

## Laboratory 2. Signs and Symptoms and Koch's Postulates

<b>OBJECTIVES:</b>
1) Use Koch's postulates to determine the cause of a disease
2) Learn symptoms and signs associated with plant diseases
<b>SKILLS:</b>
1) Isolation of pathogens from diseased plants

### Continued from last week:

**Exercise 1**—Three-way streak and moist chamber from last week (Individual Activity)

**Three-way streak:** Take the plate used for the three-way streak and the moist chamber out of your drawer. Describe what you see. Did you isolate a pure strain? How do you know?

**Moist chamber:** Describe what you see. What differences are there between the Clorox treatments and the water treatment? Are they what you expected? Examine with dissecting microscope. Draw what you see.

# Symptoms and Signs of Plant Disease

## How do you determine the health of a plant?

Usually this decision is based on observations of physical characteristics that are different from what we consider to be normal for that plant. As we know from our experience with diagnosis of our own illnesses, these determinations can be quite complex. Just as doctors have a set of terms used to describe human illnesses, plant pathologists have developed a set of descriptive terminology to describe plant disease characteristics. These characteristics can be divided into:

- **symptoms** (deviations in the normal morphology or physiology of the plant); and
- **signs** (structures of a pathogen).

**Symptoms** may either be **localized** (restricted to a particular part of the plant) or **systemic** (distributed throughout the plant by internal movement). In addition, symptoms may be further characterized as **primary** (direct changes to the tissues initially infected by the pathogen) or **secondary** (indirect or subsequent changes in tissues away from the site of initial infection).

**Signs** of the pathogen, which commonly include reproductive structures (as in the case of fungi) or bacterial ooze, are often extremely beneficial in diagnosis by identifying the pathogen group responsible for the observed disease.

## How do you determine the cause of poor plant health?

It is important to treat causes rather than symptoms. For example, wilting may indicate lack of water, OR blockage of vascular tissue by pathogen infection. Through correct diagnosis of a plant disease, we can determine the most effective treatments and control measurements, predict the economic impact, and limit further spread of the disease.

## Exercise 2– Describing signs and symptoms (Group Activity)

**PURPOSE:** One main goal for this class is to help you learn to recognize the common signs and symptoms of each pathogen group. Examining plant specimens and describing signs and symptoms is something you will be asked to do often in lab. This week we will be examining more closely the plant diseases we saw on the disease tour. This activity gives you the chance to learn and use the terminology associated with signs and symptoms as well as practice using the microscope to examine specimens.

Each group should choose four plant specimens with different diseases from the side bench. Describe the signs and symptoms you see using plant pathology terms. Use the summary of plant pathology terms to determine what terms relate to the type of signs and symptoms you observe. The terms are defined in the glossary.

Can you tell whether the symptoms are local or systemic, and whether they are primary or secondary? Use the following sheets to record your observations. Draw what you observe for future reference.

**Plant name:** \_\_\_\_\_

Symptoms \_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

Local or systemic \_\_\_\_\_

Primary or secondary \_\_\_\_\_

Drawings

Signs: \_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

Drawings

**Plant name:** \_\_\_\_\_

Symptoms \_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

Local or systemic \_\_\_\_\_

Primary or secondary \_\_\_\_\_

Drawings

Signs \_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

Drawings

**Plant name:** \_\_\_\_\_

Symptoms \_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

Local or systemic \_\_\_\_\_

Primary or secondary \_\_\_\_\_

Drawings

Signs: \_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

Drawings

**Plant name:** \_\_\_\_\_

Symptoms \_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

Local or systemic \_\_\_\_\_

Primary or secondary \_\_\_\_\_

Drawings

Signs: \_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

Drawings

### **Exercise 3** – Koch’s Postulates – Proof of Pathogenicity (Group Activity)

Correlating the presence of a particular microorganism with a diseased plant is quite easy. Determining which microorganism actually **causes** a particular disease is a more exacting process. Working with a bacterial disease in the late 1800s, Robert Koch developed a series of experimental steps to prove disease causation. The so-called “Koch’s Postulates” form the basis of the science of plant pathology and medicine. For the next several weeks you will be going through all the steps outlined by Koch to prove which microorganism causes a given disease. Today you will start by examining plants for symptoms and attempting to isolate the organism from the plant.

Symptomatic (diseased) plant tissue has many different organisms associated with it, but this does not mean that any of these organisms cause the disease (correlation vs. causation). Questions that may be asked include: Are the organisms capable of causing disease? What classes of organisms are present? Could the symptoms be caused by an abiotic factor?

Koch’s Postulates to demonstrate proof of pathogenicity are:

#### **1. Association with Symptomatic Tissue**

The (suspected) pathogen must be (consistently) found associated with the disease (symptoms) in all plants examined.

#### **2. Isolation from Symptomatic Tissue**

The (organism) pathogen must be isolated and grown in pure culture on nutrient media and its characteristics described (nonobligate parasites), or it must be grown on a susceptible host plant (obligate parasites), and its appearance and effects recorded.

#### **3. Inoculate Host with Isolated Organism**

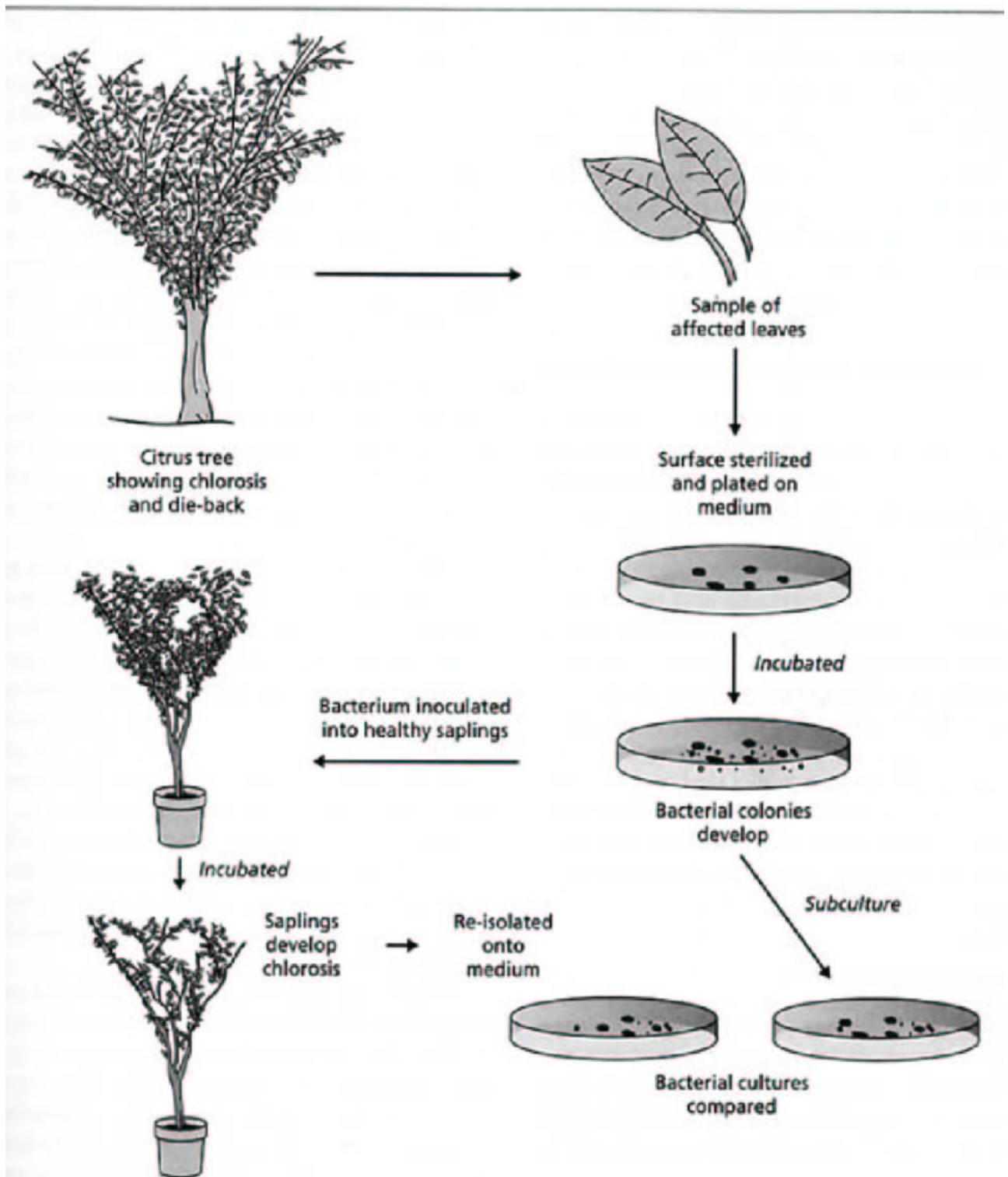
The pathogen (organism) from pure culture must be inoculated on healthy plants (with proper controls) of the same species or variety on which the disease (symptoms) appears, and it must produce the same disease (symptoms) on the inoculated plants.

#### **4. Symptoms on Inoculated Host**

Symptoms should develop on the inoculated plants that are identical to those observed on the original plants.

#### **5. Reisolation from Inoculated and Symptomatic Tissue**

The pathogen must be reisolated in pure culture and its characteristics must be exactly like those observed in step 2.



## Postulates 1 and 2

### Symptoms

You will be given a diseased and a healthy plant. Examine the diseased plant for common signs and symptoms of plant diseases. Use the healthy plant as a comparison, it will tell you what a healthy plant of this type normally looks like.

List the name of the plant you were given and then describe in detail the signs and symptoms you observe.

**Microbial isolation on solid media** - General tips on how to isolate the pathogen from the plant

1. *Isolate from symptomatic tissue.* Your objective is to find the pathogen and transfer it to media. Take samples of plant tissue from the margin of the symptomatic tissue (the edge between diseased and healthy tissue). This is where the pathogen is most actively growing. If you choose plant material that is dead, the pathogen may be dead too or overgrown by secondary invaders.

2. *Surface sterilize the plant material.* This will ensure that any microbes you isolate are found inside the plant and not simply ectophytic fungi living on the plants surface. It will reduce the number of microbes that grow so you can more easily isolate the actual pathogen. **Refer to last week's lab for methods on how to do this.**

3. *Fungi.* Fungal pathogens are best isolated by placing pieces of infected plant on media. Cut a few small pieces of plant tissue and place on the surface of the media. A general medium that many fungi can grow on is potato dextrose agar (PDA). Since PDA is not selective, other organisms may grow on the plates. If you suspect you have a fungus you can use PDA with streptomycin added to inhibit bacterial growth.

4. *Bacteria.* A common technique for bacterial isolation is to plate plant sap. Cut through the symptomatic tissue and rub the edge on one side of the media plate. Then continue with a three way streak. Alternatively, symptomatic tissue can be chopped up in sterile water to release the bacteria into the water. After sitting for a few minutes that water can then be used for a three way streak. A general medium that many bacteria can grow on is nutrient agar (NA). Since NA is not selective, other organisms may grow on the plates. If you suspect you have a bacteria you can use NA with cycloheximide added to inhibit fungal growth – note from last week that this would then become a somewhat selective medium.

NOTE: Today you will work with fungal or bacterial pathogens that are fairly easy to isolate on media. Nematodes, Viruses and other obligate parasites that do not grow easily (or at all) in culture require other methods to complete Koch's postulates.

**Materials:** Solid media (PDA+streptomycin and NA+cycloheximide) and standard lab tools.

**Method:** Based on the signs and symptoms you have observed and given the general tips above, design an experiment to determine the cause of disease in your sick plant. Use the following questions to help you design your experiment. **REMEMBER TO USE STERILIE TECHNIQUE AND LABEL ALL YOUR PLATES!**

1. Do you think you have a fungal or a bacterial pathogen? Why?
2. What media will you use to isolate?
3. How will you surface sterilize?
4. Where, specifically, will you sample plant tissue?
5. What plant tissue will you use and how much will you place on a plate?
6. Will you do any repetition?
7. Do you need a control treatment? If so what will it be and why do you need it?
8. What do you expect to see on your plates next week?

Next week you will examine your plates and attempt to create a pure culture of the suspected pathogen.



## LAB 2 NOTES