Koch's Postulates

Lesson plan submitted for EDG6905
Communicating Science: Topics in Emerging Pathogens

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(Heinrich Hermann) Robert Koch

- 1843-1910
- German physician
- Isolated *Bacillus anthracis*, *Tuberculosis bacillus,* and *Vibrio cholera*
- One of the founders of microbiology
- Nobel prize Physiology or Medicine, 1905

Koch’s Postulates

- The causal agent must be present in every diseased host organism examined,
- and be isolated in pure culture.
- A pure culture is inoculated into a healthy host.
- The same causal agent must be re-isolated from the host.

Exploring Plant Diseases in the Lab

- Host range of causal agents are limited to plants, with few exceptions.
  - Students may conduct experiments with plant pathogens with no health risk to themselves.
- Diseased plants are readily collected and many pathogens are easily cultured in the classroom.
- Students are unfamiliar with plant pathology, even though it affects their daily lives through its impacts on agriculture and food quality.

Primary Lesson Objectives

Students will:

- Be able to describe the concept of Koch's postulates.
- Collect leaves from their neighborhood or around the school that appear diseased and describe the pathogen signs and symptoms.
- Perform Koch's postulates.

Advanced Lesson Objectives

The students will:

- Draw fungal plant pathogen structures.
- Learn terminology related to fungal plant pathogens.
- Be able to identify complications of Koch's postulates.
- Explain the importance of a mock-inoculation.

Overview of Lesson Plan

- Four laboratory sessions take students through each step of Koch's Postulates
  - Observing signs and symptoms
  - Isolating potential pathogen in a pure culture
  - Inoculation and mock-inoculation of healthy host tissue with the pure culture
  - Observation of signs and symptoms in inoculated tissues
  - Re-isolation of pathogen from diseased tissue
- Four sessions are required for incubation times

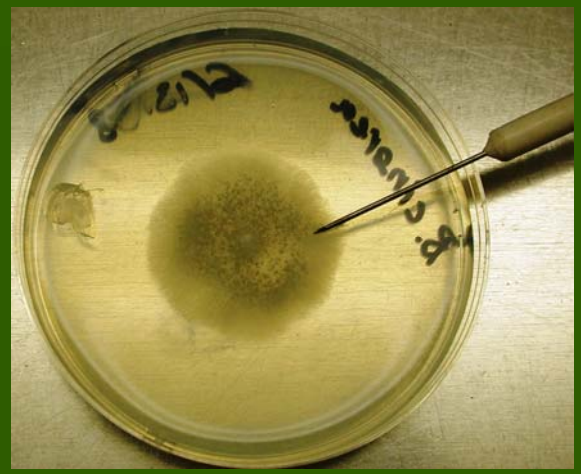
Botrytis Fruit Rot of Strawberry

- Causal agent is the fungus *Botrytis cinerea*
- Causes pre- and post-harvest fruit loss in Florida
- Disease continues to develop during transit and delivery to consumers.
- Infects a wide range of fruit, vegetable and weed species

The lab

Infected strawberries

Starting a culture



Inoculum

Surface sterilization

Treatments

Day 1: 30 sec sterilization

Concepts Included In The Assessments

- Terminology for culturing and testing a pathogenic fungus.
- Use of a laboratory notebook.
- Process for identification of the causal agent of a disease (Koch's Postulates).
- Plant pathogens live around us!

Topic: Koch's Postulates

Lesson plan by Sue Latshaw & Asha Brunings

Objectives:

Students will:

1. Be able to describe the concept of Koch's postulates, the scientific method used to determine the infectious cause of disease.

Figure 1 Grey mold on strawberry.

2. Collect leaves from their neighborhood or around the school that appear diseased and describe the pathogen signs and symptoms.
3. Isolate and identify a plant pathogen from fruit, inoculate, and re-isolate the same pathogen.
4. Draw the structures of a fungal plant pathogen.
5. Learn fungal plant pathogen terminology.
6. Identify complications of Koch's postulates and explain why it is important to do a mock inoculation.

Background:

The same kinds of organisms that cause infectious disease in people and animals also cause diseases of plants: bacteria, fungi, and viruses, for example. Students may not be aware that the spots on the surface of leaves and fruit, or the curling of leaves are often the result of an infectious disease. Scientists use Koch's postulates to determine the cause of infectious disease by culturing, identifying, and inoculating healthy organisms with suspect microbes. This is crucial for the identification of emerging pathogens. Every month, scientific articles are published about pathogens that cause new disease, or disease on new plant hosts. Experiments with plant pathogens are safe and simple ways to introduce the concept of Koch's Postulates to emerging pathogens.

Additional Resources:

Teachers can use the K-12 program from the website of the American Phytopathological Society (APS) at http://www.apsnet.org/education/K-12PlantPathways/TeachersGuide/Top.html. This website gives additional information and lesson plans which focus on plant pathology.

For more glossary definitions, go to the APS glossary:
http://www.apsnet.org/education/IllustratedGlossary/default.htm

Agrios, G.N. (2005) Plant Pathology, 5th Ed., Elsevier Academic.

Mertely, G.C. and Peres, N.A. (2006) Botrytis Fruit Rot or Gray Mold of Strawberry. Document PP-230, Plant Pathology Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida. Online at: http://edis.ifas.ufl.edu/PP152

Peres, N.A. (2006) 2006 Florida Plant Disease Management Guide: Strawberry. Document PDMG-V3-50, Department of Plant Pathology, Florida Plant Disease Management Guide; Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida. Online at: http://edis.ifas.ufl.edu/PG056

Overview of Activities:

There will be 4 labs associated with this lesson. In the first lab, the students will observe, draw and isolate *B. cinerea* from strawberry, in the second they will inoculate fresh strawberries, in the third they will re-isolate the pathogen from the strawberries, and in the fourth they will compare their new isolate with the original one.

Materials needed:

- 10% (v/v) bleach solution in water with a drop of dish detergent
- Dissecting and compound microscopes
- Dissecting needles and alcohol or sterile (autoclaved) toothpicks
- Bunsen burners or candles, if using dissecting needles
- Microscope slides and cover slips
- Paper towels
- Petri dishes
- Plastic containers with lids (2 per group) or plastic bags and twist ties
- Potato dextrose agar (PDA), or V8-juice agar
- Sterile water (autoclaved, or treated with 1 tablespoon of bleach per gallon overnight)
- Strawberries (old ones, kept at room temperature, 6 per group fresh healthy ones)

Lab 1. Observe infected strawberries and culture the fungal pathogen.

Preparation: Strawberries need to be bought a few days (no more than a week) before the initial class, and left at room temperature in a closed plastic container. The fungal Grey Mold pathogen (*Botrytis cinerea*) occurs naturally on strawberry, and under the right conditions (warm, moist) will grow profusely and form spores. PDA (or V8-juice agar plates need to be prepared (1 per group) or purchased, toothpicks and water need to be sterilized.

Initial Knowledge Assessment:

Ask students to write down their description of a healthy plant and then a sick plant. What would they do to determine what made the plant sick?

Lecture: Introduce plant pathology (abiotic vs biotic causes of disease; viruses, bacteria, fungi, nematodes, insects), symptoms and signs, sterile technique and microscope use, mycelium and spores, optimal condition for growing pathogens.

Activities: The students will look at the strawberries under the dissecting microscope, and draw what they see in their notebook (mycelium and spores, tissue degradation). They will take a tiny bit of fluff (fungal tissue), and place it in a drop of water on a

microscope slide, cover it with a cover slip, and examine it under the dissecting scope, again drawing what they see in their notebooks.

They will then use a flamed dissecting needle or sterile toothpick, to transfer a little bit of the fluff to the center of a PDA (or V8-juice agar) Petri dish. The plates can be kept at room temperature, but will grow faster if they are kept in closed plastic containers or plastic bags closed with twist ties, where temperature and humidity are higher.

Discussion questions: Signs, symptoms, sterile technique

1. What signs were evidence of the *B. cinerea* infection? Fluffy white mycelium and brown or grey spores visible under the dissecting scope and tree-like sporophores visible under the microscope.
2. What symptoms of disease were observed? Fruit tissue collapse and browning and brown calyx lesions.
3. What does sterile technique accomplish during transfer of the fungus to the PDA (or V8-juice agar? Microbes present in the environment are not transferred to the culture medium—the pathogen is transferred as a pure culture. Sometimes, several rounds of fungal transfer are required to achieve a pure culture if multiple microbes are present in the lesion, or if improper sterile technique occurs.

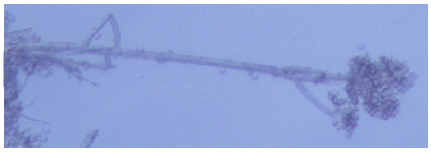


Figure 2 *B. cinerea* mycelium and spores.

Glossary:

- Plant pathology-the study and control of plant disease and the causal organisms, including bacteria, viruses, fungi, other microbes, and abiotic factors.
- Abiotic-a disease not caused by a living organism, including nutritional and environmental factors.
- Biotic-a disease caused by a living organism.
- Symptoms-the observable evidence of the plant's response to the disease process.
- Signs-visible pathogen structures.
- Sterile technique-a laboratory method that prevents unintentional introduction of microbes to surfaces by using sanitation and sterilization techniques.
- Mycelium-the mass of strand-like hyphae that form the body of the fungus.
- Spore-the reproductive unit of a fungus; can be asexual or sexual.

Lab 2. Inoculate and mock-inoculate fresh strawberries with the cultured fungus or water.

Preparation: A fresh batch of strawberries needs to be bought no earlier than a day before the class. The plastic containers should be large enough to hold 3 strawberries each and should be bleached and rinsed with sterile water before starting. Prepare sterile toothpicks, or dissecting needles and sterile water.

Lecture: Introduce terms infection, inoculation, and incubation, discuss the necessity of surface-sterilization, mock-inoculation, and using sterile technique.

Activities: The students will observe their PDA (or V8-juice agar) plates with a compound microscope and draw pictures. Students will work in groups of 3, and get 6 strawberries per group. Each strawberry should be dipped in a 10% bleach solution with a drop of dish detergent and stay covered for 2-4 minutes. The strawberries should then be dipped in sterile water to remove excess bleach solution. Afterwards, the strawberries are placed in a clean plastic container or plastic bag with a paper towel wetted with sterile water on the bottom. The purpose of this step is to kill the naturally occurring pathogen and other microbes on the surface of the strawberries.

The students will use the flamed dissecting needle or sterile toothpick to pick up a small amount of spores and mycelium from the PDA (or V8-juice agar) plate, and poke a hole into one of the surface-sterilized strawberries in the plastic containers or bags. It may help to mark the position of the hole by drawing a circle around it with a marker. They will each take turns, until the same has been done to the other strawberries in the same container. The 3 strawberries in the other container will be mock-inoculated in the same way using a sterile toothpick or a flamed dissecting needle dipped in sterile water.

Discussion questions: Pure isolate, mock-inoculation, surface sterilization

1. Was your cultured fungus a pure isolate? Why, or why not? Why is this important? Examine the plate for variants in the hyphal mass or sporophores, or for other types of microbes. If more than one type of microbe or fungus is observed, it is not a pure culture. This would result from improper sterile technique, a mixed infection, or transfer of other non-pathogenic microbes from the fruit surface. It is important to have a pure culture at this point in order to properly confirm causation of disease symptoms by the suspect microbe. For groups that do not have pure cultures, you may want to have them use isolates from another group's successful plate for the inoculation, if it matches the observations of what the errant group cultured. Alternatively, they could do another round of culturing the suspect fungus by transferring the ends of some isolated hyphae to a fresh PDA (or V8-juice agar) plate.
2. What is the purpose of the mock-inoculation? The mock-inoculated fruit should display no signs or symptoms of disease if the isolated pathogen causes the disease. Secondly, this demonstrates that you used good sterile technique and that all laboratory methods are effective.
3. What does surface sterilization accomplish? It sanitizes the fruit by killing all microbes on its surface. *B. cinerea* is almost always present on strawberries, so without this step, disease symptoms would result on all berries, regardless of whether they were inoculated or not. Also, plant surfaces are rich niches for microbes that do not cause disease normally, but could invade the wound at the inoculation site.
4. What do you expect to see on your inoculated and mock-inoculated strawberries during the next lab? The inoculated fruit will have actively growing *B. cinerea* and the mock-inoculation will have no fungal growth.

Glossary:

- Hypha(e)-tube like filaments that make up the body of the fungus.
- Surface sterilization-the process of sanitizing the exterior plant tissues.
- Inoculation-the process of purposefully transferring viable microbes to tissues or culture media.
- Infection-the presence of viable and actively growing pathogens on or in host tissues.
- Incubation-the period of time in the right conditions required for signs and symptoms of disease to emerge or for cultured pathogens to grow.
- Mock-inoculation-mimics the inoculation process, except that no microbes are transferred. This is a control for sterile technique and for association of symptoms with the suspect causal agent.

Lab 3. Observe disease signs and symptoms on inoculated fruit and re-isolate the fungus on PDA (or V8-juice agar). Share student collections of potential diseased plant materials collected from home, yards, and woods.

Preparation: PDA (or V8-juice agar) plates need to be prepared (1 per group) or purchased, toothpicks and water sterilized by autoclaving. Symptoms will show up on the strawberries 1-4 days after inoculation. Have each student bring in 1-3 plant parts that show symptoms and/or signs of disease.

Lecture: Reinforce terms introduced in labs 1 and 2. Show a slide show of plants infected with various pathogens to represent the range of disease symptoms. A resource for this is on the APS website: http://www.apsnet.org/education/LessonsPlantPath/Top.html . Alternatively, invite your local IFAS extension agent or plant pathologist to share their slides in class.

Activities: If possible, the students can observe the strawberries every day to mark how many days after inoculation the symptoms show up. In the lab they will observe and then isolate the pathogen on PDA (or V8-juice agar) as in lab 1. Students will also draw and observe a selection of the plant parts they collected and discuss how they would determine the causes of the apparent diseases.

Discussion questions: Plant disease signs and symptoms

1. Name some types of damage associated with plant diseases. Wilting, lesions, color changes, growth abnormalities, leaf drop, water soaked tissues, enzymatic tissue degradation, etc. Which did you observe in your collected materials?
2. How would you determine whether the symptoms were caused by a plant pathogen? Follow Koch's Postulate!
3. Describe signs of the pathogens observable on the collected materials. Can you guess what type of pathogenic agent caused the symptom?

Lab 4. Final observations, data recording, and discussion.

Activities: The students will observe their PDA (or V8-juice agar) plates, draw pictures using the compound microscope and compare their pictures with those drawn in lab 2. Do word search activity.

Final Discussion:

1. What are the fundamentals of Koch's Postulates? Re-isolating the same pathogen from the inoculated strawberries, together with the observation that the mock-inoculated ones do not have the disease, completes Koch's Postulates, and establishes that the pathogen is the cause of the disease.
2. Other than the inoculation, all strawberries were treated in exactly the same way. If any mock-inoculated strawberries did get infected, discuss the ways in which that could occur, and allow the students to make suggestions on how to improve the experimental technique.
3. What are the limitations and complications of Koch's Postulates? Unculturable organisms such as viruses and fastidious bacteria; organisms that need a vector for spread like citrus greening; presence of more than one pathogen on the infected tissue; secondary infections; regulated or quarantined pathogens; ethics of performing Koch's Postulates on humans.

Final Assessments:

Repeat the Initial Assessment: Ask students to write down their description of a healthy plant and then a sick plant. What would they do to determine what made the plant sick?

Collect and grade laboratory notebooks. Students should have recorded observations of disease signs and symptoms, fungal growth on the fruit and in culture, and successes and failures of lab procedures. The notebook should also include reports on Koch's Postulates, results of each lab session, predictions of outcomes for upcoming labs, and discussion of results.

Each group may present their disease collections to the class and describe the signs and symptoms of disease, thus demonstrating mastery of these concepts.