Plant Disease Clinic Sampling and Shipping Guidelines

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*****Please complete a submission form and send it with your sample*****

1. Plants for disease diagnosis:

- Dead plants are not suitable for disease diagnosis. The further a disease progresses, the more likely secondary pathogens and saprophytes are present. This makes diagnosis difficult.
- Select several plants, if possible, showing representative symptoms and stages of disease.
- Send the entire plants, including roots, if possible. Above ground symptoms can be the result of root rots.
- Wrap plants or plant parts in newspaper or paper towel (tying roots & soil off separately to reduce contamination) and put in a plastic bag or container.
- Do not add moisture to the sample. Moisture will promote growth of secondary organisms and make diagnosis difficult.
- Do not ship over the weekend.
- During extreme temperatures in summer or winter, please send plants in a styrofoam container to help preserve them during shipping. Plants that are very wilted from heat stress during shipping or that freeze during shipping are not suitable for diagnosis.
- It is very helpful if you record your concerns on the submission form:

- What is your concern: Wilting? Leaf spots? Root rot?
- Do you suspect a specific disease because you have had it in the past? Please tell us.
- The plants appear healthy but you would like to check for a specific disease(s)? Please tell us.
- You would like us to run a specific test? Please tell us.

2. Insects for identification:

- Select insect identification on the sample submission form. Insects are identified by morphological characteristics unless you state clearly on the submission form that you require identification by DNA sequencing.

- Record on the sample submission form the location where the insect was found. If the insect was associated with a plant, record the species of plant on the form.

- Live insects should be sent in a sturdy container (e.g. pill vial) and should have sufficient food to survive until arrival. Clearly label the package with 'Live Insects'.

- Dead insects should be sent cushioned in a sturdy container.

- Do not send insects in water. Do not tape insects to paper. Do not send insects loose in an envelope.

- Because ethanol is considered a dangerous good and there are regulations concerning shipping ethanol, we suggest that you do not ship insects in ethanol.

- If you are sending an insect for identification by DNA sequencing, do not store the insect in ethanol.

3. Turf for disease diagnosis

- Sample from the edge of symptomatic turf so there is a mixture of healthy and symptomatic turf. Do not sample from an area where all the turf is dead.

- Sample a 7 to 10 cm diameter core that is deep enough to include the roots (at least 5 cm).

- Wrap the core in newspaper or paper towel before placing it in a plastic bag. The wrapping will absorb excess moisture and help prevent soil from dislodging and covering leaf symptoms. Do not add moisture to the sample.

4. Turf for nematode counts

- Using a 2.5 cm diameter soil corer to take samples to a depth of 10 cm (or the depth of the root system) works well. Each core is considered a subsample and

subsamples can be combined in a plastic bag to make one sample. Do not add moisture to the sample.

- For areas where there is damage suspected to be caused by nematodes, collect at least 10-15 subsamples throughout the affected area and combine them into one sample. It is helpful to collect a second sample, from an adjacent healthy area, for comparison. Remember to clearly label the samples as ‘damaged’ or ‘healthy’.

- For areas where there is no obvious damage but nematode counts are desired, collect at least 10-15 subsamples from throughout the area to be tested. Combine subsamples into one plastic bag. Do not add moisture to the sample.

5. Grapevine virus testing

Dormant canes:

- For testing grapevine leafroll associated virus (GLRaV) and grapevine red blotch associated virus (GRBaV), dormant canes can be collected whenever the canes are fully dormant (completely hardened off). Collect 3-5 canes per sample.

- Wrap the canes with paper towel or newspaper, before putting them in a plastic bag. Do not add moisture to the sample.

- Label the sample clearly.

- Fill out a sample submission form (you can attach a list of your sample IDs if multiple samples are submitted). Attach it to your sample.

- Ship the sample to the lab as soon as possible, preferable by overnight delivery. If there is a delay, refrigerate samples until they can be shipped. Do not ship over a weekend. Do not expose samples to direct sunlight or to high temperatures.

Leaves:

- Collect the leaves at the appropriate physiological stage. For testing GLRaV, collect well-developed mature leaves from the lower part of the plant late in the growing season. For GRBaV, we recommend matured leaves with petioles.

- Each sample should contain 5 leaves (with petioles). Wrap the leaves in newspaper or paper towel before putting them in a plastic bag. The wrapping will absorb excess moisture. Do not add moisture to the sample.

- Label the sample clearly.

- Fill out a sample submission form (you can attach a list of your sample IDs if multiple samples are submitted). Attach it to your sample.

- Ship the sample to the lab as soon as possible, preferable by overnight delivery. If there is a delay, refrigerate samples until they can be shipped. Do not ship over a weekend. Do not expose samples to direct sunlight or to high temperatures.

6. Water for DNA water scan and other testing

- Samples of 1000mL are recommended but smaller amounts can be tested.
- Samples should be collected into clean bottles, preferably sterile plastic water bottles, but clean empty drinking water bottles also work.
- Send samples to the Plant Disease Clinic immediately after sampling if possible. Keep the water samples cool. Refrigerate samples if they cannot be shipped immediately. If you are not able to bring the sample to our lab, ship the samples using overnight courier.
- Do not ship over the weekend.

7. Sampling soil for DNA soil scan

Note: When symptomatic plants are present it is better to sample plants, including roots, than to sample soil.

When sampling soil, it is important to gather a representative sample.

- To sample an entire field, take sub-samples from ten or more locations throughout the field. Mix the subsamples together thoroughly in a clean container or plastic bag. Send approximately 1 pound of the soil mixture for testing.

- If only a certain area(s) of the field is being affected, you can collect subsamples from affected and unaffected areas. Keep the samples separate and send two samples for comparison.

- When sampling soilless mix or other growth media, collect subsamples and mix them thoroughly in a clean container or plastic bag to get a representative sample. Send one to two cups of the growth media for testing.

For shipping/ submission, place samples in a plastic bag or plastic container and label clearly with a waterproof marker or label.

Shipping samples immediately after sampling is best. Samples can be stored at 4^{o}C until they can be shipped. **DO NOT** freeze samples or expose them to heat.

8. Nematode counts

From roots:

- Root samples can be taken whenever the soil is not frozen. In Ontario, nematode populations are generally highest in May-June and again in September-October.

- For small plants, the entire root system and surrounding soil of plants should be submitted.

- For large plants, 20-30 grams of fresh weight roots should be dug from the feeder root zone.

- For problem areas, root samples should be dug from the margin of the problem area where plants are alive. For comparison, samples should be taken from healthy areas.
- For shipping/submission, place samples in a plastic bag or plastic container and label clearly with a waterproof marker or label.

- Shipping samples immediately after sampling is best. Samples can be stored briefly at 5-10°C until they can be shipped. **DO NOT** freeze samples or expose them to heat.

From soil:

- Soil samples can be taken whenever the soil is not frozen. In Ontario, nematode populations are generally highest in May-June and again in September-October.

- For most plant parasitic nematodes, soil is sampled to a depth of 20 cm (8 in.); for bare soil areas, the top 2 cm are removed prior to sampling. ***For bulb and stem nematodes, the top 2.5-5 cm (1-2 in.) of soil is sampled.*** If your concern is bulb and stem nematode, please indicate this on the submission form because a different extraction procedure is used.

- One sample should equal about 1 litre of soil which is obtained from a mixture of 10 or more sub-samples or soil cores (use soil sampling tube, trowel or narrow bladed shovel).

- The number of sub-samples taken depends on the size of the area. For example, if the area is less than 500 m^2, take at least 10 sub-samples; if the area is 500 m^2 to 0.5 ha, take at least 25 sub-samples; and if the area is 0.5 to 2.5 ha, take at least 50 sub-samples.

Note: No one sample should represent more than 2.5 ha.

- Each soil sample should be from an area of uniform soil type.

- See figures below for sampling patterns.

- For bare soil areas, the top 2 cm are removed prior to sampling. If crops are present, sampling should take place around the feeder roots or, in the case of trees, around the drip line.

- For problem areas, soil samples should be dug from the margin of the problem area where the plants are alive. For comparison, samples should be taken from healthy areas.

- Subsamples should be mixed in a clean pail or plastic bag.

- For shipping/submission, place samples in a plastic bag or plastic container and label clearly with a waterproof marker or label.

- Shipping samples immediately after sampling is best. Samples can be stored briefly at 5-10°C until they can be shipped. **DO NOT** freeze samples or expose them to heat.

Nematode Sampling Patterns

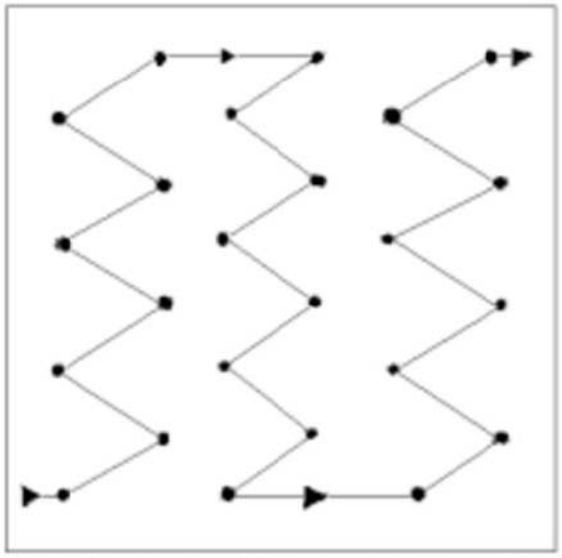

Figure 1. Bare soil/cover crops – Example of how to sample throughout the field.

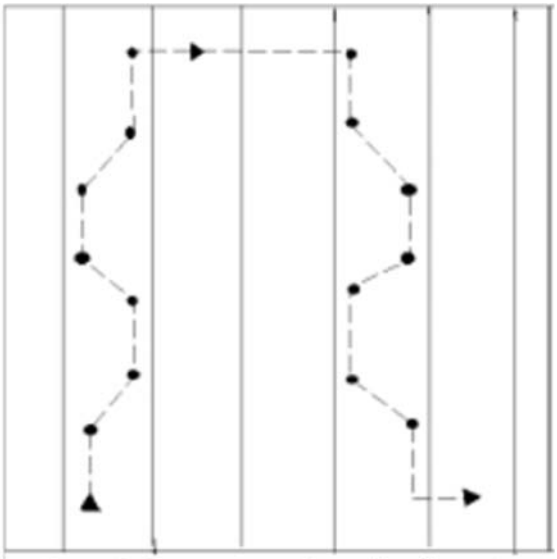

Figure 2. Row crops – Example of how to take samples in a field (samples are taken from feeder roots).

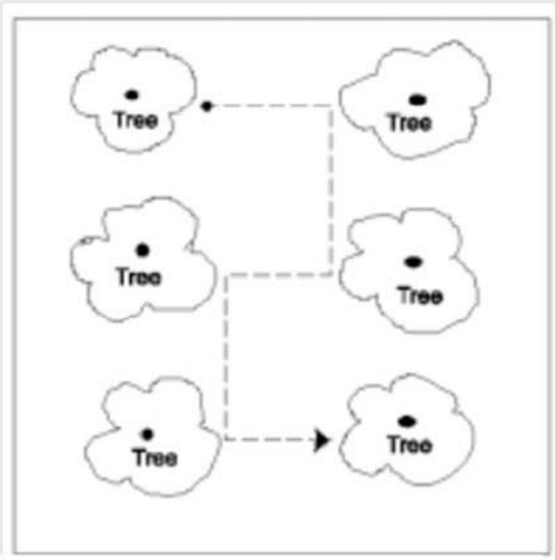

Figure 3. Row trees – Example of how to sample in an orchard.

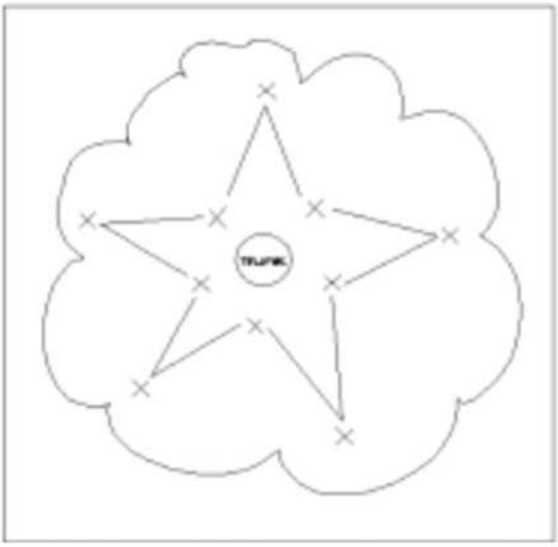

Figure 4. Individual trees and shrubs – Example of how to sample around one plant.