

## **Isolating a Potato Killer: Using Aseptic Laboratory Techniques and Koch's Postulates to Isolate and Transfer *P. infestans* from an Infected Sample to a Healthy Sample**

**Objective/Problem:** In this activity, you will use the scientific method in laboratory exercise to isolate and transfer *P. infestans* the causal agent of potato late blight from a affected potato to a healthy potato. This pandemic resulted in the Great Famine of Ireland and causes potato crop failure today. Using a field sample you will create a “sandwich” to determine how this pathogen infects its host and causes potato disease.

**Observations:** Watch the following PowerPoint presentation and take notes on the following processes of Koch's postulates, Oomycetes, and the Disease Triangle. These will help you to understand the process of how pathogens attack their hosts. Make sure you take notes either before, during or after the lecture session.

**Materials (First Day):** Infected late blight (*P. infestans*) potato lesion, Sterile forceps, bleach solution, distilled water, sharpie pen, gloves, rubbing alcohol, safety glasses, sealable plastic bag, paper towel, forceps/tweezers, timer, ruler, small healthy potatoes, sterilized pipette, Lab Sheet, and Lab Procedure sheet.

**Safety Considerations:** Even though *P. infestans* is a plant pathogen, procedures should be followed to make sure that it is properly killed before disposal to maintain a sterile working environment. Use the alcohol swabs to clean microscope eye pieces before and after each use to prevent the spread of communicable eye diseases. Wear your safety glasses.

**Hypothesis:** Create a hypothesis about how *P. infestans* attacks its host and interrupts normal plant homeostasis. Put your response in an “if...then” format.

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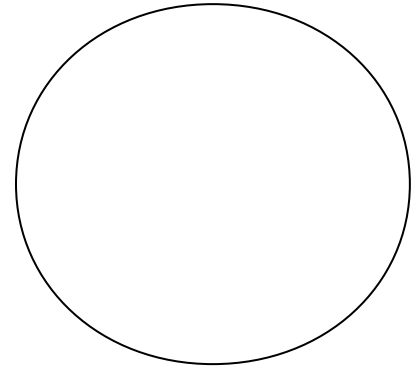
**Materials (Seventh Day):** Plastic bag containing potato samples from Day 1, Gloves, Safety goggles, dissection microscope, Watch glass, Sterile probe or forceps, Lab sheet, Lab Procedure sheet, rubbing alcohol and bleach solution (for clean up)

**Data:** Use the following laboratory technique to isolate *P. infestans* using the isolate “sandwich” method. For both sessions, your teacher will provide you with the laboratory procedures. From Day Seven, sketch an image from the slide you made under the dissection microscope below.

**Magnification:** \_\_\_\_\_

**Label your Mycelium sketch with the following:**

Sporangia, Sporangiohores



**Analysis & Conclusions:** Using your data, answer the following questions.

1. Why is it important to create an isolate from the field? What are some microorganisms that could contaminate your sample? Why do you think we grew three separate “sandwiches?” How would this affect further (DNA) analysis?
2. How does Koch’s postulates identify this as a true potato pathogen? Explain.
3. Was your hypothesis supported or rejected? Why or why not?
4. Based upon your knowledge of how *P. infestans* invades its host, how does this affect the potato’s process of homeostasis? (Hint: Think about infection of the stomata and its function in the plant.)
5. Why is it important to study how a pathogen infects their host and the disease triangle? How could this knowledge be applied to a method in which to control *P. infestans* or other types of pathogen outbreaks?