

A SAFRINET MANUAL FOR NEMATOLOGY

Collecting and Preserving Nematodes



**Compiled by the
Biosystematics Division, ARC-PPRI, South Africa**

Sponsored by SDC, Switzerland

Collecting and Preserving Nematodes

A Manual for Nematology

by

SAFRINET, the Southern African (SADC) LOOP of
BioNET-INTERNATIONAL



Compiled by the
National Collection of Nematodes
Biosystematics Division
ARC – Plant Protection Research Institute
Pretoria, South Africa

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Sponsored by
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Preface

This manual is a guide to a course in practical plant nematology for technical assistants of the SADC countries of the SAFRINET-loop of BioNET-INTERNATIONAL.

The course is presented by the staff of the National Collection of Nematodes of the Plant Protection Research Institute and comprises lectures, discussions and practical sessions, aimed at teaching students to recognise the major groups of plant nematodes and the plant damage symptoms that they produce. Also included are techniques to collect, process and prepare nematodes for study and to preserve them in reference collections. The manual contains information on basic handling procedures, plant nematode morphology, and naming and classifying nematodes. A list of pertinent literature is provided.

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Introduction

Damage by plant nematodes is one of the most important constraints to sustainable crop production that farmers in Africa have to contend with. Nematodes cannot be eradicated from agricultural soils but control, although expensive, is possible once the role of each species in specified disease situations is understood and predictable. The basic programme for nematode research should include surveys to collect and identify harmful nematodes, determination of the prevalence and economic importance of nematode-related crop-growth problems, and initiation of control measures. These operations rely heavily on accurate identification of all species involved in crop-damage situations. Taxonomy, which precedes identification, is a fundamental and highly specialised branch of biology and is based on well-curated reference collections. In turn, such collections are based on effective techniques for the collection, extraction and preservation of nematodes.

1. **W**hat are nematodes?

- Nematodes are generally minute, colourless, unsegmented and worm-like animals that lack eyes, appendages and circulatory and respiratory systems but have well-developed excretory, digestive, reproductive and nervous systems.
- The sexes are separate and reproduction takes place by cross-fertilisation or parthenogenesis.
- Nematodes are aquatic animals and require wet surroundings for movement and other activities.
- Nematodes inhabit virtually every environment that can support life, and are found in fresh water, in salt water, in all kinds of soil, and as internal parasites of humans and animals.
- Within the animal kingdom nematodes are treated as the phylum Nemata and placed close to the phyla Rotifera, Kinorhyncha, Gastrotricha and Nematomorpha in the subkingdom Metazoa.

Plant nematodes

Most of the nematodes that inhabit the soil are microbial feeders or predators of other soil organisms, but some parasitise plants and are known as plant nematodes or eelworms (Fig. 1).

Plant nematodes differ from non-parasitic forms basically in the possession of a movable, needle-like structure, the stylet, in the mouth cavity, and in oesophageal characteristics (Fig. 2, Table 1).

The stylet is used to puncture plant cells to inject digestive juices and to draw the modified cell contents into the oesophagus.

Plant nematodes move in the film of water that surrounds soil particles and the moisture contained in plant tissues.

Because of their small size (average length about 1 mm) and translucent bodies, plant nematodes cannot readily be seen in soil or plant tissues with the unaided eye.

Plant nematodes are important in agriculture because they inhibit root growth and eventually overall plant development. This results in poor crop performance and sometimes crop failure.

The three plant nematode orders can be separated by morphological characters (Table 2).

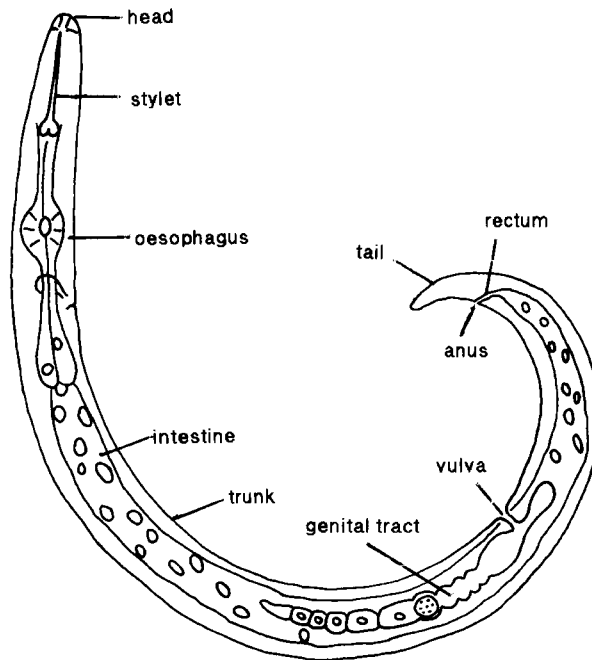


Fig. 1. Typical Tylenchida plant nematode

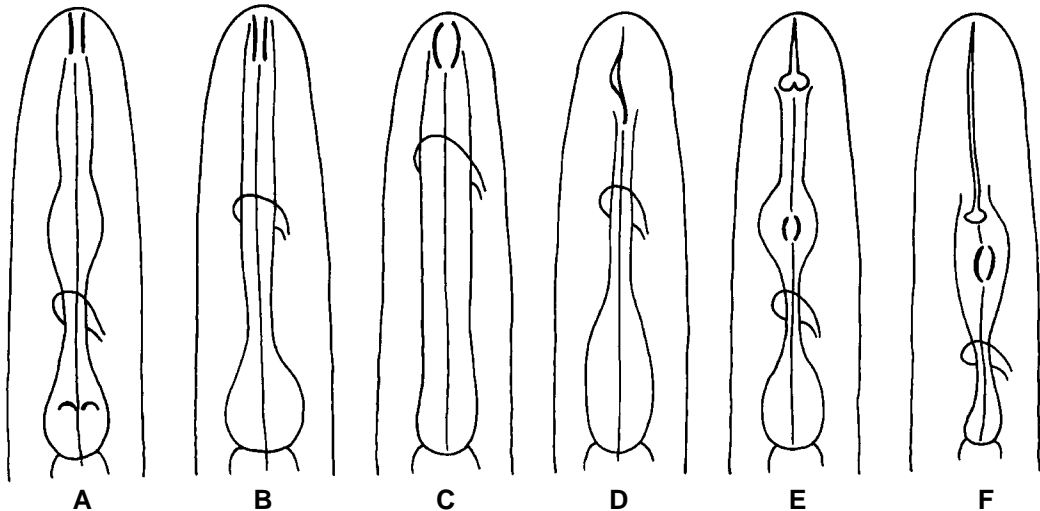


Fig. 2. Oesophagus types among soil nematodes.

A, Rhabditida; B, Araeolaimida; C, Monhysterida, Enoplida, Mononchida; D, Triplonchida (dorylaimoid); E, Tylenchida (tylenchoid); F, Tylenchida (criconematoid)

Table 1. Differences between plant nematodes and other soil nematodes

Nematode orders	Characters
Plant nematodes	
Order Triplonchida	} Mouth cavity with stylet Valves, when present, in metacarpus
Order Dorylaimida	
Order Tylenchida	
Other soil nematodes	
Order Enoplida	} Mouth cavity without stylet, may have plates or teeth Valves, when present, in postcorpus
Order Mononchida	
Order Monhysterida	
Order Araeolaimida	
Order Rhabditida	

Plant nematodes are formally classified as follows:

Phylum Nemata

Class Secernentea

Subclass Diplogasteria

Order Tylenchida

Suborder Tylenchina

Superfamily Tylenchoidea

Families Anguinidae

Dolichodoridae

Belonolaimidae

Pratylenchidae

Hoplolaimidae

Heteroderidae

Superfamily Criconematoidea

Families Criconematidae

Tylenchulidae

Suborder Aphelenchina

Superfamily Aphelenchoideoidea

Family Aphelenchoididae

Class Adenophorea

Subclass Enoplia

Order Triplonchida

Suborder Diphtherophorina

Superfamily Trichodoroidea

Family Trichodoridae

Order Dorylaimida

Suborder Dorylaimina

Superfamily Dorylaimoidea

Family Longidoridae

Table 2. Diagnostic features of plant nematode orders

Character	Order Tylenchida	Order Triplonchida	Order Dorylaimida
Cuticle	annulated	not annulated	not annulated
Cephalic framework	present	absent	absent
Excretory pore	usually present	present	usually absent
Oesophagus	tylenchoid or criconematoid	dorylaimoid	dorylaimoid
Nerve ring	around postcorpus	around corpus	around corpus
Prerectum	absent	absent	usually present
Testis	single	single	paired
Copulatory supplements	absent	present	present
Phasmids	present	absent	absent
Typical representative	<i>Pratylenchus brachyurus</i>	<i>Paratrichodorus minor</i>	<i>Xiphinema index</i>

2. **M**orphology and taxonomy

Basic plant nematode morphology (Figs 1–10)

External features

- The body is usually elongate-cylindroid (Fig. 10c), but may be spindle-shaped (Fig. 10a) or saccate (Fig. 10b,d) and is divisible into a head region (Fig. 3), a neck region, a trunk and a tail.
- A colourless, multi-layered, semi-permeable cuticle covers the body and together with the hypodermal cell layer forms the hydrostatic skeleton.
- In Tylenchida the cuticle is annulated, i.e. it bears regular transverse grooves which delimit the annules (Fig. 4).
- The longitudinal lateral fields bear smooth or scalloped lateral lines and various sense organs, e.g. deirids in the neck region and phasmids on the tail.
- In Triplonchida and Dorylaimida lateral fields and annules are absent.
- Tylenchida males have paired caudal alae on the tail; these are absent in Dorylaimida and present in some Triplonchida.



Fig. 3. Head (*Dolichodoros* sp.)

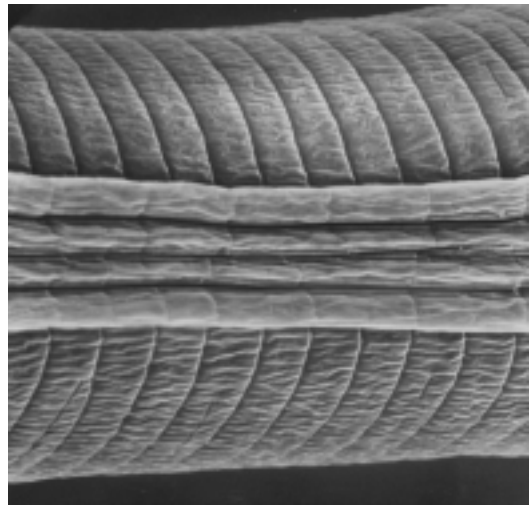


Fig. 4. Lateral field and annules (*Meloinema* sp.)

Digestive system

- Anteriorly the head bears the mouth cavity or stoma which opens on a raised labial disc, surrounded by the subdorsal and subventral lip pairs and two lateral lips, or by structures derived from the lips, e.g. submedian lobes and circumoral ridges.
- In Tylenchida the head is supported internally by a sclerotised cephalic skeleton, consisting of a basal plate, the cylindrical wall of the anterior stoma (vestibule) and six arms or blades that connect the basal plate with the stoma wall (Fig. 6).
- In Tylenchida the stylet is called a stomatostylet, and consists of an anterior conical part (conus), a cylindrical shaft and three basal knobs (globi) to which the protractor muscles responsible for stylet movement are attached. The central canal of the stylet opens anteriorly near the tip of the conus, on the ventral side, and passes posteriorly into the oesophagus, behind the globi (Figs 5, 6).
- In Triplonchida the stylet is called an onchiostylet, and lacks a lumen but is partly hollow and attaches posteriorly to its extension, the onchiophore (Fig. 7).
- In Dorylaimida the stylet is known as an odontostylet; the lumen opens dorsally near the tip and the posterior extension, the odontophore, may bear flanges for attachment of the protractor muscles (Fig. 8).
- The oesophagus consists of an anterior corpus and a posterior glandular postcorpus. In Aphelenchina and most Tylenchina the oesophagus is 'tylenchoid' (Fig. 2e), the corpus consisting of an anterior procorpus and a muscular, usually valved metacarpus. In some Tylenchina the pro- and metacarpus are amalgamated to form a 'criconematoid' oesophagus (Fig. 2f). The metacarpus is followed by the isthmus, which is encircled by the nerve ring, and a postcorpus that secretes digestive juices into the lumen of the oesophagus in the procorpus (Tylenchina) or metacarpus (Aphelenchina).

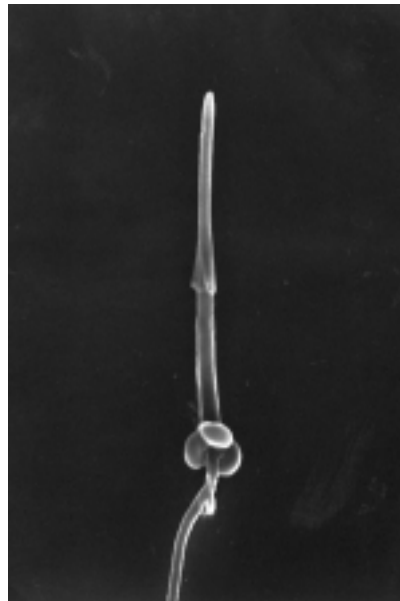


Fig. 5. Stylet (*Meloidogyne* sp.)

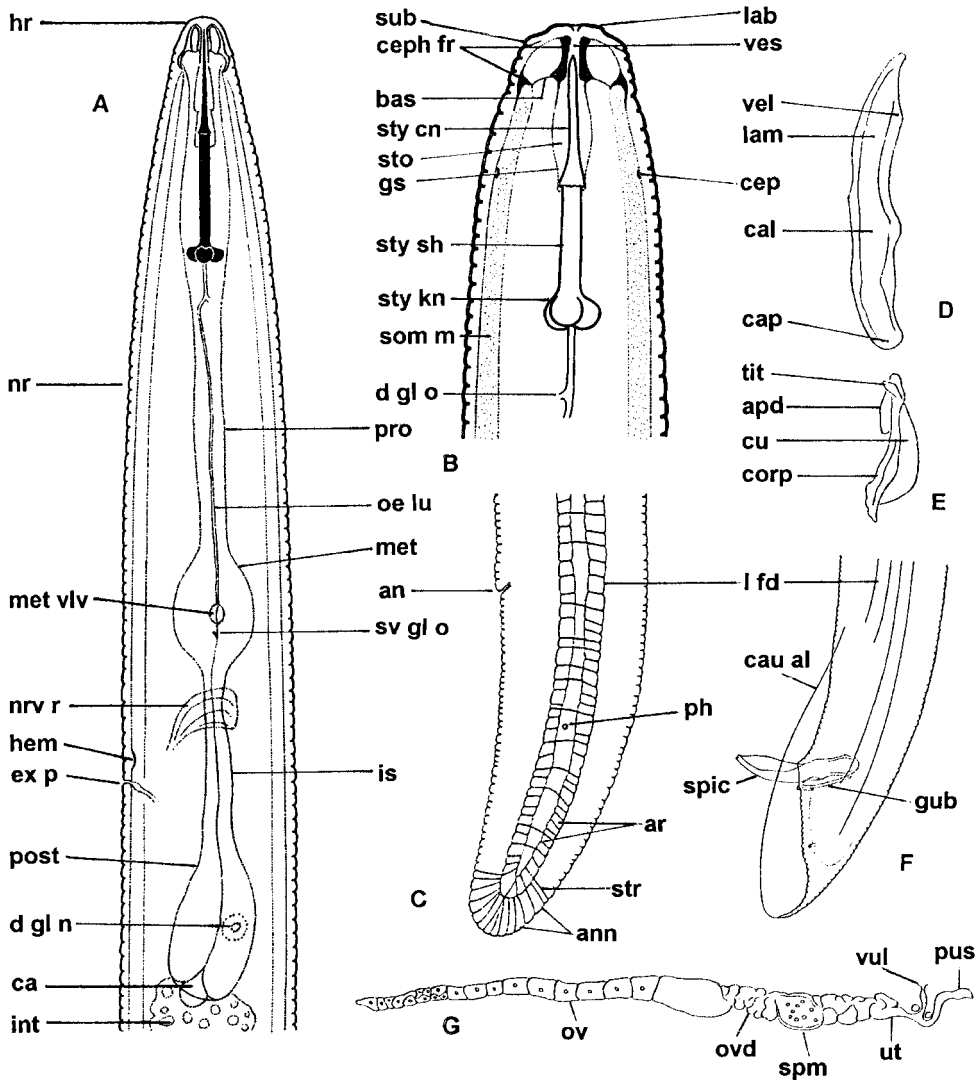


Fig. 6. Morphology of Tylenchida.

A, anterior region; B, head region; C, female posterior region; D, spicule; E, gubernaculum; F, male posterior region, G, female genital tract.

an = anus; **ann** = annule; **apd** = apodeme; **ar** = areola; **bas** = basal plate; **ca** = oesophageal-intestinal valve; **cal** = calomus; **cap** = capitulum; **cau al** = caudal alae; **cep** = cephalic; **ceph fr** = cephalic framework; **corp** = corpus; **cu** = cuneus; **d gl n** = dorsal oesophageal gland nucleus; **d gl o** = dorsal oesophageal gland outlet; **ex p** = excretory pore; **gs** = stylet guiding sheath; **gub** = gubernaculum; **hem** = hemizonid; **hr** = head region; **int** = intestine; **is** = isthmus; **lab** = labial disc; **lam** = lamina; **l fd** = lateral field; **met** = metacarpus; **met vlv** = metacarpus valve; **nr** = neck region; **nr v** = nerve ring; **oe lu** = oesophageal lumen; **ov** = ovary; **ovd** = oviduct; **ph** = phasmid; **post** = postcorpus; **pro** = procorpus; **pus** = postvulval uterine sac; **som m** = somatic muscles; **spic** = spicule; **spm** = spermatheca; **sto** = stoma; **str** = stria; **sty cn** = stylet cone; **sty kn** = stylet knobs; **sty sh** = stylet shaft; **sub** = submedian pseudolips; **sv gl o** = subventral oesophageal gland outlet; **tit** = titillae; **ut** = uterus; **vel** = velum; **ves** = vestibule; **vul** = vulva. (A, C–F, after Kleynhans, 1992, *Phytophylactica* 24, B, after Kleynhans, 1988, *Phytophylactica* 20, reproduced in terms of the Government Printer's copyright permit 10026 dated 19 July 1995; G, original).

- In Longidoridae and Trichodoridae the oesophagus is 'dorylaimoid' (Fig. 2d), with the slender corpus encircled by the nerve ring and the large, often muscular postcorpus secreting directly into the oesophageal lumen.
- In all groups an oesophageal-intestinal valve ('cardia') at the proximal end of the oesophageal lumen prevents regurgitation of food from the intestines.
- The intestine consists of a layer of large endodermal cells that are lined internally with a sheet-like layer of cells called microvilli. The rectum is separated from the intestine by an intestinal-rectal valve in Tylenchida and Triplonchida; in Longidoridae a prerectum is present between the intestines and the rectum (Fig. 8).

Nervous system (Figs 6–8)

- This system comprises a nerve ring and various smaller ganglia that serve various sense organs. The arms of the nerve ring amalgamate in a large ventral commissure, the hemizonid.

Reproductive system (Figs 6–8)

- In females the system comprises a vulva, a vagina and one or two genital tracts, each tract consisting of an ovary with an apical germinative zone and a proximal growth zone, an oviduct, a uterus and sometimes a spermatheca. One tract, usually the posterior tract, may be reduced, leaving a remnant, the postvulval uterine sac.
- The male system consists of one or two testes, a seminal vesicle, a vas deferens and an ejaculatory duct that joins the rectum in the cloaca. The cloaca houses the usually paired, extrusible spicules which are guided during protrusion by a gubernaculum in Tylenchida and Triplonchida. In Longidoridae a lateral guiding piece is present near each spicule tip. Triplonchida and Dorylaimida males have a series of ventromedian copulatory papillae (supplements) in front of the cloacal opening.

Excretory system (Figs 6–8)

- This system is usually asymmetrical and includes an excretory cell, collecting tubules and an excretory duct and pore. The cuticle and prerectum may also be involved with excretion of waste products.

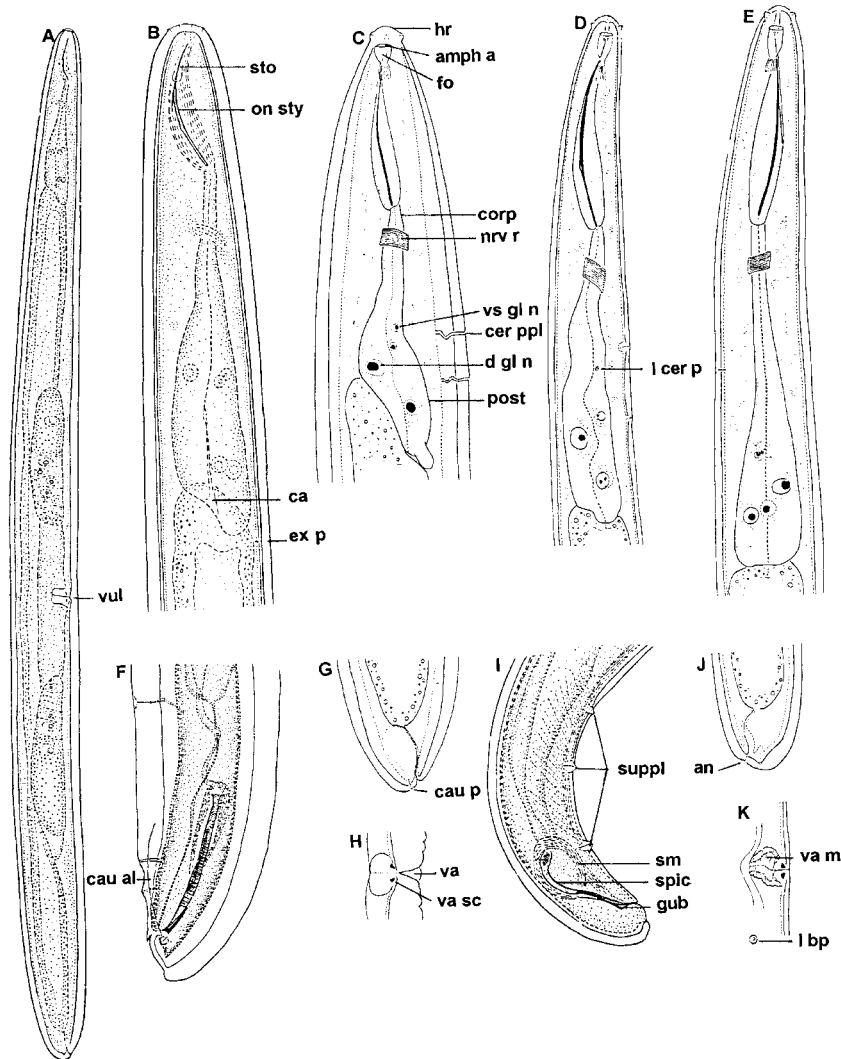


Fig. 7. Morphology of Triplonchida.

Paratrichodoros spp. Female **A**, whole specimen; **B**, anterior region; **G**, posterior region; **H**, vulva and vagina, lateral. Male: **C**, anterior region; **F**, posterior region.

Trichodoros spp. Female: **E**, anterior region; **J**, posterior region; **K**, vulva and vagina, lateral. Male: **D**, anterior region; **I**, posterior region.

amph a = amphid aperture; **an** = anus; **ca** = oesophageal-intestinal valve; **cau al** = caudal alae; **cau p** = caudal pore; **cer ppl** = cervical papilla; **corp** = corpus; **d gl n** = dorsal oesophageal gland nucleus; **ex p** = excretory pore; **fo** = fovea; **gub** = gubernaculum; **hr** = head region; **l bp** = lateral body pore; **l cer p** = lateral cervical pore; **nrv r** = nerve ring; **on sty** = onchiostyle; **post** = postcorpus; **sm** = spicule suspensor muscle capsule; **spic** = spicule; **sto** = stoma; **suppl** = copulatory supplements; **va** = vagina; **va m** = vaginal constrictor muscle; **va sc** = vaginal sclerotisation; **vs gl n** = ventrosublateral oesophageal gland nucleus; **vul** = vulva. (C–E, G,H,J,K, after De Waele & Kilian, 1992, *Fundamental and Applied Nematology* 15, Gauthier-Villars Publishers; F, after Vermeulen & Heyns, 1983, *Phytophylactica* 15, 1, after Vermeulen & Heyns, 1984, *Phytophylactica* 16, reproduced in terms of the Government Printer's copyright permit 10026 dated 19 July 1995; A,B, after Heyns, 1975, *CIH Descriptions of Plant-parasitic Nematodes* No. 69, Commonwealth Agricultural Bureaux).

Morphology and taxonomy

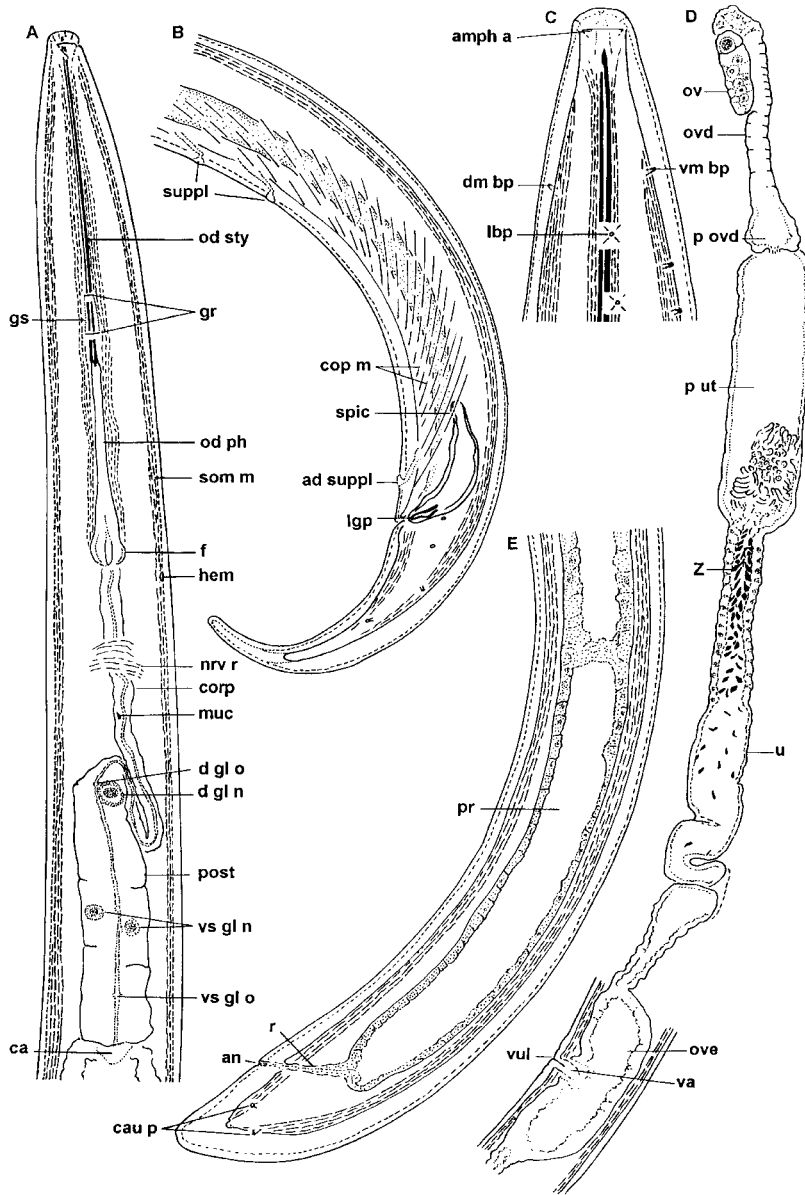


Fig. 8. Morphology of Dorylaimida.

A, anterior region; B, male posterior region; C, head region; D, female genital tract; E, female posterior region. **ad suppl** = adanal copulatory supplements; **amph a** = amphid aperture; **an** = anus; **ca** = oesophageal-intestinal valve; **cau p** = caudal pore; **cop m** = copulatory muscles; **corp** = corpus; **d gl o** = dorsal oesophageal gland nucleus; **d gl p** = dorsal oesophageal gland outlet; **dm bp** = dorsomedian body pore; **f** = flange; **gr** = guiding ring; **gs** = guiding sheath; hemizonid; **lbp** = lateral body pore; **lgp** = lateral guiding piece; **muc** = mucro; **nrv r** = nerve ring; **od ph** = odontophore; **od sty** = odontostyle; **ov** = ovary; **ovd** = oviduct; **ove** = ovejector; **p ovd** = pars dilatata oviductus; **p ut** = pars dilatata uterus; **post** = postcorpus; **pr** = preectum; **r** = rectum; **som m** = somatic muscles; **spic** = spicules; **suppl** = copulatory supplements; **u** = uterus; **va** = vagina; **vm bp** = ventromedian body pore; **vs gl n** = ventrosublateral oesophageal nucleus; **vs gl o** = ventrosublateral oesophageal gland outlet; **vul** = vulva; **Z** = Z-differentiation. (Original)

Taxonomy

General

Taxonomy is the branch of biology that deals with the identification and arrangement of species into related groups on the basis of common characteristics, and with the description and naming of new species. It is the tool with which the immense biodiversity of living organisms may be understood and recorded.

- Identification of species and subspecific groups is basic to all research on nematodes. Advisory, regulatory and pest-control services rely heavily on accurate identification in order to inform growers of potentially harmful species, to prevent exotic species from entering a country, or establishing and spreading within a country, and to develop nematode-resistant crop cultivars.
- Once the identity of a species is known, published information concerning the species can be retrieved from the literature and new information can be reported.

Zoological nomenclature

- Most languages have common or vernacular names for animals but these have a restricted use in science; formal, unique scientific names provide the required order and stability in research, facilitate communication between scientists and assure the status of each animal species.
- Zoological nomenclature is the system by which scientific names are given to animals. The system works according to a set of rules, the International Code of Zoological Nomenclature, which maintains stability in name-giving and standardises the procedures. The rules are administered by the International Commission on Zoological Nomenclature and may be suspended by the Commission when their strict application results in confusion.
- Animals are named according to the binomial system of nomenclature, introduced by Linneaus in 1758, whereby an animal receives a generic name and a specific name which together form the scientific name, e.g. *Meloidogyne javanica* (Treub, 1885) Chitwood 1949, which is always underlined or printed in italics or bold.
- The genus name starts with a capital initial letter and like the species name

is usually a Latinised word or a combination of letters which is Latinised. The name should be unique, i.e. it should be used for only one group of animals, and has a grouping purpose, indicating that its members are adapted to a particular mode of life and share certain consistent characteristics. Each genus is based on a type species.

- The species name is a unique handle by which these natural objects, the species, are known and is based on an actual specimen, the holotype, the name-bearer of the species and a fixed standard of reference for the name. Other specimens that are available at the time that a species is described are known as paratypes.
- The scientific name is followed by the name of the person who described and named the species, and the date of publication of the description. The author's name fixes responsibility for the species name, assists in tracing original descriptions and helps to recognise homonyms. When the author's name is enclosed by brackets and followed by another author's name, the species has been transferred from the original genus to the present one by the second author.
- Scientific names proposed in accordance with the Code are valid and have status in science. An important ruling under the Code is the law of priority of publication, e.g. when a particular animal has been described more than once and under different names within the same genus, the first properly published name is valid (the senior synonym) and the other names become invalid junior synonyms of it; when the same genus name or full scientific name is used for two or more different animals the first properly published name is valid (the senior homonym) and the other names become invalid junior homonyms of the older name.

Taxonomic characters

Classification is that process by which organisms are ordered into taxonomic categories (species, genera, families etc.) and the categories arranged in a hierarchical scheme based on relationships between the organisms. In practice these relationships are considered to be shown by differences and similarities in taxonomic characters.

- Taxonomic characters are attributes of organisms by which organisms in a given taxonomic category resemble each other more than they resemble organisms in other equivalent categories. Morphological characters

express the visible appearance of a species and may have significance in biology and evolution; other taxonomic characters are physiological, ecological, geographical or molecular in nature.

- Taxonomic characters function as indicators of difference as well as indicators of resemblance, and by inference, relationships. Some express differences between species within a genus, others express resemblances between the species that allow them to be placed in the same genus.
- In taxonomic descriptions and for identification purposes the most useful characters are those that are consistently similar, or vary within narrow limits, among organisms within a given category and are consistently different between organisms in different but equivalent categories.
- Taxonomic characters may be arranged in keys for identification purposes but not all the characters used in nematology have the same value; characters used in combination are more useful than when used singly.

Higher categories

- Above the genus category are the higher categories (family, order, class and phylum) which, like the genus, are collective in nature. A family includes one or more genera with a supposed common origin and is separated from other families in an order by clear biological and structural characters of its members. Each family is based on a particular genus, the type genus, which is the fixed standard for the family.
- An order includes one or more families, and a class one or more orders.

3. **B**iology

Habitats

- All plant nematodes spend at least part of their life cycle in soil where they are influenced by conditions such as soil moisture, soil temperature, soil type, aeration, organic-matter content, distribution of host-plant roots, cultivation practices, and parasites, predators and pathogens of nematodes. The success of nematodes as plant parasites depends on these conditions being favourable to them:
 - The optimum soil temperature range for activity is 16–29 °C; above and below this range nematodes become inactive, and temperatures below 4 °C and above 40 °C are lethal.
 - The optimum soil moisture range is between 40 % and 80 % of field capacity; dry soils do not allow movement and saturated soils lack oxygen and also inhibit free movement.
 - Nematodes inhabit all types of soil but prefer sandy soils in which soil-water film thickness, pore diameter and particle size are favourable, aeration is adequate and drainage to field capacity is rapid.
 - Host-plant root exudations orientate nematodes towards roots and may stimulate the juveniles to hatch but may also inhibit hatch and repel or kill some species.
 - Cultural practices such as monocropping with nematode-susceptible crops may eventually result in severe crop damage whereas rotation with susceptible and resistant crops inhibits multiplication of the nematodes. Frequent turning of the soil exposes the nematodes to the drying effect of sun and wind.
- In cultivated soils most nematodes occur in the upper cultivated layer, in the root zones of plants. Some species are long-established, having transferred from wild hosts to crop plants when cultivated lands were first established, other species are more recently introduced, usually through the activities of man.
- In cultivated soils the horizontal distribution of nematodes is determined by the effects of host-plant roots, e.g. root exudations, by the distribution of previous and current host root systems, by cultivation practices such as ploughing that spreads nematodes in the direction of tillage, and by clustering of eggs in egg masses and cysts in some species. The vertical

distribution is determined among others by soil type, seasonal migration of the nematodes and vertical reach of host-plant roots.

In practice, nematodes are effectively spread to new habitats by chance and passively, over both short and long distances, by irrigation and flood water, dust storms, insects, birds, rodents, livestock, farm equipment, clothing, footwear, farm produce and containers, and planting stock. Some species are readily spread in dried and cut flowers, bulbs, tubers, rhizomes and in soil adhering to plant parts, and in packing materials and debris in containers.

Life cycles

- The nematode life cycle is direct and uncomplicated and includes an egg stage, four juvenile stages (three in some Longidoridae) and the adult stage. Eggs are deposited singly or in clusters in the soil or plant tissues or are grouped in egg masses and cysts.
- The first-stage juvenile develops in the eggshell and, in Tylenchida, moults in it to produce the second-stage juvenile which remains in the eggshell until stimulated to hatch by favourable temperature and moisture conditions, and in some species by host-root exudations (Fig. 9). In



Fig. 9. Cyst-nematode eggs and juveniles

Longidoridae and Trichodoridae the first-stage juvenile is the hatching stage. Each juvenile stage moults once and the adults appear after the last moult.

- The life-span varies from several days to several years. Many species winter in the egg stage, others survive in roots of perennial plants. Some species survive prolonged adverse conditions in inactive, dormant states.

Feeding habits

- All parts of plants are attacked by nematodes, most feeding on the underground parts but some invading the leaves, stems, seed and flowers.
- Usually all the post-embryonic life-stages feed but in some groups all or some of the juvenile stages and sometimes the males do not feed.
- During feeding the stylet is inserted into a plant cell and secretions of the oesophageal glands are injected into the cell. The secretions have an extra-oral digestive function and modify the cell contents for ingestion by the nematode. The modified cell contents is drawn into the oesophagus through the stylet by the action of the oesophageal musculature, or into the stoma, as in Trichodoridae.
- Ectoparasitic species are either vermiform and migratory (Fig. 10a), e.g. *Criconema*, *Tylenchorhynchus*, feeding from outside the plant and sometimes entering the plant tissues temporarily with the anterior body, or sedentary and saccate (Fig. 10b), with the anterior body permanently embedded in the plant tissues, e.g. *Tylenchulus* and *Rotylenchulus*.

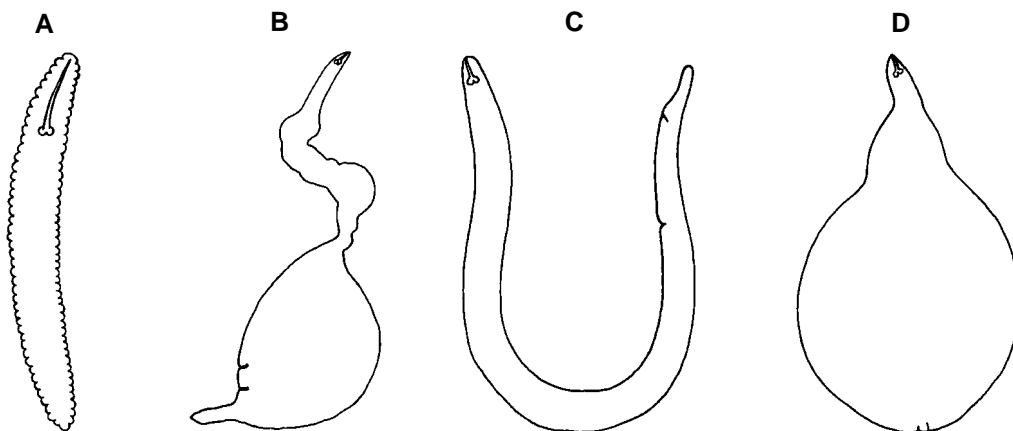


Fig. 10. Outline diagrams of Tylenchida females.

A, migratory ectoparasite (*Criconema* sp.); B, sedentary ectoparasite (*Tylenchulus* sp.); C, migratory endoparasite (*Pratylenchus* sp.); D, sedentary endoparasite (*Meloidogyne* sp.)

- Endoparasites enter plant tissues entirely to feed and are either vermiform and migratory within the tissues, e.g. *Pratylenchus* (Fig. 10c), or saccate and sedentary, e.g. *Meloidogyne* (Fig. 10d).

Effects of nematodes on plants

- Nematode damage to plants as a rule only becomes apparent several years after establishment of an infestation, by which time the nematodes have increased their numbers and widened their distribution.
- Damage is often not obvious, especially during the early stages of an infestation, and non-distinctive, being obscured by symptoms produced by cultural conditions such as low soil fertility, poor drainage, drought stress, herbicides and by other plant pathogens such as root-rot fungi and soil insects. Low and below-average yields often go unnoticed, or are accepted as normal, or are ascribed to more obvious causes. Because of their small size and secluded habitats, nematodes are inconspicuous and are not readily associated with plant growth problems.
- Nematodes injure plants directly and indirectly. Mechanical injury is caused by movement of endoparasites through the tissues and by stylet action, resulting in cell-wall injury and loss of cell contents. Chemical injury occurs when oesophageal secretions kill cells or incite abnormal cell enlargement or cell proliferation, causing the overall physiology of the plant to become impaired.
- Nematodes damage plants also by acting as vectors of other plant pathogens, transmitting plant viruses and carrying and protecting fungal spores and creating wounds such as injured cell walls through which fungi and bacteria can enter to give rise to necrosis and disease complexes. Nematodes also reduce natural plant resistance to some plant pathogens.
- Crop conditions such as lack of vigour, reduced yields and poor produce quality are indications of water and nutrient deficiencies, which in turn indicate disfunctioning root systems and possible nematode involvement.
- Other above-ground indications of possible nematode involvement include tree toppling, twig die-back, stunting, early flowering and fruiting, below-average fruit quality and quantity, yellowing of foliage, defoliation, increased sensitivity to stress conditions such as excessive wilting during periods of drought, and premature plant ageing and death.
- Above-ground symptoms that are more specific to nematode action include leaf and stem malformations (*Ditylenchus* spp.), leaf discolouration (*Aphelenchoides* spp.), leaf and seed galls (*Anguina* spp.), and dead and deformed flowering parts and growing points.
- Below-ground symptoms of nematode damage (Figs 33–42) include root

lesions (*Pratylenchus* spp., *Radopholus* spp.), root galls (*Meloidogyne* spp., *Xiphinema* spp., *Hemicycliophora* spp.), galls on tubers (*Meloidogyne* spp.), lesions on tubers (*Pratylenchus* spp.) wet and dry rot of tubers (*Ditylenchus* spp.), excessive root branching (*Paratrichodorus* spp.), root-tip death (*Paratrichodorus* spp., *Xiphinema* spp.) and unhealthy appearance of roots due to soil particles and debris adhering to the gelatinous matrixes of *Tylenchulus* sp. females.

- In fields the presence of nematodes is often indicated by defined, more or less circular patches of poor crop growth, often with abundant weed growth, that enlarge with time, spreading outwards in perennial crops and in the direction of tillage in annual crops.
- Host plants are plants that are susceptible to one or more nematode species, i.e. they allow the nematodes to feed and reproduce. Good or efficient hosts allow the nematodes to reproduce freely and poor or inefficient hosts allow only limited reproduction. Tolerant hosts can support a large nematode population without suffering damage whereas intolerant hosts are severely damaged under the same conditions. Non-host plants are either resistant to nematodes, allowing some feeding but little or no reproduction, or immune, allowing neither feeding nor reproduction.
- Nematode populations increase when roots are available and soil temperature and moisture conditions are favourable, and decrease when these conditions become unfavourable. However, for any set of conditions every crop can support a specific number of individuals of a given species without being damaged, but will suffer damage when the population density exceeds this tolerance limit.

4. **C**ollecting nematodes

General

- Nematodes are collected by means of soil and plant tissue samples for regulatory, advisory, control, survey and taxonomic study purposes.
- The nematodes in a sample must truly represent the underlying population at a given time, and this requires due attention to sample size and the depth, timing and pattern of sampling, and handling and storage of samples.
- For a sample to be considered representative at least some sampling precision is required, and this must be balanced against the time and effort expended in gathering the sample.
- Because of soil and plant influences and clustering of eggs in egg masses and cysts, the horizontal distribution of nematodes in soil is clumped or aggregated; the vertical distribution also varies, being influenced by soil type, crop root distribution, the nematode species involved, and season.
- This uneven distribution makes collection of truly representative samples difficult, as it introduces sampling errors.
- Other sources of sampling error are low population densities, latent infestations, subsampling and counting of nematodes.

Absence of nematodes in a sample may indicate their absence in the sampled field, but may also indicate that the populations are too low to be detected by sampling.

- To increase the probability of detecting nematode infestations, roots of weeds and volunteer crop plants should be collected when bare soil is sampled.
- Always obtain permission before collecting samples from state or private land.

Sampling equipment

- For collecting soil samples to a depth of 20 cm, a sampling tube (Fig. 11) can be constructed from a 40 cm-length of water-pipe with an internal

Collecting nematodes

diameter of about 15 mm and one side cut away. Scrape the soil from the tube with a screwdriver (Fig. 12) to avoid compression of the soil and mechanical injury to the nematodes.

- For deeper sampling an Edelman soil auger is used (Fig. 13).
- In rocky soil small garden spades or garden trowels are useful (Figs 14, 15).
- Use protective rubber or plastic gloves when handling soil samples collected on pesticide-treated lands.
- Clean all equipment and footwear of adhering soil before leaving a sampling site to reduce the risk of spreading nematodes to uninfested areas.

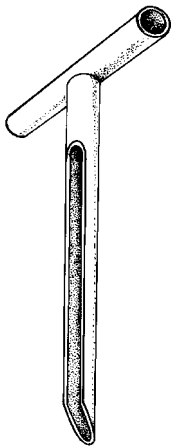


Fig. 11. Soil-sampling tube



Fig. 12. Screwdriver



Fig. 13. Edelman soil auger



Fig. 14. Garden spade

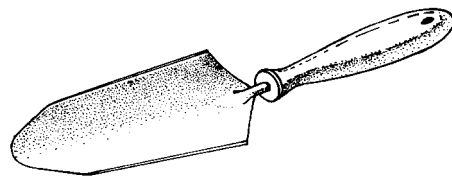


Fig. 15. Garden trowel

Sample size

- Sample size refers to the number of soil samples or separate plant-tissue samples that are combined to make up a composite sample which is eventually processed.
- A comparatively large number of samples will increase the probability of detecting small populations and will therefore reflect the nematode situation in a field more precisely than a small number of samples.
- The number of samples collected depends on the particular sampling pattern used, but broadly the minimum number of samples for fields less than 5 m² in size is ten, for fields between 5 and 100 m² in size, 20, and for larger fields, 30.
- Any number of samples can be combined to form a composite sample.
- If an entire composite sample cannot be processed, subsample by mixing the soil or plant tissue sample very gently but thoroughly by hand, avoiding excessive handling to prevent mechanical damage to the nematodes (Fig. 16).
- Cut roots and other plant material smaller (about 1 cm pieces) before mixing.
- The size of a subsample depends on the extraction procedure used but should be at least 100 ml of soil and 50 g plant material.

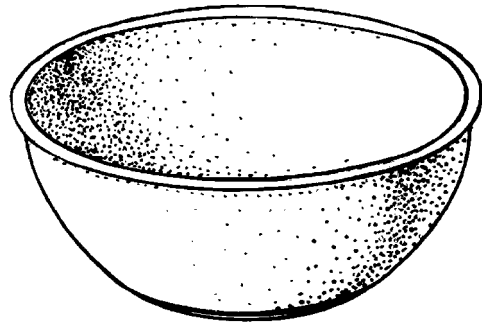


Fig. 16. Mixing bowl for soil samples

Sampling pattern

- Divide large fields into units of 1 hectare.
- Collect soil samples systematically at equally-spaced points according to a grid pattern that covers an entire field; the length of the intervals between sampling points will depend on the sampling precision required, e.g. a 2 × 2 grid pattern by which samples are collected two paces apart along parallel rows also two paces apart will reflect the nematode distribution more precisely than a 10 × 10 pattern (Fig. 17).
- Samples can also be collected at equally-spaced points along a line running diagonally across a field.

Collecting nematodes

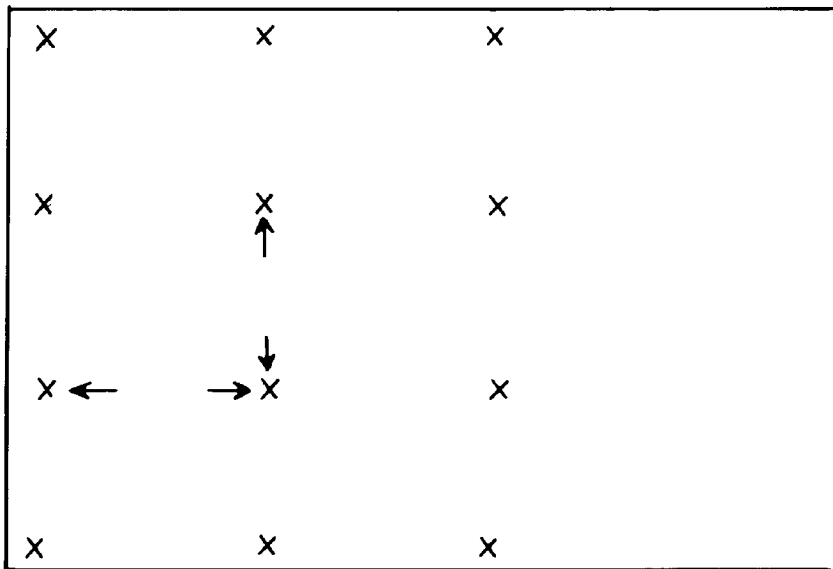


Fig. 17. Sampling pattern for field crops and bare soil

- Sample annual row crops along the rows in small plots and across the rows in larger plots, 10–20 cm from the stem, in the root zone; also collect roots of crop plants, weeds and volunteer crop plants.
- Sample patches of poor plant growth along the borders of the patches.
- Sample tree crops in the drip line, on alternate sides of the trunks, and include feeder roots (Fig. 18).

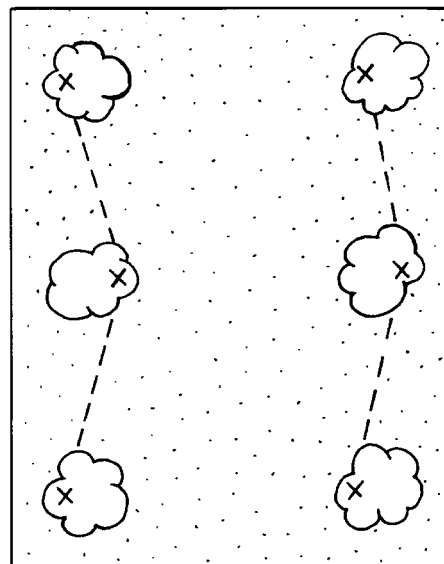


Fig. 18. Sampling pattern for tree crops

Sampling depth

- Soil samples collected to a depth of 20 cm are adequate for most crops.
- Sample to different depths around deep-rooted perennials, e.g. 15, 30 and 60–100 cm.
- Sample shallow-rooted perennials to a depth of 8–12 cm.
- In regions with hot, dry summers collect samples 30–45 cm deep to allow for vertical migration of nematodes.

Sampling time

- In agricultural soils, samples should be collected close to planting time, as the nematodes present at this time can generally be related to yield in annual crops.
- Samples can also be collected between mid-season and harvest as nematode numbers increase towards harvest.

Care of samples

- Collect samples in sturdy plastic bags and close bags firmly with string (Fig. 19).

Handle bagged samples very carefully and especially do not drop or expose to direct sunlight or other sources of heat.

- Place above-ground plant material and soil samples in separate bags but place roots together with soil in the same bag and cover with the soil.
- Do not moisten soil samples collected in dry soil.

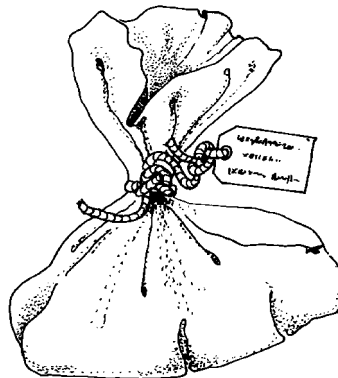


Fig. 19. Bagged soil sample

- Transport samples in cool-boxes or other insulated containers, preferably among ice-packs.
- Process samples within a week of collection or store in a cool place or in a refrigerator at 10–15 °C and keep storage time as short as possible.

Labelling of samples

- Attach labels to the outside of bagged samples or place a bag inside an empty bag and insert label between bags.
- The information appearing on the label should include at least a sample number or another identification symbol.
- In a field book record the sample number, name and postal address of grower, name of collector, locality, the recent cropping history of sampled fields, previous nematode and other pest-related problems and control measures taken, below and above-ground symptoms of the presence of nematodes, the standing and intended crops, size of area sampled, type of irrigation and source of water, and date of sample collection.

Dispatching of samples

- Dispatch bagged samples tightly packed, but without undue pressure, in insulated containers such as cool-boxes or in cardboard boxes among balled newspapers or packing materials such as Styrofoam chips (Fig. 20).
- Label containers clearly with names and addresses of consignee and sender and add warning notices, e.g. 'handle with care' and 'live biological material'.
- Write a covering letter with information about the samples and place a copy inside the container with the samples.

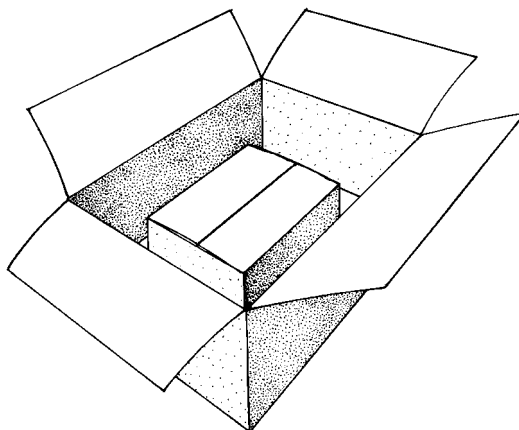


Fig. 20. Container for dispatching samples

5. **E**xtracting and detecting nematodes

EXTRACTING NEMATODES

Various techniques to extract nematodes from soil and plant tissues have been developed. Some are rarely used, others have been adapted to suit local conditions and requirements and availability of equipment.

Baermann tray

This modification of the Baermann-funnel technique extracts only live, active nematodes from soil and plant material, in a clear suspension. Very large and sluggish species are generally poorly extracted.

☛ Soil

- Break up lumps and remove stones and plant debris.
- Spread 50 ml of soil evenly on a circle of single-ply paper towel supported on a coarse-meshed plastic screen standing in a plastic container (Figs 21, 22).

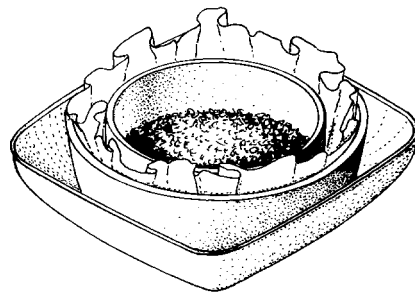


Fig. 21. Baermann tray set-up



Fig. 22. Baermann tray set-up

- Add water to the container until the soil is thoroughly wet but not immersed.
- Cover the container with a large Petri dish top to reduce evaporation of the water.
- Leave set-up undisturbed for at least 24 hours.
- Remove the soil, discard and pour the nematode suspension from the container for examination.

☛ Plant material

- Rinse free of soil and cut smaller.
- Spread 50 g of material evenly on paper filter and continue as described above.

Sieving – centrifugation – flotation

With this technique both active and sluggish species are extracted from soil and plant material. Results are quickly available but the equipment is expensive and considerable expertise is required.

☛ Soil

- Wash soil (250 ml) through a coarse-meshed sieve (2 mm apertures) into a 5-litre bucket (Figs 23, 24).
- Add water to bucket to increase suspension to 5 litres.
- Stir suspension, then allow to settle for 30 seconds.
- Pour suspension through a 45 μm -aperture sieve (Fig. 25).
- Repeat procedure with soil in bucket two more times, but shorten settling times to 20 and 10 seconds.
- Transfer residue on 45 μm sieve to four 50 ml centrifuge tubes.

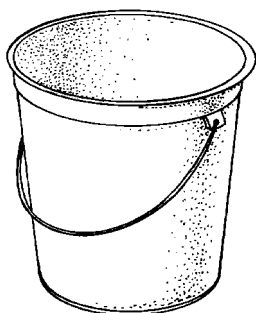


Fig. 23. Bucket

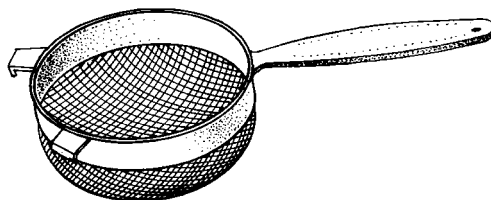


Fig. 24. Coarse-meshed sieve

- Centrifuge for 7 minutes at 1750 rpm
- Decant supernatant from tubes and discard.
- Add sugar solution (450 g/l water) to tubes.
- Shake tubes, centrifuge for 3 minutes at 1750 rpm
- Pour suspension through the 45 μm sieve.
- Rinse residue from sieve for examination.

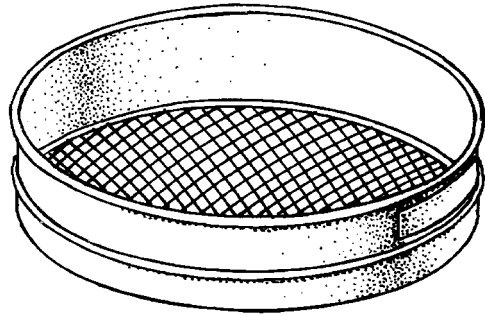


Fig. 25. Fine-meshed sieve (45 μm aperture)

☛ Plant material

- Shred material (50 g) in a food blender or cut into 1 mm pieces, cover with water.
- Leave material for 24 h.
- Add material to centrifuge tubes together with 50 g of kaolin powder and centrifuge and decant as described above.

Sieving – sedimentation

This technique provides quick results but small nematodes may be trapped by the flocculating debris.

- Wash and decant a 50 ml soil sample as described for the previous technique.
- Wash residue on 45 μm sieve into a 100 ml measuring cylinder (Fig. 26).
- Add five drops of a flocculant, e.g. Superfloc N100 (SA Cyanamid), to contents of cylinder.
- Close cylinder with hand and invert several times.
- Allow suspension to settle for 20 seconds.
- Pour supernatant into a 200 ml cylinder.
- Again add flocculating agent to contents of 100 ml cylinder and invert.

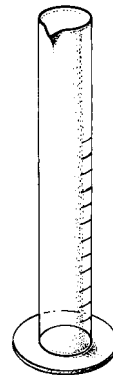


Fig. 26. Measuring cylinder

- Allow suspension to settle for 10 seconds.
- Pour supernatant into 200 ml cylinder.
- After 50 minutes siphon off most of contents of 200 ml cylinder to leave about 20 ml of nematode suspension for examination.

Seinhorst cyst-elutriator

With this apparatus heteroderid cysts are extracted from both wet and dry soil. The cysts are kept in suspension by a controlled upward current of water (Figs 27, 28).

- Close clamp on lower downpipe and close cylinder with rubber bung.
- Adjust speed of upward current of water in cylinder to about 4 cm per second by manipulating feeder-tank stopcock.
- Let water out of apparatus, place both downpipes in 150 μ m-aperture bucket sieve.
- Close cylinder with rubber bung and open feeder-tank stopcock to desired setting.
- As water rises in cylinder, wash a 100 ml soil sample through coarse-meshed sieve (2 mm apertures) into top bowl.
- As water spills over collar and into bucket sieve, wash residue on sieve with strong fan-shaped spray of water.



Fig. 27. Seinhorst cyst-elutriator

- After about 30 seconds open clamp on lower downpipe, continue spraying residue on sieve to prevent clogging of sieve meshes.
- Rinse top bowl and cylinder until water in cylinder is clear.
- Let water out of apparatus and rinse.
- Wash residue on sieve onto a 120 mm square of fine gauze supported on a coarse-meshed plastic screen clamped to a retort stand.
- Fold gauze containing residue into a small packet and leave to dry (residues with cysts other than *Globodera* cysts should not be allowed to dry completely) (Fig. 29).
- Line a large glass trough (19 cm diameter) with a 7 × 60 cm strip of blotting paper (Fig. 30).
- Add water to trough until lower half of paper is submerged and place dried residue into water.
- Slowly push a large conical flask, half-filled with water, down into trough (Fig. 31).

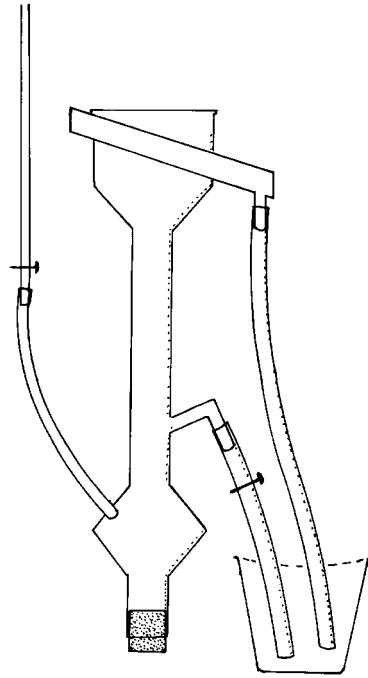


Fig. 28. Seinhorst cyst-elutriator

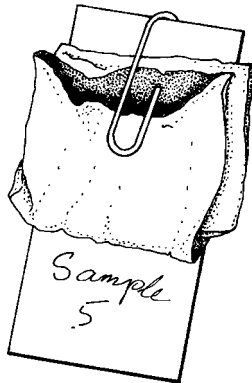


Fig. 29. Folded gauze square with residue

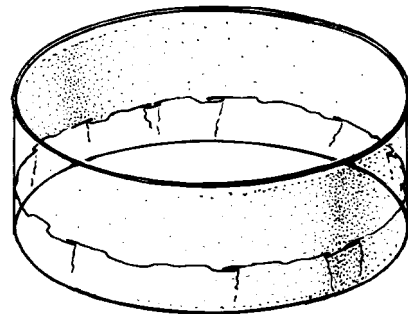


Fig. 30. Glass trough

Extracting and detecting nematodes

- After about 15 seconds lift flask out of water and rinse adhering debris into trough with a wash-bottle (Fig. 32).
- Siphon off water in trough, remove paper strip and lay flat on a strip of Perspex.
- Under a dissecting microscope separate cysts from debris with a dissecting needle and collect with a wet camel-hair brush.

Alternatively

- Crumble 50 ml of air-dried soil into a 2-litre conical flask half-filled with water.
- Swirl suspension vigorously.
- Fill flask with water to just below rim and let stand for some minutes.
- Decant debris and cysts into a glass trough lined with a strip of blotting paper and continue as described above.

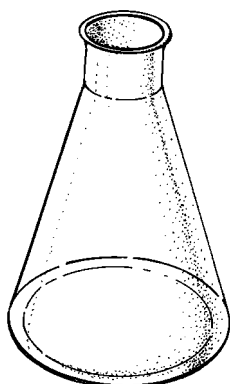


Fig. 31. Conical flask



Fig. 32. Wash-bottle

DETECTING NEMATODES

Bioassay

A useful technique to detect very low populations, or populations in the egg stage, of certain species.

☛ Root-knot nematodes

- Half-fill several 15 cm pots with steam-treated sandy soil (30–50 % sand).
- Top up each pot with field-collected soil.
- Transplant a three-week-old tomato seedling (cvs UC82B, Rutgers or Moneymaker) into each pot.

- Grow plants in a greenhouse at about 25 °C, irrigating from drainage saucers and fertilising as required.
- After six weeks remove plants from pots, cut off tops and gently rinse roots free of soil in running water.
- Immerse washed root masses in phloxine B vital stain (0.15 g/l water) for about 15 minutes, then rinse.
- Egg masses show bright red on root surface.
- If an indication of the severity of the original infestation is required, count the egg masses and rate according to the following scale: no egg masses = 0; 1–2 egg masses = 1; 3–10 egg masses = 2; 11–30 egg masses = 3; 31–100 egg masses = 4; more than 100 egg masses = 5.

☛ Cyst-nematodes

- Grow appropriate host plants in 15-cm pots in greenhouse.
- During first few weeks of active growth, periodically water plants to excess and collect about 50 ml of the run-off from each pot.
- Pool the run-off, which contains the root exudations and store at 5 °C until required.
- Soak cysts in tap water for 24 hours.
- Immerse about 100 cysts in 1 ml of the run-off water in a cavity block.
- Cover block with lid and keep at 25 °C in an incubator.
- Remove and count hatched juveniles and replace fluid weekly.
- Continue process until hatching ceases.

Direct examination of plant material

- Wash roots free of soil in running water.
- Cut material smaller as required.
- In water under a dissecting microscope tease material apart with dissecting needles.
- Collect freed nematodes from the water and sedentary females of ecto- and endoparasitic species from roots.

Alternatively

- Keep 5–10 g of cut plant material or seed in shallow water in a closed container for several days at about 25 °C.
- Migratory endoparasites and infective stages of sedentary ecto- and endoparasites leave the material for the water, where they can be observed and collected.

Examples of nematode damage (Figs 33–42)

- Yam tubers with cracked, spongy surfaces (*Radopholus similis*, *Pratylenchus coffeae*, *Scutellonema bradys*).
- Potato tubers with dark, dry spots or pimples on surface (*Pratylenchus* spp.) (Fig. 38) or with dark, cracked areas covering necrotic tissue (*Ditylenchus destructor*) or with irregular, knobby surface protuberances (*Meloidogyne* spp.) (Fig. 40).



Fig. 33. Galled tomato roots (*Meloidogyne* sp.)



Fig. 34. Damaged tobacco (*Meloidogyne* sp.)

- Ginger rhizomes with sunken, brown watery lesions on surface (*R. similis*, *Meloidogyne* spp.).
- Banana corms and roots with internal dark, necrotic lesions (*R. similis*, *Pratylenchus* spp., *Helicotylenchus multicinctus*).
- Garlic and onion bulbs with internal rot, sometimes with 'nematode wool' (dried fourth-stage juveniles of *Ditylenchus dipsaci*).



Fig. 35. Damaged (left) and healthy (right) groundnut pods (*Ditylenchus africanus*)



Fig. 36. Damaged (left) and healthy (right) groundnut seed (*D. africanus*)

Extracting and detecting nematodes

- Vegetable roots with yellow or white spherical bodies at about flowering time (Fig. 41) (cyst-nematodes) or with galls (*Meloidogyne* spp.) (Fig. 33).
- Black, misshapen grass and wheat seed (*Anguina* sp.).
- Shrivelled, discoloured bean seed, sometimes with 'nematode wool' (*D. dipsaci*).
- Shrivelled groundnut seed with dark testas (Fig. 36) or pods with surface lesions (Fig. 35) (*Aphelenchoides arachidis*, *Ditylenchus africanus*, *Pratylenchus brachyurus*).



Fig. 37. Damaged citrus roots (*Tylenchulus semipenetrans*)



Fig. 38. Damage to potato (*Pratylenchus brachyurus*)

- Citrus roots with adhering soil particles and debris (*Tylenchulus semipenetrans*) (Fig. 37).

Waste disposal

- All solid and liquid material remaining after processing of samples must be disposed of in such a way as to prevent the introduction and spread of exotic and other species that may pose threats to local agriculture.



Fig. 39. Hyacinth bulb damage (*Pratylenchus penetrans*)

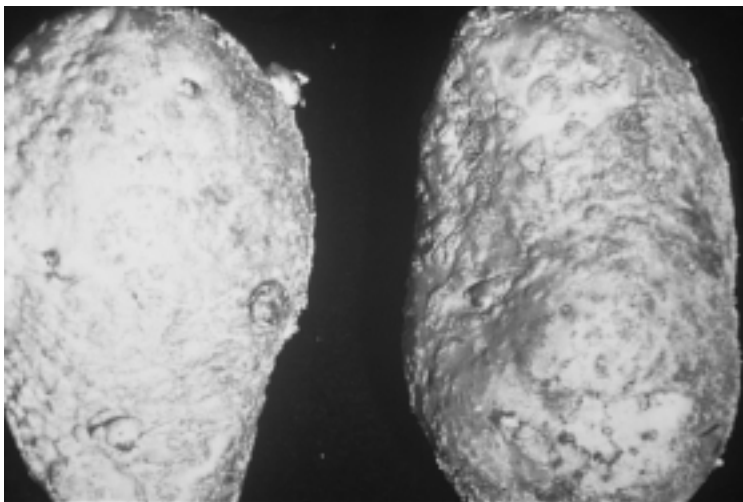


Fig. 40. Damage to potato (*Meloidogyne hapla*)

Extracting and detecting nematodes

- Destroy infested plant material and contaminated containers, packing materials, etc. by burning.
- Treat liquids with 5 % formalin and soil with steam, or spread soil thinly on a plastic sheet in full sunlight to kill the nematodes.

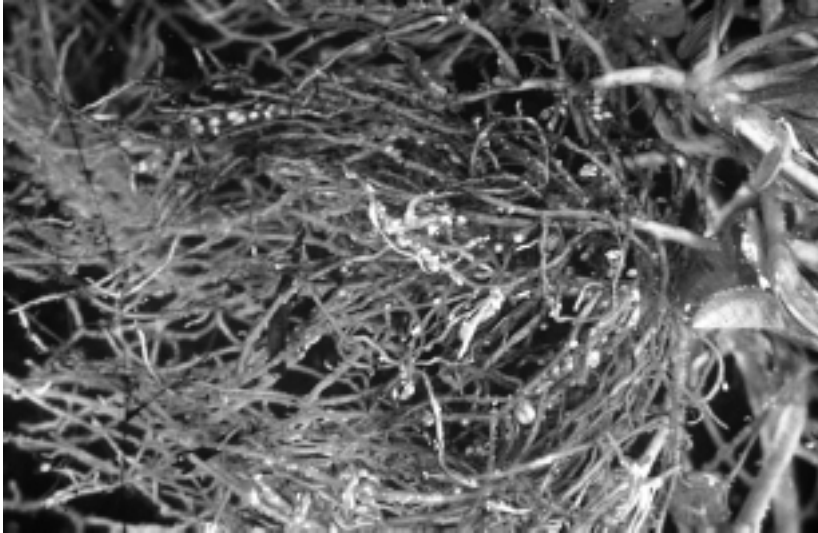


Fig. 41. *Heterodera trifolii* females on clover roots



Fig. 42. Damage to sweet potato (*Pratylenchus* sp.)

6. **G**eneral techniques

Handling nematodes

Because of their small size, nematodes are always handled in a fluid medium under a dissecting microscope. Removing nematodes from a nematode suspension may be difficult to master; for individual specimens a handling tool is used, and batches of specimens can be transferred with a Pasteur pipette (Fig. 47).

Worm-like specimens (Fig. 10a,c)

- A handling tool can be made from a tooth-brush bristle, an eyebrow hair or a No. 0 insect mounting-pin with a recurved tip, all mounted in pin-vices or attached to penholders (Figs 43, 44).
- With a dissecting microscope set at a convenient magnification and using transmitted light, manipulate a specimen in a nematode suspension with the handling tool until the specimen rises in the fluid, then lift it out of the water.



Fig. 43. Pin-vice handling tool

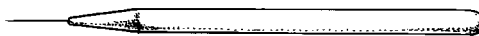


Fig. 44. Dissecting needle

Swollen specimens (Figs 10b,d, 48, 49)

- Under a dissecting microscope and with direct lighting remove root-knot nematode and cyst-nematode females and other swollen species from fresh roots in water or from roots softened and fixed in lactophenol, by breaking away the root tissue surrounding the specimen with dissecting needles and forceps (Fig. 45), taking great care not to puncture the bodies.
- Lift the specimen out of the liquid with a camel-hair brush (Fig. 46) or dissecting needle with the tip bent into a small loop.

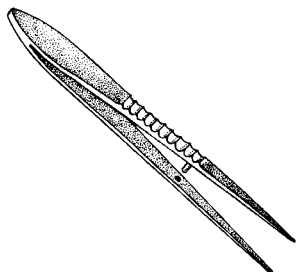


Fig. 45. Forceps



Fig. 46. Camel-hair brush

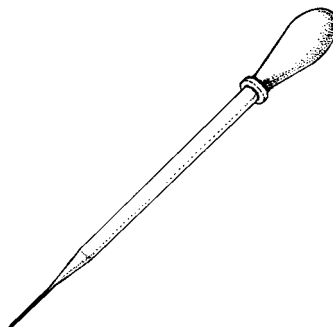


Fig. 47. Pasteur pipette

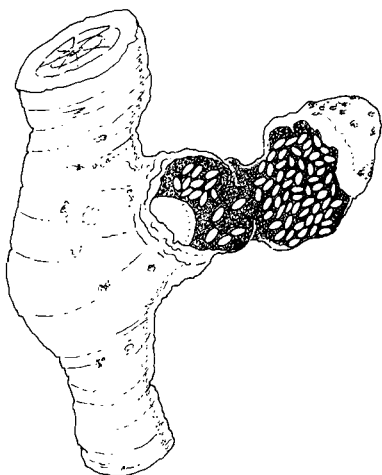


Fig. 48. *Meloidogyne* sp. female with egg mass in root

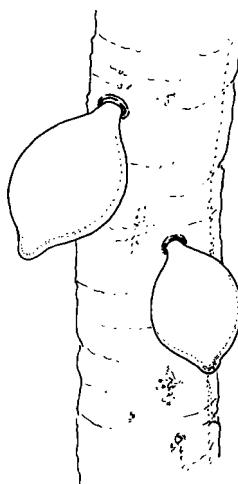


Fig. 49. Cyst-nematode females on root

Counting nematodes

- The tedious task of counting large numbers of nematodes can be eased by extracting several fixed-volume subsamples of nematodes from a suspension and counting them in graduated Perspex or glass counting dishes or slides, e.g. Peters' 1 ml slide, a Doncaster dish or a De Grisse dish (Fig. 50).

- With a nematode suspension in a 250 ml beaker, allow specimens to settle for about 10 minutes.
- Adjust volume of suspension to 100 ml by adding or withdrawing water.
- Agitate suspension by blowing through it for about 15 seconds using a pipette.
- Immediately withdraw a 10 ml subsample of the suspension with a graduated 25 ml pipette (Fig. 51) and transfer to a counting dish.
- Under a dissecting microscope, with transmitted light, identify all plant nematodes to generic level and count the number of specimens in each genus, using a counter (Fig. 52).
- Return subsample to nematode suspension and repeat counting procedure two more times.
- Multiply all counts by ten and calculate the average number of specimens in each genus.

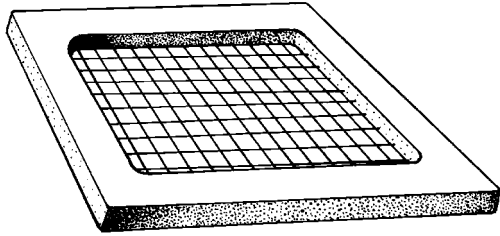


Fig. 50. De Grisse counting dish

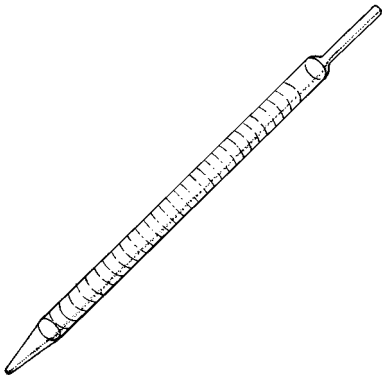


Fig. 51. Graduated pipette

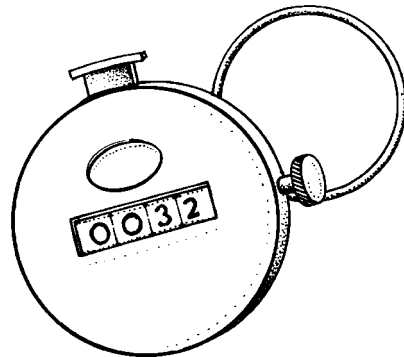


Fig. 52. Hand-held counter

Differentiating between live and dead nematodes

For experimental work it may be necessary to know whether free nematodes and specimens contained in seed galls, egg masses and cysts are alive or dead.

Vital stains

- Free nematodes and specimens contained in seed galls, egg masses and cysts are stained in new blue R to distinguish live specimens from dead ones.
- Soak cysts and seed galls in water for 24 hours.
- Cut seed galls to release specimens.
- Immerse material in the stain (15 mg/l water) in a covered cavity block (Fig. 53) for several days.
- After staining, squash egg masses and cysts in a drop of water between two glass slides to release juveniles and eggs.
- Examine specimens in water under a dissecting microscope – dead specimens stain dark blue or purple, live specimens remain unstained.

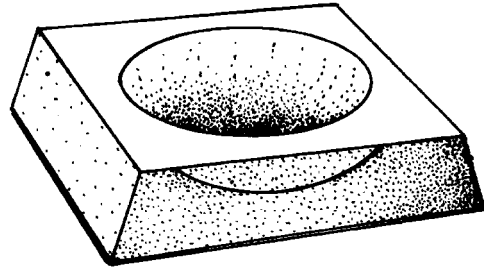


Fig. 53. Cavity block

Internal body pressure

- With a surgical eye scalpel (Fig. 54) cut single specimens transversely in half in water in a cavity block.
- The internal body pressure of live specimens forcibly extrudes the body contents at the wound, whereas dead specimens show no reaction.



Fig. 54. Eye scalpel

Body posture

- Expel infective juveniles of root-knot nematodes and cyst-nematodes from eggshells by slight pressure on the shells with a fine needle or in a drop of water under a coverslip on a glass slide.
- Live specimens immediately assume the typical smoothly-curved body posture (Fig. 55b) and may show some movement whereas dead specimens retain the kinked or angular posture (Fig. 55a) caused by folding of the body in the eggshell.

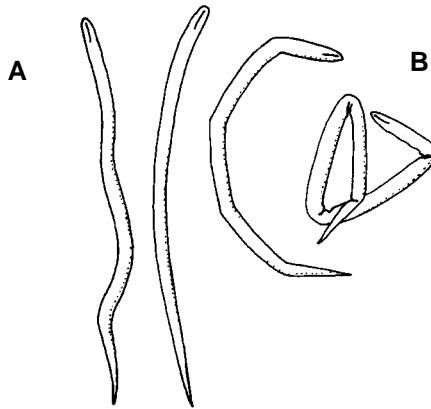


Fig. 55. Dead (A) and living (B) *Meloidogyne* sp. juveniles

Establishing greenhouse populations

For many aspects of nematological research a constant supply of nematodes is required. Because of their wide distribution, known plant-pathogenic abilities and the research that they generate, root-knot nematodes are preferred laboratory animals.

- To establish single-species populations, collect plants with galled roots containing mature females with attached egg masses from infested fields.
- Gently wash roots free of soil in running water.
- Cut 20 root pieces from root mass, each with a single female and egg mass.
- Place root pieces separately in water in numbered cavity blocks.
- Dislodge egg masses from roots and leave in cavity blocks.
- Collect, process, mount and identify females to species level.
- Combine egg masses of each species.
- Wash egg masses into 2 cm-deep holes made around three-week-old tomato seedlings (cvs Rutgers, UC82B or Moneymaker) growing in steam-treated sandy soil (30–50 % sand) in several 15 cm plant pots (Fig. 56).

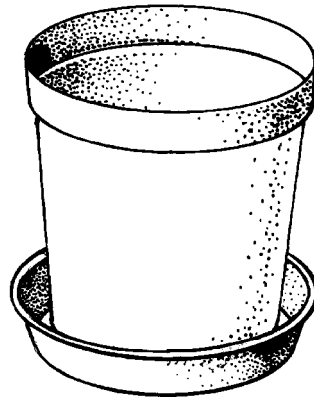


Fig. 56. Plant pot with saucer

- Fill holes with soil and water plants lightly.
- Maintain plants in a greenhouse at 25 °C, widely spaced on metal-mesh surfaces, and irrigate from drainage saucers to avoid cross-contamination; fertilise as required.
- Periodically cut off plant tops and transplant fresh seedlings into pots.

Alternatively

- Place dislodged egg masses on a piece of tissue-paper resting on a circle of plastic mesh in a small Petri dish (Fig. 57).
- Add enough tap water to dish to wet tissue-paper.
- Stand dish in an incubator at 25 °C for 7–10 days.
- Withdraw nematode suspension daily from the dish.
- Pool juveniles of each species and inoculate tomato seedlings as described for egg masses.

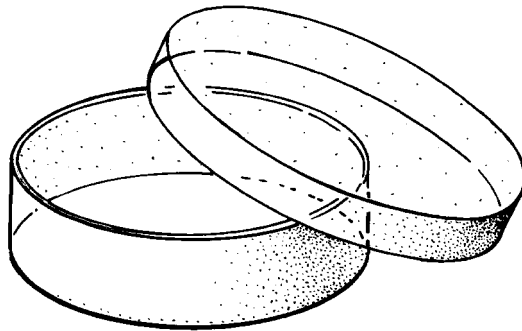


Fig. 57. Petri dish

7. **P**reserving nematodes

Nematodes are preserved as slide mounts, unmounted in fixatives in vials, and frozen and stored in liquid nitrogen. Nematode cultures are also maintained in the greenhouse as live collections. Proper methods of relaxing (killing), fixing and mounting nematodes are essential to ensure that specimens will remain in good condition for many years.

Killing and fixing

Fixation kills and hardens tissues and preserves cellular structure. Specimens should be fixed immediately after killing, or killed and fixed simultaneously.

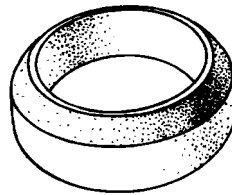


Fig. 58. Syracuse dish

Worm-like specimens

- Transfer live specimens to a cavity block or small Syracuse dish (Fig. 58) in a very small amount of water.
- Heat FA 4:1 fixative (plus 2 % glycerol) to 90–100 °C in a small tube standing in boiling water in a small beaker (Fig. 59) over a spirit flame (Fig. 60).
- Quickly pour hot fixative in excess over specimens.
- Cover container and keep for 2–3 weeks at room temperature to allow fixative to evaporate, leaving specimens in glycerine.
- Store container with specimens in a desiccator (Fig. 61), with calcium chloride as desiccant, until specimens are required for mounting.

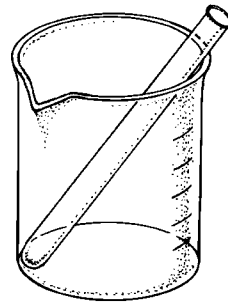


Fig. 59. Glass beaker

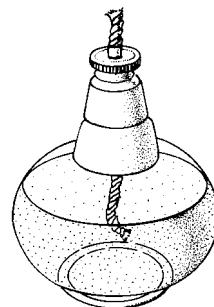
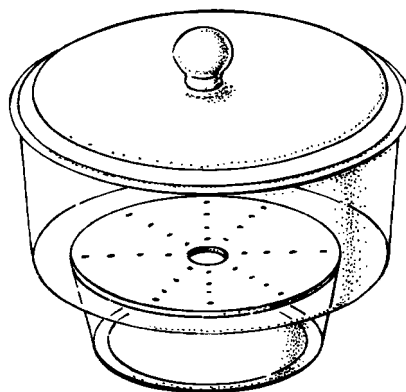
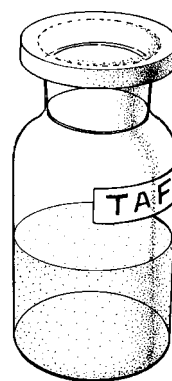


Fig. 60. Spirit lamp

Alternatively

- Transfer live specimens to a small amount of water in a small tube.
- Stand tube in a beaker with warm water (60 °C) for about 2 minutes over a spirit flame.
- Fix specimens in hot FA 4:1, incubate and dehydrate as described above, or
- Fix specimens by adding cold TAF (Fig. 62) in excess to tube.
- Refrigerate specimens for 7 days in the TAF.
- Transfer specimens to 1.25 % glycerol in distilled water in a small Syracuse dish.
- Keep the dish with specimens in an incubator for six weeks at 25 °C.
- Store dish with specimens in a desiccator with calcium chloride until mounting is required.

**Fig. 61. Desiccator****Fig. 62. Container for fixatives****Alternatively**

- Heat live specimens in water in a small Syracuse dish over a spirit flame (do not boil water).
- Check specimens frequently for movement, stop heating as soon as specimens stop twitching.
- Draw off most of the water in dish with a Pasteur pipette and fix specimens with hot FA 4:1 or cold TAF as described above.

Swollen specimens

- Immerse whole root masses or pieces of fresh roots containing root-knot nematode and cyst-nematode females in fuming (not boiling) lactophenol for several minutes. **Caution:** inhalation of phenol fumes is dangerous to health.
- Transfer roots to cold lactophenol, remove females and store in lactophenol in vials until required for mounting.

Mounting

Temporary preparations

For quick identification or study of features best seen in unfixed specimens:

- Transfer live specimens to a small drop of water on a glass slide.
- Briefly heat slide over spirit flame, checking frequently for nematode movement.
- Stop heating as soon as specimens stop twitching.
- Apply coverslip and seal around edge with nail varnish, using a fine camel-hair brush.

Permanent preparations

Worm-like species

- Place a small drop of glycerol in the centre of a glass slide.
- Arrange several pieces of fibreglass rods around edge of drop. The rods must match the nematodes in thickness.
- Place several fixed specimens in drop, gently pressing them down onto surface of slide.
- Briefly warm a 19 mm coverslip over spirit flame.
- Lower coverslip onto glycerol drop and fix it down at three points with nail varnish.
- Allow drops to set, seal coverslip with nail varnish.

Swollen species

- Place a fixed mature root-knot nematode or cyst-nematode female in a small drop of 100 % lactic acid on a glass slide. **Caution:** lactic acid is corrosive.
- Puncture body with a sharp dissecting needle and cut transversely in half with a surgical eye scalpel.
- Transfer anterior half of body to a small drop of glycerol on a glass slide.
- Remove body contents from posterior half with a dissecting needle and trim away the cuticle around the perineal pattern or vulva area with the scalpel (Fig. 63).
- Transfer cuticle piece to glycerol drop and press down onto slide surface, with outer surface uppermost and next to anterior end.
- Apply coverslip and seal with nail varnish.

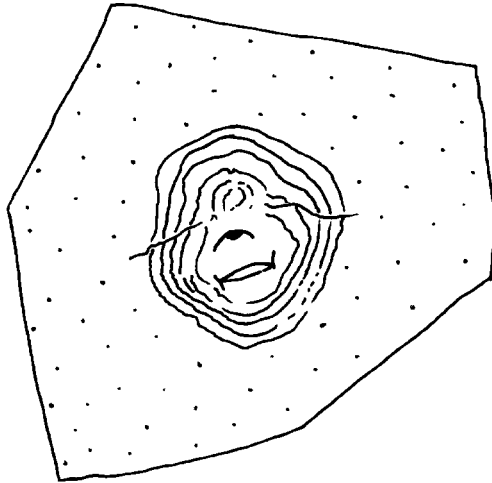


Fig. 63. Cuticle piece with perineal pattern

Cysts

- Soak cysts in tap water for 24 hours.
- Transfer cysts to fuming lactophenol and then to 100 % lactic acid.
- In the acid cut off the posterior end of a cyst with an eye scalpel, remove body contents with a dissecting needle without disturbing structures in vicinity of vulva, and trim away cuticle around vulva area.
- Transfer cuticle piece to water, then to 96 % alcohol and then to xylene for clearing.
- Transfer cuticle piece to a very small drop of Canada balsam on a glass slide, with outer surface uppermost.
- Keep slide in a horizontal position in a closed, dust-free container for about a week, to allow balsam to set.
- Add more balsam to drop and apply coverslip.

Unmounted material

- As a back-up collection, preserve fixed specimens in vials (Fig. 64) in FA 4:1, glycerol or 3–5 % formalin with 2 % glycerol added to prevent specimens from deteriorating should the preservative evaporate.

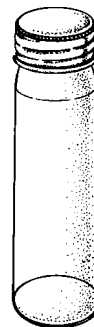


Fig. 64. Screw-top vial

Swollen species can be stored in lactophenol.

- Seal vial tops with parafilm and label vials with same information as on slide mounts.

Fixatives

FA 4:1

Formalin (40 % formaldehyde) — 10 ml

Glacial acetic acid — 1 ml

Glycerol — 2 ml

Distilled water up to 100 ml

CAUTION: formaldehyde is carcinogenic

FAA

Formalin (40 % formaldehyde) — 6 ml

Glacial acetic acid — 1 ml

Ethanol (96 %) — 20 ml

Distilled water — 40 ml

TAF

Formalin (40 % formaldehyde) — 7 ml

Triethanolamine — 2 ml

Distilled water — 91 ml

Lactophenol

Liquid phenol — 20 ml

Lactic acid — 20 ml

Glycerol — 40 ml

Distilled water — 20 ml

**CAUTION: phenol is caustic and toxic;
lactic acid is corrosive**

8. **R**eference collections

Collections of slide-mounted and otherwise-preserved nematodes represent an essential and permanent record of the species that occur in a particular country or region and provide a basis for taxonomic studies, for maintaining regulatory control, for developing identification and advisory services and for teaching.

Accessioning

- When preserved material is added to a collection a unique accession number is assigned to every slide and container. This number and all information available about the material are recorded on an index-card system or computer database, or other accessible systems for recording and retrieving information.
- The information recorded must include the accession number, date of collection, name of country, a grid reference, names of rivers, farms, mountains, distance from nearest town, details of habitat, host plants, infestation symptoms, soil type, altitude, purpose of sampling, name of collector, number and sex of specimens, genus and species names, fixing and mounting techniques, and other data recorded in field books.
- As much as possible of this information should be displayed on labels that accompany specimens.

Slide mounts

- Cobb double-coverslip aluminium slides are lighter than glass slides, can withstand rougher handling and are easily stacked, but are difficult and more expensive to produce.
- Slides are labelled with the date of collection, name and life stages of specimens, host plant, locality, accession number, name of collector and mounting medium. Water-proof ink should be used (Fig. 65).
- Slides are stored horizontally in slide boxes or trays that are stored in steel cabinets in fire-proof rooms.
- After use, remove immersion oil from slides with alcohol and cotton wool, to avoid damage to the sealant.
- Periodically check slides for leakage of mounting medium, and remount specimens or replenish medium.

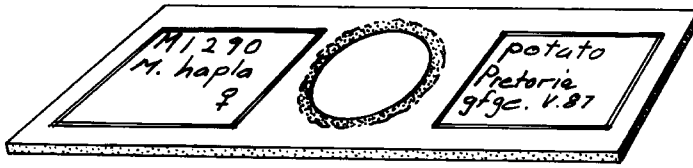


Fig. 65. Labelled slide mount

Unmounted material

- For teaching and demonstration purposes, preserve roots and other infested plant material that show clear symptoms of nematode damage in FAA in appropriate glass containers with tight-fitting lids.
- Label containers with name of host plant, nematode species, locality, date of collection and collector's name.
- Periodically check containers for leakage.

Packaging and dispatching of material

- Fixed, unmounted specimens are dispatched in FA 4:1, 3–5 % formalin, TAF or glycerol in glass screw-top vials or Eppendorf capsules (Fig. 66). Swollen species are placed in lactophenol.
- Seal vial and capsule tops with parafilm or paraffin wax to prevent leakage.
- Label containers individually with pertinent information on a slip of paper protected with transparent sticky tape wrapped around the container.
- Wrap containers individually in tissue paper and pack in a sturdy cardboard container, making up a small parcel.
- Place parcel in a larger container, e.g. a cardboard box (Fig. 20), and fill surrounding space with balled newspaper or Styrofoam chips.
- Alternatively, affix Eppendorf capsules to a piece of thick cardboard with sticky tape and cover with another cardboard piece for bracing; dispatch in a padded envelope.

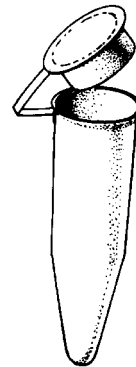
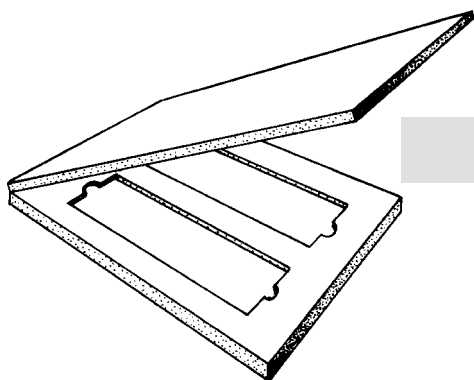
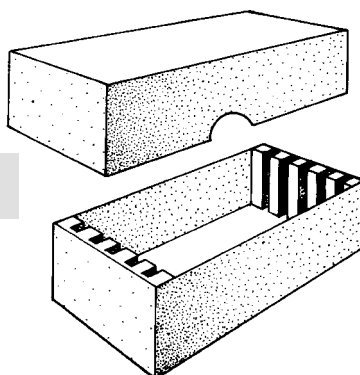


Fig. 66. Eppendorf capsule

- Write names of sender and consignee clearly on container and add warning notices, e.g. 'preserved biological material' and 'handle with care'.
- Protect slide mounts in wooden, plastic or cardboard slide holders (Fig. 67) or slide boxes (Fig. 68), with cotton wool or foam plastic padding to restrict movement, and dispatch in padded envelopes.
- Alternatively, stack slides back-to-back with thick cardboard spacers between labels to separate slide pairs.
- Tape slides together and dispatch in a large container as described above.
- Wrap fresh material such as root pieces with root-knot nematode females and attached egg masses in a few layers of moist tissue paper, place in a small unsealed plastic bag and dispatch in a regular or padded envelope.
- Alternatively, place dislodged egg masses in 0.9 % saline solution in Eppendorf capsules and pack and dispatch capsules as described above.
- All consignments must be accompanied by copies of covering letters and import permits.
- Register parcels containing type or other valuable material, or send live material by courier service to ensure rapid transit.

**Fig. 67. Slide holder****Fig. 68. Slide box**

Glossary

annule - the interval between two transverse grooves on cuticle

apical - at furthest point from base

attribute - a property or quality that makes a thing what it is

caudal alae - ventrolateral extensions of the cuticle on male tail

cephalic - pertaining to the head

circumoral - around mouth opening

cloaca - a common chamber for products of digestive and reproductive systems in males

commissure - a bundle of nerve fibres that connect ganglia

cultivar - a cultivated plant with characteristics that are significant in agriculture and are retained on reproduction

cuticle - the non-cellular external layer of the body

cylindroid - cylinder-like in shape

cyst - the hardened body wall of a dead female of the family Heteroderidae

deirids - paired sense organs in neck region

excretion - separation and discharge of waste products of metabolism from body

exotic - introduced; not native

flanges - expansions at base of stylet for attachment of muscles (Order Dorylaimida)

galling - abnormal growth of plant tissues, caused by stimuli external to plant and appearing as pronounced localised swellings

ganglion - a defined concentration of nerve-cell bodies and cords, forming a nerve centre

germinative zone - area where growth of egg cells begins (ovary)

glandular - containing or consisting of glands

gubernaculum - a sclerotised structure that guides spicules during protrusion

hierarchical - in classification, indicating a series of successive taxonomic categories of increasing rank

hypodermal cells - cell layer just below cuticle

isthmus - portion of oesophagus between metacarpus and postcorpous

labial disc - the raised area of cuticle around mouth opening

latent infestation - a nematode population predominantly in egg stage or sedentary in roots and therefore not collected during conventional sampling

lumen - the enclosed space or cavity of an organ or duct

migratory - able to move freely between feeding sites in or on plant

- morphology** - study of form and structure of organisms
- moult** - periodic shedding of cuticle to permit growth
- necrosis** - death and decay of cells or tissues
- oesophagus** - muscular tube between stoma and intestines
- parthenogenesis** - reproduction by development of embryo from unfertilised egg cells
- perineal pattern** - pattern formed by folds and annules of cuticle around anus, vulva, phasmids and tail in *Meloidogyne* females
- phasmids** - paired sense organs on tail
- post-embryonic** - period or stage after hatch from eggshell
- prerectum** - part of digestive tract between intestines and rectum
- proximal** - nearest to middle of body or to point of attachment of structure
- regurgitation** - back-flow of food from intestines to oesophagus
- saccate** - swollen, bag-like
- scalloped** - edged with semicircular projections
- sclerotised** - a hardened condition
- secretion** - production and release of substances useful to secreting gland or to organism of which gland is a part
- sedentary** - feeding and reproducing at one locality in or on plant
- seminal vesicle** - portion of male reproductive tract for temporary storage of sperm
- spermatheca** - portion of female reproductive tract between oviduct and uterus, for storage of sperm
- spicules** - paired, sclerotised copulatory organs in male
- spindle-shaped** - tapered towards each end
- stunting** - suppression of growth; dwarfing
- vas deferens** - a muscular tube between ejaculatory duct and testis
- vermiform** - an elongated, cylindrical body; worm-shaped

R

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