

EXERCISE NO. 8**COLLECTION AND STORAGE OF SAMPLES AFFECTED BY
PLANT PARASITIC NEMATODES****PRACTICAL AND THEORETICAL CONSIDERATIONS**

When making a sampling plan for estimating nematode numbers, biomass and species composition, a number of practical and theoretical considerations must be observed. The design of the sampling plan will partly depend on the purpose of the study, the required accuracy, the time frame, as well as the costs involved. Besides, when making the plan, knowledge about the variation in space and time of nematode populations, their biology, and the influence of abiotic factors has to be considered, to arrive at a correct choice regarding timing and depth of sampling, tools, number of samples and the sampling pattern.

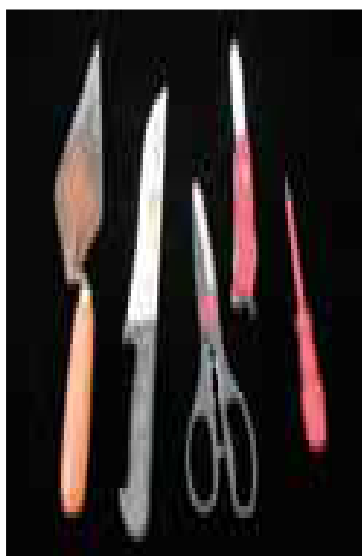
THE SAMPLING PROCESS**TOOLS**

Sampling can be carried out using a variety of tools, ranging from an ordinary spade to special soil samplers or augers. The type of sampling device depends on the purpose of sampling. One sample consists of several cores. Since the level of accuracy increases with the number of cores, it makes sense to take out only a small amount of soil per core, thus limiting total sample size. However, the diameter of the soil sampler or auger should not be too small, since too much friction harms the nematodes. A 17 mm auger is generally used. If vulnerable nematode genera of the *Trichodoridae* and *Longidoridae* families are to be sampled, it is recommended to use a sampling tube with a very wide diameter, e.g. a preserving can with both lids removed. The soil sampler must be inserted at a straight angle and be given a quarter or half turn before retracting, so that the soil will stick in the tube. Equipment always needs to be clean to avoid contamination from a previously sampled plot and to prevent a 'second degree error' (wrongly conclude that nematode species attached to a dirty tool is present in the plot). Apart from the described sampling tools, sketch paper and a pencil are used to record other details as the condition of the crop, the weather, date, locality, host crop, extent of damage and collector etc.

TRANSPORT AND STORAGE

To avoid dehydration, samples are preferably collected and stored in plastic bags. Use a permanent waterproof marker for writing on the label or bag, to avoid discoloration and bleaching. Avoid exposure of samples to high temperatures; a cooler

box is preferred. The samples must be handled with care because nematodes may die from shock and friction. The samples must be processed as soon as possible, because storage may cause changes in nematode numbers and species composition. Data on storage effects mostly refer to plant parasites; increases and decreases in numbers have been observed. Bacteriovorous *Rhabditidae* multiplies at temperatures as low as 4°C. For a general guideline Samples are stored at 4°C. Keep storage time as short as possible. Samples can be stored up to half a year in wet without considerable effects on nematode numbers.



PRE-PROCESSING OF THE SAMPLE

a. MEASURING: VOLUME OR WEIGHT

The size of a sample can be described in terms of volume or weight, both having advantages and disadvantages. In research for advisory purpose or phytosanitary certification, sample size is generally given as volume (100 or 200 ml soil), while in other studies sample size is usually based on weight (100 or 200 g). Sampling and mixing destroy the natural structure of the soil, altering the degree of soil compaction. For that reason it is difficult to find the amount of soil that would correspond with a particular volume of soil under undisturbed field conditions. Using weight to express sample size has the disadvantage that large differences in the amount of soil per unit of weight occur, due to variations of soil moisture content. This problem, however, can be solved by making a simple measurement of dry matter content.

b. TAKING SUB-SAMPLES; MIXING

To achieve sufficient accuracy, a relatively large sample size is often needed. Usually, these cannot be processed completely, making it necessary to take sub-samples

(Note: this represents an extra source of variance!). Before taking a representative sub sample, the sample needs to be homogenised by mixing. If homogenisation is carried out thoroughly, analysis of one sub-sample is sufficient (Carbonell & Angulo 1979). Mixing the sample can be done mechanically or manually. The sample should be mixed with care as each treatment causes mortality of nematodes. Mixing by hand causes less damage to the nematodes and is therefore preferred to mechanical mixing. Manual mixing is a useful method for most samples, except for heavy clay soil. Disadvantages of manual mixing are its labour intensity and difficulty to standardize.

A procedure suitable for soil or other fine materials (e.g. seed) is to pass the sample through a coarse sieve (mesh size of 0.5-1 cm) to remove stones, roots, etc. Mix the sample on a small sheet ($\pm 1 \times 1$ m) by lifting one sheet corner making the soil roll over to the opposite corner. Do the same with all corners and repeat a fixed number (generally six) of times. Take a sub-sample of the desired volume or weight, using small scoops from different parts of the sample. It is possible to obtain a representative sub-sample by taking small scoops from different parts of the sample without previous mixing. This may be preferred when vulnerable nematodes are expected, e.g. *Trichodorus*. Mechanical mixing is generally not used for taking sub-samples, but for homogenizing or making a suspension. For plant material, a blender can be used, whereas soil samples are usually mixed with a mixing machine such as the Hobart dough mixer used at bakeries, which consists of a large batter bowl with a dough blade. Prior to mixing, water is added to the sample until it is just submerged. For mixing heavy clay or large soil samples, sodium hexametaphosphate or other appropriate chemical is added to the sample (a dose of 1% of the soil weight) to break bonds between (clay) particles. Sodium hexametaphosphate is not harmful to most of the Tylenchid nematodes, but its effect on other nematodes is not known.

QUESTIONS

1. Why the infected plants are not pulled out?
2. How the samples are collected from citrus?
3. Why the data about locality, date of visit and crop is recorded during sampling?