

## **DIAGNOSIS OF PLANT VIRUS DISEASES**

### **A METHOD**

To correctly diagnose plant disease problems, follow a few basic steps. View the plant and its environment from various perspectives.

### **CLOSE-UP VIEW**

The most obvious place to look first is up close. Use a hand lens if necessary. Don't stop at the first or most obvious symptom; check for more. You want to find all of the symptoms.

Look for symptoms on leaves, stems, roots, flowers and fruits. Cut open a branch or stem to look for vascular problems. Vascular problems show as discoloration of vascular tissue, leaf or stem wilting and sudden wilting of a section or a total plant.

### **GENERAL VIEW**

Stand back and look at the overall picture. Consider the total environment: weather, soil, stage of development for plant and pathogens, cultural practices and condition of other plants in the area. A plant growing in the wrong location may be stressed. Consider pesticide applications, recent construction or digging, and weather conditions.

### **TIME**

Determine when the symptoms became apparent. The onset of a problem may be due to a cultural practice, the seasonal appearance of a disease or insect, or a weather-related event. Remember that long-term stress is slow to appear, taking a year or more at times.

Is the problem spreading? This may indicate it is a pathogen. Are plants of other species affected? Diseases are usually species-specific. Problems caused by environmental factors do not spread, although the symptoms may become more severe.

### **KNOWLEDGE AND EXPERIENCE**

You must know what the plant should look like to be able to determine abnormalities. Check the references to see what problems are typical for a particular species. Gather all the information you can to help you make the diagnosis.

Remember that there is usually no single cause. There may be a primary cause; however, it may be associated with cultural or environmental conditions. Just as there is probably no single cause, there is usually no single symptom. Search for all of the symptoms. Orderly thinking and good questions are the key to accurate diagnoses.

When in doubt about a diagnosis, turn to agents, state specialists or the Pest and Plant Disease Clinic for assistance or a second opinion.

### **NONLIVING OR ABIOTIC AGENTS**

Nonliving or abiotic agents can indirectly result in plant problems. Additionally, several factors in the plant's environment can produce disease-like symptoms: weather extremes, high winds, high or low temperatures, nutrient deficiencies, physical damage and poor cultural conditions.

Frost often damages buds and leaves in early spring. Hail can cause leaf spotting or holes. Drought and high winds result in wilting and in extreme cases, browning and curling.

Air, water and soil pollution affect plant health and can produce disease-like symptoms. Soil imbalance, resulting from construction or other dumping, or misapplied garden chemicals can cause damage and disease-like symptoms. Dumping of household, automotive and industrial chemicals can also produce plant damage.

Plant disease can result from a combination of abiotic agents and biotic agents. Plants may be initially placed under stress by nonliving agents. This creates a susceptibility in plants for attack by living agents. Drought may damage roots which then are more likely to be infected by fungal diseases.

### **RANDOM OR UNIFORM PATTERNS**

Random distribution of symptoms on injured plants is usually caused by a biotic factor, such as infectious disease pathogens or an insect/animal. Uniform patterns are

generally associated with abiotic or noninfectious agents like pesticides, fertilizers, environmental or site stress and mechanical damage.

## **COLLECTING SPECIMENS**

Plant specimens that are to be diagnosed should be taken from the area where symptoms are showing on living tissue. Dead plants are often invaded by secondary pathogens which may hide the original problem. Collect several representative samples showing various stages of disease development. A generous sampling will assist in diagnosis. If possible, collect the entire plant, including roots.

Wrap the specimens in dry paper. Do not moisten them or seal them in plastic wrap or plastic bags. Never mix different specimens in a single bag. A fresh sample is required. Complete the diagnostic form as thoroughly as possible. This will result in better diagnosis.

## **Strategies for Control**

Resistance: Conventional approaches

- Breeding for Resistance to Plant Viruses
- Control of Plant Virus Diseases by Cross-protection of Virus Infection in Crops Through Breeding Plants for Vector Resistance: Biochemical and molecular approaches
- Biochemistry of Resistance to Plant Viruses
- Protein and Replicase-mediated Resistance to Plant Viruses
- Antisense RNA and Ribozyme-mediated Resistance to Plant Viruses
- Satellite RNA-mediated Resistance to Plant Viruses: Are the Ecological Risks Well Assessed?
- Alternative Strategies for Engineering Virus Resistance in Plants
- Controlling Mosaic Virus Diseases Under Field Conditions by Using Multiple-gene Strategies in Transgenic Plants Special topics related to resistance
- Releasing Genetically Engineered Virus Resistant Crops - Risk Assessment
- Antiviral Substances of Plant Origin Resistance to Plant Viruses: an Overview

## **Control of Vectors**

- Forecasting Aphid-borne Virus Diseases
- Chemical Control of Insect and Nematode Vectors of Plant Viruses
- Chemical Control of Fungal Vectors of Plant Viruses

## **Quarantine and Certification**

- Exclusion of Plant Viruses
- IPGRI's Role in Controlling Virus Diseases in Plant Germplasm
- Seed Certification for Viruses
- Control of Viruses Affecting Potatoes Through Seed Potato Certification Programs
- Virus Certification of Grapevines
- Virus Certification of Ornamental Plants - The European Strategy
- Virus Certification of Fruit Tree Propagative Material in Western Europe
- Virus Certification of Deciduous Fruit Trees in The United States and Canada
- Indexing for Viruses in Citrus
- Virus Certification of Strawberries
- Certification for Plant Viruses: an Overview

## **Elimination**

- Heat Treatment of Perennial Plants to Eliminate Phytoplasmas, Viruses, and Viroids While Maintaining Plant Survival
- Virus Elimination by Meristem Tip Culture and Tip Micrografting

## **Detection**

- Advanced Diagnostic Tools as an Aid to Controlling Plant Virus Diseases
- Identification and Detection of Recalcitrant Temperate Fruit Crop Viruses Using dsRNAs and Diffusion Antisera

- Detection and Identification of Plant Viruses and Viroids Using Polymerase Chain Reaction (PCR)
- Ultra Microscopic Detection of Plant Viruses and Their Gene Products
- Detection and Management of Plant Viroids

By the time disease symptoms appear, disease pathogens are inside the plant and usually beyond control. Therefore, it is important to prevent penetration of pathogens. Consider the following methods of disease control:

### **AVOID**

Avoid certain diseases through choice of appropriate site and planting time, purchase of disease-free stock and cultural practices that do not favor disease infection. Insect control can be important for controlling spread of viruses, mycoplasmas, bacteria and fungi. Mulching can help control diseases by preventing the contact of foliage with soil.

### **REMOVE**

Remove all diseased plants, including alternate hosts. Immediate removal of diseased plants or plant parts reduces the chance of the disease spreading. Selective pruning and careful sanitizing of pruning equipment can prevent spread of disease. Rotate crops to avoid soil-borne diseases.

### **PROMOTE VIGOROUS GROWTH**

Use best cultural practices. Follow recommended cultural practices.

### **Plant Disease Evaluation**

#### **Parasitism and Disease development**

Diseases of plants, like diseases of humans and animals, are complex phenomena. It may be difficult to provide a comprehensive definition of disease, but one important characteristic of plant diseases is that they are injurious, causing harm to plants in some way. Disease also is progressive, which helps distinguish disease development over time from an instantaneous injury such as lightning striking a tree. Plant diseases are a challenge to people interested in maintaining and producing healthy plants. Plant health can be achieved only with an

understanding of plant diseases. We need to know what causes them, how they spread, and how we can prevent them or at least minimize the damage they inflict.

Diagnosis is the first step in addressing the challenge of plant diseases. We are familiar with symptoms and signs of animal and human diseases, but symptoms of plant diseases are very different from human symptoms. However, a better understanding of disease development can help minimize the problems caused by plant diseases.

## **Plant Disease Triangle**

The disease triangle represents the three fundamental elements required for disease in plants:

- (i) a susceptible plant
- (ii) a pathogen capable of causing disease
- (iii) a favorable environment

### **The Plant**

Susceptibility to disease can be affected by many factors, including the growth stage of the plant, its genetic predisposition and stress. By careful observation of a diseased plant, one can begin to speculate about what basic functions of the plant are affected and, therefore, what kind of disease it may be.

Symptoms are the expression of disease by a plant as a response to the activities of the pathogen. It may be localized, such as leaf spots or systemic such as stunting that affects the entire plant. Some symptoms develop first on young and otherwise healthy tissues, other occur first on senescent tissues, such as aging flowers or lower leaves that are turning yellow. Many symptoms of plant diseases involve the death of plant tissues. Symptoms alone are not enough for accurate diagnosis of many plant diseases.

### **The Pathogen**

The second vertex of the plant disease triangle is the pathogen. The word *pathogen* comes from the root word *pathos* (“suffering”) and the suffix *-gen* (“origin” or “genesis”). Together they mean “the cause of a disease”.

Most plant diseases are caused by parasites. A parasite is an organism that obtains its nutrients from another living organism. Parasites of plant include fungi, bacteria, nematodes, viruses and even other plants. Parasites become pathogens when they do not merely live on and obtain nutrients from a host organism but actually cause harm to the host, resulting in disease

symptoms. Pathogens that are also parasites are infectious. They can be spread (transmitted) from plant to plant, potentially causing an epidemic. These living organisms that cause diseases are sometimes called biotic pathogens. There are also abiotic (nonliving) agents that cause disease. Noninfectious factors may affect many plants in an area, but the diseases they cause are not “contagious”, i.e. they are not transmitted from one plant to another.

### **The Environment**

An environment favorable for disease development (the third vertex of the disease triangle) consists of factors affecting the plant, factors affecting the pathogen, and sometimes additional organisms, such as vectors. The environment in which plant disease occurs consists of a wide range of factors that should be investigated when a diagnosis is being made. These factors include recent temperature (such as extreme highs and lows), rainfall or irrigation (amounts, timing and source), and light intensity or shade. Characteristics of the soil, such as drainage, soil type, and pH, are also important.

### **Determination of disease causing organism: Koch's postulate**

- i. The suspected pathogen must be consistently associated with diseased plants
- ii. The suspected pathogen must be isolated in a pure culture and its characteristics noted
- iii. The diseased must be reproduced in a healthy plant inoculated with the isolated organism
- iv. The same pathogen characterized in step 2 must be isolated from the inoculated plant

### **Biotrophs and Necrotrophs**

Plant diseases often involve the interaction of plants and parasites. Thus, diseases are greatly affected by the biology of the parasites.

Biotic pathogens can be divided into two categories (biotrophs and necrotrophs) that describe their approach to causing disease. **Biotrophs** require living plant tissues. **Necrotrophs** usually produce destructive toxins and enzymes that destroy plant tissues.

Some parasites are so dependent on their hosts that they are unable to exist without them. These parasites, called **biotrophs** or **obligate parasites**, can obtain nutrients only from plant cells. They often form some kind of survival structure in the absence of a host. In some cases,

biotrophs do not form a survival structure and will perish if they do not find a way to move to another living plant before the host plant dies.

Most plant pathogens are more flexible in their adaptation and can live either as **parasites** or as **saprophytes**, organism that obtain nutrients from dead organic matter. Organisms with the flexibility to switch back and forth between the parasitic and the saprophytic life style are described as facultative. **Facultative saprophytes** are better adapted to living as parasites but can survive as saprophytes when necessary. **Facultative parasites** are primarily saprophytes but can live as parasites if given the opportunity to invade compromised or senescent plant tissues. Many other bacteria and fungi are **obligate saprophytes** living only on dead organic matter and incapable of causing plant disease. These organisms may act as secondary invaders of dead and dying tissues. They contribute to the decay of plants and other organisms. At the other end of the spectrum of parasitism are the facultative parasites and facultative saprophytes, or **necrotrophs**. As the name suggests, they obtain nutrients from dead organic matter or from dead or dying cells of living plants. In contrast to the more delicate invasion by biotrophs, necrotrophs tend to produce toxins and enzymes that rapidly kill and degrade plant tissue and inhibit defense mechanisms by the plant. Necrotrophs commonly penetrate plants through wounds or natural openings.

### **The Disease Cycles**

Pathogens, like their hosts plants, have life cycles. These may be as simple as that of a bacterium, in which a single cell divides into two cells. When interaction between a plant and a pathogen result in disease, the interactions are described by the **disease cycle**.

A disease cycle may be simple or complex, but all disease cycle follow a pattern of discrete steps occurring in a predictable order. Disease cycles may be completed in as little as a few days or (in some tree diseases) as long as several years. It is important to be able to identify the stages of the disease cycle of each disease, because they will suggest ways to prevent or manage the disease.

The life history of a pathogen consists of two phases: (i) pathogenesis and (ii) survival

Pathogenesis – is the chain of events whereby disease occurs

Pathogenicity - is the ability of the parasite to interfere with one or more of the essential functions of the plant, thereby causing disease.

Each cycle begin with the introduction of the pathogen into a plant environment and terminate with the cessation of its activities. Primary cycle begin only with after a period of rest or dormancy or seasonal inactivity, while secondary cycle originate during the growing season and there is neither a period of rest or dormancy.



Pathogenesis in both primary and secondary cycles proceeds in the following stages.

- (i) Inoculum – Inoculation is the initial contact of a pathogen with a site of plant where infection is possible. The pathogen(s) that lands on or is otherwise brought into contact with the plant is called the **inoculum**.
- (ii) Penetration – Is the entrance of the pathogen into a plant and this usually takes place through wounds, natural openings e.t.c.
- (iii) Infection – Implies the establishment of the pathogen within the tissues of the plant.
- (iv) Incubation – This is the interval of time between the infection by the pathogen and production of disease symptoms.
- (v) Invasion – This refers to the latter stages of infection in which the pathogens invade and ramify the tissues and cells of the plant.
- (vi) Reproduction – After establishing itself between the plant cells and tissues and obtaining nourishment from them, a pathogen may increase in size and number.
- (vii) Dispersal – After reproduction, a plant pathogen or its propagules must be dispersed and disseminated to other plants.
- (viii) Survival – After passing through its pathogenic phase during crop growth and during the growing season. A pathogen must survive until the next growing season. Many pathogens produce hardy structures with which they can survive from one growing season to another.

#### **Measurement of Plant Disease**

- (i) Incidence of the disease i.e. the number or proportion of plant units that are diseased (i.e. the number or proportion of plants, leaves, stems and fruit that show any symptom) in relation to the total number of units examined. Measuring disease incidence is relatively quick and easy, and this measurement is the one that is used commonly in epidemiological studies to measure the spread of a disease through a field, region or country.

Severity of the disease i.e. the proportion of area or amount of plant tissue that is diseased.

Disease severity is usually expressed as the percentage or proportion of plant area or fruit

volume destroyed by a pathogen. More often, disease assessment scales from 0 to 10 or 1 to 4 are used to express the relative proportions of affected tissue at a particular point in time.