PP-186

UF UNIVERSITY of FLORIDA
IFAS Extension

Fungal and Bacterial Disease Diagnoses for Distance Diagnostic and Identification System (DDIS)[1]

Tim Momol, Tom Kucharek and Hank Dankers[2]

Introduction

In this Fact Sheet we are reviewing the necessary techniques and tools that will help to identify plant diseases caused by pathogens through DDIS. This document will also make you familiar with fungal and bacterial plant pathogens and symptoms of plant diseases.

If the fungal structures (signs) are already present on the plant tissue, the county faculty who is submitting images should use "Guidelines for Submitting Plant Disease Samples Using DDIS" (Fact Sheet PP-185) and submit images to a plant pathologist on the specialist list from the UF/IFAS/DDIS web site. If fungal structures are not present, a sample should be sent to the Plant Diagnostic Clinic (PDC) in addition to the digital image. For a few bacterial diseases, there are practical tests that could be performed. These also will be reviewed.

The Concept of Disease in Plants

A plant is healthy when it can carry out its physiological functions to the best of its genetic potential. Some of the essential physiological functions that plants carry out are growth, absorption of water and nutrients from the soil, photosynthesis, and production of seeds or other reproductive organs.

A plant disease is a "Series of invisible and visible responses of plant cells and tissues to a **pathogen** or **environmental factor** that result in adverse changes in the form, function, or integrity of the plant and may lead to partial impairment or death of the plant or its part" (Agrios, 1997).

Pathogens (Infectious, biotic): Fungi, bacteria, viruses, viroids, nematodes, algae, mycoplasma-like organisms, protozoa and parasitic plants.

> FUNGUS (Fungi, pl.): An organism that lacks chlorophyll, has a vegetative body consisting of hyphae (microscopic threadlike filaments) or plasmodia (an amorphous, jelly-like slime) or budding cells (the yeasts), and usually has

1. This document is PP-186, one of a series of the Plant Pathology Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida. Original publication date September 1999. Revised March 2001. Reviewed May 2008. Visit the EDIS Web Site at http://edis.ifas.ufl.edu.

2. Tim Momol, assistant professor, North Florida Research and Education Center (NFREC), Quincy, FL 32351; Tom Kucharek, professor, Plant Pathology Dept.; and Hank Dankers, senior biologist, NFREC, Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida, Gainesville, FL 32611.

the potential to form spores. Beneficial and harmful fungi exist.

BACTERIUM (Bacteria, pl): One celled microorganism that reproduces by cell division and is without chlorophyll. Plant pathogenic bacteria are typically 0.5 to 1-3 microns in size. In mass, bacteria may be grown on agar as a slimy colony. Xylem-limited bacteria tend to be smaller in size, more difficult to culture, and are typically transmitted by leafhoppers; Pierces disease of grape is one example. Some plant pathogenic bacteria are systemic in plants. Beneficial bacteria abound in soil and water.

VIRUS: An ultramicroscopic entity (some say organism) without cells that is obligately parasitic. The structure of viruses includes a nucleic acid (RNA or DNA) surrounded by a protein coat.

VIROID: An infectious agent of a low molecular weight nucleic acid (e.g. RNA) similar to virus but lacking a protein coat around the nucleic acid

Environmental factors (non-living, abiotic): Air pollutants, nutrients imbalances, soil pH, low temperature, high temperature and various environmental factors.

Diagnosis of Plant Diseases

Successful plant disease management starts with the correct identification of the causal agent. Careful and accurate observation is essential. Note symptoms on plants and distribution of diseased plants in a field. Use a hand lens and/or microscopes to observe signs (structures of the pathogen). Symptoms alone are not diagnostic of a certain disease.

Symptoms are the external and internal reactions or alterations of a plant as a result of infection. They may be either localized or generalized throughout the plant (systemic). In addition, symptoms may be direct changes to tissues infected with the pathogen, or indirect and subsequent changes in host tissue at a distance from the site of the pathogen. Frequently, the sequence of symptom development characterizes a particular disease.

Signs are observable structures or products of the pathogen. The most common signs are reproductive structures of a pathogen (e.g., fungal spores, sclerotia) and are of greater diagnostic value than symptoms because different pathogens can cause the similar appearing symptoms.

Steps in Diagnosing Plant Diseases

1. Identify the host plant (common and botanical names). It is important to know the cultivar and levels of resistance to plant diseases. One should be aware of what a normal, healthy plant looks like.

2. Visualize symptoms of common diseases and signs of the pathogen on specimen: Symptoms can be internal as well as external. You should attempt to determine what part of the plant was affected first. The presence of general decline or wilt may indicate root problems. Look for signs such as fungal fruiting structures, mycelia in or on tissue, sclerotia or bacterial ooze. Not all fungi produce fruiting structures readily, but many do.

3. Use reference books to determine what diseases are recorded for the host plant. Refer to monographs, compendia, bulletins, host indices, and Fact Sheets that describe the common diseases and problems of the host plant.

4. Other information: Determine the distribution and location of diseased plants in the production area, recent treatments, and other practices. Collect historical information about the field or production area (e.g. greenhouse, herbicide, fertilizer and pesticide applications, cropping history, rainfall, irrigation, etc.).

Use of Stereo Microscope

With a stereo microscope, magnification ranges between 25-75x. Use this microscope for initial examination of a disease sample. Always start with the lowest magnification and gradually increase the magnification as needed.

Examine the affected plant part, where chlorotic or necrotic tissues exist. Fungi produce their structures on or in the affected tissues and that could

be helpful for identification. Fungi in the Deuteromycetes (Imperfect or asexual fungi), which consist of many of the fungal pathogens, produce conidia (spores) on conidiophores or in specialized structures such as sporodochia, synnemeta, pycnidia, or acervuli. Many of these structures are visible with a stereo microscope. These findings are important for the identification of fungi because they indicate where the spores are produced and what constitutes their color and physical characteristics. Take several pictures of these structures for submission through DDIS.

While focusing on the fruiting structures, cut out several small sections of the tissue that include the fruiting structures and prepare a wet mount for viewing with the compound microscope. The key to preparing a good wet mount is to cut very small pieces or sections of infected tissue. Also try clear-tape mounts for viewing under a compound microscope.

Pycnidium (Pycnidia, pl.): An asexual spherical or flask-shaped fruiting body lined inside with conidiophores and producing conidia. Some Fungi Imperfecti produce their spores (conidia) in pycnidia (Fig. 1). Examples, *Phyllosticta*, *Cytospora*, *Sphaeropsis*, *Diplodia*, *Septoria*, *Phoma*, *Ascochyta*

Figure 1. Pycnidia of *Didymella bryoniae* (anamorph *Phoma cucurbitacearum*). Pathogen of gummy stem blight of watermelon.

Acervulus (Acervuli, pl.): An imbeded or superficial, saucer-shaped, asexual fruiting body producing conidia on short conidiophores. Some Fungi Imperfecti produce their spores (conidia) in acervuli (Fig. 2). Examples, *Colletotrichum*, Gleosporium, Coryneum, Cylindrosporium, Pestalotia, Entomosporium.

Conidia on simple or branched conidiophores (Naked Conidiophore): Some Fungi Imperfecti produce their conidia on simple or branched conidiophores (Fig. 3). Examples, *Oidium*, *Monilia*, *Botrytis*, *Cladosporium*, *Cercospora*, *Pyricularia*, *Alternaria*, *Bipolaris*.

Figure 2. Acervulus of *Entomosporium* is produced within the center of each spot.

Figure 3. Powdery mildew pathogen *Oidium neolycopersici* on a leaf surface of greenhouse tomato.

Use of Compound Microscope

The magnification range of the compound microscope is typically 100-1000x. Each objective provides a different magnification (e.g. 10x, 20x, and 40x). For most fungi the highest magnification used is 400x which is obtained by the 40x objective. Another 10x magnification is obtained through ocular (eyepieces). The use of 1000x magnification is not

necessary for most fungal identifications. Always start with the lowest magnification and gradually increase magnification as needed (See Simone et al, 1988 for details).

Wet mount or clear-tape mounts will be used to view fungal fruiting structures under compound microscopes. Some fungi can be indentified based on spore shape, size, color and ornamentation, for example, *Alternaria*, *Fusarium* and *Entomosporium* (Fig. 4, 5 and 6).

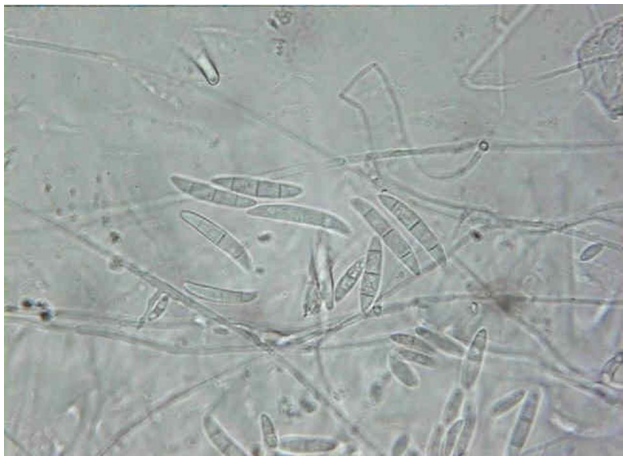

Figure 4.a

Figure 4.b

Many fungi have similar appearing spores. Therefore it is not always possible to identify each fungus based on spore characteristics. For accurate identification of the fungus, in many cases, you must see where the spores were produced. One example is Phoma and Gloeosporium spores which have similar color and shape. In order to distinguish them, it is critical to see where the spores are produced. Phoma

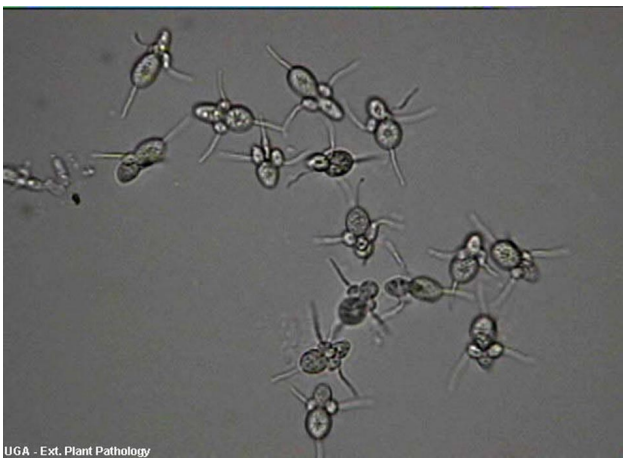

Figure 4.c

Figure 4. a) *Fusarium*, b) *Alternaria*, c) *Entomosporium*

spores are produced in pycnidia and Gloeosporium spores are produced in acervuli.

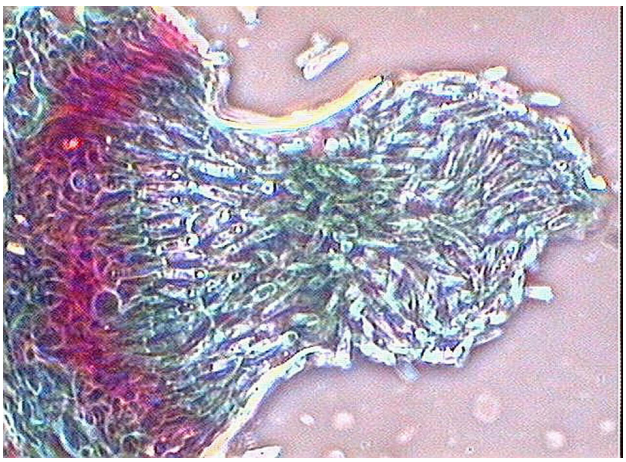

Figure 5. *Colletotricum* - acervulus and light-colored 1-celled conidia. Texas A&M Univ. WWW site. Credits: Texas A&M University WEB Site

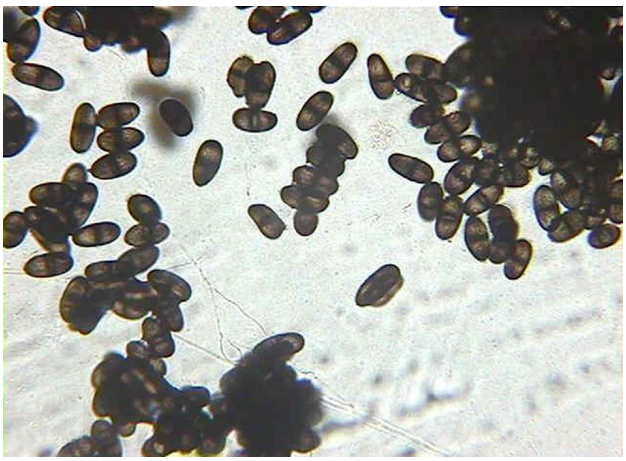

Figure 6. *Diplodia* spores (conidia), dark, 2-celled.

Practical Tests for Bacterial Disease Diagnosis

Bacterial Wilt of Tomato

Pathogen: *Ralstonia solanacearum*. Bacterium survives in the soil. It is a disease of high temperature and humidity. If a transverse cut is made through the stem, and dipped in water, bacterial streaming is often visible within minutes (Fig. 7).

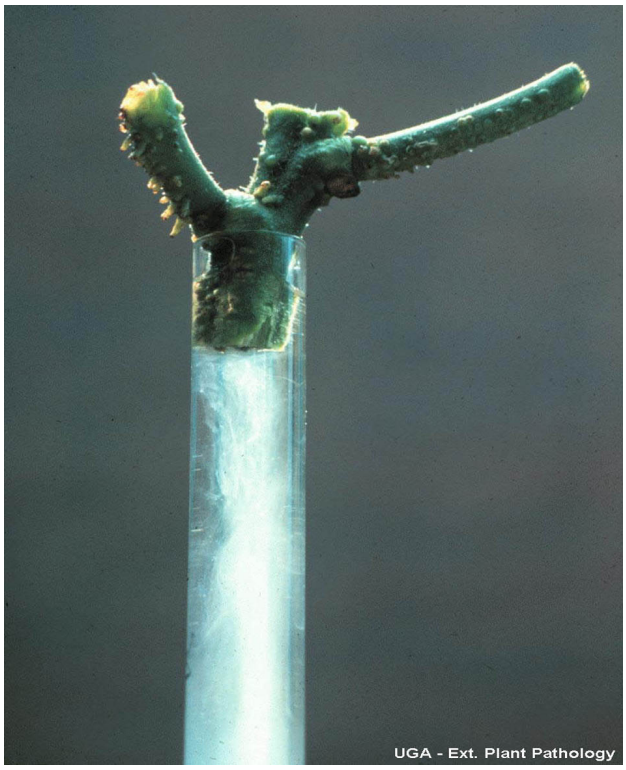

Figure 7. Milky white streaming of bacteria from cut tomato stem.

Bacterial Wilt of Cucurbits

Pathogen: *Erwinia tracheiphila* (rarely reported in Florida). The bacterium survives for a short time in infected plant debris, but it survives over winter in the intestines of stripped cucumber beetles (*Acalymma vittata*) and spotted cucumber beetles (*Diabrotica undecimpunctata*). Cucumber, muskmelon, squash, and pumpkin are affected and it has been detected to a lesser degree in other cucurbits. Bacteria propagate in the vessels of plants. Wilting of several leaves are the first symptoms then the whole plant will wilt. If a transverse cut is made through the stem, a sticky substance will be found, which will form threads if two parts of the stem are separated gently (Fig. 8).

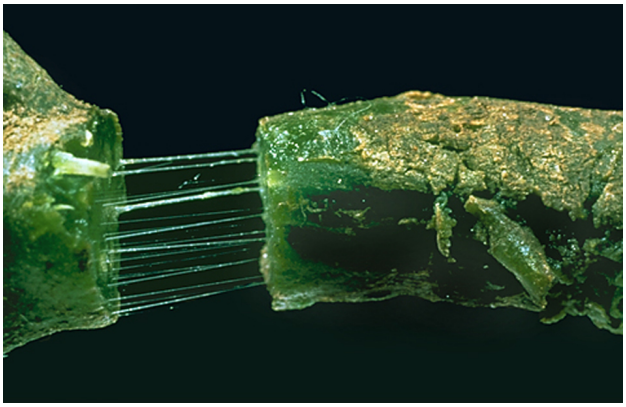

Figure 8. Bacterial wilt of cucurbits (masses of bacteria streaming from xylem). Credits: APS WWW Site

References

Agrios, G.N. 1997. Plant Pathology, 4th Edition. Academic Press.

Barnett, H.L. and Hunter, B.B. 1998. Illustrated Genera of Imperfect Fungi, 4th Edition. APS Press.

Simone, G.W., Pohronezny, K.L. and Blake, J.H. 1988. Training in Microscope Diagnosis of Plant Diseases. July 26-27.

Shurtleff, M.C. and Averre, A.W. 1997. The Plant Diseases Clinic and Field Diagnosis of Abiotic Diseases. APS Press.

The Disease Compendium Series of the American Phytopathological Society.

Related Web Sites

American Phytopathological Society (APS) http://www.scisoc.org/

The Plant Pathology Internet Guide Book http://www.pk.uni-bonn.de/ppigb/ppigb.htm

University of Florida / IFAS DDIS http://ddis.ifas.ufl.edu/

University of Florida, Department of Plant Pathology http://plantpath.ifas.ufl.edu/

University of Georgia, Extension Plant Pathology

http://plantpath.caes.uga.edu/