What are Koch's Postulates?

- How would you prove that a particular organism was the cause of a plant disease? How could you be sure you had found the right microorganism and not just confused it with another of the millions of microorganisms that occur on a plant?

- This problem challenged scientists for decades and it eventually led to "Koch's Postulates" as the accepted scientific method for identifying the causal agent of a plant disease. In the following experiments, you can repeat Koch's Postulates" and learn how scientists still identify which microorganisms cause which plant diseases.

KOCH'S POSTULATES

- Three rules for experimental proof of the pathogenicity of an organism were presented in 1883 by the German bacteriologist, Robert Koch; a fourth was appended by E. F. Smith (1905). Briefly, these rules state:

1. The suspected causal organism must be constantly associated with the disease.
2. The suspected causal organism must be isolated from an infected plant and grown in pure culture.
3. When a healthy susceptible host is inoculated with the pathogen from pure culture, symptoms of the original disease must develop.
4. The same pathogen must be re-isolated from plants infected under experimental conditions.

- These rules of proof are often referred to as Koch's Postulates.

An example of the use of Koch's Postulates to study a disease of wheat leaves:

- Steps to prove that the organism isolated from infected plant tissue caused the original infection.

Isolation of pathogen from infected tissue.

1. Cut between 5 and 10 sections (1 cm) of infected leaf tissue
2. Surface-sterilize (SS) leaf tissue
3. Place SS leaf tissue in humidity chamber in plastic bag for 5 days
4. Examine SS leaf tissue for fungal spores
5. Place a few spores in the centre of a petri dish containing agar medium
6. Allow to incubate at room temperature for 5 to 7 days
7. Examine culture under microscope to check that they are the spores of the pathogen

Inoculation of a susceptible host.

1. Grow healthy wheat plants in soil for 3 weeks
2. Make inoculum with spores collected from pathogen on agar plate
3. Spray spore suspension on to leaves of wheat plants until run-off
4. Place large, clear plastic bag over plants to retain high humidity for 24 hours, and place in the dark.
5. Remove plastic bag and leave at room temperature for 7 days in normal light/dark conditions.

Re-isolation of pathogen from wheat plants.

Follow steps 1 to 4 in isolation of the pathogen above.

Where to get materials

Wheat tissue infected with the spot blotch organism and wheat seed

Contact: J. Gilbert **(Note: at least four weeks notice is required)**
Cereal Research Centre
AAFC
195 Dafoe Road
Winnipeg, MB R3T 2M9

Planting materials. Buy potting soil and plant pots, or use 2 litre milk cartons cut down to approximately15 cm height.
A small (500 ml or 1 litre) garden pump sprayer

Petrie plates and potato dextrose agar (PDA). Malt agar or Nutrient agar also may be used. Or purchase prepared agar plates.
Filter paper

Order from: Fisher Scientific
Tel: 1 800 234 7437
Fax: 1 800 463 2996
Web: www.fishersci.ca

What to do

Plant seedlings of wheat
Surface-sterilize infected leaf tissue

Surface-Sterilization

Dilute bleach 1:20 with cooled boiled water.
Immerse cut sections of leaf tissue in bleach solution for 3 mins and then rinse in cooled boiled water.
Use alcohol or a bunsen burner to sterilize needles and loops.

Humidity Chambers

Place one circle of filter paper on the bottom of a petrie plate and another in the lid. Moisten the filter paper in the lid with cooled boiled water. Leave the other piece dry. Place the SS leaf tissue on the lower, dry surface. Label plates with date and ID etc.. Put plates in plastic bags to retain humidity. Leave at room temperature in the dark for 5 to 7 days, but check every 2-3 days that the filter paper in the lid of the plate is still moist.

Identification of pathogen

The spores of *Cochliobolus sativus*, the spot blotch pathogen are relatively large, black and shiny. They develop either singly, or more usually, in clusters of 2 or 3. Use a sterile needle to pick one or two spores from the infected tissue and place in the centre of an agar plate. Expose the agar surface for as little time as possible to prevent contamination. Label plate

with date and ID of pathogen. Place on a window sill and keep at room temperature (20 - 23 C) for 5 to 7 days.

Inoculation

When the wheat seedlings are approximately 25 cm tall (21 days or so) inoculate with a spore suspension. Wash spores from the agar plates with cooled boiled water. Spores can be dislodged with a sterile loop. Add one drop of detergent to reduce surface tension and allow spores to spread on the surface of the wheat leaves. If a haemacytometer is available you may standardize the spore concentration to 3000 sp/ml. Otherwise add sterile water and pour the spore suspension into the pump sprayer. Spray wheat plants with the spore suspension until run-off. After 30 min to 1 hour cover the plants with clear plastic bags secured around the pots with elastic bands and leave for 24 hours. Remove bags the next day. Leave plants on a window sill for 5 to 7 days, then examine leaves for disease symptoms.

The final step in Koch's postulates is to re-isolate the pathogen from the infected tissue and identify it under the microscope.

In the interests of time, the teacher may prepare the wheat plants and inoculum ahead of time, so that students start with inoculation and then re-isolate from the newly-infected tissue