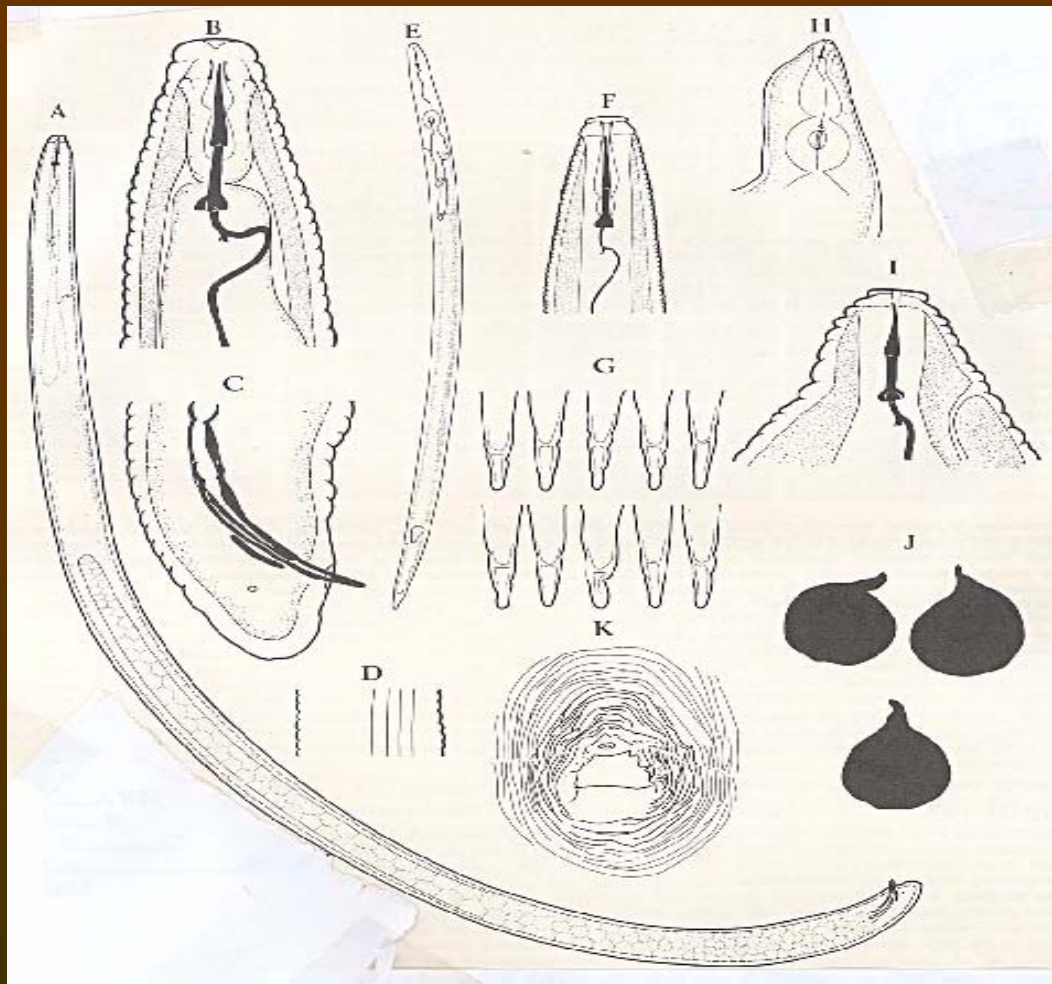


**NEMATODES:
Sample collection,
Extraction and Preservation**

**BAIMEY Hugues (PhD)
(IITA-Cotonou Station, BENIN)**



Mâle

- A = Adulte mâle
- B = Partie anterieure
- C = Partie posterieure
- D = Champ lateral

Larve de second stade

- E = Larve entiere
- F = Partie anterieure
- G = Queue

Femelle

- H = Partie anterieure
- I = Partie anterieure
- J = Femelle entiere
- K = Coupe annale

Meloidogyne chitwoodi





1

2

3

4

5



6

7

8

9

10

- 1 = Poivron (*Capsicum annuum*)
- 2 = Grande morelle (*Solanum macrocarpum*)
- 3 = Celosie (*Celosia argentea*)
- 4 = Glasseman (*Talinum triangula*)
- 5 = Vernonia (*Vernonia anygdalina*)

- 6 = Carotte (*Daucus carota*)
- 7 = Tchiayo (*Ocimum gratissimum*)
- 8 = Egusi (*Citrulus lanatus*)
- 9 = Laitue (*Lactuca sativa*)
- 10 = Aubergine (*Solanum melongena*)

Some vegetable crops infected with root-knot nematodes

Dry rot caused by *Scutellonema bradys* on yam tuber



Nematodes:

- They are very important in the world agriculture
- In association with fungi, bacteria, viruses etc. they induce complex diseases
- Yield loss of major world crops due to nematodes is estimated at 12.3%
- Monetary losses: more than \$77 billion annually
- Necessity of management, but knowledge of nematode involved is important for choosing the appropriate management option

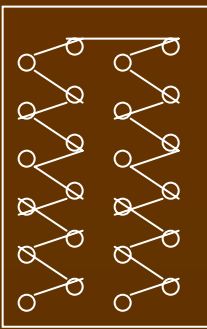
Sampling

* **Goals**

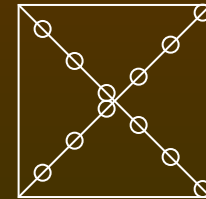
- **Collect the nematode community present at a given place (no precise tools should be considered)**
- **Diagnostic purpose (more attention should be taken)**

* Sampling pattern

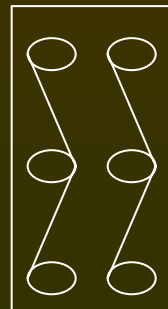
- Systematic at equally-spaced point according to the grid pattern (Fig1)



- At equally-spaced points along a line running diagonally across a field (Fig2)



- Sample tree crops in the drip line, on alternate sides of the trunks and include feeder roots (Fig3)



- ★ For annual crops, collect samples at 10-20 cm from the stem, in the root zone.
- ★ Collect samples of weeds and volunteer crop plants
- ★ Collect sample from: soil, roots, leaves, stems, buds, flowers, seeds
- ★ Number of samples: 10 for 5m² in size or less fields, 20 for 5m²-100m² fields, 30 for larger fields

- * **Sampling depth:** 20 cm for most crops, 20-100 cm for perennial crops according to type and age of crops, 30-45 cm in regions with hot and dry summers
- * **Timing:** prior to planting (relation of population density with yield), between mid season, at harvesting

★ Material

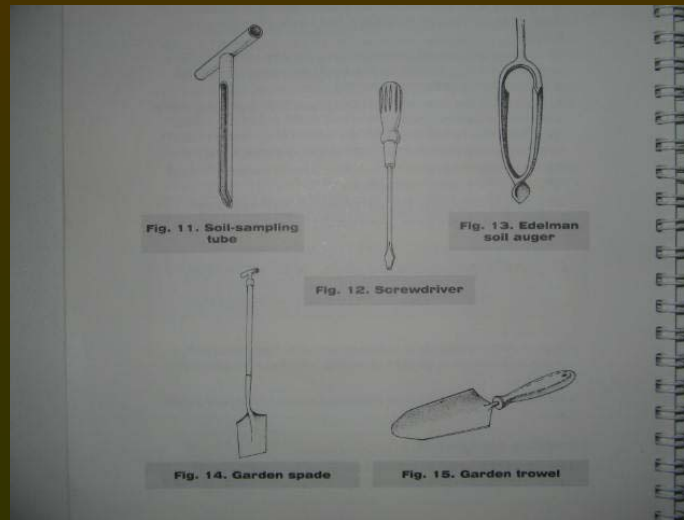
~~Hand~~: never uproot plants or take soil samples

Soil sampling tube (15-20 cm)



Soil auger (more than 20 cm)

Spade or garden trowel (in rocky soil)

Screwdriver (to scrape the soil from the sampling tube)



★ Care of samples

- Plant material and soil in the same bag 
- Moisten soil samples collected from dry soil 
- Nematodes should be extracted immediately after collection or stored in a fridge at temperatures between 4-15°C, optimum = 10°C.
- Keep storage time as short as possible

Extraction

- ★ **The technique depends on:**
 - **nematode type: ectoparasite, endoparasite, soil nematode, sedentary, cyst, female of RKN**
 - **the nematode size: small or large**
 - **nematode movement: slow, rapid**
 - **dead or live nematode**
 - **Consequence: Each technique has advantages and limitation**

* The Baermann techniques (for soil and plant material)

- a) Funnel technique

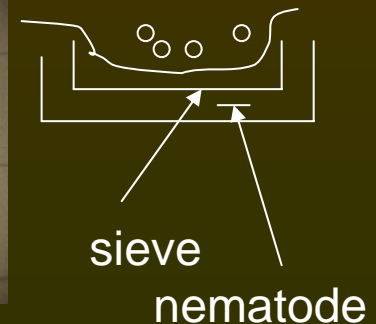
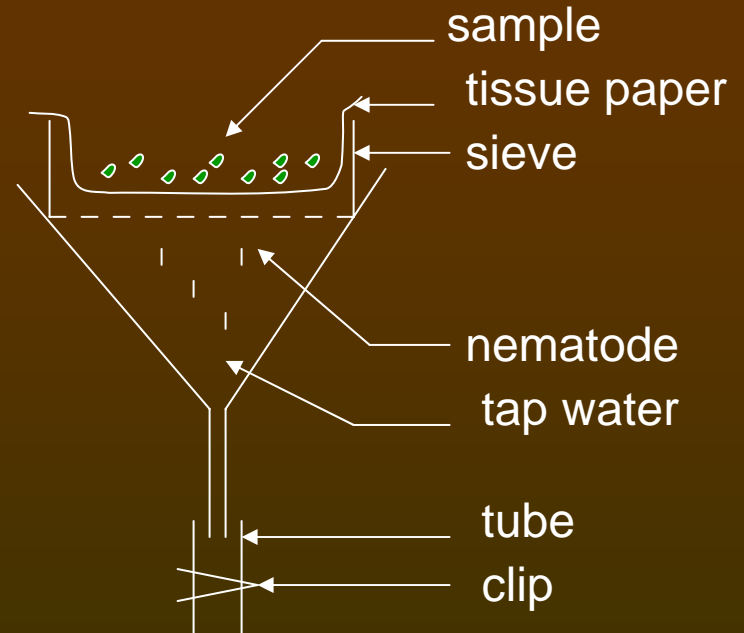
- Cut root pieces (1-0.5-1cm)

- **Advantages:** simple material, can be practiced everywhere

- **Disadvantage:** not suitable for inactive or large nematodes

b) Modified technique

Time: 48h

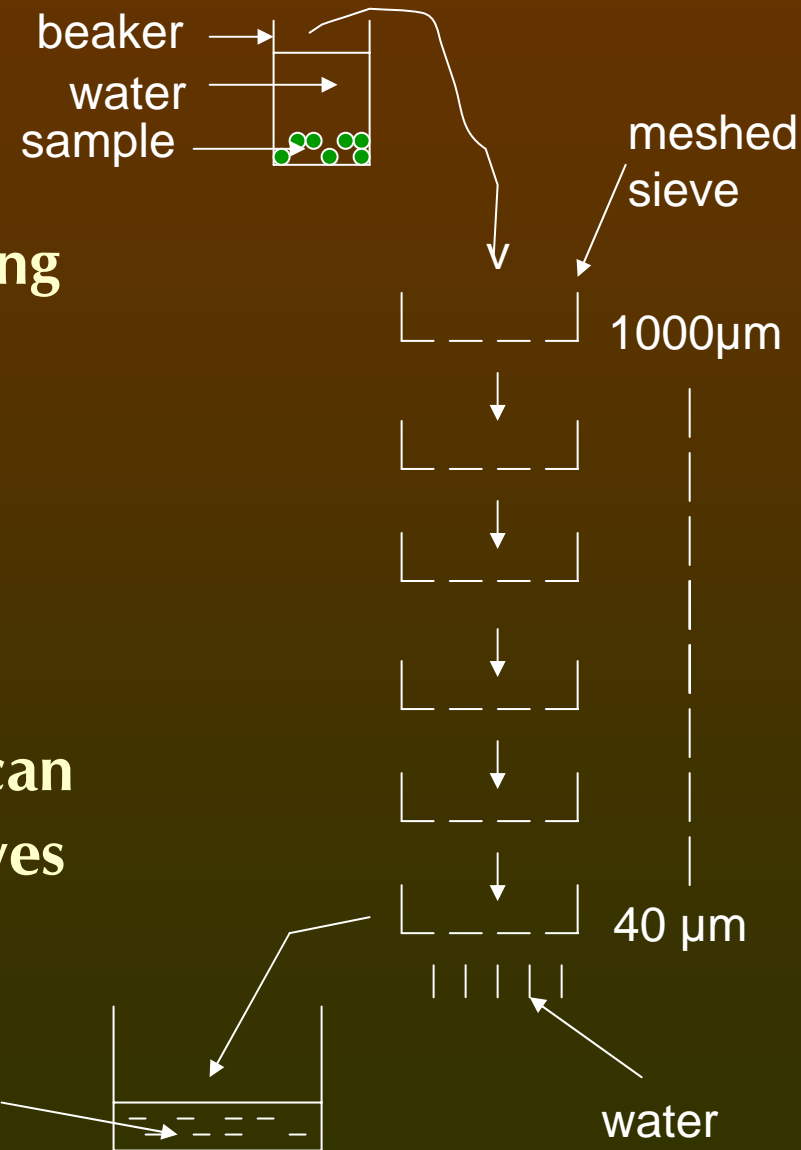


* Cobb's decanting and sieving method (for soil and plant material)

NB: blind plant material before sieving

- **Advantages:** does not require sophisticated material, active and sluggish nematodes are extracted

- **Disadvantage:** large nematodes can stay in some sieves



- * **Sieving – centrifugation – flotation (for soil and plant material)**
 - Sieve soil samples + nematodes
 - Centrifuge the suspension for 7 and then 3 min at 1750 rpm
 - NB: for plant material blend 1 cm pieces in a food blender before sieving
 - **Advantages:** fast, active and sluggish species are extracted, small and large nematodes are extracted
 - **Disadvantages:** the equipment is expensive and expertise is required

★ **Seinhorst cyst-elutriator**



- ★ **Best method for extracting heteroderid cyst nematodes**
- ★ **The cysts are kept in suspension by a controlled upward current of water**
- ★ **Cysts (except larvae) can not go through sieves, the Baermann techniques are not suitable**

Preserving nematodes

- ★ **Nematodes are preserved as:**
 - slide mounts (Cobb double-coverslip aluminium slide), unmounted in fixatives in vials, frozen and stored in liquid nitrogen, in a greenhouse as live collections
 - **Killing and fixing: nematodes are killed and fixed in hot fixatives**
 - **FA 4:1 = Formalin (10ml), Acetic acid (1ml), Glycerol (3ml), H₂O (100ml)**
 - **FAA = Formalin (6ml), Acetic acid (1ml), Ethanol 96% (20ml), H₂O (40 ml)**
 - **TAF = Formalin (7ml), Triethanolamine (2ml), H₂O (91ml)**
 - **lactophenol = Liquid phenol (20ml), Lactic acid (20ml), Glycerol (40ml)
H₂O (20ml)**

THANK YOU

Matériel & Méthodes

Résultats & Discussion

Résultats & Discussion

Résultats & Discussion

Résultats & Discussion

Perspectives



Merci pour votre aimable attention