

# FACT SHEET DIAGNOSIS OF COMMON NEMATODES IN HAITI AND PRACTICAL METHODS FOR NEMATODE EXTRACTION FROM SOIL AND PLANT TISSUES

Demesyeux, Lynhe, MS.c.<sup>1</sup>

#### **General introduction on nematodes**

#### Structural aspect and lifestyle

Nematodes are thread-like roundworms, generally microscopic animals, with a serpentine mode of locomotion that live in a wide range of environments including soil, fresh and salt water (Kumar and Yadav, 2020). Their length varies from 0.3 mm to 8 m and their width is between 0.01 to 0.05 mm (Coyne et al., 2018). They are the most numerous organisms on earth with over a million of species identified (Lambshead, 2004). They are highly diverse, complex, specialized metazoans and feed on multiple organisms Some species are free living and others feed on fungi, bacteria, insects, animals, humans, other nematodes and plants (De Ley, 2006; Kumar and Yadav, 2020).

Plant parasitic nematodes differ from other nematodes by a special feature called a stylet that they use for penetration, suction of plant juice and for secretion of enzymes inside the plant tissues they parasitize. Their stylets can be short and strong (Stomatostylet), long and slender (Odontostylet) or long and curvy at the base (Onchiostylet) depending on the feeding mode of the species (Figure 1). All plant parasitic nematodes are microscopic. They can be as short as 250  $\mu$ m, but can reach up to 4mm, with an average of 1 mm long. They are generally slender with a width between 15 to 35  $\mu$ m (Lambert and Bekal, 2002). They are transparent and have a cylindric body shape. However, the female of some species become swollen and rounded in their adult stage due to their sedentary lifestyle (Lambert, K. and Bekal, S. 2002).



Figure 1: Mouth part structure of different trophic group of nematodes and different types of stylets of plant feeding nematodes. Photo credit: (Brantley, 2017).

I. Lynhe Demesyeux, Feed the Future Haiti Appui à la Recherche et au Développement Agricole (AREA) project.

Plant parasitic nematodes feed on all parts of the plant including the roots, flowers, the seeds. Usually, foliar nematodes cause damage that is more aesthetic than lethal. In severe infections, foliar nematodes can cause injuries, inducing defoliation and inhibiting or limiting flowering. The symptoms caused by foliar nematodes differ depending on the stage of the plant's development. For example, they can cause the young leaves to curl, twist, and stunt. On older, mature plants, they can induce small blotches with an angular and vein limited shape. In advanced stages on these mature leaves, the blotches become brown and dry which may leave a hole on the leaf and the leaf may eventually fall off of the plant (Meyers and Hudelson, 2014; Southey, 1993). Other typical symptoms of foliar nematodes are gall formation on the leaves or the seeds of the affected plant; leaf stripes; bleaching and discoloration of leaves; internal stem necrosis and inflorescence necrosis (Coyne et al., 2018).

Virulent plant parasitic nematodes feeding on roots and other underground parts of plants results in the reduction of the root mass and the alteration of the root structure. This morphological modification reduces the ability of the roots to absorb water and nutrients from the soil causing yellowing of the leaves, fewer and smaller leaves, nutrient deficiency, wilting, stunting, yield reduction, and in the most drastic stage, the death of the plant (Lambert and Bekal, 2002).

## Types of nematodes based on their feeding habit

Plant parasitic nematodes feeding on roots and other underground parts of plants are generally separated into three different categories based on their feeding habits and their mobility. The categories are: migratory endoparasites, sedentary endoparasites and ectoparasites (Figure 2).

Migratory endoparasites (ex: *Radopholus similis, Pratylenchus* spp.) are mobile nematodes that feed on cells located inside the plant root tissue. They burrow inside the root from one cell to another to look for new feeding sites. They may even migrate outside the plant tissue to find a fresh spot to feed on. All the juvenile and adult stages of nematodes belonging to this group are mobile, except for the eggs. The eggs are usually laid inside the plant cortical tissue during their migration and in the rhizosphere of the affected roots. The damage caused by the migration of the nematodes leave an entry for fungi and bacteria that usually causes a secondary infection in the plant (Figure 2) (Coyne et al., 2018).

The sedentary endoparasite nematodes (*Meloidogyne* spp. or root knot nematodes, *Heterodera* spp. or cist nematode) start feeding on the roots as early as the newly hatched females reach the second juvenile stage. They migrate from the soil to look for a feeding site inside the root of a host plant. They become sedentary for their total lifetime once they find a good feeding site inside the roots. In time, the slender young nematode develops into an adult female with a swollen body that is either spherical, lemon, kidney or ovoid shape. The males do not infect the plant but instead feed on the surface of the roots of the host plant. Their role is to fertilize the females and then they die a few days later in the soil. There is a subcategory of this group that includes nematodes that are also sedentary but only part of their body is inside the root tissue (*Rotylenchulus* spp. or reniform nematodes). The other half of their body stays in the soil where they lay their eggs. This category is called semi-endoparasitic (Figure 2) (Coyne et al., 2018).

The ectoparasites (*Helicotylenchus* spp.) are nematodes that feed directly on the outer surface of the root hair or on the cortical tissues but live in the soil. Even when they are found in high densities, they are generally not considered as a major problem except when the plant is already affected by other organisms. Some of these types of nematodes are associated with being vectors of plant viruses as they feed from one plant to another (Figure 2) (Coyne et al., 2018).



Figure 2: Types of plant parasitic nematodes. A) migratory endoparasitic nematode (Scutellonema bradys) in yam. B) sedentary endoparasite (Root-knot nematode: Meloidogyne spp.) in rice root. C) semi-sedentary endoparasite (Reniform nematode: Rotylenchulus spp.). D) ectoparasite (Aulosphora spp.) feeding on root rice. Photos credit: Bridge, J.

#### **Economic Importance of Plant parasitic Nematodes**

More than 4100 species of plant parasitic nematodes have been identified and most feed on the roots and other underground parts of plants. They reduce the ability of the plant to absorb water and nutrients from the soil resulting in a reduction of the general agronomic performance of the crop (Bernard et al, 2017). They constitute a huge liability for agricultural production since sizeable crop losses have been associate to their presence in field production. On a worldwide basis, their economic impact is estimated to be over US \$157 billion annually. They are found in every crop and it has been reported that a single species can lead to the loss of up to 85% of an entire plantation (Gowen et al., 2005) (Singh et al., 2015). Therefore, it is important to perform diagnosis for plant parasitic nematodes in crops and develop a proper management plan for these agricultural pests.

## **Diagnosis of Plant Parasitic Nematodes**

Plant parasitic nematodes are microscopic and often their negative impacts to food production is underestimated by producers and researchers worldwide. Often, their unspecific symptoms lead to misdiagnosis on the part of agronomist and agricultural managers who frequently attribute their damage to other causes plant pests (insect, fungi, bacteria and even abiotic causes). For optimal crop production, it is important to test for plant parasitic nematodes as well as other agricultural pests (Coyne et al., 2018). Moreover, an assessment of plant parasitic nematodes is necessary in order to determine the spectrum of the nematodes present in the field and to help decide which control method is needed before planting and during the crop cycle.

Diagnosis of plant parasitic nematodes includes the following steps: sampling, extraction from plant tissues and from soil, identification and quantification.

#### Sampling for nematodes

Sampling for nematodes should be performed once symptoms of potential plant parasitic infestation is observed in the field. A number of considerations should be taken prior starting the process.

- 1. Accuracy of sampling: the level of accuracy required depends on the purpose of the sampling. For diagnosing plant parasitic nematodes presence in the field, the level of accuracy is typically lower than for ecological studies or for issuing a phytosanitary certificate. More intensive sampling is more costly due to the number of samples necessary. For diagnosis purposes, a simple plan is generally sufficient (Bezooijen, 2006)
- 2. **Variation in space**: the sampling plan should take into consideration the variation of the nematode population across the field, and in the soil. The samples should be taken following the distribution of the plants in the field but and the depth of the root zone of the crop. Thus, when sampling, it is

recommended to remove the first 5 cm of top soil (since the nematodes may not live in this environment due to compaction, dryness and other factors) and then, take the roots and soil samples from 15 to 20 cm deep. This depth is generally acceptable for vegetables and fruit trees (Jagdale & Cross, 2011) (Bezooijen, 2006).

- 3. **Variation in time**: It is important to sample at an appropriate time in order to have a good representation of the nematode population in the field as it fluctuates throughout the year. Some species are in abundance in autumn while others proliferate more in summer. However, nematode populations are generally higher at the end of the growing season and from late summer to the beginning of winter. In general, any time during the growing season is considered as an appropriate time to sample for diagnositic purposes (Jagdale & Cross, 2011) (Bezooijen, 2006).
- 4. **When**: Samples should not be taken when soil is extremely dry or wet as it affects the nematode population

#### Sample number

To successfully estimate the diversity and occurrence of the nematode population in a selected area, taking sufficient number of samples is important to have an accurate representation of field. Since nematode distribution can be haphazard and patchy, the site should be divided into blocks depending on the size of the field. For larger fields, the size of a block can be one to two hectares and separated according to their cropping history; the crop that is being grown in the field during the survey, the stage of the crop, the soil type and level of soil compaction. From each block, enough samples should be taken to have an accurate representation of the nematode population in the site. Ten to fifty (ideally at least 20) subsamples should be taken and then combined to form a composite/bulk sample. Once all samples are combined into a composite sample for the site, a subsample should be taken once the composite is manually mixed in an effort to reach homogeneity (Coyne, 2018). The mixed sample from each block should be properly identified and separately stored for analysis.

#### How to sample

There are numerous patterns one can follow when screening for nematode populations in a field. They can be categorized into random and systematic patterns. Some are more appropriate for a given crop and field than others. For instance, for a perennial crop, it is recommended that the samples are taken following a systematic pattern since overall it ensures a better chance of obtaining the nematode distribution and the variability of the field. Random sampling can be used too, but it is generally not recommended as they do not consider the patchy nature of the nematode populations. It can be used only for sampling small areas. (Figure 3). (Coyne, 2018; lagdale, G. B., & Cross, J. (2011).)



Figure 3: Sampling pattern for plant parasitic nematodes, where "a" is a random pattern and "b to d" represent systematic patterns (Coyne, 2018).

To sample single plants such as a fruit tree, the subsamples should be taken under the canopy drip line of the plant. The subsamples of each tree should be mixed into a bulk sample from which a sample will be taken to the laboratory (Jagdale & Cross, 2011)

# Tools

The tools needed for nematode's sampling are very simple and as follows:

- A soil auger can be used if available, but a simple shovel is as efficient as the soil auger.
- A plastic bucket to place and mix the subsamples taken from a block
- Plastic bags
- Permanent markers
- Recording sheet and pen

# Sample handling

Samples collected from different areas (blocks) should be stored into separate plastic bags that are properly identified with the location of the field (GPS coordinates when possible), the name of the owner of the property, the block number, the sampling date and the sampled crop in the plot. Samples should be stored in an insulated cooler to prevent dehydration of the sample and damage of the nematodes before delivery to the laboratory for examination.

## Nematode extraction

Immediately after sampling, the nematodes should be extracted from plant tissue and soil samples to prevent deterioration of the nematodes in the samples. There are various extraction methods. Some are more specific to plant tissues or to soil and others can be used to extract nematodes from both soil and root samples. Moreover, some methods are based on factors such as the nematode mobility, the rate of settling, size and shape. Choosing an extraction method will depend on the sample type, the nematode types and the materials available for the process (Bezooijen, 2006) (Coyne, 2018).

For the purpose of this work, we will describe the Baermann funnel/tray/ pie-pan method, which is used for mobile nematodes. It is an affordable, simple and practicable method for laboratories with small budgets. Also, it can be used to extract nematodes from both plant tissues and soil samples.

## Extraction Method for Nematodes within Plant Material using Baermann tray/Pie-pan method

This low-cost method is suitable for underdeveloped countries because the equipment and materials needed for extraction are easy to find or fabricate. Nonetheless, its efficiency at capturing all nematodes in a sample is lower compared to other extract methods. The slower and larger nematodes are generally not extracted easily with this method and the extraction liquid is dirty which makes identification tedious and more time consuming compared to other methods.

# **Equipment needed**

- Beakers
- Screen with coarse mesh or domestic used sieve
- 38µ sieve
- Tray/plastic bowl slightly bigger than the screen

- Coffee filter or tissue paper
- Knife or scissors
- Wash bottle
- Kitchen blender
- Kitchen scale
- Permanent markers

# Procedure

- I. Label each tray/bowl with the same information that appears on the label for each sample
- 2. Prepare the tray: place the screen/ domestic sieve into the tray/bowl and place the coffee filter into the screen/domestic sieve (Figure 4).
- 3. If you are sampling plant root or tuber tissue, first rinse the plant roots or peeled tubers with tap water.
- 4. Towel dry the washed plant roots and/or tubers.
- 5. Using the knife or scissors, cut the plant tissues into small pieces of 1-2 cm and place them into a dish properly labeled with the same indications as for the sample.
- 6. Weigh out a subsample from the cut material (10-25 g). If you are testing multiple subsamples, make sure all of them are the same weight.
- 7. Place each subsample in the blender and pour just enough water in the blender to cover completely the blades of the blender and the subsample.
- 8. Blend the subsample. For the fine roots, let the blender run for 5 seconds twice. For larger roots, blend for 20 seconds, two separate times (10 seconds each time). Wait for the suspension to settle between the two blending periods. If you are running more than one sample, make sure to blend all subsamples separately and to rinse the blender after each subsample.
- 9. Pour the blended material into a beaker. Rinse the blender with a wash bottle to collect all the root's debris.
- 10. Pour the root's suspension onto the coffee filter that is supported by the screen/domestic sieve in the tray.
- 11. The content of the coffee filter should always be moist. Check on the samples often to check on their humidity and to add just enough water in the tray if needed to keep it humid.
- 12. Leave the sample on a lab bench undisturbed to incubate from 24 to 72 hours.
- 13. After the incubation period, discard the contents of the coffee filter. Keep the liquid containing the sample of nematodes that has been collected in the plastic bowl.
- 14. Gently pour the water and nematode sample in the plastic bowl through a 38µ sieve into the sink. Then, use a wash bottle to wash and collect the nematodes held in the 38µ sieve into a properly labeled beaker.
- 15. The content of the beaker can be prepared for nematode identification and quantification.
- 16. For more visual reference, watch the following video https://www.youtube.com/watch?v=GrBrbJtvCfc

# Extraction Method for Nematodes in Soil using Baermann tray/Pie-pan method

The procedure to extract nematode from soil samples using the Baermann tray method is similar to the root's extraction method with slight modifications.

# **Equipment needed**

- Beakers
- 38µ sieve
- Screen with coarse mesh or a domestic use sieve

- Tray/plastic bowl, slightly bigger than the screen
- Coffee filter or tissue paper
- Knife or scissors
- Wash bottle
- Kitchen blender
- Kitchen scale
- Permanent markers

# Procedure

- 1. Prepare the tray: place the screen into the tray/bowl and place the coffee filter into the screen/sieve (Figure 4).
- 2. Label each tray with the same information that appears on the label for each sample.
- 3. Place a soil sample in a coarse sieve and remove all the debris such as rocks or organic matter in the soil samples.
- 4. Use a 50- or 100-ml beaker to take a subsample from the soil sample. If you plan to take multiple subsamples, make sure you use the same volume of soil for each subsample. If desired, you can use a bigger beaker to mix the soil thoroughly with water before pouring it into the tray.
- 5. Pour the soil suspension into the prepared tray that contains the collection bowl and coffee filter.
- 6. Follow step 10 to 14 previously described above (Figure 5).



#### Illustrations



10)



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Figure 4: Visual guide for nematode extraction using the Baermann tray method. Photo credit: Demesyeux, L,2019; Coyne et al., 2018; Govindasamy et al. 1) Drying roots with paper towel. 2) Cut roots in small pieces. 3) Weigh roots prior blending. 4) Macerate roots in kitchen blender. 5) Place the screen in the tray/bowl. 6) Place the coffee filter in the screen. 7) Gently pour the root's suspension in the prepared tray. 8) After incubation, discard the content of the coffee filter. 9) Concentrate the nematodes in the 38  $\mu$  mesh. 10) Collect the nematodes from the 38 by using a wash bottle.



Figure 5: Visual guide for extracting nematodes from soil samples. 1) removing debris from sample. 2) taking subsample from cleaned soil sample. 3) mixing sample with water (optional). 4) incubating sample in tray. Photo credits: Demesyeux, L, 2019; Coyne et al., 2018.

## Conclusion

Once nematodes have been extracted from the plant tissue or soil, the sample can be analyzed by a technician who is familiar with the characteristics/features of the major plant pathogenic nematodes. This diagnosis is a crucial step for managing plant parasitic nematodes in agricultural fields. Proper extraction and identification will provide information on the level of infestation of nematodes in the crop and the species present in the sample. The Baermann tray method is a suitable extraction method that can be used in Haiti to provide farmers and researchers with information about the types of nematodes impacting agriculture crops. Agronomists and other agricultural professionals can use this information to provide management recommendations to farmers as it does not require sophisticated equipment.

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