

Using Sterile Techniques as a Tool in Studying Plant Diseases

Developers:

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Grade Level:

Seventh Grade

Discipline:

Life Science

Goals:

1. To introduce the concept of sterile technique.
2. To utilize sterile techniques to isolate and identify plant pathogens.

Objectives:

After completion of this lab activity, the students should be able:

- To understand the concept of sterile techniques.
- To realize that plant diseases can be identified and classified in various way.

Introduction:

The science that studies the nature, cause, and management of plant diseases is called Plant Pathology. The identification of common plant diseases is very helpful to anyone who desires to grow vegetables and fruits.

Throughout recorded history, man has noted problems with plant diseases. There are biblical references to molds and mildews of crops. There have been mass migrations due to famines. The potato famine in Ireland led to the immigration of about one million people; roughly another million died of starvation as a result of the potato late blight fungus. St. Anthony's fire which afflicted Europe in the Middle ages was caused by a fungus which attacked rye and when consumed caused serious illnesses in the people that ate it.

It is important to understand that plant and animal diseases do not arise spontaneously. Diseases are usually caused by 'infectious agents.'

Plant diseases are caused either by living, infectious agents or environmental, non-living, abiotic agents. Examples of environmental agents are ozone damage, over-watering and frost. Examples of infectious agents are viruses, bacteria and fungi, i.e. molds and mildews.

To enable the student to learn about the diseases, we will introduce the concept of sterile technique. We will use collected plant materials to enable us to develop the sterile techniques that are required to identify some of the diseases that attack the garden plants.

Materials:

Petri plates
Potato dextrose agar
V-8 juice® (optional)
Scalpels
Collected leaf, root or fruit samples

Hand lenses
Microscopes
Microscope slides and coverslips
10% Chlorox® solution (10 mL bleach and 90 mL water and 1 drop of liquid detergent)

Preparation:

The students will collect the leaves of various plants that appear to be infected with some type of disease. The students will observe that the leaves are discolored, have large spots or in some way do not appear to be healthy.

The type of plant will be identified and a description of the particular leaf spot will be recorded. The leaves will then be surface sterilized and parts of the leaf will be placed on the agar plates to observe the growth of the fungus or bacteria that may be present.

Procedures:

The following is a generalized protocol for reisolating fungi from diseases plants, i.e., leaves, stems, roots, fruits and flowers.

Prior to the lab exercise, prepare the sterile tools, sterile water, sterile petri plates and the sterile media. The sterilization of the tools can be done in two ways:

- 1) You can set a kitchen oven to 140 – 150 degrees F.; wrap the tools in foil wrap and place them in the oven. Two to four hours should be adequate for sterilizing.
- 2) You can soak the tools in alcohol for 5 minutes prior to using. The tools should be soaked periodically during the process.

The petri plates are shipped in sterile sleeves. If you remove them carefully, they will be sterile.

1. Select a plant part that has a clearly separate diseases spot or lesion. Isolation of just one fungus is easier if the disease spot is not mixed up with bruises or many other spots.
2. Sterilize the plant leaf, flower, fruit or root in a jar with a screw-cap lid: fill the jar about one-half full with the 10% bleach solution and add the plant parts. Close the jar tightly and shake gently for 2 minutes.
3. After the time is up, use sterile forceps to remove the plant parts and place them in a sterile petri plate with sterile water. Swish the tissue gently in the sterile water and then remove it to a second rinse. Repeat the rinse 3 times, then place the tissue in a dry sterile petri plate.
4. Use a sterile scalpel to cut the plant tissue into small pieces. Try to cut the tissue so that each piece will have part of the lesion and part of the green or healthy tissue outside the lesion. Usually, the fungus will grow from the edge of the lesion across the nutrient medium. Try to select 4 – 5 pieces about the size of the end of a pencil eraser.
5. Place the prepared pieces onto a plate of nutrient medium using sterile forceps. If possible, use several different media for each kind of fungus. Some fungi do better on an amended medium. Try 3 – 4 pieces of tissue on each plate.
6. The fungus will usually grow out and away from the plant tissue after several days on the lab bench top. Use a sterile knife tip or needle to cut a tiny piece of the leading edge of the fungal colony out of the medium and place it on a fresh, new plate of medium. It will be a nearly pure culture of the fungus. After a week or so, you can examine its spores with a microscope. There are manuals with pictures of spores and taxonomic information that will help to identify the organism.

Preparation of Selected Media:

Autoclave or sterilize time is 15 minutes for 100 – 500 mL; 20 minutes for 500 – 1000 mL. An ordinary home canning sterilizer will work instead of an autoclave. Use the same time that is recommend in the recipe.

Water agar:

20 grams of agar
1 Liter of distilled water

Potato dextrose agar:

39 grams of the powdered pre-mix
1 Liter of distilled water

V-8 juice agar:

200 mL of V-8 juice® from the can
20 grams of agar
800 mL of distilled water

Nutrient agar:

23 grams of powdered pre-mix
1 Liter of distilled water

Sources:

Agar – 91/92 Fisher catalog; pg 1016
0.25 lb jar DF0013-02-3 potato dextrose agar
0.25 lb jar DF00140-02-9 bacto agar
0.25 lb jar DF001-02-7 nutrient agar

Petri plates – 91/92 Fisher; pg 684
size 100 x 20 mm Falcon optilux 08-757-103C

Ordinary unflavored gelatin that is available at the grocery store can be used as a substitute for the agar. The appropriate nutrients can be added to obtain the desired media.

Summary:

After preparing the agar media, allow it to cool. Have the students pour it into the petri dishes. Direct the student to follow the outlined sterile technique procedures. Specifically allow the students to prepare the cultures from infected tissue. As the students cut out the diseased portion of the specimen, be sure that they also leave a little of the uninfected area on the sample.

Supervise the students as they place the specimens on the prepared petri dishes, so that there is no accidental contamination of the tools or the specimens.

After the specimen samples are isolated place them in a cool dry place. Have the students observe the specimens on a daily basis. After several days when a fungus or bacteria is present, it will be very noticeable. Use the following extensions as activities to enhance the lesson.

Extensions:

1. Isolation of the pathogen can be done easily. Have the students cut a small piece of the leading edge of the fungal or bacterial growth and place it on a prepared petri dish. Allow the culture to incubate for several days and the student will be able to observe a "pure" culture of the organism.
2. Have the students place the specimen on a microscope slide and observe the bacteria or mycelia and spores of the fungus. Instruct the students in the use of reference manuals to identify the culture.
3. Have the students use a portion of the "pure" culture to infect an uninfected plant. If the organism is a pathogen, it will cause the new plants to show the same type of symptoms that were displayed on the original leaf sample.
4. Additional studies can be conducted on the environmental conditions needed for

the growth of the fungi or bacteria. An example could be the effect of light and dark on the growth of the organism, or the effect of temperature by using a refrigerator with the cooler temperature versus the room temperature. There are several variations that can be developed for this study.

References:

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5. “Identifying Diseases of Vegetables”, 1983., Pennsylvania State University, College of Agriculture, University Park, PA.
6. Large, E. C. “The Advance of the Fungi”, Dover Publications, Inc., New York
7. The Yearbook of Agriculture, 1953, “Plant Diseases”, U.S. Government Printing Office. It is out of print so be sure to check old bookstores and libraries.

Additional Bibliography:

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2. Dhingra, Onkar D. and James Sinclair., “Basic Plant Pathology Methods”, Boca Raton: CRC Press Inc., 1985.
3. Tuite, John., “Plant Pathological Methods, Fungi and Bacteria”, Minneapolis: Burgess Publishing Company, 1969.
4. “The Ortho Problem Solver”, Edited by Michael D. Smith, Chevron Chemical Company.

Insect Information:

1. Davidson, Ralph H. and Lyon, William F., “Insect Pests of Farm, Garden and Orchard”, John Wilet and Sons, New York.