

## C. Sampling for Non-chemical Analysis Purposes

### 1. Pathology Diagnosis

The following field collection procedures will ensure that all samples collected for pathological investigation are received in the best possible condition for symptom examination, isolation and identification.

- Select specimens to illustrate as completely as possible the various parts of the plant affected by the disease or injury agent. Whenever possible, roots, tubers, bulbs, stems, leaves, flowers and fruits should be collected.
- It is important that all plant material arrive at the laboratory in good condition with a completed field label and assigned number. Samples should be collected in perforated plastic bags (large or small) so as to minimize dehydration/decay during transit and storage.
- All symptoms or indications of pathological agents should be recorded in a note book.

### 2. Nematode Disease Diagnosis

Field sampling for nematode identification should include soil as well as plant root or shoot components.

Notes of observed symptoms should be recorded as they may be helpful to the pathologist. Sampling should preferably be conducted late in the growing season (near harvest) as nematode populations are at their highest then. Paired sampling is necessary for comparing nematode numbers and kinds in diseased vs healthy plants. The first sample should be from the vicinity of healthy plants adjacent to diseased plants. Collect 10-30 soil cores (using a soil tube, or small-bladed or garden shovel) to give a volume of about 500-1000 cm<sup>3</sup> of soil. A similar second sample should be collected from stunted or debilitated plants in one or more areas in the field.

Soil should be collected using a "W" pattern in the affected row for annual row crops. The collection of soil cores on perennial crops, such as ornamentals should be within the area of the drip line of the foliage.

The depth of soil cores should be the rooting zone, generally the upper 15-20 cm of soil. Deep-rooted plants, such as fruit crops or ornamentals may require sampling depths as great as 1 meter.

Each soil sample should be placed in a water-tight container which is then placed in an insulated container to prevent exposure to heat or direct sunlight during transportation to the pathology laboratory. Sample handling should be conducted as gently as possible (ie do not drop or handle samples roughly) as this could kill the nematodes which must remain alive for extraction and identification.

### 3. Diagnosis of Insect Pests

Although investigators may be familiar with a variety of common insect pests, samples should be collected and referred to a pathologist for confirmation. Diagnosis can sometimes be made in the absence of the insect, therefore detailed notes of the symptomatology observed should be recorded and made available to the pathologist. In addition to any insects found, samples submitted to the pathologist should include all relevant components, eg. eggs, larvae, and adult stages, casings, characteristically injured foliage, twigs, bark etc. Live insects should be placed in a screw capped (perforated) jar. Plant samples should be placed in perforated plastic bags. All relevant observational notes should be copied for the pathologist.

### 4. Histology Diagnosis

#### a) WHMIS Requirements

The procedure used in the past for preserving samples in FAA (formaldehyde/ethyl alcohol/acetic acid) for histological diagnosis is strongly discouraged. FAA is toxic, and it is difficult to avoid inhalation of vapours when it is being transported in a vehicle, particularly during warm weather. If, however, samples need to be preserved in the field, then steps should be taken to avoid personal contact and/or inhalation of FAA fumes. Also, if FAA is to be transported in a vehicle, the bottles must have appropriate WHMIS workplace labels and must be accompanied by MSDS sheets for formaldehyde, ethyl alcohol and acetic acid.

#### (b) Sample Collection Procedures

The following important points are suggested to all field investigators when collecting samples for histological observation.

- Select very typical specimens which represent the injury that you are investigating. Although very little plant material is required, the sample should be typical. Specimens should include all stages of injury symptoms (incipient, intermediate and advanced).
- Specimens as "control" comparisons must be collected. These should include samples from healthy plants at the same stage of development but remote from the affected area.
- Record all relevant information and provide a copy of this information to the histopathologist.

**5. Herbarium Preservation**

A plant may be collected for the herbarium for one of three reasons:

- a) the specimen is a permanent one which is properly pressed, dried and mounted, labelled, and clearly illustrates the particular causal agent symptoms;
- b) the specimen collected may be for personal reasons, may show a peculiar symptomatology or may be required for a court case. It too is properly pressed, dried, mounted and labelled, but this specimen is placed in the herbarium;
- c) the specimen is collected for personal reasons by the investigator and is of value only to the collector. This plant is not mounted on herbarium paper but kept for only a short time then discarded.

The following is a guide to collecting plant material in the field to ensure good specimens for identification and for the herbarium. Complete details on pressing drying labelling and mounting are available in various herbarium procedures manuals.

- a) In collecting small herbaceous plants, the whole plant should be collected including inflorescence (if present) and roots, keeping in mind the size of the herbarium sheet (30 X 45cm).
- b) With larger plants, trees and shrubs, 25-30 cm long samples should be taken from the end of the plant or branch with several leaves intact and showing terminal and lateral buds, flowers and fruits.
- c) In collecting, care should be taken to select a good representative specimen which illustrates typical plant conditions.
- d) When collecting samples of injured plants, always include adequate material, either less injured or uninjured for positive identification of the host.

More detailed information concerning the collection, preservation, labelling and care of herbarium specimens may be found in various procedures manuals.

## CHAPTER 6: SAMPLE COLLECTION IN RELATION TO LITIGATION

Sample collection in cases where legal action may arise requires special care due to the influence this sampling may have on the defense of the case. In general, the sampling methods that have been described previously may be used. However, the following additional points and techniques should be fully read and understood before any samples are taken. The investigator must be able to guarantee the integrity of the sample up to the point of submission to the laboratory through proof of possession.

- i) The area where soil or plants are to be sampled should be carefully examined so that the investigator will be completely familiar with the overall geographic location of the sample. A sketch map showing the location of the samples is recommended.
- ii) The investigator-in-charge should obtain existing information on the alleged type of contamination involved and sample accordingly with respect to suitable containers and processing requirements.
- iii) As legal samples must be analyzed in duplicate, it is recommended that at least twice the normal sample volume be collected.
- iv) Samples should be collected from as many locations as necessary to effectively rule-out contamination from other sources; this will include the collection of control samples to establish background contaminant concentrations for similar soil types or plant species in at least two areas remote from the influence of the alleged source.
- v) It is preferable that the actual sampling be performed with the assistance of a witness who is willing to sign an affidavit and appear in court as a witness, if necessary.
- vi) All necessary forms and paper-work should be completed in detail and cross-checked to ensure that the sample numbers coincide with the material collected.
- vii) As the investigator-in-charge must be able to swear that the samples were in his/her possession and control before signing them into the processing laboratory, the following procedures must be followed:
  - the vehicle used to transport the samples must be locked whenever the investigator-in-charge is visibly removed from it.
  - on overnight investigations the samples must be kept either in the locked vehicle or, by special arrangement, in a cold room or refrigerator which can be locked. The investigator must maintain the sole key in his/her possession at all times.
  - All legal samples should have a legal seal attached, in addition to the regular sample identification number.
- viii) Once samples have been signed into the processing laboratory, it is the responsibility of the laboratory supervisor (or designate) to ensure that they are stored in a locked area and processed or delivered promptly to the analyst using the "locked-box" procedure.

## CHAPTER 7: INTERPRETATION OF CHEMICAL ANALYSIS RESULTS

Guidelines have been developed for a variety of contaminants, for both soil and vegetation. Appendices E and F provide two types of guidelines, "Upper Limit of Normal Guidelines" and "Clean-up Guidelines for Soils", as well as a brief explanation of the derivation and application of these guidelines. A more complete description of the development of the Upper Limit of Normal (ULN) Guidelines is found in Report No. ARB-138-88 Phyto. Application of the soil clean-up guidelines is discussed in considerable detail in the Waste Management Branch policy document entitled "Guidelines for the Decommissioning and Cleanup of Sites in Ontario".

The Ontario Ministry of Environment and Energy is currently in the process of replacing the ULN guidelines with a more extensive soil and vegetation background data base to be referred to as OTRs (Ontario Typical Range). It is expected that this initiative once approved by the Advisory Committee on Environmental Standards (ACES) will take several years. During that time, OTRs will gradually replace ULNs.

Appendix E contains the MOEE's Soil Clean-up/ Decommissioning Guideline and includes a discussion of the manner in which the various soil guidelines should be interpreted.

Lastly, Appendix F presents a table of conversion factors to enable the investigator to convert contaminant concentration results for garden produce on a dry weight basis (i.e. as received from the analytical laboratory) to a fresh weight (as consumed) basis. Fresh weight results enable comparisons with health-based consumption guidelines. This appendix also contains an "Note Regarding Significant Figures".

## APPENDICES