

BASIC CONCEPTS OF PLANT DISEASE AND HOW TO COLLECT A SAMPLE
FOR DISEASE DIAGNOSIS

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This circular represents a departure from the usual format in which a particular plant disease is treated. The problem of inadequate sampling has always been one of the major obstacles in determining an accurate disease diagnosis. Ideally the diagnostician should collect the sample, but this is not always practical. Training of personnel responsible for sample collection to improve the quality of submissions would be incomplete without a concise discussion of basic concepts of plant disease, in order to rationalize the sample collection guidelines and procedures.

What is a plant disease?

A modified definition of disease from Webster's Third New International Dictionary is: "Disease is an impairment of the normal state of the living (plant) that interrupts, modifies, (or stresses) vital functions. Disease is a response to specific causal agents (biotic or abiotic), inherent defects of the organism, or combinations of these factors".

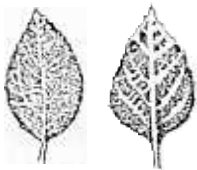
Diseases are recognized by the resulting symptoms expressed by the host, and signs (actual manifestations) of the causal agent. Taken together, symptoms expressed by the host and signs of the causal agent are called the disease syndrome. The causal agents, as the term is used in the definition of disease above, can be biotic (living or infectious), or abiotic (non-living or non-infectious). Biotic causal agents of disease are termed pathogens. Examples of biotic causal agents (pathogens) of plant disease are fungi, bacteria, mycoplasmas, spiroplasmas, viruses, viroids, nematodes, protozoans, and parasitic higher plants. Examples of abiotic causal agents of plant disease are temperature extremes, moisture extremes, nutrient extremes, light extremes, soil pH extremes, air pollution, oxygen deficiency, pesticide phytotoxicity, cultural malpractices.

Various symptoms of plant diseases are illustrated diagrammatically in Table 1 together with possible causal agents and certain considerations to be weighed during the sample collection process. It is important to remember that even though a disease may appear to be static, as a rule diseases are dynamic, and the disease syndrome will change over time. Symptoms are usually not reversible, though with some abiotic causal agents such as drought stress, symptoms (wilting) can be reversible. Of course, healing of the damage done by biotic and abiotic causal agents, or even by inherent defects in the host plant can occur, though some evidence of the disease usually remains.

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Table 1. Symptoms, Possible Causes, and Sampling Considerations

Symptom	Possible Causes, Sampling Considerations	
	leaf spot	Fungal, bacterial, or viral leaf spot pathogen, chemical phytotoxicity. Collect all stages of development. Note pesticide schedule.
	leaf tipburn or scorch	Root or stem dysfunction, water stress, excess soluble salts, herbicide or other chemical injury. Fungal, bacterial or other pathogenic infection. Collect whole plant with roots and soil. Note pesticide and fertilizer schedule plus other components of cultural program.
	distortion	Fungal, bacterial, viral or other pathogenic infection, herbicide or growth regulator injury, insect or mite damage, chemical phytotoxicity, mechanical damage. Collect representative sample of all symptoms. Note cultural program.
	vein enation	Viral infection, growth regulator injury. Collect representative range of symptoms. If possible, keep tissue fresh or submit whole plant to permit observation and testing to take place over a period of time.
	edema	Excess moisture, chemical phytotoxicity, fungal infection. Collect all representative stages. Note soil moisture, preceding weather conditions (prolonged cloudy, humid to wet weather?).
	vein clearing	Viral infection, growth regulator or herbicide injury. Collect all representative stages, note pesticide schedule on or near crop. Keep sample very fresh (essential for many viral diagnostic tests). Whole plant helpful.
	mosaic ringspot	Viral infection, reaction to cold or hot water on foliage, chemical phytotoxicity. Note watering facilities, pesticide schedule. Collect very fresh sample or whole plants.
	mildew	Fungal infection. Collect symptomatic tissues.



leaf
blister

Fungal infection, insect or mite injury. Collect range of symptomatic tissues.



scab

Fungal or bacterial infection, insect or other injury, chemical damage. Collect range of symptomatic tissues.



defoliation

Root dysfunction, water stress, excess soluble salts, cold, heat, normal senescence, chemical or insect damage. Collect whole plants. Note recent cultural treatments.



chlorosis

Macro or micronutrient deficiency, low or excess light, growth regulator or herbicide injury, natural pigmentation/variegation, root dysfunction, soil pH problems. Collect representative samples of all symptoms, soil and roots.



wilt

Fungal, bacterial viral or other pathogenic wilt, root rot or canker pathogen. Water stress, excess soluble salts, high temp., wind, cold, insect injury. Implies sudden onset. Collect whole plant with roots and soil.



tip
dieback

Slower or later expression of same factors as above, plus cold injury, insect damage.



blight

Localized or generalized branch or twig disorder caused by fungi, bacteria, insects, mechanical injury. Collect sample which includes transition zone from diseased to healthy tissue.



stunt

Fungal, bacterial, viral or other pathogenic infection, nutrient deficiency, water stress, nematode or insect injury, growth regulator damage. Collect whole symptomatic and asymptomatic plant. Note cultural program.



fasciation

Unknown. Usually considered a genetic abnormality. Collect symptomatic tissue of all stages, whole plants if possible. Note pesticide and growth regulator schedule.



proliferation
witches' broom

Fungal, bacterial, viral, or MLO infection. Mite infestation. Micronutrient deficiency, herbicide or growth regulator phytotoxicity. Collect whole plants if possible. Note pesticide schedule.



girdle

Fungal disease, mechanical constriction, insect injury. Collect symptomatic tissues of all stages of development.



**canker
diffuse
and/or
calloused**

Fungal or bacterial infection, mechanical injury, heat or cold injury, sunburn, chemical injury. Collect symptomatic tissues, especially transition zones between healthy and symptomatic tissue. Check pattern of symptom expression.



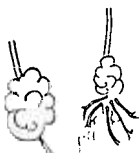
**Initiation
of root
primordia**

Excess water, mechanical injury or pathogenic infection at ground line. Enough vigor to induce root primordia implies sudden onset of disorder to otherwise healthy plant. Collect whole plant or all representative plants parts.



pustule

Fungal infection (rust), excess moisture (exploded lenticels). Collect symptomatic tissue.



gall

Fungal or bacterial infection, mechanical injury, hormonal disruption, insect injury. Collect samples of all stages of gall development, including surrounding soil if below ground.



root rot

Fungal or bacterial infection, excess moisture, excess soluble salts, chemical injury. Collect whole plant with soil and roots.



**nodule
root galls**

Nodulating N-fixing bacteria, actinomycetes, bluegreen algae, root-knot nematode, insect injury, fungal, bacterial or viral pathogenic infection. Collect symptomatic tissue.



**crown rot
bud rot**

Crown rot affects oldest tissues. Bud rot affects youngest tissue. Fungal, bacterial infection. Excess soluble salts. Collect entire plant with roots and soil.



dry rot

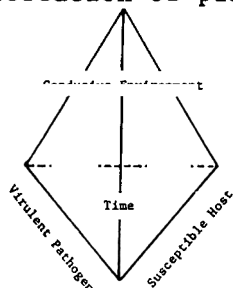
Fungal infection, mechanical injury, heat or cold damage, chemical injury. Collect representative samples of all stages.



smut

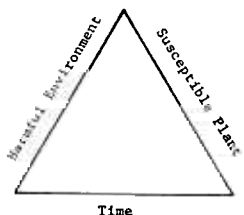
Fungal infection. Collect representative samples of all stages.

The disease pyramid, historically a staple in fundamental plant pathology courses, is very useful to remind the inspector/collector of all of the factors involved in the manifestation of plant diseases caused by pathogens. The volume of the pyramid, representing the amount of disease, would be proportional to the quantified value of each of the four sides of the pyramid.



Where the three factors of virulent pathogen, susceptible host, and conducive environment occur simultaneously for an adequate period of time, disease will occur. (Incidentally, studying this disease pyramid will also bring to mind several options for controlling plant disease.).

Diseases caused by abiotic factors can be similarly represented diagrammatically by this two dimensional model:



In this case, the area of the triangle representing the amount of disease would be proportional to the quantified value for harmful environmental condition, susceptible plant, and time. Therefore, where the two factors of harmful environmental conditions and susceptible plant (no longer a host, as such) coincide for a sufficient period of time, disease will occur.

Inherent plant defects which result in disease symptoms can be expressed under stable, innocuous environmental conditions, or may require some normally harmless abiotic or biotic stimulus for expression. A few documented cases of disease caused by exopathogens (pathogen not in actual intimate contact with susceptible plant) are known (7), and may have been mistakenly attributed to inherent plant defects or abiotic diseases in the past.

While considerable literature has been generated on disease diagnostic techniques useful once the sample has been received in the clinic, (1,2,3,4,5), surprisingly little attention is given to the important initial step of the diagnostic process, the collection of the sample. **Without an intelligent approach to the acquisition of the sample, the accuracy of the entire diagnosis is suspect.**

Whereas it is helpful to know the mechanisms by which causal agents and inherent plant defects cause the symptoms of a disease, this information is not required in order to obtain a good sample for diagnosis. Good observational and detective skills are necessary. The inspector/collector should have a good concept of the normal appearance of the plants under scrutiny, so that samples of deviations from normal can be collected. Some disease is to be expected in a plant population even if (sometimes especially if!) there is human interference. If symptoms are seemingly undetectable, the disease is either not serious enough to warrant attention, or observational skills need improvement. Some suggestions to improve observational skills are given in Table 4.

One common omission in inspection is examination of underground or ground level plant parts. Avoid the temptation to collect only symptomatic tissues, as these do not necessarily harbor the pathogen. Sampling techniques are unavoidably destructive to the host plant, sometimes quite so, using even the most sophisticated diagnostic methodology. If there is ever any doubt about what normal plant appearance should be, or what to include in a sample for diagnosis, send whole plants including roots and soil (about a liter of soil and roots), or representative samples of all plant parts and soil from plants that are too valuable, too large, or otherwise impractical to send to the clinic. (Other considerations for collecting samples are given in Table 1.)

Some diseases will inevitably defy complete diagnosis, therefore resampling may be necessary. Even then, no thorough resolution of the problem may be forthcoming. The diagnostic team should come closer to the answer(s) with each attempt, however. Diseases of complex etiology (more than one causal agent) often fall into this refractory category, and are actually quite common (6).

Procedure for Sample Collection, Packaging and Submission

After deciding what to include in the sample, the following procedures for obtaining, packaging, and submitting the sample are suggested:

- 1) Obtain fresh material in reasonable quantity, several examples of the various symptoms being expressed. Be certain to include as many identifiable stages of the disease as are represented. Most recently developed symptoms usually afford the best material for diagnosis. Be sure to include suitable plant material for botanical identification, since occasionally field identifications may be in error or the host plant identity may not be known.
- 2) Lift roots carefully so as not to leave feeder roots or rotted roots behind. Include about a liter of soil for pH, soluble salts, and possibly a nematode assay.
- 3) Place samples in appropriately sized plastic bags, including a paper towel for a blotter if sample is very wet.
- 4) Wrap a wire twist-tie around stem at ground line to keep soil off of above-ground plant parts. Accurately label samples.
- 5) Keep samples cool and moist, protected from crushing.
- 6) Gather appropriate information from the grower (see Table 3) and make pertinent observations (see Table 4). Include this information on sample submission form. Complete mailing addresses and map locations are necessary, if owners want to be informed of diagnosis or site must be revisited. Also, clerical work in the clinic is greatly simplified when a properly completed submission form is included with the sample.

Table 2. Contents of a Field Kit for Plant Disease Sample Collection

Plastic bags	Pruning shears, pruning saw	Wire twist ties
Paper towels	Knife, hatchet	Plastic/rubber gloves
Trowel, shovel	Rubber bands	Hand lens 5-10X
Forms, pencil	Labels	Vials/jars with lids
Padded collection boxes	Camera, film, macro lens flash equipment	Large ice chest/cooler
Maps	Field manuals, compendia	Disinfectant for hands & tools

Table 3

Examples of information inspector should obtain from grower:

1. Host: cultivar/variety; age; propagation method; site prep; dates and rates of fertilizer, pesticide*, growth regulator applications; pruning; pinching; transplant; omissions or additions to usual/conventional culture program.
2. Irrigation: frequency, rate, timing; water quality; determination of need.
3. Soil/media: pH, soluble salts, texture, drainage, homogeneity, aeration, temperature, planting depth, cultivation, cropping history, earthmoving/construction, burial/disposal site, trash burning.
4. Environmental conditions: temperature, humidity, ventilation, wind, lightning, exposure, light intensity, air quality, method of heating and cooling, etc., all preceding and during syndrome development.
5. Rate of syndrome development, coincident with any treatment or environmental event.
6. Recent human, animal, insect, mite activity around or on symptomatic plants.
7. Idiosyncracies of plants in question, predisposition (plant made more susceptible) by cultural or environmental conditions.

*Take safety precautions appropriate for pesticides used on crop.

Table 4. Examples of observations to be made by inspector:

1. Spatial pattern of disease in crop, on individual plants. Are symptoms different or consistent, uniform or scattered?
2. What is the frequency and intensity of the syndrome?
3. Any signs of pathogen/causal agent? (fruiting structures, chemical residues, insects/mites, frass, etc.).
4. Any evidence of host recovery?
5. Nearby plants (same or different) show any symptoms? Are root zones shared?
6. Inspect interior, crown, roots of plant. Cut open stems, crowns, flowers, fruits, and roots. Are there any hidden, internal symptoms/signs of disease?

Conclusions: The whole process of disease sample collection for diagnosis is of pivotal importance. Unless done correctly, the entire diagnostic process may be side-tracked, and the subsequent recommendations for controlling or correcting the disease may be of limited effectiveness. Sample collection can be a difficult, time-consuming process, and there is no guarantee that the plant problem in question will be resolved with a single sampling. With all the variables involved in plant production, every plant problem demands different sampling techniques. By justifying and summarizing the various phases and techniques of the plant disease sample collection process, inspectors should enjoy greater efficiency, and more accurate diagnosis and resolution of the plant disease problems they encounter in the field.

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