Soil Sampling for plant parasitic nematodes

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Effective nematode management begins with soil sampling to determine genera and or species and their densities present within a given area. Nematode distribution is seldom uniform or consistent, but rather tends to be patchy within the field. Thus, sampling should be conducted on a regular basis, especially when nematode populations are medium to high. If the population level is low, monitoring every 3-5 years is recommended, depending on the crops grown and nematode genera present. Nematode distribution patterns should be taken into consideration for accurate nematode estimation. When deciding how many samples to collect from a field, remember that sample numbers are directly correlated with the precision of nematode densities, i.e. more samples will provide more precision.

Why Soil Sampling? Soil samples provide an estimate of plant parasitic nematode populations in the soil and may be preventative or diagnostic**:**

1. Preventive: Since nematodes are widespread across the region, you may have interest knowing pre-planting nematode estimates to determine any future potential problems and/or management needs. If a field is greater than 5 acres, sub-divide the field into several sections based on soil type, yield pattern, crop rotation etc. A soil probe, trowel, or shovel may be used for sampling. Within each section, collect 10-25 subsamples and mix well to make one representative sample/bag per field.

Although the objective of detection seems simple, careful sampling is needed for accurate detection, especially when populations are low. A negative result does not necessarily prove absence of nematodes, but only indicates that a population level is below the detection level. The best time for preventative sampling is towards the end of the growing season or immediately after crop harvest when population densities are near their maximum levels.

2. Diagnostic: A diagnostic sample can be collected if a problematic patch or patches within the field are showing abnormal symptoms that cannot be explained by other causes. This type of sampling can also be conducted to investigate the cause of observable yield decline over time. Sampling for nematodes has been increasingly important for plant disease diagnosis, especially for high value crops, golf greens, vegetables, and nursery stock because nematode damage can be difficult to distinguish from nutrient deficiencies, some fungal and bacterial infections, viral infection, or some abiotic stresses.

Soil sampling may vary with the nematode feeding behavior. Nematodes can be both endoparasitic (feeding within the root) or ectoparasitic (feeding outside of the root). Ectoparasitic nematodes such as spiral or dagger nematodes actively feed within the root zone. When dealing with endoparasitic nematodes such as root-knot or root lesion nematodes, soil samples alone may not be enough for the correct estimation of population densities. When collecting diagnostic samples for endo or ecto-parasitic nematodes, soil samples should be collected near the root zone and you may also need to include root and or foliage tissue

along with soil. When submitting diagnostic samples, it may be helpful to also include soil and/or plant samples from adjacent, healthy-appearing plants/areas nearest to the most severely affected plants/areas. Numbers of diagnostic samples to be collected vary with field size and type of problem suspected. If the severity of the symptoms varies in the field, include samples representing various severity categories. Include at least three samples in each category and submit these samples along with sample from healthy looking plants/areas.

When to sample? Sampling can be conducted at any time of year; however, careful interpretation of results is critical as populations can vary based on soil type, season, and crop grown. To improve sample quality:

a) Sample at the right time of year: The optimal time to sample is early fall, usually before harvest in preparation for the following season/crop.
b) Sample in the right environmental conditions: When collecting nematode samples the soil should be moist, but not excessively wet or frozen.
c) Keep record of chemical applications: If nematicides were applied, samples collected following chemical application, but before planting, can be compared to end of season samples to determine the effectiveness of a treatment. This type of sampling can help to estimate the change or serve as baseline knowledge of population levels for the next crop or treatment.

Where to sample? For preventive sampling, collect random samples in a zigzag or w pattern across the field. Figure 1 demonstrates recommended soil sampling patters for annual and perennial crops. For diagnostic sampling, collect samples from problem areas and healthy areas. Check with the lab, if they accept and process root and foliage samples, these may be helpful in addition to the soil submission.

Soil samples should be collected from the plant root zone, 6-12” deep in annual crops/plants or turf, and 12-18” deep in trees. If possible, include some roots along with the soil sample. If no crop is planted, take samples to fit the intended root zone. Before planting annual crops, sample cores from a fallow field should be taken by first removing the upper two inches of soil. If roots of a previous crop are present deeper in the soil, the topsoil is dry, or if the site is high in organic matter, then sampling to a depth of 15 to 20 inches is recommended. Deeper cores, up to 30 inches, should be taken after prolonged fallow, dry or freezing conditions, or in fruit tree orchards. Do not forget to include all soil, root, and foliage samples along with similar healthier area as well.

A. and B. Patterns for annual crop or fallow field.
C. and D. Patterns for perennial plants.

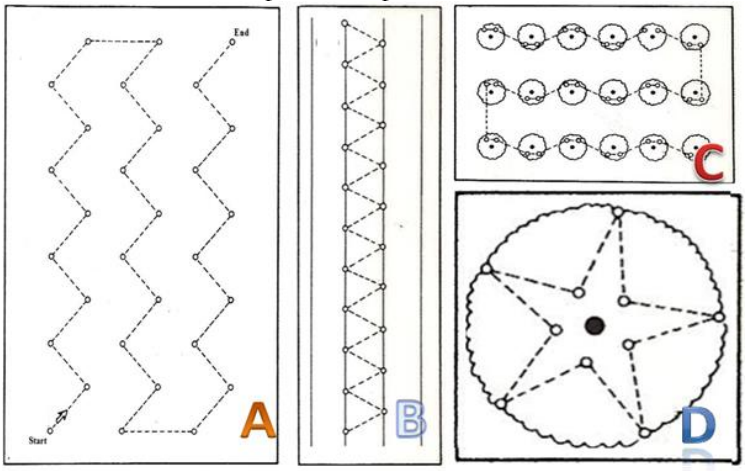

Fig.1. Suggested soil sampling pattern for nematode analysis from: A) larger field up to 5 acres, B) two center rows out of four rows of crops, C) perennial crop, and D) feeder root zone, adapted from Baker, 1988.)

Sampling Pattern: The sampling pattern should be designed to obtain a reliable representative sample with as little sampling error as possible. An example of a recommended soil sampling pattern for fallow ground or an established crop is shown in Figure 1. Sample size depends on the area and crop being sampled, the crop's value, and any supplementary information to be gathered during sampling. Sample size is measured in terms of the number of cores of soil constituting the sample. The precision of the resulting nematode estimates can be improved by increasing the number of soil cores in a sample. This is less expensive than increasing the actual number of samples. A useful rule-of-thumb for estimating cost is to consider allocating 1 percent of the crop's total production expense on nematode sampling. A good approach in sampling is to bulk soil cores in a clean bucket, mix thoroughly, and submit one quart of the mixture for processing. Place the sample in a sturdy, moisture-retaining bag and clearly identify with a tag attached to the outside of the bag. Tags or labels inside the bags may get wet and discolor easily.

Factors to consider while designing sampling procedure:

1. Because of the clumped distribution patterns of nematodes, large fields should be subdivided, to avoid biased results.
2. Since nematode species move very short distances in the soil, the majority of their movement is enabled by humans or their activities.
3. A majority of PPNs are present where active roots are found in the soil. Thus, while sampling, collect soil near to, as well as along with, some feeding roots.
4. Biology, feeding habits, and environmental interactions of the nematode genera/species may influence distributions and densities.
5. Crop types, rotations, soil type, and cultural practices all affect nematode genera/species present and their densities. These factors should be considered while sampling.

Soil sampling equipment. Soil sampling for both plant nutrients and nematodes are similar except that soil for nematode assays should be moist when collected; avoid heating and drying while handling and storing samples. Equipment needed for nematode estimation (soil probe, bucket, bags) complements tools needed for soil nutrient analysis, so it is often convenient to collect both at the same time.

Tips for Nematode Samples. Take the initial sample from a healthy area and then sample symptomatic areas. Clean sampling tools when moving between different symptomatic fields. Use re-sealable, clean, convenient sized plastic bags. Provide about half a pound to a pound of representative soil. Label each bag clearly (using your ID system) and keep a log of the ID numbers on a separate form, for reference.

Keep samples cool in the field by placing samples in shade or preferably in a cooler while in the field and during transport. An insulated cooler is convenient for sample protection. Do **not** expose soil samples to high temperatures/direct sunlight, do not let the samples dry out (make sure bags are sealed tightly); do not leave in a car trunk, or other area that may heat excessively; do not put the soil samples in a freezer; and **DO NOT ADD WATER**.

Deliver or mail the samples to the processing laboratory as soon as possible preferably overnight mail. Write instructions to protect from direct sun and high temperatures during transport and delivery. Include the nematode diagnosis request form with appropriate information.

Storage and delivery of samples.

It is very important to handle field collected soil samples carefully. Inappropriate handling, storage, and delivery result in inferior or misleading results, even if samples were carefully taken. During collection, storage, and transportation, samples should be kept cool (ideally, 50 to 55^{o}F), and moist**.**

Samples must be clearly labeled and accompanied by complete background information.

Generally, each laboratory processes 100 to 500 cc. of soil per sample. Reducing soil samples into smaller volumes that provide representation of the field is very important.