Nematode Laboratory Work

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October 2, 2015

AVRDC Workshop "Management of Nematodes in Vegetable Crops"
October 2, 2015

Soil Sampling



Spade, hand trowel, scissors, screwdriver, soil auger or corer with a blade of 20-30 cm in length and 20-25 mm diameter (either complete cylinder or half cylinder).

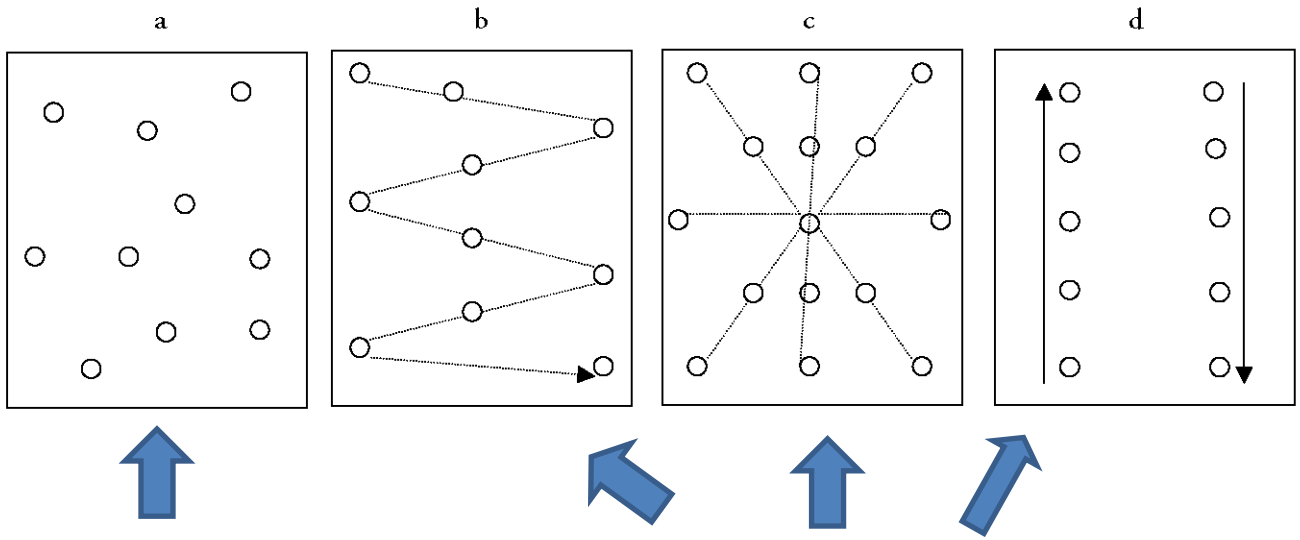
Sampling Pattern

Facts:

1. Nematodes are rarely distributed evenly in the field; patchy distribution

2. Samples should be collected from several areas in the field

3. Collect samples from both the poor growth and relative good growth areas



Random sampling
(Good only in small areas)

Systemic Sampling

Sampling Times

For predictive sampling:

- Before planting ,or at the end of the previous cropping season

For diagnostic sampling:

- At the middle of the season and/or at the final harvest

For perennials

- During the active growing period (during rainy/growing season)

Number of Samples

- Take enough samples to ensure

"representative of the situation in the field"

- The greater the number of soil samples, the more accurate the assessment will be.

However, the availability of time and resources needs to be balanced

e.g. Take 10 or up to 50 soil samples in an area of 0.5-1 hectare

Make sure to take samples between rows.

Once the corer is full, carefully remove it from the ground.

The sample should represent a cross section of the soil from the surface (0 cm) to around 20–30 cm below.

Place the corer over a large flat and sturdy box (preferably plastic).

With a strong blunt instrument, scrape **all** the contents of the corer into the box. **Make sure you thoroughly shake out any excess soil before taking another sample**.

Place samples in sturdy bags with a tie at the top. Label clearly with a card written with **pencil** (not pen as it smudges).

Or even easier, just label the bag on the outside with a permanent thick marker.

Figure 23. Insulated cool box for sample storage.

Taking Root Samples

- Collected at the same time with soil
- Put in the same bag as soil; soil helps preserve the roots

- Take 25-100 g of root per sample

- Avoid sampling dead plants or in advanced stages of senescence (nematodes already migrate out!!)

Nematode Extraction

1. Extraction tray method
2. Root or leaf maceration method
3. Sieving method
4. Incubation method

Note: Done as soon as possible as samples deteriorate over time

1. Extraction tray method

Advantages:

1. Simple equipment (plastic basket etc.)
2. Good for mobile nematodes
3. Well adapted to local circumstances

Disadvantages:

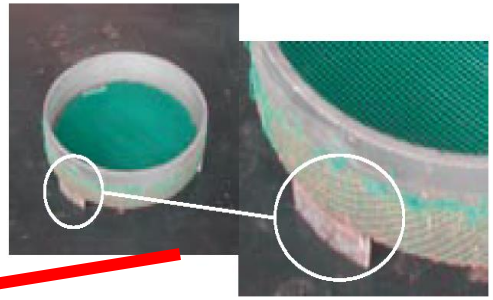
1. Not good for slow moving nematodes
2. Dirty extractions (if work with high clay content soil)
3. Take 3-4 days

Extraction tray method

1. Coarsely sieve the sample to remove debris and lumps.

2. Measure out a standard sized sub-sample by volume, e.g. 100 ml.

3. Line a sieve with tissue paper and place it on a plastic plate.



Three or four small feet glued to the base of the sieve (or basket)

4. Place soil on the tissue ensuring that the soil remains on the tissue and does not to spill over the edges.

5. Carefully pour water into the tray making sure you pour the water down the gap between the tray and the sieve.

6. Store extract from samples over 2 days, constantly checking that the samples remain wet and do not dry out due to evaporation.

7. Carefully drain and remove the sieve from the tray and discard the tissue paper and soil.

8. Pour the water containing the nematodes into a labeled beaker/cup.

9. Thoroughly rinse the tray into the beaker.

10. Leave samples to settle for a few hours or overnight.

11. Reduce the suspension by decanting or using a small aperture (i.e. 28 µm) sieve and collect in a beaker ready for assessing nematodes.

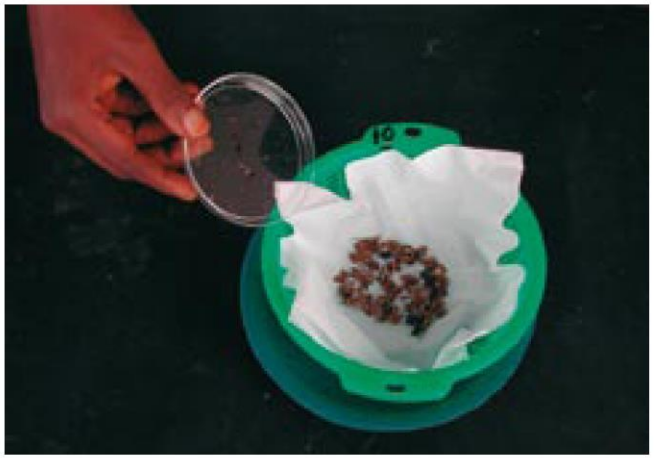
12. The sample can be stored in a tube if not observing immediately.

13. If sending away for assessment, the nematodes can be removed from the bottom of the large tube with a pipette, after settling, for storage/dispatch in small tube.

1. Extraction tray method (with root samples)

1. Chop roots and/or tuber peel and place in labeled dish.

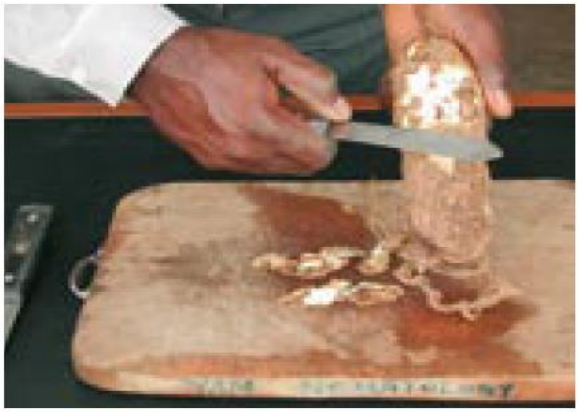
2. Weigh out root sub-samples.



3. Place root sub-sample in sieves for extraction.

Then follow steps 5-10 in the Extraction tray method for soil sample

2. Root or leaf maceration method



1. After rinsing the tubers, peel them thinly with a knife or peeler.

2. Chop the peelings or roots coarsely with a pair of scissors or a knife.

3. Weigh out sub-samples.

4. Macerate roots/peel using a blender.

Five-ten second burst for two times

5. Pour blended suspension into a labeled beaker and rinse out blender into the beaker.

6. Gently pour the sample onto the tissue paper in the sieve as for extraction tray method.

Then follow the steps in the Extraction tray method for root sample

2. Root or leaf maceration method

- Advantage:
  - Does not require specialized equipment
  - Used when extracting nematodes from large plant parts

- Disadvantage:
  - Time for macerating is critical, must be sufficient to allow nematodes to move out of plant tissues, not damage nematodes

3. Sieving method

1. Measure water in a beaker to a known volume, e.g. 200 ml.

2. Measure soil by adding clumps from each bulk sample and displacing water to marked volume.

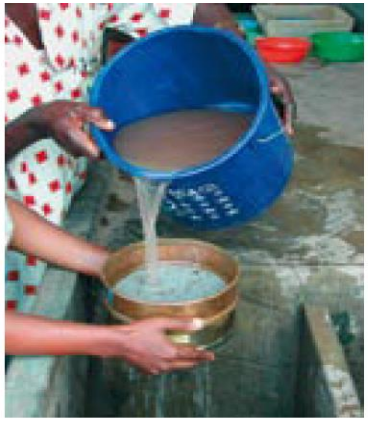
3. Measure a set volume of water using a pre-marked line on the inside of a bucket.

4. Pour pre-measured soil sub-sample into the water.

5. Mix thoroughly.

6. Leave the soil to settle for 30 seconds.

7. Slowly pour three-quarters of the water through the nested sieves (such as 90 and 38 µm size) with the 90 µm sieve on the top.



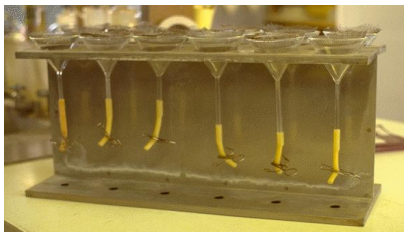
8. Refill bucket and repeat steps 5, 6 and 7.

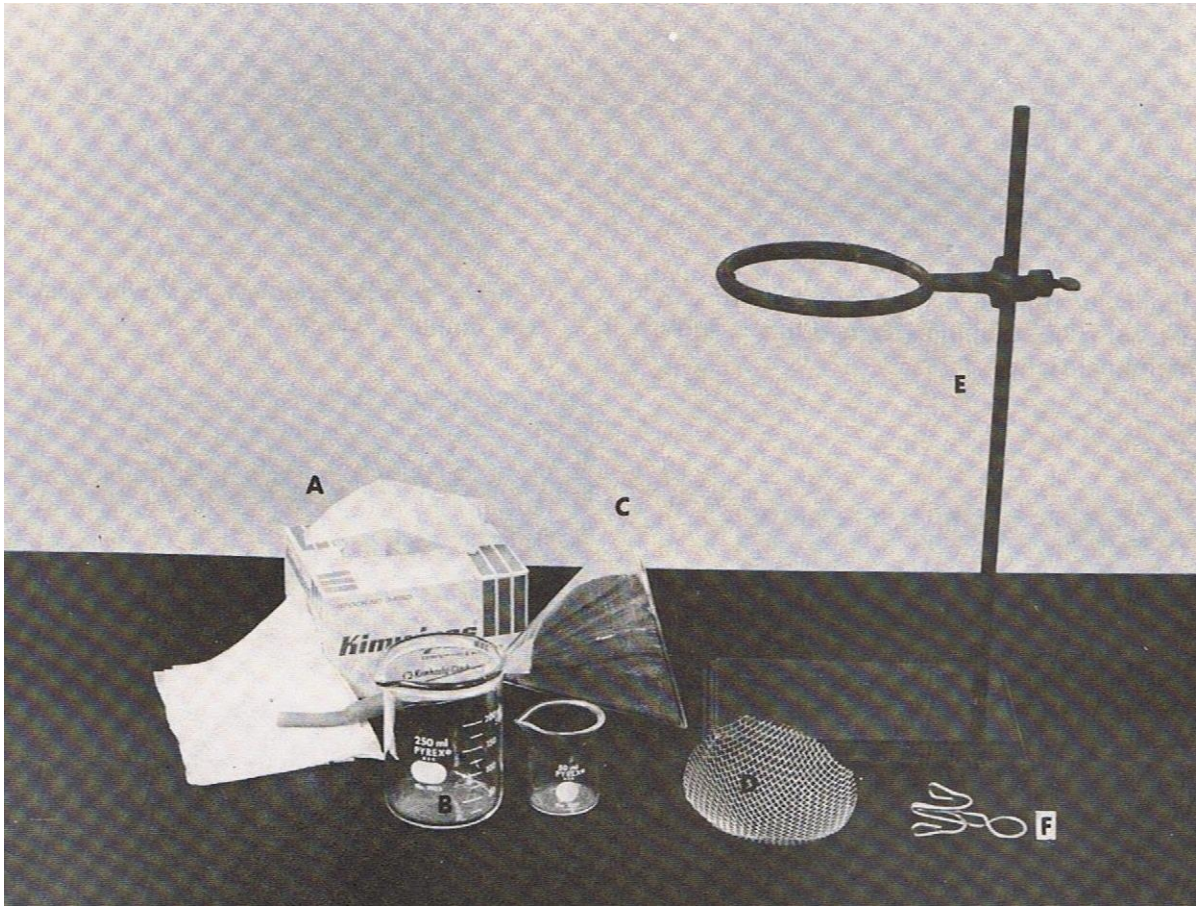
9. Condense the extract debris by **gently** rinsing the sieves thoroughly with a hose, mainly from the back.

10. Ensure the sieves are back-washed properly and all debris and nematodes are collected from the sieve surface at the bottom point of the sieve.

11. Gently wash debris from the 90 µm and the 38 µm sieve into a labeled beaker.

Baerman Funnel Method





Baerman Funnel Method

2. Incubation method

1. Chop the roots and weigh the sub-sample.

2. Place weighed sub-sample of roots in jar, conical flask or plastic bag with water and leave for 2–7 days. Take care not to fully seal the container, but loosely cover.

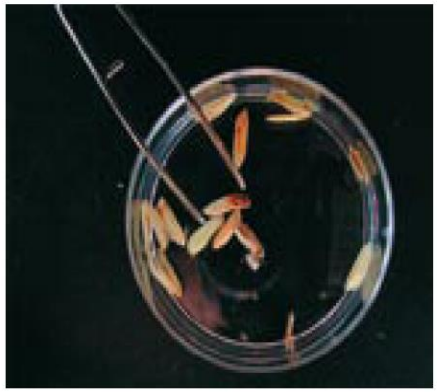
3. Each day, shake/swirl the container and gently pour the suspension into a beaker, leaving the plant material in the container.

4. Replenish with fresh water after pouring off suspension.

5. Concentrate the suspension and collect nematodes for further assessment, e.g. using a small aperture sieve, or leave the beaker to settle and pour off the excess. Then follow Fig 26, steps 12 and 13.

Direct Examination from Plant Tissues

Sheaths teased apart.



Seed coats broken to allow nematodes free movement.

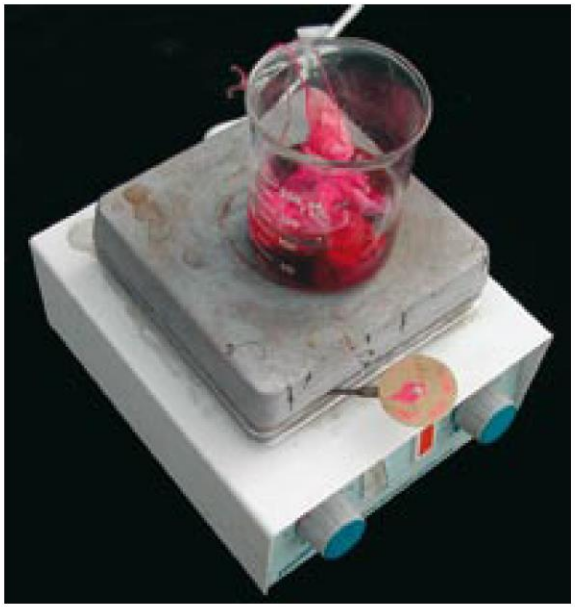
Using a dissection stereo-microscope with understage lighting.

Using a compound stereo-microscope with understage lighting.

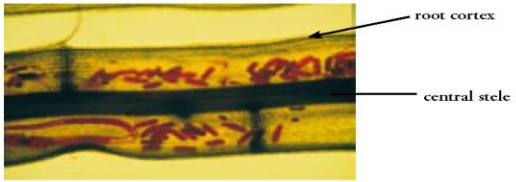
- To easily assess whether nematodes are present in plant tissues

e.g. Females of cysts & root-knot nematodes

Staining Nematodes in Plant Tissues



Scutellonema bradys in yam.



Hirschmanniella in rice [JB].

Staining color: lactoglycerol+0.1% cotton blue or 0.05-0.1% acid fuchsin

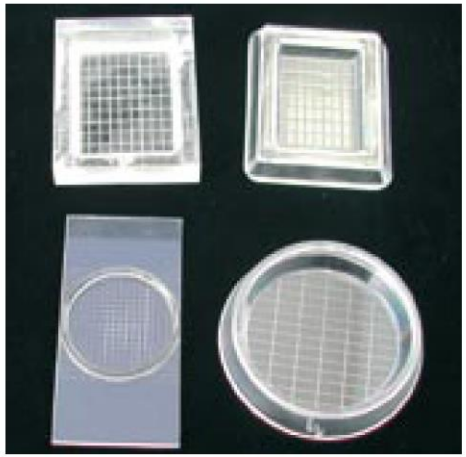
Destaining: Equal volume of glycerol and distill water + few drops of lactic acid

Procedures

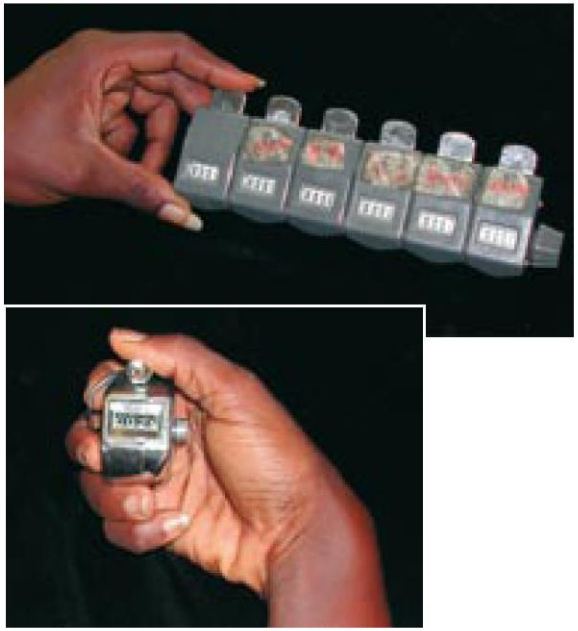
1. Slice thinly thick and bulky roots
2. Transfer the roots in muslin cloth
3. Place muslin cloth in boiling staining solution on a hot plate for 3 minutes
4. Remove muslin cloth and rinse in running water
5. Place stained roots in destaining solution

Staining Egg Masses by Phloxine B

- **Increasing the visibility of egg masses and enabling rapid count of egg masses of *Meloidogyne***
- **Egg masses are still viable after staining**
- **Mixing 15 mg of Phloxine B + 1 liter of water (staining solution)**
- **Place roots into the staining solution for 15-20 minutes**



A sample of counting dishes.

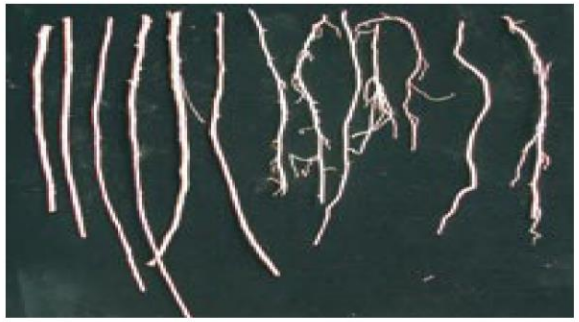


Multiple and single tally counter.

Root-knot gall (*Meloidogyne* spp.) scoring on cassava

Use a score combination of both roots and tubers when assessing mature harvested plants or a score of roots only for assessment of roots removed from standing plants.

Cassava roots

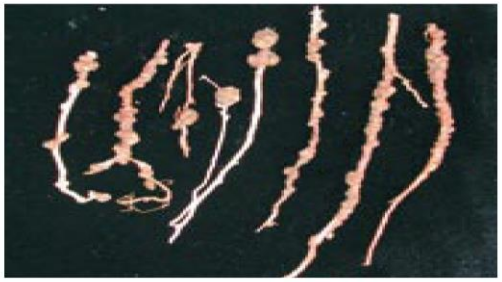


1. No galls observed, feeder roots intact.

2. At least one gall observed.

3. Numerous galls, about 50% of roots affected.

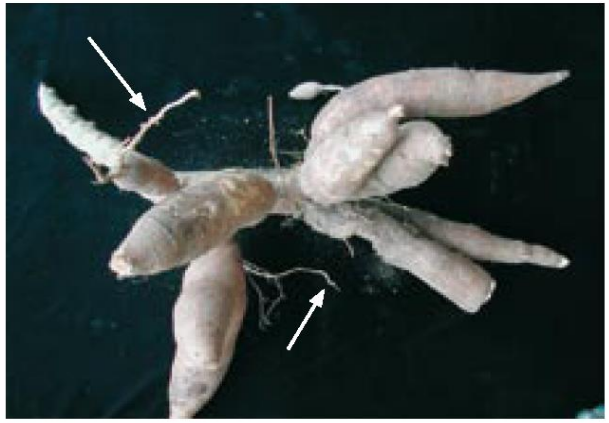
4. Numerous galls, most roots affected.



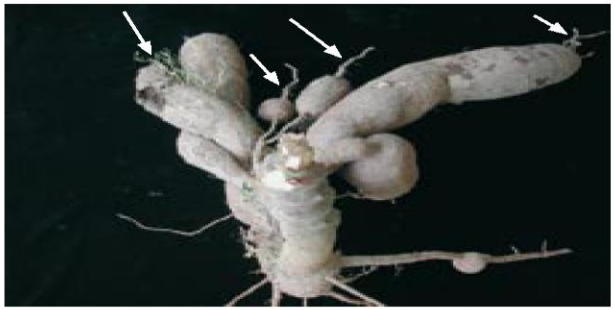
5. Heavy galling on most roots, with necrosis, and feeder roots heavily affected or absent.

Cassava plants

1. No galls observed, healthy feeder roots and tubers.



2. At least one gall observed on roots.



3. Galls obvious on roots, a few feeder roots and tubers reduced in size.

4. Numerous galls, roots necrotic, and tubers reduced in size.

5. Heavy galling on most roots, feeder roots largely absent, and few tubers.

Root-knot gall scoring on carrot

1. No galling damage.

2. Slight galling.

3. Mild galling.

4. Moderate galling.

5. Severe galling.

Root-knot gall scoring on lettuce

1. No galling damage.

2. Slight galling.

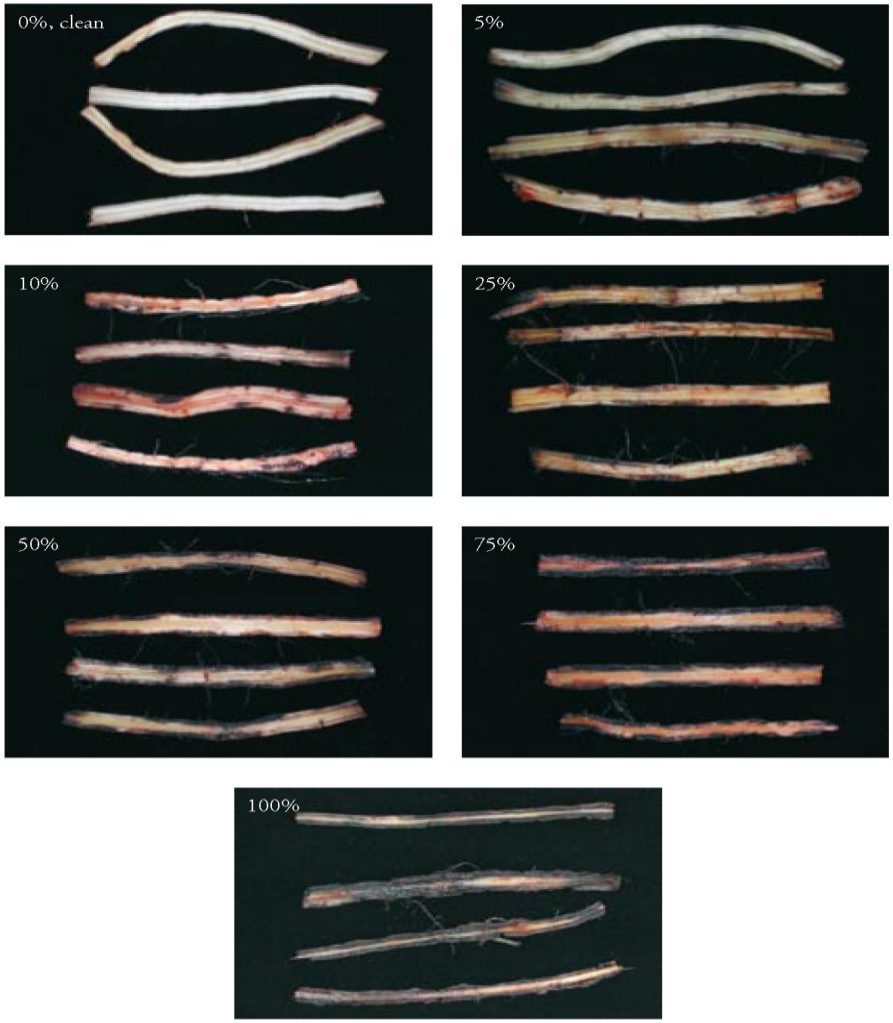
3. Mild galling.

4. Moderate galling.

5. Severe galling.

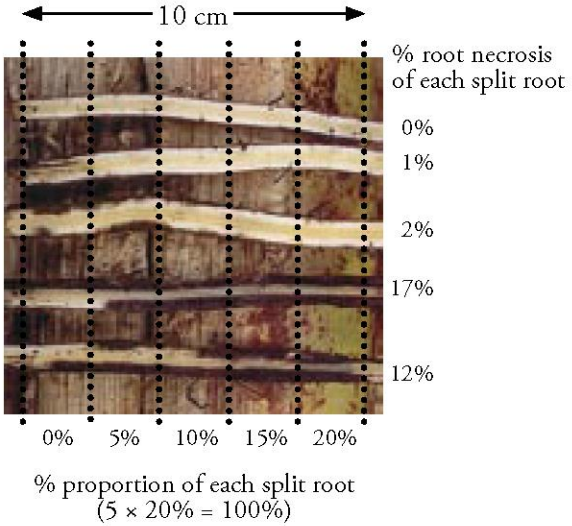
Lesion scoring for banana roots

Adapted from Paul Speijer and Dirk De Waele (1997).



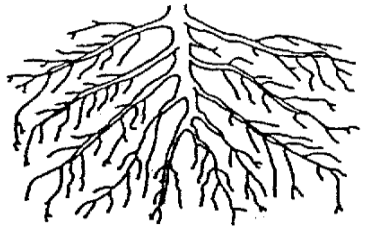
Lesion scoring for *Musa*

Example of scoring five lengthwise sliced banana roots for root necrosis (%) of root cortex surface showing necrosis caused by migratory lesion endoparasites (courtesy of Paul Speijer and Dirk De Waele, 1997).

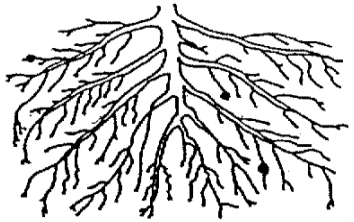


Diagrammatic root-knot scoring chart

Courtesy of John Bridge and Sam Page (1980).

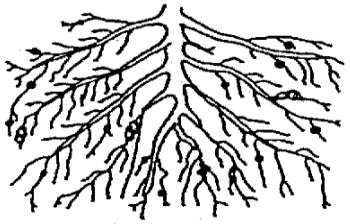


0 – No knots on roots.



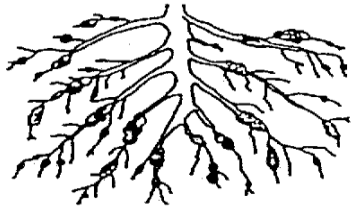
1 – Few small knots, difficult to find.

2 – Small knots only but clearly visible. Main roots clean.

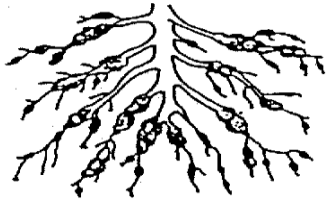


3 – Some larger knots visible. Main roots clean.

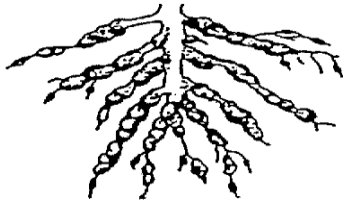
4 – Larger knots predominate but main roots clean.



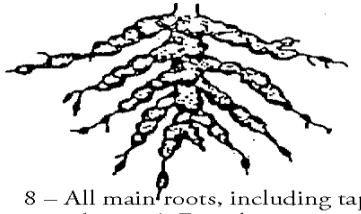
5 – 50% of roots affected. Knotting on some main roots. Reduced root system.



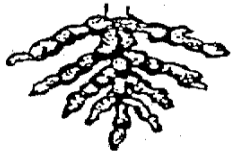
6 – Knotting on main roots.



7 – Majority of main roots knotted.



8 – All main roots, including tap root, knotted. Few clean roots visible.



9 – All roots severely knotted. Plant usually dying.

10 – All roots severely knotted. No root system. Plant usually dead.