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Plant Problem Diagnosis

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Agents, consultants and others who advise clients on plant health issues need to have background training not only in plant sciences, plant pathology and entomology, but also in plant problem diagnosis. The sciences are used in diagnosis, but there is also another component which some might call an art. It is this component that combines the sciences with the detective work required to make a correct diagnosis. This article presents the various steps and activities associated with accurate plant problem diagnosis. The process may vary with different situations but the overall process is relatively consistent. The steps, outlined below, all require careful observations and inquiry.

Know what is normal

Proper plant identification Identification of affected plants is one of the first steps in diagnosing a plant problem. Both scientific and common names of the plant should be noted. Common names should not be relied upon since some distinctly different plant species may have the same common name, and the common name used in one area may be used for a completely different species in another area. For example the common name "cedar" is used to describe eastern red cedar (*Juniperus*), western red cedar (*Thuja*), Port Orford cedar (*Chamaecyparis*), incense cedar (*Libocedrus*), and Atlas cedar (*Cedrus*). In addition to knowing the common and scientific names of an affected plant, it is important to know the specific variety or cultivar, whenever possible. A great variation in susceptibility to a specific disease may occur within different cultivars of a plant species. For example, when we look at the susceptibility of wheat to wheat stem rust caused by *Puccinia graminis* f. sp. *tritici*, we find that not all wheat cultivars are susceptible to all races of *P. graminis*.

Recognize healthy plant appearance. It is important to know what the normal appearance of a plant is before you decide there is a problem. Each plant species has special growth habits, foliage colors and growth rates and if you don't know what to expect of the plant you cannot recognize when something is wrong. Also the overall appearance can vary with different cultivars. Some cultivars have naturally yellow to pale green leaves (e. g. certain hosta cultivars) which at first glance appear to have symptoms of under-fertilization, root stress or soil pH problems.

Once the "normal" appearance of the plant is determined, comparisons can be made between the problem plants and healthy plants of the same type. Compare characteristics such as overall size, shape, and coloration; leaf size, coloration, and distribution; root distribution and coloration; and bark, stem or trunk texture and coloration. It is also important to note normal events, such as leaf drop, that may occur in a healthy plant. For example, some holly species normally drop leaves in the spring.

Literature resources for Disease Diagnosis

Knowing the identity and other characteristics of the affected plant allows one to utilize various resources that contain lists of plant diseases associated with specific plants. The following lists are very helpful in suggesting possible pathogenic agents. Refer to the citation section for details.

-Common Names of Plant Diseases on The American Phytopathological Society (APS) web site (http://www.apsnet.org/online/common/top.asp).
-Westcott's Plant Disease Handbook[7]
-Diseases and Pests of Ornamental Plants[9]
-Fungi on Plants and Plant Products in the United States[4]
-Index of Plant Diseases in the United States. Agricultural Handbook No. 165[13],

-APS Press Compendium series on diseases and disorders for specific plant species[8]
-Diseases and Disorders of Plants in Florida[3]

In some cases these lists of plant diseases may suggest potential disease possibilities or they may lead the diagnostician to rule out certain diseases. One factor to keep in mind, however, is that these lists are often incomplete or the disease may be new and unreported on the plant or in a specific region. The best possible option is to utilize several different resources.

Check for symptoms and signs

First, the affected parts of the plants should be noted. Are there symptoms on the roots, leaves, stems, flowers, or fruit? Is the entire plant affected or are symptoms limited to one limb or one to side of the plant?

Identify characteristic symptoms. Once the affected plant parts are noted, the type of symptoms they exhibit must be determined. Symptoms can often be grouped as follows:

- **Underdevelopment of tissues or organs.** Examples include such symptoms as stunting of plants, shortened internodes, inadequate development of roots or leaves, inadequate production of chlorophyll and other pigments, and failure of fruits and flowers to develop.

- **Overdevelopment of tissues or organs.** Examples include: galls on roots, stems or leaves, proliferation of shoots (witches' brooms) and callous tissue around cankers.

- **Necrosis or death of plant parts.** These may be some of the most noticeable symptoms, especially when they affect the entire plant, such as with wilts or diebacks. Other examples include shoot or leaf blights, leaf spots, and fruit rots.

- **Alteration of normal appearance.** Examples include mosaic patterns of light and dark green, yellowing of leaves in more uniform patterns, malformation of leaves or stems and altered coloration of flowers.

Once the general type(s) of symptoms are noted, it is sometimes necessary to determine the source of these symptoms within the plant's system. For example, if a plant is wilting, this may be due to a disruption of the vascular system, so the vascular system should be checked for the discoloration that results by scraping off the outer tissue with a knife. If vascular discoloration isn't present, the root system may be malfunctioning and should be checked for abnormalities such as rots, decreased feeder roots or improper planting technique. On the other hand, if only individual branches show wilt symptoms, these should be checked for cankers and/or discolored wood indicative of dieback pathogens. The symptoms and/or signs of some diseases are most commonly seen on specific plant parts and this observation can be important in diagnosis. For instance, in some rust diseases, spore masses develop only on leaves of the host, while in others they form on both leaves and stems.

Determine if symptoms have progressed. Diseases involve a progression of symptoms that can vary significantly. The progression of symptoms is one of the most important characteristics associated with problems caused by biotic, or living, agents. Diseases often exhibit primary and secondary symptoms. For example, decayed tree roots are a primary symptom, while the yellowing of leaves due to damaged roots is a secondary symptom. As the tree declines, the symptoms become progressively worse and in the end the tree may die. During later stages of a disease, secondary invaders may cause additional symptoms which are not typical of the symptoms developed in response to the original pathogen. These secondary invaders can also complicate the detection of the primary pathogen.

Since a progression of symptoms is typical for biotic agents, it is important to look for a lack of such progression when abiotic, or nonliving, causes are suspected. For instance, in cases involving herbicide damage, the initial symptoms, such as leaf distortion caused by 2,4-D, may be similar to those typically observed as a result of an infectious agent like a virus. However, with herbicide injury, the symptoms usually appear suddenly and when new leaves form, they will generally be free of symptoms, indicating a lack of symptom progression.

Be aware of symptom variability. Variations in symptoms expressed by the affected plants may lead to an improper diagnosis. These variations can occur when there is more than one problem present, and in some cases there may be more than one pathogen infecting a plant. For instance, a plant may have yellowing leaves due to a soil nutrient deficiency and leaf spots caused by a pathogen. From these symptoms, one might improperly conclude that the pathogen is the sole cause of the yellowing.

The symptoms exhibited when multiple pathogens infect a plant may be significantly different from the symptoms expressed in response to each of the pathogens acting separately. Other factors which influence symptom variability or expression include host genetics and physiology, pathogen virulence, environmental conditions and length of time the plants have been infected.

Look for signs of biotic causal agents. Signs of plant biotic agents are the observable evidence of the actual causal agent. Signs may include the mycelia, spores or spore-producing bodies of a fungal agent. Indications of insect pests may include the actual insect, insect frass, mite webbing, and insect eggs. Signs are much more specific to biotic agents than are symptoms and are extremely useful in the identification of the agent and diagnosis of the problem. Signs of some diseases, such as spores or fruiting bodies, can be detected in the field by using a hand lens. Mycelial mats of root and/or crown rot fungi, such as *Sclerotium rolfsii* and *Armillaria* spp., may be found at a plant's base or under the bark at the crown of the plant. Bacterial ooze can be observed on site with some bacterial diseases. With others, streaming can be detected by cutting stems and placing them in water. Powdery mildews are typically diagnosed by the observation of gray to white mycelia and conidia observed on the surface of leaves and flowers.

Observe patterns

Check distribution of symptoms. One of the first things that a diagnostician should note is how the damaged plants are distributed over the affected area. Are they distributed uniformly across an area or are they localized or scattered? Is there a definite pattern to the distribution? For example, does it occur only along the edges of a greenhouse near open windows, next to roadways, in low spots of a field, along a planted row, or is it affecting plants at random in a field? This distribution can be especially important in considering the possibility of non-infectious problems, such as improper herbicide use or various soil factors[11]. A uniform pattern on an individual plant and uniform damage patterns over a large area are generally not associated with biotic agents, but are usually due to abiotic agents.

Determine prevalence of the problem. Are all plants affected? Infectious problems generally occur over time and there is a progression of symptoms. Rarely will all of the plants be affected. Generally, problems caused by biotic agents will be observed initially when they are affecting a low percentage of plants. Even then, rarely will 100% infection/infestation be observed. When a problem appears suddenly in 100% of the plants, it more commonly results from factors such as soil conditions (deficiencies or toxicities), climatic extremes (cold temperatures, hail, drought, etc.), or toxic chemicals (improper use of pesticides or growth regulators or from air pollutants, such as ozone, etc.).

Check for host specificity. Is the problem occurring in only one plant species or are different plant species affected? If different plant species are affected, this suggests the possibility of a non-infectious agent which could be related to cultural or environmental problems. However, with diseases, such as root rots caused by *Phytophthora*, *Pythium* or *Rhizoctonia* species, disease can develop on many different plant species. Some insects also infest numerous plant species. Also, if more than one species of plant is involved and they are closely related (i.e. same genus or family), it's possible that they can be infected by a common pathogen or insect pest.

Ask Questions

Review the cultural practices and growing environment. It is vital that a diagnostician question the activities that have been conducted around affected plants as cultural and maintenance activities can be significant. Has the client irrigated the plants and if so, how often? Have pesticides or other chemicals been applied? At what rate, by whom and when were they applied? What equipment was used for the application? Careful investigation by the diagnostician is required because, in some cases, someone may have done something improperly and may be unwilling to admit their error. In addition, the problem may not be due to anything the grower has done; the problem could be related to what his/her neighbor has done.

Information pertaining to the plant's growing environment is also a vital piece of the puzzle. It is especially important to document changes in the environment. Environmental factors to consider include: extreme temperatures (freezing and heat), rainfall, hail, lightning, prolonged drought, temperature inversions (important in cases of possible air pollutant damage and pesticide drift) and prevailing winds. All these abiotic factors can be important to the problem. Site factors such as soil type, possible drainage problems, and soil pH should also be evaluated.

Laboratory Tests for Disease Diagnosis

If abiotic factors are ruled out, and field examination suggests the cause to be an infectious disease, it may be necessary to conduct laboratory tests to identify the causal agent. This can be a time-consuming and labor-intensive process that takes specialized skills. Dissecting and compound microscopes are used in the lab for observation of specific spores and spore structures that may be present on the sample. If signs aren't present on the plant material upon arrival in the lab, other procedures are used to make the diagnosis.

Incubation of plant material One of the first steps in the laboratory may be to place a sample of the diseased tissue under conditions which allow a pathogenic fungus to produce spores. This can be accomplished by placing plant tissue in a moist chamber[11,13]. A sterile petri dish containing wet filter paper can be used as a moist chamber. To prevent tissue decay, the sample should be elevated above the wet paper in some way. For leaf spot diseases, the upper surfaces of some leaves should be exposed, while exposing the lower surfaces of others. The edges of the plate should be sealed to maintain moist conditions, or the plate could be placed in a sealed plastic bag. This type of moist chamber will work for small, relatively flat specimens such as leaves. Plastic bags containing moist paper towels may be necessary for larger specimens. Saprophytes that are present on the specimen are also encouraged to grow in a moist chamber so a brief surface swab with 70% isopropyl alcohol may be useful for reducing their population. Moist chambers are generally incubated at room temperature with exposure to light.

Isolation and identification of biotic plant disease agents Isolating the plant pathogen from the tissue is another step that often takes place concurrently with moist chamber incubation. It is best to attempt to isolate the plant pathogen from the margins of the diseased tissue where it is usually more concentrated and more active than the saprophytes that quickly colonize recently killed tissue. Isolation of fungi is usually accomplished by placing pieces of infected plant tissue on various nutrient media [11]. To reduce saprophytes on the tissue, it is usually surface-sterilized by exposure to 70% isopropyl alcohol or 10% bleach for one or two minutes, then rinsed in sterile water prior to placement on the medium. The organism that grows out of this tissue is then isolated in pure culture[1,13]. Bacteria are often isolated by grinding up infected tissue in a small amount of sterile water. This water:bacteria suspension is then streaked onto a bacteriological medium. Despite surface sterilization, saprophytes may outgrow the plant pathogen on the nutrient medium, obscuring the pathogen. In cases where a specific plant pathogen is suspected, a medium that is selective for the suspected pathogen, but retards or prevents the growth of other microorganisms, may be utilized.

Once an organism is isolated, it must be identified. The characteristics upon which their identification is based are often complex and specialized training is necessary to identify them. Experienced diagnosticians can often identify the most common pathogens. However, they often turn to manuals, such as *Illustrated Genera of Imperfect Fungi*[2], which contain identification keys and pictures accompanied by descriptions of fungi which aid in identification. Once the organism has been identified, one must determine if it is the true cause of the problem. Conducting Koch's postulates[1] may be necessary to conclusively answer this question, especially if the organism has not been previously reported as a plant pathogen on that host. Koch's postulates are seldom conducted for routine diagnoses, but may be extremely important for new diseases and for research.

Additional diagnostic tests for identification of biotic causal agents A major problem in identification of some biotic causal agents is the inability of some pathogens to grow on artificial media. Viruses, as well as some fungi (e.g. powdery and downy mildew fungi), parasitic nematodes and some prokaryotes (e.g. phytoplasmas), require a living host in order to grow. In these cases, other methods may be used for pathogen detection. For instance, viral identification is often accomplished utilizing a serological test called ELISA (enzyme-linked immunosorbent assay). This test is based on the binding of an antibody produced in reaction to a specific virus with that virus in the infected plant material[1]. Other techniques used for the identification of viruses include negative staining and electron microscopy to view the viral particles in plant tissue or in suspensions. More tests are currently being developed using the polymerase chain reaction (PCR) for detection of specific organisms[5,10]. These types of reactions take specialized equipment and reagents, and the tests are not commonly done outside diagnostic and research laboratories. Additional tests may include analysis of fatty acids of organisms, carbohydrate utilization (i.e. BIOLOG test), and enzyme activity testing (i.e. pectinase, isozyme patterns)[5]. The detection of nematode pathogens involves several different techniques to recover them from soil, root or tissue samples. Then the pathogenic species must be identified and their population determined by a trained nematologist.

Diagnostic tests for identification of abiotic plant disease causal agents It is extremely important to look for abiotic factors that may be causing the observed symptoms. Soil and water tests may be necessary to determine pH, nutrient composition, salinity, and other factors, such as pesticide residues, that may induce various symptoms. It may also be important to analyze samples of plant tissue for nutrient content to determine if there are macro- or micronutrient deficiencies or toxicities.

Final Diagnosis

Diagnosis is a form of hypothesis testing, where the hypothesis is simply the identity of the problem. A good diagnostician goes through multiple iterations of the scientific method, i.e. seeking evidence through testing that supports or refutes the hypothesis that s/he generates. These hypotheses are generated through examination of the plant, observations about the environment, and information gathered from the grower. When all of the information is collected, literature sources are consulted to determine what is already known about the specific problem. Information on plant diseases can be obtained from the published and online resources listed earlier. Other sources include commodity newsletters and personal communication with plant disease experts. This type of communication has been made easier with the advent of digital cameras. Digital images showing field conditions, symptoms and signs can be sent instantly via electronic mail to experts or other diagnosticians[6] for an opinion.

When no information is available on the specific plant, information regarding related plants may be useful. There may also be rare cases where no information is available related to the problem. Then, extensive testing may be necessary to make the final diagnosis. In these cases, control measures may have to be based on diseases or problems of similar etiology.

A plant disease diagnostician can be compared to a detective investigating an assault or murder case, only in this case, the victim is a plant. All clues should be investigated and exceptions to general rules must also be considered. Some clues may lead down blind alleys while others will lead down the correct path. It is the compilation of the information and clues that will ultimately lead to the most accurate diagnosis.

Citations

1. Agrios, G. N. 1997. Introductory Plant Pathology. 4th ed. Academic Press, New York, NY.
2. Barnett, H. L., and B. B. Hunter. 1998. Illustrated Genera of Imperfect Fugi, 4th edition. American Phytopathological Society, St Paul, MN
3. Alfieri, S. A., Jr., K. R. Langdon, J. W. Kimbrough, N. E. El-Gholl, and C. Wehlburg. 1994. Diseases and Disorders of Plants in Florida. Fla. Dep. Agric. Consumer. Serv. Div. Plant Ind. Bull. No. 14.
4. Farr, D. F., G. F. Bills, G. P. Chamuris, and A. Y. Rossman. 1989. Fungi on Plants and Plant Products in the United States. American Phytopathological Society, St Paul, MN.
5. Hansen, M. A. and R. L. Wick. 1993. Plant disease diagnosis: present and future prospects. Advances in Plant Pathology 10:65-126.
6. Holmes, G.J., E. A. Brown, and G. Ruhl. 2000. What's a picture worth? The use of modern telecommunications in diagnosing plant diseases. Plant Dis. 84:1256-1265.
7. Horst, R. K. 2001. Westcott's Plant Disease Handbook. 6th ed. Kluwer Academic Publishers, Boston, MA.
8. Plant Disease Compendia. American Phytopathological Society, St Paul, MN. http://www.apsnet.org
9. Pirone, P. P.1978. Diseases and Pests of Ornamental Plants. 5th edition. John Wiley & Sons, New York.
10. Putnam, M. L. 1995. Evaluation of selected methods of plant disease diagnosis. Crop Protection 14:517-525.
11. Shutleff, M. C. and C. W. Averre. 1997. The plant disease clinic and field diagnosis of abiotic diseases. American Phytopathological Society, St. Paul, MN.
12. United States Department of Agriculture. 1960. Index of Plant Diseases in the United States. Agricultural Handbook No. 165.
13. Waller, J. M., B. J. Ritchie, and M. Holderness. 1998. Plant Clinic Handbook. CAB International, New York, NY.

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