

EXERCISE # 2**COLLECTION AND PRESERVATION OF PLANT DISEASE SAMPLES**

For practice in recognizing and identifying plant diseases, their symptoms, and their signs, look for diseased plants around your campus and vicinity. Spring through autumn is good times to do this, and parks, farms, orchards, gardens, and fencerows are good places to look. Don't overlook non-crop plants: you may find many diseases on landscape plants, weeds, etc.

The purposes of the collection are to:

- (1) Increase your awareness of and ability to detect plant diseases in the environment;
- (2) Develop your diagnostic abilities through firsthand practice;
- (3) Increase opportunities for diagnostic discussions, observations, and sharing of ideas in laboratory;

FROM WHERE THE SAMPLES SHOULD BE COLLECTED:**1. COLLECT FROM A VARIETY OF HABITATS**

You are encouraged to collect off campus as much as possible in order to see diseases in areas of crop production. Get permission to collect on private property, collect only where appropriate (e.g., don't collect in nature preserves or botanical gardens), and avoid excessive or harmful collecting, for instance, of rare plants.

2. STRIVE FOR A VARIETY OF

- a. Hosts (e.g., ornamentals, field and vegetable crops, fruits, etc.).
- b. Pathogens/causal factors (infectious and noninfectious; fungi, bacteria, etc.; insect-related specimens may be included if the insect injury has resulted in disease-like symptoms).
- c. Types of diseases (e.g., root rots, leaf spots) and symptoms.

3. COLLECT ENOUGH OF EACH SPECIMEN

To show representative symptoms, symptomatic stages, and signs (if any). If the sample amount is limited by practicality, grower reluctance, or other reason, check and note the condition of various parts of the plant/crop so that you can recall the overall disease "picture." Remember the elements of the disease triangle.

4. FIELD NOTES

Identification of hosts and diseases often requires information that must be recorded when the specimen is collected. Any soluble pigments such as the colors in flower petals or around disease spots, may fade or disappear, and should be noted when the specimen is still fresh. Also record, preferably in a field notebook:

- a. Name of the host (collect some "normal" plants or parts for verification).
- b. Location and habitat: state, country: Vegetation type (field, woods, garden, etc.)
- c. Cultural and environmental conditions that may be helpful in diagnosis.
- d. Date and collector's name.
- e. Severity of the condition. Are similar symptoms present on adjacent plants of the same or other species?
- f. Preliminary diagnosis.

5. MAINTAIN THE FRESHNESS OF SPECIMENS

By sealing them in plastic bags, by refrigeration, and by processing them promptly. Specimens that are moist, succulent, or otherwise prone to rot or degeneration should be wrapped in paper before being placed in sealed plastic bags. Materials that are dry when collected should be placed in paper bags. Keep your specimens away from heat at all times. Refrigerate specimens prior to submission or processing, particularly if more than overnight storage is needed before a class meeting. If refrigeration is not possible or convenient, keep the specimens as cool as possible. If specimen degeneration is expected because of moisture, partial drying between papers may be helpful.

6. ATTEMPT A DIAGNOSIS.

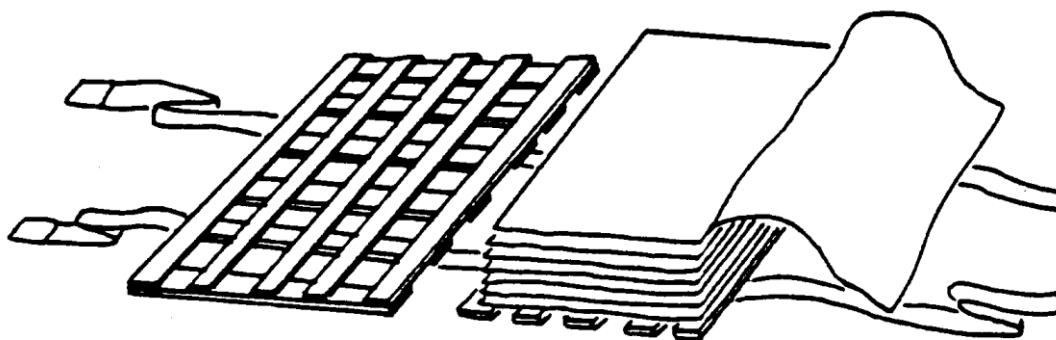
- a. Many leaf-inhabiting, and some stem-inhabiting, fungi produce sori or fruiting bodies that can be seen with a 10X hand lens. If you find a diseased leaf or stem with fruiting structures visible in the disease spot, bring it into the laboratory and try simple scrape mounts or free-hand sections to remove the spores and/or fruiting bodies. On other plants, you may find spots that may be disease spots, but no spores are visible. One to three days in a moist chamber (closed container or plastic bag with moist filter paper or towel) may induce production of spores on the surface of the plant tissue so that you can remove them to make your microscope mount. It is often not necessary to culture the pathogens any further than this.
- b. The **presence of bacteria may be detected by an ooze-test** (i.e., place some infected tissue in water and look for bacterial ooze issuing from the tissue).

7. PRESERVATION.

You may need to dry the plant parts in a plant press so they will keep without rotting until you can study them. A plant press is a "sandwich" of layers of blotting paper to absorb moisture from the specimen, cardboard ventilators to allow airflow

through the press to remove excess moisture, and a wooden frame, which is tightly tied together to put pressure on the specimens, driving out the moisture.

Remove any soil or trash attached to the plant material. Arrange the specimen neatly inside a folded half sheet of newspaper between two sheets of blotter paper, then between two pieces of corrugated cardboard. Repeat for additional specimens. Place the pile of specimens, blotters, and cardboard between the wooden frames and bind it tightly together with cord or straps. Set in a dry place or, preferably, over a source of warm air until the specimens are flattened and dry like tissue paper. Then store the specimens in paper envelopes. However, a plant drier does not have to be fancy: you can place leaves between layers of paper towels or newspaper under a stack of heavy books or inside an old telephone book. Remember to change to dry blotters or paper towels every day for the first 2 or 3 days, then every other day until the specimens are dry.



Succulent or fleshy specimens such as mushrooms or fruits can be dried whole by placing them on a screen over a source of warm air (about 40 °C or 100 °F is sufficiently warm) for a few days, or they may be "pickled" in a solution of 5-12% formaldehyde (toxic!) in water or in 30-50% alcohol with a small amount of glycerin added.

In actual research, plant pathologists keep these preserved specimens of the diseased host plant as "vouchers" for the characteristics and identity of the organism they are studying.

6. SUBMISSION PROCEDURE FOR PLANT DISEASE COLLECTION

1. Maintain the freshness of specimens by sealing them in plastic bags, by refrigeration, and by submitting them promptly. Specimens in a condition that precludes proper observation will not be accepted. Protect specimens from heat at all times. Refrigerate specimens prior to submission, particularly if more than overnight

storage is needed before a class meeting. If refrigeration is not possible or convenient, keep the specimens as cool as possible. If specimen degeneration is expected because of moisture, partial drying between papers may be helpful. Keeping leaf samples in a moist chamber for about 2 days and then drying those increases the chances that sporulation by fungal pathogens will be present.

2. Attempt a diagnosis on your own prior to submission and be prepared to discuss reasons for your decision. Wrong determinations will not be held against you but will, together with your stated reasons, be used by the professor to examine and develop your diagnostic skills.

Diagnosis is a gradually learned skill. The professor should comment periodically on the suitability and progress of the student's collection.

3. Bring collected specimens together with the Plant Disease Collection submission sheet to laboratory meetings. Fill in the submission form (may use a blank sheet of paper) and obtain the instructor's approval of each submission. For each submission, record the host (it is up to you to determine its identity), briefly describe the disease/disorder, and give the cause (to the extent possible). Explain and document your diagnosis! Draw a line across the page under each entry and then make the next entry immediately below, and so on. Keep your submissions list until the due date. Unless instructed to do otherwise, you may discard approved specimens.
4. On the due date, submit your Plant Disease Collection list to the professor. You are encouraged to work throughout the term to complete your collection, so get started as soon as possible.
5. You may wish to use specimens you collected yourself also for the diagnosis reports.

QUESTIONS

1. Why striving for variety of samples is important?
2. At what temperature we should store our samples in the laboratory?
3. Is the preservation of sample is important? If yes than why?