mini Case Study

1. Soon after you graduate, you take a job with a company set up by indigenous people in the Amazon Basin in South America. The company’s goal is to help local peoples find economic value in their forests, to help them preserve their way of life and conserve biodiversity. A few days later, you find yourself climbing out of a large dugout canoe onto a dock with stairs that lead into a tall, lush tropical forest.

 As you walk through the forest with your boss and the leaders of the local community, you learn that one project they are working on is *bioprospecting.* The goal of bioprospecting is to discover new commercial uses for natural products. “Your goal,” your new boss tells you, “is to help to find new *bioactive* compounds from the plants here.”

 One of the indigenous leaders adds, “We use many of the plants in our daily lives. But there are plants we don’t use, and they may help cure diseases that we’ve never encountered.”

 As you climb back into the dugout canoe to head downriver to your lab, you realize the enormity of the task: there are thousands of plant species in just this one small forest site. How are you going to effectively search for bioactive compounds? When you mention this to your new boss, she smiles. “This is why we hired you.”

**Questions:**

A. What do you think bioactive compounds are?

Based on the context, students will likely suggest that they are compounds in plants that can act as medicines. The term, however, is usually used more generally, for compounds that affect biological activities such as the growth, division, and survival of cells, or the broader physiology of an organism.

B. How could you determine if a compound is bioactive?

The goal with this question is to get students to speculate, hopefully in an informed manner, on how “bioprospectors” could evaluate different compounds. Typically, the first step is to expose extractions from plants (or other organisms) to cells (such as bacteria or cancer-cell lines) or to whole organisms (such as brine shrimp).

C. Can you give examples of plants that make bioactive compounds?

Students often know that about aspirin from Willow trees, capsaicin from chili peppers, taxol from Yew trees, and nicotine from tobacco plants. Answers might also include herbs and spices, which often have anti-microbial properties.

D. What are some potential uses of bioactive compounds? Medicines, herbicides, pesticides, dietary supplements, etc.

2. Back in your lab, you ponder the question: How are you going to effectively search for bioactive compounds among thousands of plant species? Didn’t you discuss something about this in your Ecology course? Fortunately, you still have your class notes. Flipping through them, you read:

“The Resource Availability Hypothesis argues that the amount that plant species invest in defenses depends on the resources available to them and the value of the tissue. For example, plant species that evolved to grow in light gaps (such as tree falls that open a hole in the canopy of the forest) have lots of resources available to them and can grow very fast. Consequently, they invest less in plant defenses because their leaves are not very valuable to them, since they can be quickly replaced. Shade-tolerant species (that normally live in the shaded understory of the forest) have to grow slowly, since light is limited. This means that their leaves are valuable to them, and they should have evolved to invest a lot in chemical defenses against insects and pathogens.”

First, you are impressed by how well you took notes as a student. Second, you try to decide what this means for your search for bioactive compounds in plants.

Questions

A. Why might plant chemical defenses sometimes function as bioactive compounds, killing bacteria or inhibiting the growth of cancer cells?

From the lecture or the readings, the students should know that many plant chemical defenses kill or are toxic not just for the expected herbivores or pathogens, but other organisms as well. For example, nicotine and caffeine affect the behavior of humans and can (at high doses) have toxic effects. The goal here is to have students connect the idea that compounds often have effects on species that they did not evolve to target.

B. In addition to increased light, how else might light gaps be an easier place to be a plant?

Light gaps usually have higher soil nutrients (since they are often formed by tree falls that then decay away) and higher water availability (more through fall and often less root competition).

C. Based on the Resource Availability Hypothesis, what is your prediction for which types of trees (light-demanding vs. shade-tolerant) are more likely to have bioactive compounds in their leaves? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ shade-tolerant

D. Explain why:

Shade-tolerant species have fewer resources to invest in growth. Therefore, each leaf produced represents a greater investment. This means that the cost of replacing leaves is higher for shade-tolerant plants (that is, the opportunity cost is greater). Chemical defenses to defend their leaves are, relatively speaking, cheaper for them to make, than plants that evolved to grow in light gaps.

3. Your next task is to figure out how you are going to detect potential bioactive compounds. You plan is to return to the forest and collect leaves from shade-tolerant and light-demanding plants. You know from your Biochemistry class that you can extract the contents of cells using water or ethanol. These extracts could contain bioactive compounds. But how can you test them?

 Back in your college notes, you come across the Kirby-Bauer Disk Susceptibility Test, which you learned about in your microbiology class. In the assay, round filter paper disks are saturated with a compound (such as an antibiotic or the extract from a leaf) and placed on a petri dish newly covered in a “lawn” of bacteria. The compound diffuses out of the disk and into the agar. If the compound kills or inhibits the bacteria, then a “zone of inhibition” forms around the disk. This means that it is (at least potentially) bioactive. If the compound is harmless to the bacteria, then no zone of inhibition forms, and the compound appears to be bio-inactive, at least against this strain of bacteria.

**Questions**

A. The figure below illustrates a Kirby-Bauer test. Which compound appears to have the biggest effect (or is the most bioactive)? \_\_\_\_\_\_\_\_ C

B. Which compound appear to have least effect? \_\_\_\_\_\_\_\_ A

Figure by Sommer36. CC by-SA 4.0 license. Wikipedia.org.

C. A more precise way of assessing these tests is to measure the diameter of the zone of inhibition. Larger zones mean that the compound is having a bigger effect.

 C

Control

 A

What is the diameter of the zones for:

A \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

B \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

C \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

D. Which of these is the most bioactive

when tested against this type of bacteria? \_\_\_\_\_\_\_\_

 B

4. Feeling prepared, you return to the Amazon forest to collect leaf samples for your first trial in finding plants with bioactive compounds. The local people show you to gaps in the forest, where light-demanding species with short-lived leaves are found. Next, you walk through the darker understory, where you are shown species that are shade-tolerant. You collect leaves from eight species, four that are light-demanding and four that are shade-tolerant. You return to your lab and set up the Kirby-Bauer Disk assay. After 24 hours, you measure the zone of inhibition. (See the data pages.)

**Questions**

A. Fill in the following chart for the average diameter of the zone of inhibition for the light-demanding (LD) plant species:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Light-demanding species | LD species 1 | LD species 2 | LD species 3 | LD species 4 |
| Sample 1 |  |  |  |  |
| Sample 2 |  |  |  |  |
| Sample 3 |  |  |  |  |
| Sample 4 |  |  |  |  |
| **Average** |  |  |  |  |

B. Fill in the following chart for the average diameter of the zone of inhibition for the shade-tolerant (ST) plant species.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Shade-tolerant species | ST species 5 | ST species 6 | ST species 7 | ST species 8 |
| Sample 1 |  |  |  |  |
| Sample 2 |  |  |  |  |
| Sample 3 |  |  |  |  |
| Sample 4 |  |  |  |  |
| **Average** |  |  |  |  |

C. What is the overall average diameter of the zone of inhibition for the light demanding species? \_\_\_\_\_\_\_\_\_\_\_\_

D. What is the overall average diameter of the zone of inhibition for the shade-tolerant species? \_\_\_\_\_\_\_\_\_\_\_\_

E. Use the frame below, make a bar graph of the average values for both the light demanding and shade tolerant species. Be sure to label the X and Y axes. Include tick marks on the Y axis.

F. Do the results support your prediction in question 2D above? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

G. What could you do next to improve your method for finding bioactive compounds?

1. Sample more plant species.

2. Use a different species of bacteria in the plates.

3. Use eukaryotic cell lines, like cancer cells.

4. Test other hypotheses for figuring out which plant species have defensive chemicals (like Plant Apparency Theory).

5. Consider the effects of other environmental factors (soil differences, water availability) on the evolution of plant defenses.

6. Sample from young leaves, flowers, roots and fruits.

Take Home Questions

Answer ONE of the following questions, remembering what we have previously discussed in class. Double-space your answers and limit yourself to a half a page.

1. How do you think the types of plant defenses might be different between plants that have evolved in soils with high levels of nitrogen versus soils with low levels of nitrogen? Why?

2. Do you think the types of defenses that plants evolve are affected by the herbivores present? For example, do you think plants on an island without any mammalian herbivores present might have different types of defenses from a mainland site with lots of deer and elk? Explain your reasoning.

3. Imagine that you transplant a shade tolerant plant to a light gap. How and why do you think its investment in plant defenses might change? Is this an example of adaptation or acclimation? Why?

Data: Below are images of the petri dishes, 24 hours after they were set up. Measure the diameter for each zone of inhibition. These are the **light-demanding species.**

Sample 1

Sample 2

Species 1

Species 1

Species 4

Species 4

Species 2

Species 2

Species 3

Species 3

Sample 4

Sample 3

Species 1

Species 1

Species 4

Species 4

Species 2

Species 2

Species 3

Species 3

These are the **shade-tolerant species.**

Sample 2

Sample 1

Species 5

Species 5

Species 8

Species 8

Species 6

Species 6

Species 7

Species 7

Sample 4

Sample 3

Species 5

Species 5

Species 6

Species 8

Species 8

Species 6

Species 7

Species 7