Practical plant nematology: A field and laboratory guide

D.L. Coyne, J.M. Nicol and B. Claudius-Cole

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This guide has been produced by the International Institute of Tropical Agriculture (IITA) and the International Maize and Wheat Improvement Center (CIMMYT) as part of the strategy of the Systemwide Program on Integrated Pest Management (SP-IPM) to improve the quality and usefulness of pest management research. IITA and CIMMYT are supported by the Consultative Group on International Agricultural Research (CGIAR; www.cgiar.org). For the first print run, contributions were made by ACP-EU Technical Centre for Agricultural and Rural Cooperation (CTA) for printing. Syngenta funded the 2009 reprint and DuPont funded the 2014 reprint. The current 2018 reprint has been undertaken to provide a resource for the International MSc in Agro- and Environmental Nematology and pest management activities in East Africa, which has been funded by Austrian Development Agency (ADA), Koppert Biological Systems, RealIPM, USAID IPM Innovation Lab and VLIR-UOS.

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The project "Healthy seedling systems for safer, more productive vegetables in East Africa", funded by the **Austrian Development Agency (ADA)** with funds of Austrian Development Cooperation, is a 3-year project (2015–2018) focused on plant health and the reduction of prominent vegetable (tomato, pepper) diseases, increasing vegetable production by resource-poor farmers, and making safe and affordable nutritious vegetables available for consumers and producers in Ethiopia and Uganda. The research aims to sustainably increase food production and agricultural productivity through biologically-based pest damage reduction and increased awareness, including through the production of suitable resource manuals.

Koppert Biological Systems is the international market leader in the field of biological crop protection and natural pollination. Koppert contributes to better health of people and the planet. In partnership with nature, they make agriculture healthier, safer and more productive. They provide an integrated system of specialist knowledge and natural, safe solutions that improves crop health, resilience and production. Koppert has a reputation internationally for reliability, innovation and quality. Their commitment to sustainability is demonstrated by the choice to keep all their products as 100% natural and safe. They are currently present in over 27 countries and their products are used in more than 100 countries around the globe.

The ongoing attempt to discover and utilize natural principles forms the backbone of Koppert's activities. Koppert's strength lies in its ability to turn this knowledge into practical applications, which contribute to finding solutions to prevailing problems. In Africa for instance, climate change continues to greatly impact food production and security. Through our cutting-edge innovations, Koppert endeavours to use their extensive know-how to enable farmers, communities and nations improve food production, quality and sustainability. This remains especially challenging in the face of increasingly frequent entry of new exotic and invasive pests and diseases. The particular role of healthy soils in enabling sustainable food production and security cannot be over-emphasized. That is why Koppert continues to study and develop new ways to restore and protect this most valuable asset.

Koppert seeks to not only offer ready solutions, but rather to also act as an enabler. Indeed, numerous solutions are developed through strategic partnerships, and disseminated through training and education. That is why past successes have inspired the Koppert Foundation to work on education, health and economic development, with focus on three areas: smallholder farmers, the company's and employees' social responsibility and innovative/educational projects. Join Koppert in their partnership with nature, to make agriculture healthier, safer and more productive.

The **East African Vegetable Crop IPM Innovation Lab (EAVCIPM-IL) (USAID)** project is implemented in East Africa towards the development, implementation and scaling-up of integrated pest management (IPM) technologies for selected vegetable crops in Feed the Future (FtF) priority areas in Tanzania, Ethiopia and Kenya. The overall goal of EAVCIPM-IL is to build the capacity of the host country institutions to implement effective IPM research and locally-adapted, gender-appropriate technology transfer programs that increase environmental benefits, farm productivity and incomes, and inform national and regional policy. It will build on lessons learned from previous work to develop and transfer appropriate IPM strategies.

The **International Master of Science in Agro- and Environmental Nematology (IMAGENT)**, also known as PINC, from **Ghent University**, offers a masters programme that is globally unique and focuses on the agricultural and environmental aspects of nematology. It brings together renowned experts in the field of nematology and students from all over the world. Key objectives of the programme include: (1) disseminate knowledge of nematode effects on crops and their role in disease complexes; (2) transfer of information to implement sustainable farming practices and improve agricultural productivity under rapidly changing environmental and climate conditions; (3) facilitate the use of nematodes as biological control agents; (4) advance the knowledge of nematodes in their role as ecosystem service providers. To create a more conducive and locally appropriate link for capacity building in the South, the programme has an international mobility option and satellite programmes in Ethiopia (Summer Course in Nematology) and Kenya (Basic Nematology Crash Course).

VLIR-UOS supports partnerships between universities and university colleges, in Flanders (Belgium) and in the South, looking for innovative responses to global and local challenges. VLIR-UOS funds cooperation projects between professors, researchers and teachers. VLIR-UOS also awards scholarships to students and professionals in Flanders and the South. Lastly, VLIR-UOS helps to strengthen higher education in the South and to strengthen a specific international dimension of the higher education in Flanders. VLIR-UOS is an institution of public utility and was founded by the Flemish Interuniversity Council (VLIR), an overarching consultative organization between the Flemish universities and the Belgian Government. In 1998 the VLIR gained responsibility for the federal funds for university development cooperation of the Flemish universities and founded VLIR-UOS as the VLIR-secretariat for university development cooperation. VLIR-UOS receives funding from the Belgian Development Cooperation. In February 2016 VLIR-UOS received the 'EFQM Committed to Excellence 1 Star' label. The EFQM Excellence model is one of the most widely used organizational frameworks in Europe. The label is an acknowledgement of VLIR-UOS' dedication to improving its organizational management quality and performance.

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Preface

This guide is a simple, easy-to-follow reference for assessing plant parasitic nematode problems. It provides clear instructions, with many illustrations, on procedures for collecting and processing samples for nematode assessment, as well as information on accessing further identification and diagnosis support. The manual is aimed at technicians, field workers, extension agents and others with an interest in crop production and crop protection, particularly in those parts of the world where access to expert help and advanced facilities is limited. It has been produced in response to frequent demand from colleagues for a guide to aid diagnosis of nematode problems. This guide will hopefully simplify some aspects of nematology, and help to lessen the mystery surrounding this crop production problem.

It is sometimes said that nematodes are viewed as a crop production problem only when a nematologist is present, and that without the nematologist there would be no nematode problem. Paradoxically, the unspecific symptoms of nematode damage are often attributed to other causes, which may seem more likely or more obvious. The reality is that a number of constraints often combine to reduce crop production, and it is necessary to quantify all of the main constraints, including nematodes. Keeping the nematode threat in perspective, in relation to other pests and diseases, is a challenge, but one that will benefit enormously from better quantification of the nematode problem through improved field and laboratory procedures.

Plant parasitic nematodes are ever-present and are incidental with plant growth and crop production. They are significant constraints to sustainable agriculture and can be difficult to control. Determining the importance of individual nematode species, nematode communities and nematodes in combination with other problems is not a simple task at the best of times, but is more difficult in tropical than in temperate climates. Species previously not known to cause crop damage are continually being discovered, particularly as agriculture changes to suit changing needs, and new crops are introduced. Introduction of, or improvements in, nematological techniques and diagnostics can lead to identification of nematodes as the cause of a problem which had been present for many years, but through lack of local expertise had not been properly diagnosed.

Much remains to be learned about nematodes and the damage they cause to crops. There is, for example, a lack of reliable data on the relationship between nematode numbers and yield for many different crops and types of nematodes. In many less developed countries much basic information simply does not exist. It is therefore important that even small developments and knowledge gains are recorded for future use, through publications of the relevant networks and societies, or regional or international journals.

We hope that this guide will contribute to improving pest and disease management, particularly where nematological expertise is scarce, such as in the less developed countries of the world. An initial step to nematode management is establishing their presence through collection and relation with symptoms, and with expert help accurately identifying the species involved. This guide aims to support that initial step.

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Dr Braima James (SP-IPM Coordinator) provided the impetus and continued support to preparing this guide that we hope satisfies the demands of our partners and proves useful in increasing awareness of and reducing nematological problems. We thank Braima for his continued support in this.

Dr John Bridge's guidance and numerous contributions during the development of this guide are particularly appreciated.

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Editing assistance from Mr Duncan Scudamore and Ms Elif Sahin is gratefully acknowledged.

Introduction

Nematodes are a diverse group of worm-like animals. They are found in virtually every environment, both as parasites and as free-living organisms. They are generally minute, but some species can reach several meters in length. This guide focuses specifically on plant parasitic nematodes, which are very small or microscopic, can cause significant damage to crops, and are extremely widespread (Appendix 1).

Because nematodes are difficult or impossible to see in the field, and their symptoms are often non-specific, the damage they inflict is often attributed to other, more visible causes. Farmers and researchers alike often underestimate their effects. A general assessment is that plant parasitic nematodes reduce agricultural production by approximately 11% globally (Agrios, 2005), reducing production by millions of tonnes every year.

The amount of damage nematodes cause depends on a wide range of factors, such as their population density, the virulence of the species or strain, and the resistance (ability of the plant to reduce the population of the nematode) or tolerance (ability of the plant to yield despite nematode attack) of the host plant. Other factors also contribute to a lesser extent, including climate, water availability, soil conditions, soil fertility, and the presence of other pests and diseases. However, although we have some knowledge on the nematode–crop relationship and influencing factors, much remains to be learned. Damage thresholds for nematodes on various crops in various parts of the world, for example, are often unknown, and the threat nematodes pose often requires an educated guess.

Once nematodes are identified as significantly contributing to crop damage, management options can then be decided. These depend on the nematodes involved, the crop and cropping system, and local circumstances. If the species is identified some specific interventions can be considered, for example, using resistant crops or varieties. Otherwise more general options or combinations of options, such as rotation, chemical application, biological control and sanitation practices, may be appropriate.

The aims of this guide are to assist the reader to:

- Appreciate basic nematode biology
- Recognize the different groups and feeding behaviors of nematodes
- Identify damage symptoms
- Collect nematode samples
- Process the samples
- Dispatch nematodes and samples for more precise identification.

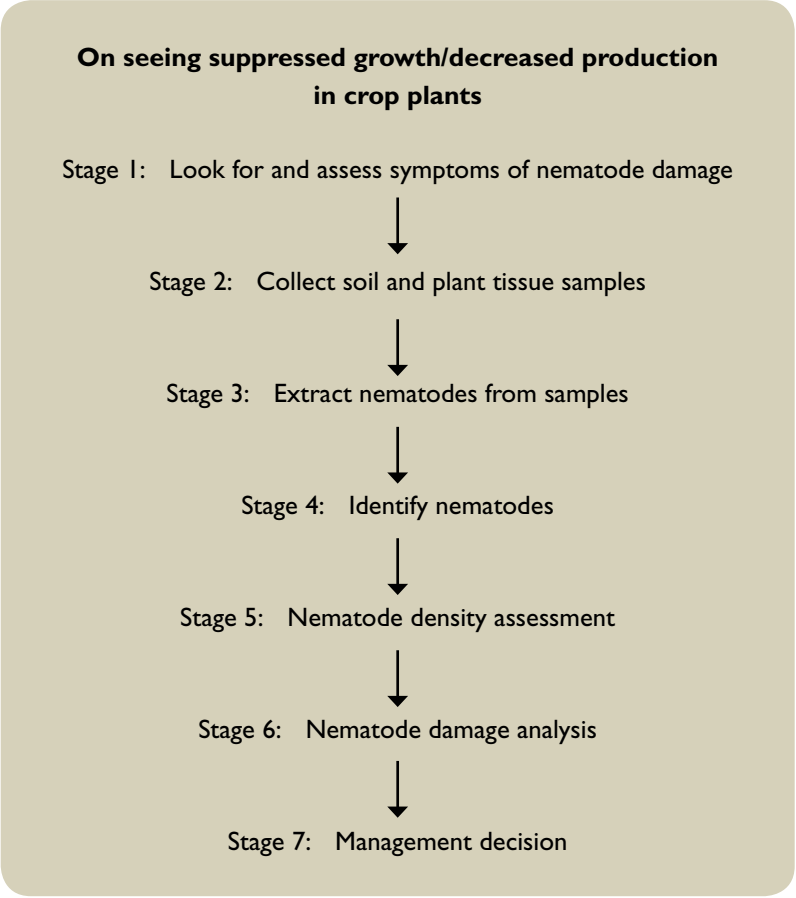


Figure 1. The stages in nematode assessment and management.

Figure 1 shows the stages in nematode assessment and management. This guide provides information on carrying out stages 1–3. Stage 4 may also be attempted, but assistance from a specialist nematologist will probably be needed, and will certainly be required for the final stages. The References and Further Reading section (page 65) includes some useful publications to take nematology tasks beyond those described in this guide.

Basic biology of plant parasitic nematodes

Appearance and structure

Plant parasitic nematodes are mostly thread-like worms ranging from 0.25 mm to >1.0 mm long, with some up to 4.0 mm. Although most taper toward the head and tail, they come in a variety of shapes and sizes (Fig. 2). Females of some species lose their worm-like shape as they mature, becoming enlarged and pear-, lemon- or kidney-shaped or spherical as adults.

Like all animals, nematodes have circulatory, respiratory and digestive systems (Fig. 3). Plant parasitic nematodes differ from nematodes that feed on bacteria and fungi in that they have a specialized feeding structure, the spear or stylet (Fig. 3). This is used to inject enzymes into plant cells and tissues and then to extract the contents, in a similar way that aphids feed on plants.

Life cycle

The nematode life cycle is typically divided into six stages: the egg, four juvenile stages and the adult (Fig. 4). The duration of any of these stages and of the complete life cycle differs for different species, and also depending on factors such as temperature, moisture and plant host. Under favorable conditions in the tropics many species have relatively short life cycles, with several generations possible per season. This can lead to rapid population build up from just one (if self-fertilizing) or two nematodes.

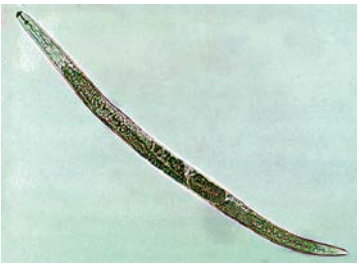
Nematodes can survive unfavorable conditions, such as a dry season or a cold winter. Different species survive best at different life stages, for example *Heterodera* species survive best as eggs encapsulated within cysts, *Ditylenchus* species as fourth stage juveniles, and *Anguina* species as second stage juveniles.

Nematode types

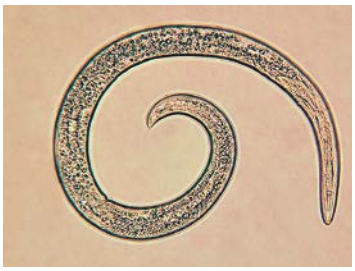
Plant parasitic nematodes can be separated into aerial parasites – those feeding on above-ground parts of plants – and root and tuber parasites – those feeding on below-ground parts.

They can also be grouped by their feeding behavior and motility into three main groups:

- Migratory endoparasites – mobile nematodes that feed inside the plant root tissue.
- Sedentary endoparasites – nematodes that, once they have reached a feeding site inside the plant, cease to be mobile and feed from a fixed location.
- Ectoparasites – nematodes that feed on the plant from the outside of the plant.

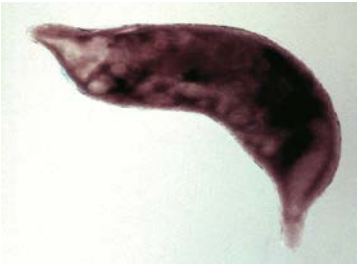


Pratylenchus (worm-like/ vermiform) [JB]

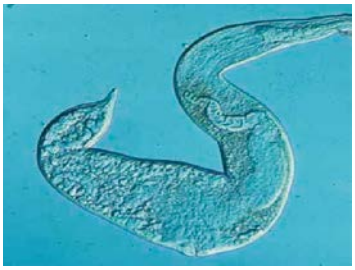


Helicotylenchus (worm-like vermiform/spiral) [GG]

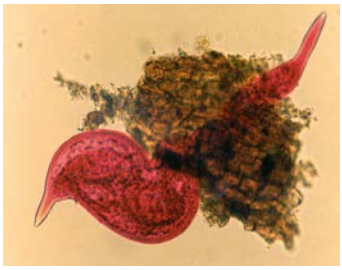
Discocriconemella (slightly swollen)



Nacobbus (swollen/fusiform) [JB]

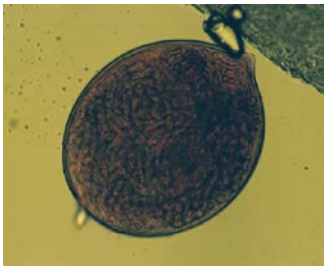


Achlysiella (swollen/fusiform) [JB]

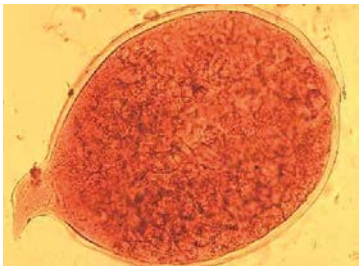


Tylenchulus (pear-shaped) [JB]

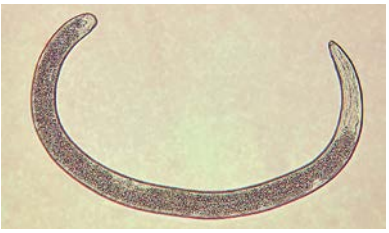
Rotylenchulus (kidney-shaped/ reniform) [JB]



Heterodera (lemon-shaped)



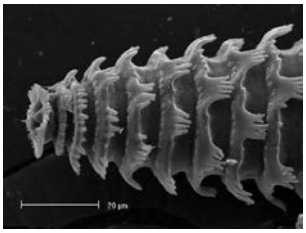
Meloidogyne (spherical/gourd-shaped) [JB]

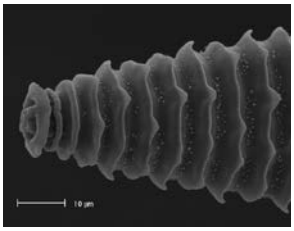


Scutellonema (worm-like vermiform/C-shaped) [GG]

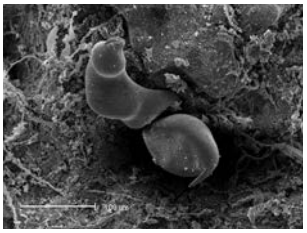
Criconematid (ribbed/ridged)

Hirschmanniella (vermiform/ long) [JB]



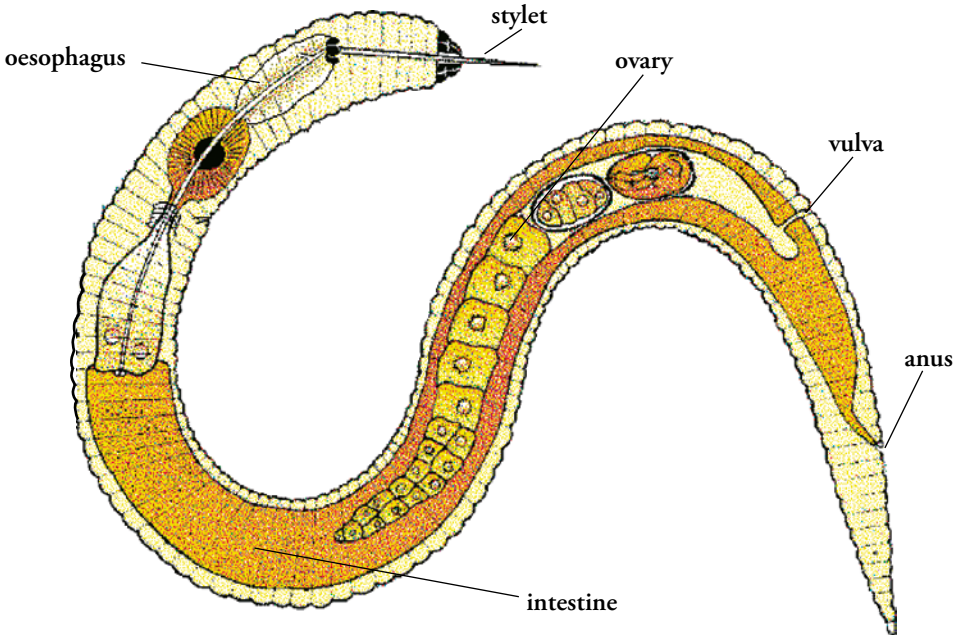


Ogma surface structure (flanged/flared ribs) [EvB]

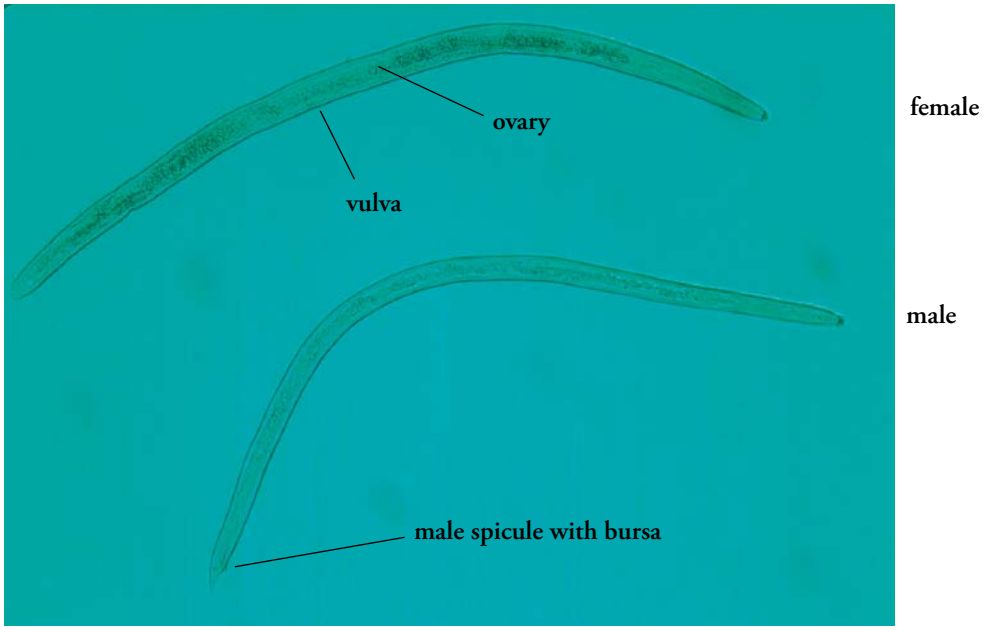


Tylenchulus physical appearance on outside of root (pear-shaped) [EvB]

Figure 2. Diverse shapes of nematodes, as seen under the microscope.
(Photographs by J. Bridge [JB], G. Goergen [GG] or E. van den Berg [EvB].)



Typical nematode structure (courtesy R. Esser).



Male and female *Scutellonema*.

Figure 3. (Top) Diagram of nematode structure as observed through a microscope. (Bottom) Example of male and female nematode (*Scutellonema bradys*) as seen under the microscope with male (spicule) and female (vulva and ovary) reproductive organs indicated. Note that not all nematode species have males. A protective bursa is found around the spicule on males of some species, as seen here, but not all species. (Photograph by H. Meerman.)

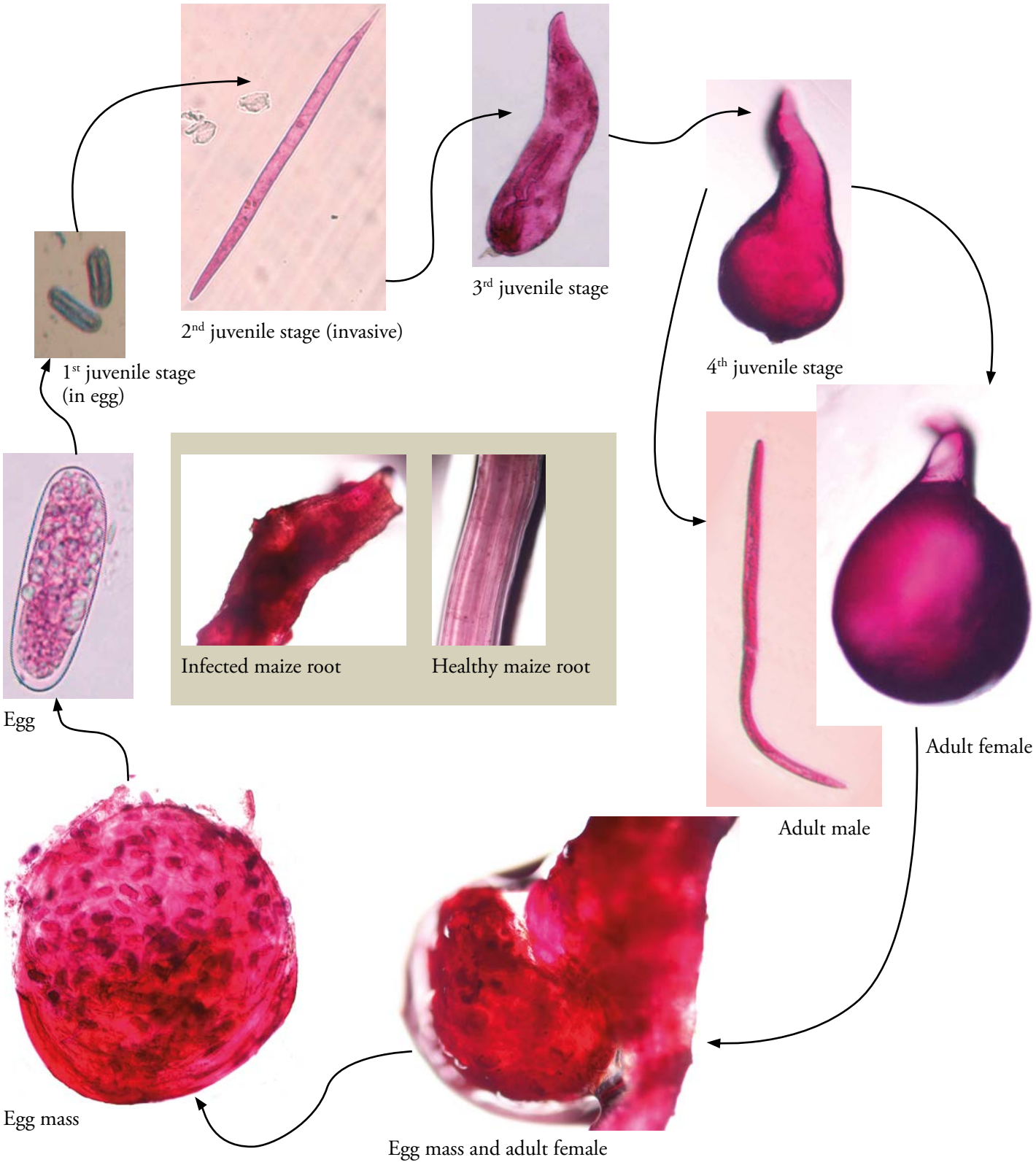


Figure 4. Nematode life cycle. *Meloidogye* used as an example on maize, as observed through the microscope. Not all stages are to the same scale. Migratory nematode lifecycles follow a similar course, but remain wormlike and do not produce egg masses.
(Photographs by E. Oyekanmi.)

Migratory endoparasites (Fig. 5)

All life stages of migratory endoparasitic nematodes are mobile except the egg. The nematodes burrow through the plant from cell to cell, or may leave the plant tissue in search of new feeding sites. Whilst feeding they commonly lay eggs both inside the plant cortical tissue and also in soil surrounding the root tissue. Damaged cells release toxins which kill neighboring cells, resulting in small spots or lesions of necrotic tissue. Root rot fungi and bacteria are often associated with infestations of migratory endoparasitic nematodes, which enter the plant tissues through areas damaged by nematodes.

Scutellonema bradys **in yam.**

Hirschmanniella **in rice** [JB].

Figure 5. Migratory endoparasitic nematode female and eggs stained red in root tissue. (Photograph by J. Bridge [JB].)

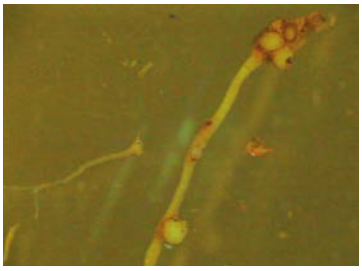
Sedentary endoparasites (Fig. 6)

Sedentary endoparasitic nematodes invade plant tissue usually as newly hatched second-stage juveniles – the 'infective' wormlike stage. They move through the soil to locate host roots, and then through the plant tissue to find a feeding site. At the feeding site the female develops, remaining permanently sited for the duration of her life. As she develops, her body swells to a spherical, lemon, kidney, or ovoid form. The nematode feeds on a relatively small number of cells, which are regulated by the nematode with growth substances. Some groups (e.g. cyst and root-knot nematodes) cause 'giant' feeding cells to form in the host plant.

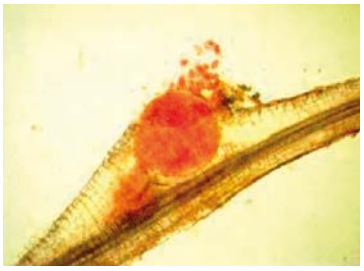
The males remain wormlike, feeding on the surface of the root for a few days, during which they may or may not fertilize the females before moving into the soil where they die.

Female sedentary endoparasitic nematodes generally produce a large number of eggs, which remain in their bodies (e.g. cyst nematodes – *Heterodera* spp.) or accumulate in egg masses (e.g. root-knot nematodes – *Meloidogyne* spp.) attached to their bodies. Some other nematodes are sedentary, but only semi-endoparasitic, such as the reniform (*Rotylenchulus* spp.) and citrus (*Tylenchulus semipenetrans*) nematodes, which become only partly embedded in the root tissue.

Cyst nematode (*Heterodera* spp.) breaking open the root cortex of rice.

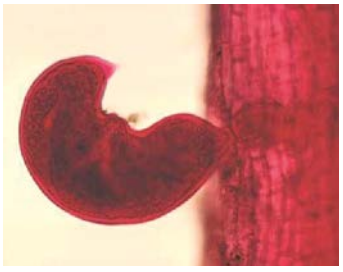


Root-knot nematodes (*Meloidogyne* spp.) bursting and protruding out of maize root.

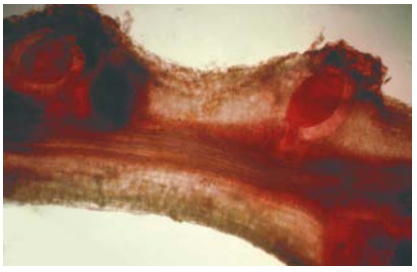


Root-knot nematode (*Meloidogyne* spp.) embedded in gourd root with eggs released outside the root [JB].

Adult cereal cyst nematode (*Heterodera filipjevi*).



Reniform nematode (*Rotylenchulus* spp.) with head buried in root tissue (semi-sedentary endoparasite) [JB].



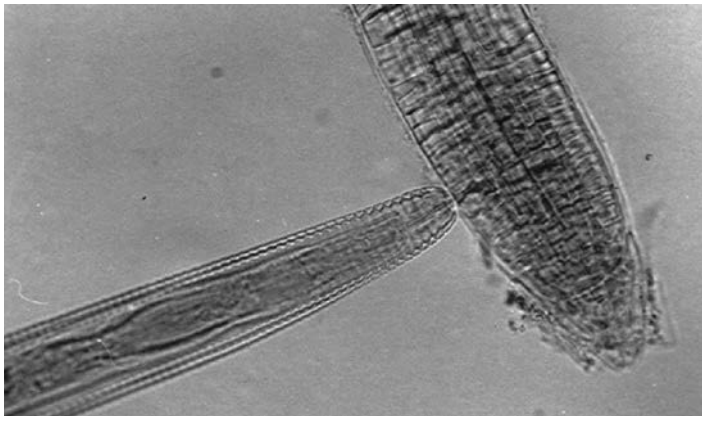
Root-knot nematode (*Meloidogyne* spp.) embedded in sweet potato root [JB].

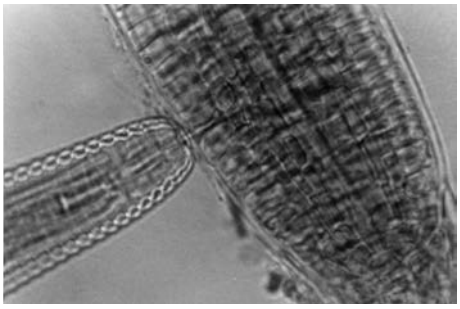
Figure 6. Endoparasitic nematodes.
(Photographs by J. Bridge [JB].)

Ectoparasites (Fig. 7)

Ectoparasitic nematodes feed externally, on the surface of the plant, usually on root hairs or cortical tissue. They are often found in high densities, but do not always pose a problem. However, they may cause serious damage if the plant is suffering from other biotic or abiotic stresses (e.g. fungal attack or low water availability). Examples of ectoparasitic nematodes are ring nematodes (*Criconemoides* spp.), spiral nematodes (*Helicotylenchus* spp.) and the aerial rice white-tip nematode (*Aphelenchoides besseyi*).

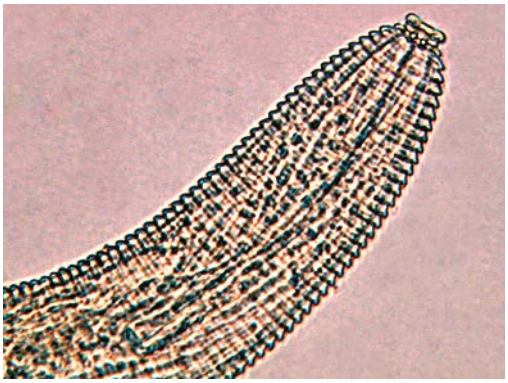
It is well recognized that some ectoparasites transmit plant viruses, for example some species of dagger nematodes (*Xiphinema* spp.), needle nematodes (*Longidorus* spp.) and stunt nematodes (*Trichodorus* and *Paratrichodorus* spp.).

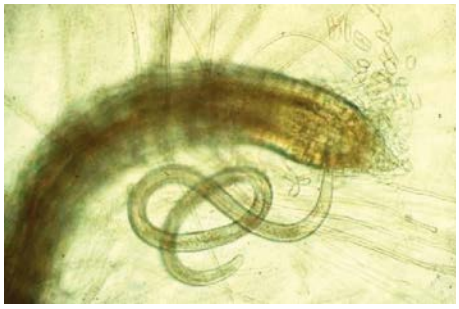




Aulosphora feeding on rice root with close-up of feeding stylet penetrating root tissue.

Discocriconemella with close-up of head region [GG].





Tylenchorhynchus feeding at the root tip [JB].

Figure 7. Ectoparasitic nematodes.
(Photographs by G. Goergen [GG] and J. Bridge [JB].)

Symptoms of nematode damage

One of the major challenges in identifying nematodes as the causal agent of crop damage is the fact that many of them do not produce highly diagnostic symptoms, which are specific and easy to identify. The damage caused by nematodes is often non-specific and easily confused with symptoms of other abiotic or biotic stresses. For example, chlorosis may be due to nitrogen deficiency or may be caused by nematodes; poor stands of growth similarly may be caused by poor soil fertility or moisture stress, or may be due to nematodes.

It is therefore highly recommended to assess for nematodes when crops are suffering yield loss and exhibiting any of the symptoms described below. Additional knowledge on the crop, cropping history, and management practices, combined with information in this guide, may also indicate the possible nematode(s) involved.

Symptoms of nematode damage are found both above and below ground.

Above-ground symptoms

Above-ground symptoms fall into two categories: those caused by aerial nematodes attacking foliage and those caused by root nematodes attacking plant roots.

Symptoms caused by aerial nematodes (Fig. 8)

These are often specific symptoms associated with the nematode pest and therefore may be diagnostic. They include:

- Gall formation, or abnormal swelling of seeds (e.g. *Anguina*) or leaves (e.g. *Cynipanguina*)
- Leaf stripe, bleaching and discoloration of leaves (especially in temperate climates) (e.g. *Aphelenchoides*)
- Swollen, crinkled and disorganized tissue growth (e.g. *Ditylenchus*)
- Internal stem necrosis, signified with a red ring (*Bursaphelenchus cocophilus*)
- Inflorescence necrosis
- Chlorosis/browning of leaves (needles in pines) and eventual tree death (*Bursaphelenchus xylophilus*).

Symptoms caused by root nematodes (Fig. 9)

Root nematodes almost always cause varying degrees of abnormal above-ground growth, but these symptoms alone are generally not enough to diagnose a root nematode problem. Most symptoms reflect or can be mistaken for other problems, such as reduced water uptake or disturbed mineral absorption. They include:

- Chlorosis (yellowing) or other abnormal coloration of foliage
- Patchy, stunted growth
- Thin or sparse foliage
- Symptoms of water stress, such as wilting or leaf rolling

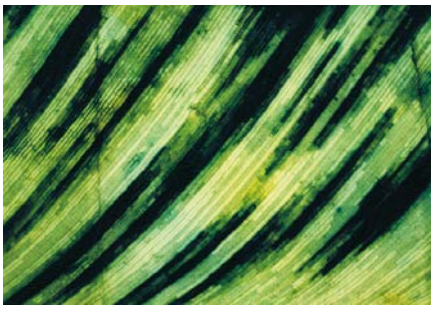
Deformed heads of barley and wheat due to seed gall nematode *Anguina tritici* [RS].

Crinkling/twisting of rice leaves by *Ditylenchus angustus* [JB].

White tip disease of rice caused by *Aphelenchoides besseyi* [JB].

Red ring symptoms (*Bursaphelenchus cocophilus*) in coconut trunk [JB].

Ufra disease on rice caused by *Ditylenchus angustus* [JB].



Bleaching and streaking discoloration of enset (*Musa*) leaf caused by *Aphelenchoides* sp. [PS].

Oats severely infected with stem nematode causing patchy growth, stunting, chlorosis (left) and basal swelling (right) associated with *Ditylenchus dipsaci* infection [HW].

Seed gall of wheat caused by *Anguina tritici* (crushed seed showing emerging eggs and juveniles) [JB].

Figure 8. Above-ground symptoms caused by aerial nematodes attacking foliage.
(Photographs by J. Bridge [JB], R. Sikora [RS], P. Speijer [PS] and H. Wallwork [HW].)

Chlorosis and stunted growth of rice plants (left) caused by *Heterodera sacchari*.

Patchy growth and chlorosis of lower leaves of wheat caused by *Heterodera* spp. [HW].

Delayed flowering/maturity and suppressed growth in a patch of solanum potatoes affected by potato cyst nematodes [DH].

Thin and sparse foliage of irrigated rice caused by *Hirschmanniella* spp.

Stunting/reduced height of plantain (plants on left) caused by *Pratylenchus coffeae*.

Patchy distribution and reduced tillering in wheat attacked by the root lesion nematode (*Pratylenchus neglectus*) [RR and RC].

Dieback of citrus caused by *Radopholus similis*.

Toppling over of banana caused by *Radopholus similis*.

Patchy distribution, stunted and chlorotic maize plants affected by root-knot nematode (*Meloidogyne* spp.) [AM].

Figure 9. Above-ground symptoms caused by root nematodes attacking plant roots.
(Photographs by R. Cook [RC], D. Heinicke [DH], A. McDonald [AM], R. Rivoal [RR] and H. Wallwork [HW].)

- Die-back of perennial or woody plants with little or no new foliage
- Reduced fruit and seed size
- Low yields.

Other symptoms that may suggest root nematode infection are:
- Failure to respond normally to fertilizers
- A tendency to react to water stress more rapidly than healthy plants, and slow recovery from wilting
- Little or no new foliage development at the onset of a new growing season
- Severe weed problems (higher density of weeds), due to the nematode-infected plant being less able to compete with weeds
- Greater disease incidence, because of suppressed resistance of nematode-infected plants.

Below-ground symptoms

These are due to root nematodes, and may be specific enough to allow diagnosis of the root nematode problem. Uprooting of plants or excavation of roots is needed to observe symptoms. Symptoms include:
- Galling
- Shortened, stubby or abbreviated roots
- Root lesions
- Root or tuber necrosis, rotting or death
- Root or tuber cracking
- Cysts or 'pearly' root
- Deformed roots
- Altered root architecture.

Root galls

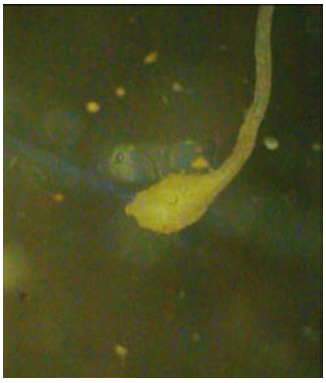
Root galls are caused mostly by the root-knot nematodes (*Meloidogyne* spp.), although other nematodes such as *Nacobbus aberrans* may also cause galling (Fig. 10). Feeding by some nematodes, such as *Xiphinema* spp., may result in swellings or less defined galls, often at the root tips.

The galls can vary considerably depending on the *Meloidogyne* species, the crop and cultivar, and whether occurring on roots or on tubers (Fig. 11). Typical appearance includes:
- Small individual bead-like swellings
- Massive clumps of fleshy tissue stuck together
- Swollen root tips
- Irregular swellings along the root
- Hook-shaped root tips
- No form of visible root swelling other than a raised surface where the nematode is embedded.

Meloidogyne spp. knobbling of cassava roots.

Meloidogyne graminicola on rice causing stunted and galled seedling roots [RP] and characteristic hooked root tips [JB].

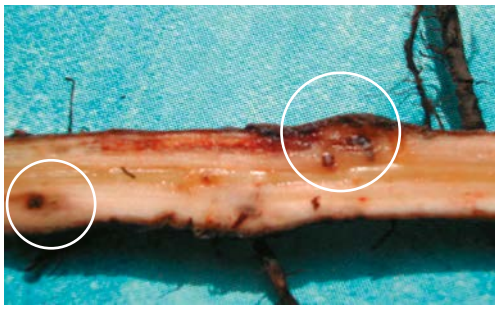
Massive clumping and galling of root tissue in vegetables caused by *Meloidogyne* spp.



Swollen maize root tips caused by *Meloidogyne* spp.

Large woody galling on tree roots caused by *Meloidogyne* spp.

Figure 10 – *continued overleaf*



Deformed banana root system with swollen roots (left) and cross section of banana root which has raised root surface with embedded female *Meloidogyne* spp. nematodes (highlighted in split root right).

Bead-like galls on lettuce caused by *Meloidogyne* spp.

Deformation, raised galls and root termination of maize roots caused by *Meloidogyne* spp.

Galling of potato roots caused by *Nacobbus aberrans* [JB].

Figure 10. Root galls and other symptoms of root-knot nematode (*Meloidogyne* spp.) and *Nacobbus* sp. (Photographs by J. Bridge [JB] and R. Plowright [RP].)

It is a common misconception that plants of the family Graminaceae (grasses and cereals) are not affected by root-knot nematodes. In fact, these species are readily affected but galling is often less visible. Small galls, however, can usually be easily observed (Fig. 10).

Tuber galls on yam (*Dioscorea* spp.).

Crenulated raised yam tuber surface with cross-section revealing female nematodes embedded in the tissue.

Galling on potato.

Galls on beetroot.

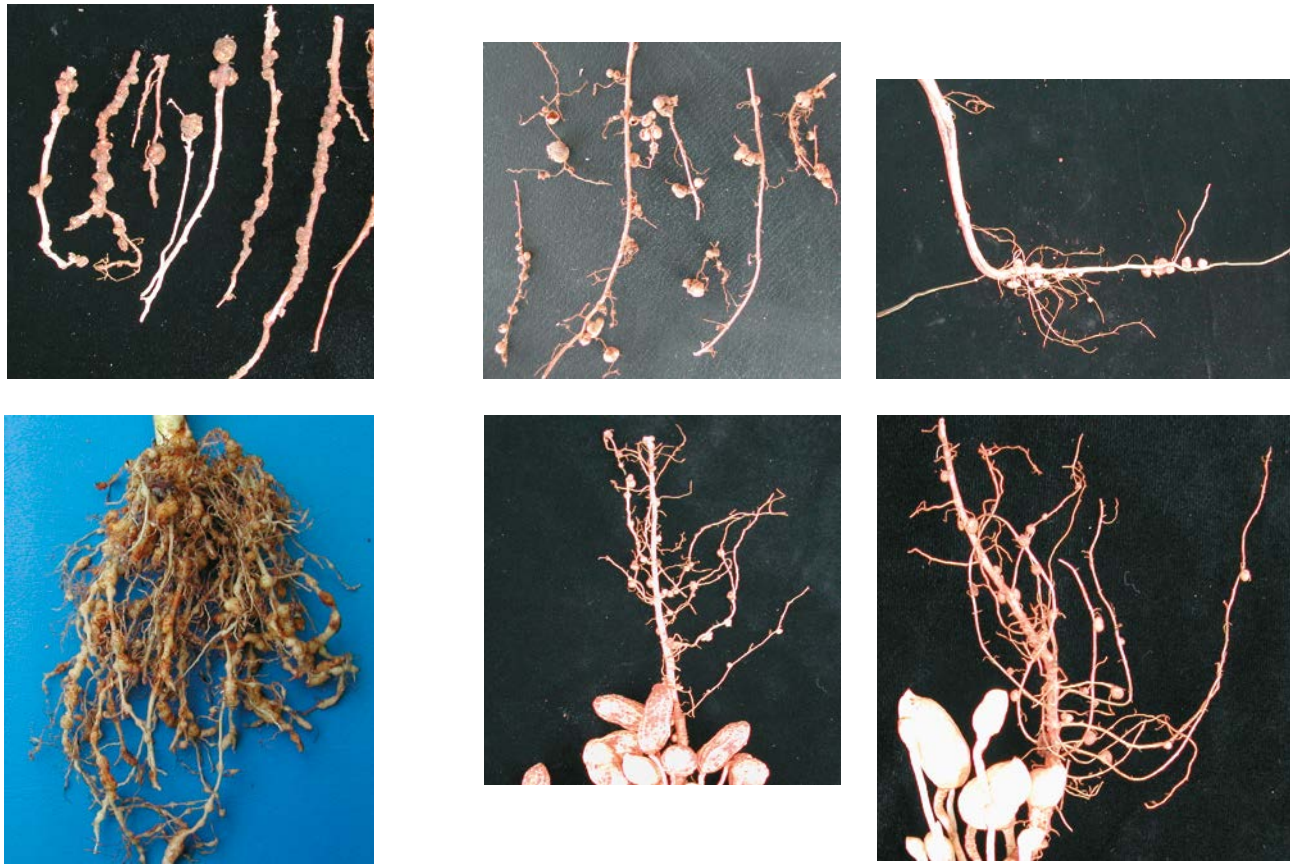
Galls on cassava roots.

Galls on carrot.

Figure 11. Galls on storage organs caused by root-knot nematode (*Meloidogyne* spp.).

Root galls versus root nodules (Fig. 12)

Other root swellings can be caused by beneficial nitrogen-fixing *Rhizobium* bacteria. These are called nodules, and are commonly seen on the roots of legume crops. They are distinguished from root galls by their contents, and by differences in how they are attached to the root. Fresh nodules will have a milky pink to brown liquid inside



Root-knot nematode galls on cassava and lettuce (two pictures left) compared with beneficial nitrogen-fixing root nodules (group on right) on soybean, bean and groundnut.

Galling-like root symptoms caused by cabbage clubroot disease on cabbage.

Figure 12. Root galls caused by nematodes, compared with other gall-like symptoms.

them, whereas galls tend to be composed of a gelatinous clear creamy solid nature, which is sometimes tough. Nodules are attached loosely to the root and can be easily removed; root-knot galls originate from within the root, and removing them is more difficult and will tear the root cortex.

Some root diseases such as the fungus clubroot (*Plasmodiophora brassicae*) can result in deformed root systems, which can resemble root-knot damage (Fig. 12).

Abbreviated root systems

Nematode activity can also cause a shortening of the roots, so that the root mass is greatly reduced or has a stubby appearance (Fig. 13).

Stunted and galled rice seedling roots caused by *Meloidogyne graminicola* [RP].

Severely abbreviated root system of olive tree saplings resulting from root lesion nematode (*Pratylenchus* spp.) infection.

Stubby root symptoms on maize, caused by *Paratrichodorus minor* [DD].

Figure 13. Root shortening.
(Photographs by D. Dickson [DD] and R. Plowright [RP].)

Root and tuber lesions

Roots and tubers may show areas of dead tissue (necrosis) as a result of nematode activity (Figs 14 and 15). As the nematodes feed and migrate within the roots they destroy plant cells and also disrupt cellular functions causing the tissue to die.

Heavily lesioned strawberry roots infected with root lesion nematode (*Pratylenchus vulnus*) [JB].

Symptoms of root lesion nematode (*Pratylenchus thornei*) on wheat roots.

Banana roots showing extended root lesioning (necrosis) caused by *Radopholus similis*.

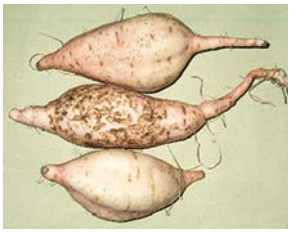
Maize roots infected with *Pratylenchus* lesion nematodes (right) compared with uninfected roots (left).

Figure 14. Lesion symptoms on roots.
(Photograph by J. Bridge [JB].)

Internal lesions and necrosis exposed below the surface of yam due to the yam nematode (*Scutellonema bradys*).

Lesions on sweet potato caused by *Scutellonema bradys*.

Internal lesions on sweet potato due to root-knot nematode (*Meloidogyne incognita*) [JB].



Lesions on sweet potato caused by *Paratrichodorus* (middle tuber) [MM].

Ditylenchus dipsaci damage on potato [FERA].

Ditylenchus destructor damage to internal potato tissue [FERA].

Figure 15. Necrosis and lesions on storage organs.
(Photographs by J. Bridge [JB], Food and Environment Research Agency, Crown Copyright [FERA] and M. Marais [MM].)

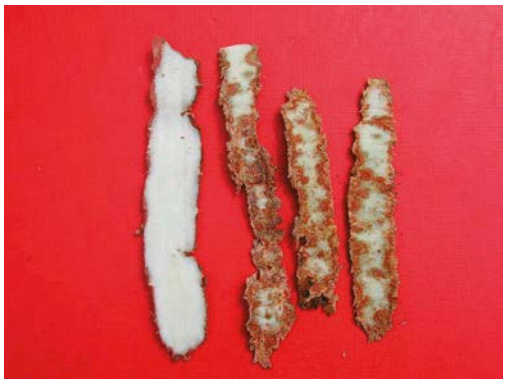
Root rot and tuber rot

Nematodes alone can cause rotting of roots and tubers through extensive burrowing leading to substantial necrosis and tissue and root death (Figs 16 and 17). The banana burrowing nematode *Radopholus similis*, the root lesion nematodes *Pratylenchus* spp., yam nematode *Scutellonema bradys*, and *Hirschmanniella miticausa* on taro are examples. Frequently, secondary fungal and bacterial infections also develop and contribute to rotting (Fig. 17).

Rot, surface cracking (left) and termination (right) of banana roots caused by a combination of *Radopholus similis*, *Helicotylenchus multicinctus* and *Meloidogyne* spp.

Necrosis and reduction of sweet potato roots due to *Scutellonema bradys*.

Figure 16. Root rot caused by nematode infection.



Sub-surface necrosis on strips of cassava storage root, caused by infection with root-knot nematode (*Meloidogyne* spp.), compared to clean strip on left.

Tuber rot of sweet potato infected with root-knot nematode (*Meloidogyne* spp.) [JB].

Internal rotting in yam tuber caused by fungal infection, probably entering as a result of *Scutellonema bradys* damage to the cortex.

Miti miti disease on cocoyams caused by *Hirschmanniella miticausa* [JB].

Dry rot of yams caused by the yam nematode (*Scutellonema bradys*).

Figure 17. Tuber rot caused by nematode infection.
(Photographs by J. Bridge [JB].)

Cracking

Roots and tubers sometimes develop a cracked surface following nematode infection (Fig. 18). This symptom may be blamed on water or nutrient stress during growth. Cracking is often seen in sweet potato tubers, caused by the reniform nematode (*Rotylenchulus reniformis*). The yam nematode can cause the same symptom on yam.

Flaking and cracking of yam tuber surface (left) caused by *Scutellonema bradys*.

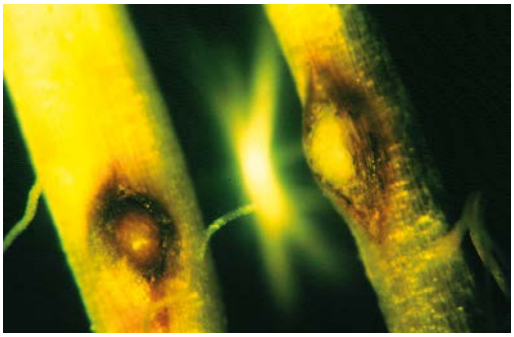
Cracking of sweet potato tuber by *Rotylenchulus* spp.

Surface cracking of potato tuber caused by *Scutellonema bradys*.

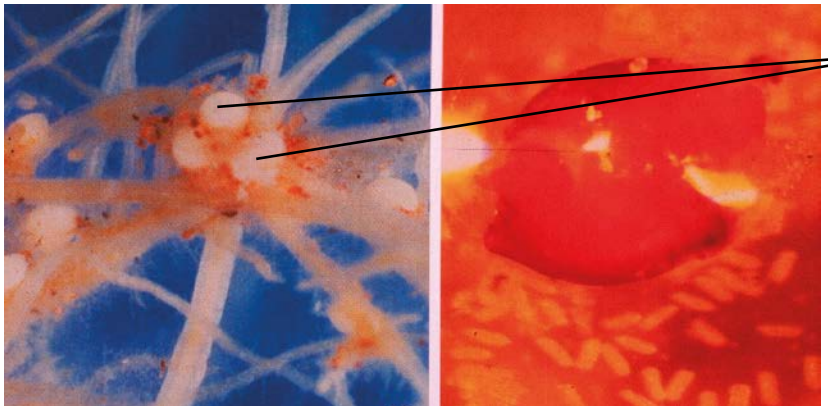
Figure 18. Tuber cracking symptoms.

Cysts or 'pearly root' (Fig. 19)

Cyst nematodes (e.g. *Heterodera* and *Globodera* spp.) can often be observed on the roots of their hosts without magnification, if the soil is gently tapped off and the observer looks very carefully. The young adult females are visible as tiny white beads, giving a pearly appearance when many are present. As the females mature, the cysts, which can contain hundreds of eggs, harden and turn brown or black.



Dark brown cyst and white female *Heterodera oryzicola* embedded in rice root tissue [RP].



White females of the *Heterodera avenae* cereal cyst nematode, each the size of a pin-head, causing knotting of wheat roots (left) before they mature to a brown cyst, shown on the right spilling eggs [HW].

Potato roots with white female *Globodera rostochiensis* attached along its surface giving it an appearance of white beads attached or being 'pearly' [JB].

Figure 19. Cyst or 'pearly root'.
(Photographs by J. Bridge [JB], R. Plowright [RP] and H. Wallwork [HW].)

Sampling

Having observed symptoms that indicate possible or likely nematode infestation, the next stage is to collect samples from the affected plants and from the soil around the roots. These are then taken to the laboratory for analysis, to determine what nematodes are present and possibly their density.

The following field characteristics have implications for the sampling method, and should therefore be considered at this stage:

- Aggregated distribution of nematodes due to host root system and the seasonal behavior of the nematode
- Crop type and history
- Areas planted to different varieties
- Soil moisture
- Soil compaction
- Soil type
- Temperature and seasonal changes.

Sampling tools

Useful tools for sampling, some of which are shown in Fig. 20, include a spade, a hand trowel, a screwdriver, a soil auger (corer), knives (for cutting roots), scissors, polythene sample bags, tags. The soil auger or corer should have a blade 20–30 cm in length and 20–25 mm diameter, and can be either a complete cylinder or a half cylinder. Half cylinders help in removing soil from the corer. Marker pens for labeling the sample bag and a pencil and notebook are also necessary for recording information.

Figure 20. An assortment of tools for sampling nematodes.

Number of samples

Take enough samples to ensure they are representative of the situation in the field. The greater the number of sub-samples/cores combined for each field sample, the more accurate the assessment will be. A balance between available time and resources is, however, necessary.

The sampling procedure and number of samples taken should allow for nematode variation or aggregation. From an area of 0.5 to 1 hectare, take a minimum of 10 core sub-samples, and even as many as 50. Combine these to make one composite sample to represent the field area sampled. Bulking of samples in this way helps to preserve them by maintaining the temperature and moisture of samples.

Sampling pattern

Nematodes are rarely distributed evenly in a field, and samples should therefore be collected from several areas within the field. Collect separate samples from both the poor growth areas and an area of relative good growth, where this is obvious, for comparison. Maintain a consistent sampling style and pattern during surveys and experiments to enable meaningful comparisons between fields, plots, treatments, etc.

Sampling patterns can be random or systematic (Fig. 21). Random sampling does not accommodate the patchy nature of nematode distribution, and is only representative if the sampling area is small. Systematic sampling is a more structured way to remove samples as it takes into consideration the nature of the field and nematode distribution.

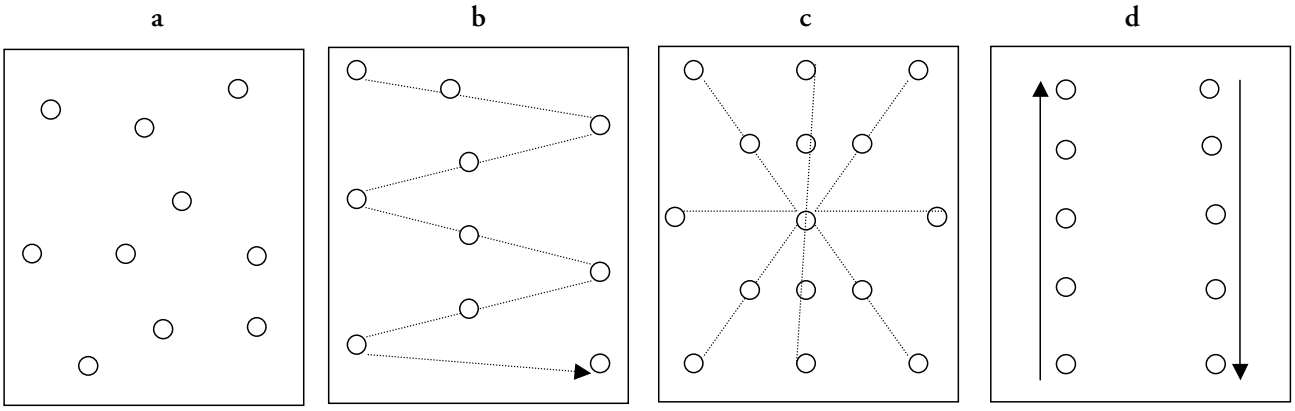


Figure 21. Sampling patterns for nematodes. (a) Random sampling; (b–d) systematic sampling.

Time of sampling

The optimum time for sampling varies between crops and is related to the growth stage of the crop and the objective of the sampling (predictive or diagnostic). Predictive sampling (not the focus of this guide) is often done early in the season, such as at or just before planting, or at the end of the previous cropping season to determine the number of nematodes (density).

Many nematode species increase to high levels during the growing season and reduce during the off (dry) season; this is easier to see in annual crops than in perennial and tree crops. Samples should therefore ideally be collected in the middle of the season and/or at final harvest for diagnostic purposes. Perennials can be sampled during the active growing period such as during the rainy/growing season to identify the problem.

Taking soil samples

As a rule, avoid sampling very wet or very dry soil. However, where crops normally grow in, for example, swamp (e.g. paddy rice) or arid conditions (e.g. sisal), these should be sampled to represent these conditions.

Divide fields larger than 1 hectare into 1 hectare (10,000 m^2) plots and sample these plots separately. Take 10 to 50 sub-samples (cores) and combine them to make a composite sample that weighs 1–2 kg. Remove the soil sub-sample from the root zone using a trowel, auger, corer, spade or similar implement that is suitable for the crop being sampled.

Carefully bag, **label** and seal samples (Fig. 22).

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.

Samples can also be taken using a trowel or other suitable implement if a corer is not available.

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 22. Taking and bagging samples.

Taking root samples

Roots can be collected at the same time and from the same locations as for soil, and in general should be combined in the same sample bag, so that the soil helps to preserve the roots.

Generally, depending on the crop, 25–100 g of roots per total sample is sufficient, but a lower weight may be collected for finer roots such as from rice, and a higher weight for thick, heavy roots such as from banana or trees. Where both fine and heavy roots occur, as on crops such as banana, it is suggested to sample these separately.

Avoid sampling dead plants or those in advanced stages of senescence, as nematodes will often have migrated from these to other food sources. For small crop plants, the whole root system of a plant can be used for each sub-sample. Lift the plants and their roots from the soil using a spade or trowel, so that a sizeable proportion of the root system is unearthed intact, and taking care not to break off the roots and leave them in the ground. After tapping soil free, randomly remove roots with a knife or scissors.

Taking samples from above-ground plant tissue

Leaf, stem, seed or other aerial material should be collected where symptoms are present and nematodes suspected. Again, it is important to select from a number of affected plants, which should be compared with non-affected plant tissue from different plants.

Care of samples

Collect samples in strong plastic bags, and **label** them clearly and systematically. Plastic labels marked with a water-resistant permanent marker or pencil can be placed in the sample bag (Fig. 22), or alternatively write directly on the plastic bag with a permanent marker pen the sample number or reference. Paper labels are best attached to the outside of the bag with wire or twine. If using paper labels, use a pencil, not a pen (which will run or smudge when wet). But remember, paper labels deteriorate quickly during wet conditions.

Record, where possible:

- The crop and cultivar
- The sampling date
- The farmer
- The location (and GPS coordinates if possible)
- A reference number (or plot) if within an experimental trial
- The previous crop(s).

Nematodes are **very sensitive and perishable**, and it is very important that appropriate care is taken to keep them in good condition. Samples should **NOT** be left in direct sunlight or in a closed vehicle in the sun. They should also not be left for long periods before processing.

After collection, samples should be placed in a coolbox (insulated container; Fig. 23), or packed in strong cardboard boxes and placed in a shaded area where conditions are cool. If unable to process immediately, samples can be stored in a refrigerator (approx. 10°C) for up to 2 weeks. Nematode survival decreases with time however, and nematodes from relatively hot environments can suffer chilling injury.

Figure 23. Insulated cool box for sample storage.

Nematode extraction

The next stage is to extract nematodes from the samples. This should be done as soon after collection as possible as samples deteriorate over time.

There are four basic extraction techniques, which are covered in this guide:

- Extraction tray method
- Root or leaf maceration method
- Sieving method
- Incubation method.

Three further methods – elutriation, the Fenwick can and centrifugal flotation – require specialist equipment and are not described in this guide. Details on these methods can be found in publications listed in the References and Further Reading section (page 65). It is also possible in some cases to examine nematodes directly from plant tissue specimens.

Choosing an extraction method

The choice of which method to use depends on the conditions and materials available, the sample type, and also the type of nematodes present. Some methods of extraction are more useful for specific types of nematode, while others are more general. This guide provides details of the most straightforward methods, including the extraction tray method, which is useful in the most basic conditions, provides a reasonable assessment of nematodes from soil, roots, seeds or plant tissue, and can be easily replicated.

Table 1 shows which extraction methods are suitable for which types of nematodes (sedentary or migratory) in soil or root/foliar samples.

Table 1. The suitability of extraction methods for different nematode types and samples.

	Soil sample		Root/foliar sample	
	Sedentary nematodes	Migratory nematodes	Sedentary nematodes	Migratory nematodes
Extraction tray (p. 34)		×		×
Sieving (p. 42)	×	×		
Root/leaf maceration (p. 40)			×	×
Incubation (p. 48)			×	×

If the target nematode is known – such as in research experimental plots – it may be possible to precisely identify which material should be sampled and processed for the nematodes (soil, root, tuber, leaf, etc.). If you are not sure though, both roots and soil should be used for extraction with both sedentary and migratory nematodes considered.

Preparation of samples

Dry soil should be properly mixed before sub-sampling for nematode extraction. Break up clumps and remove stones, roots and debris. Pass (dry) soil through a coarse sieve with holes of approx. 1–2 mm (Fig. 26, step 1) into a suitable container and then mix thoroughly. Remove a sub-sample using a beaker or container of known volume (Fig. 26, step 2); 100 ml soil is commonly used. From each bulked field sample two × 100 ml soil samples should be processed and the mean taken.

Wet soil, such as from a rice paddy, needs to be removed from the field sample using small balls or clumps from various parts of the sample or from each root base, and measured using displacement of water to get samples of the same size (Fig. 29, steps 1 and 2). Fill the beaker to a set (marked) volume (e.g. 200 ml) and raise the volume to the required marked amount using soil clumps, e.g. raising 200 to 300 ml measures 100 ml of soil. Then use the sieving method to extract nematodes.

Roots should be separated from soil and any soil adhering removed by gently tapping off, or rinsing gently under a tap or in a container of water. Dab the roots dry with paper towel, and then chop and extract according to the chosen extraction method.

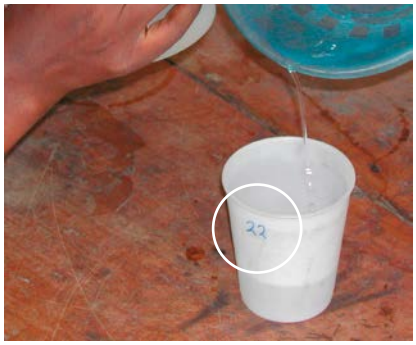
Labeling

It is important to use **clear**, **correct** and **consistent** labeling for samples (Fig. 24). Label containers with either a waterproof marker or chinagraph pencil, or use labels that can be moved along with each stage of the extraction. **Ensure all samples are correctly labeled at all times**.

Labeling samples with a paper tag and pencil.

Labeling a sample bag with a permanent black marker.

Labeling an extraction tray with both an accompanying plastic tag and also number on tray with a permanent marker or chinagraph.



Labeling a collection cup with a black permanent text or a chinagraph pencil.

Waterproof chinagraph pencil.

Figure 24. Materials for and examples of labeling.

Extraction tray method

This method (or variations of it) is sometimes also called the modified Baermann technique, the pie-pan method, or the Whitehead tray method.

Advantages:
- Specialist equipment is not required
- It is easy to adapt to basic circumstances using locally available materials
- It extracts a wide variety of mobile nematodes
- It is a simple technique.

Disadvantages:
- Large and slow moving nematodes are not extracted very well
- The extractions can sometimes be quite dirty (especially if the clay content of the soil is high) and therefore difficult to count
- The proportion of nematodes extracted can vary with temperature, causing potential variation in results between samples extracted at different times
- Maximum recovery takes 3–4 days.

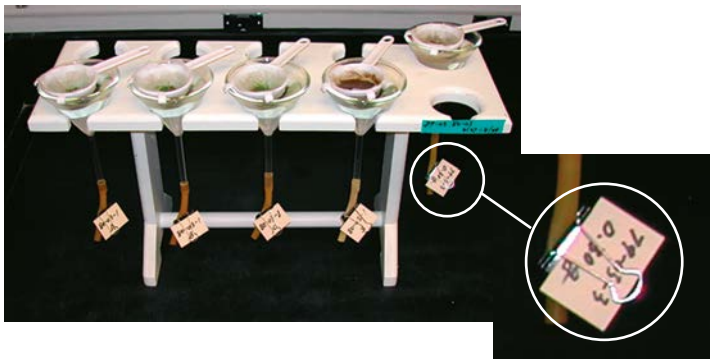
Equipment

- A basket (or domestic sieve) made with coarse mesh (Fig. 25)
- A dish/tray/plate, slightly larger than the basket
- Tissue paper
- Beakers or containers to wash the extraction into
- Wash bottles
- Waterproof pen
- Knife/scissors
- Weighing scales
- Large bench space.

Most items can be purchased ready-made or easily constructed from readily available materials (Fig. 25). Funnels can be held in a rack or stand with rubber tubing attached to the bottom and sealed with a clip (Fig. 25, top) or insect mesh attached to an ~10 cm section of ~15 cm diameter plastic piping can be used to construct the sieve (Fig. 25, middle). It is **very important** that the mesh and sieve base are raised slightly (~2 mm) from the bottom of the dish/plate using, for example, three or four small 'feet' glued to the base of the sieve (Fig. 25, bottom). If this is not done, the nematodes cannot easily migrate into the water.

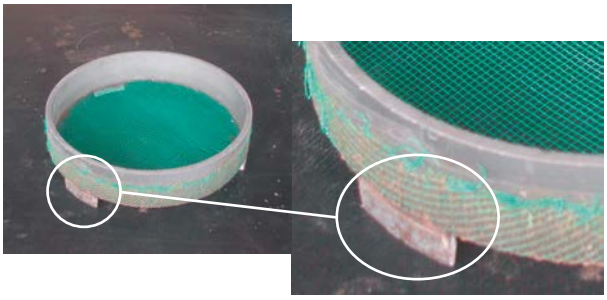
Method

It is very important to ensure good, consistent **labeling** of all containers used for each sample, as it is very easy to make mistakes. Root and soil extractions should be labeled separately.



Example of the funnel method of extraction with rubber tube clipped and labeled at the base. Nematodes will be concentrated just above the clip.

Example of different simple types of sieves and trays. The orange sample on the left uses a home-made cross section of PVC piping with mosquito mesh attached to the bottom. It is very important that the sieve does not sit flat on the bottom of the tray but is slightly raised.



Home-made PVC piping and net sieve with plastic 'feet' which ensures the nematodes can migrate easily into the water.

Figure 25. Different ways to extract the nematodes from the sample.

For soil samples:

Remove roots from sample and place in a separate dish. **Label**.

Using a coarse sieve, remove stones and debris from soil and break up soil lumps (Fig. 26, step 1).

In a plastic container (basin, bucket) thoroughly mix the soil sample.

Remove a measure of soil (e.g. 100 ml) (Fig. 26, step 2).

Place tissue paper (milk filter, paper napkin etc.) in the plastic sieve/basket (placed on a plastic plate) ensuring that the base of the sieve is fully covered by the tissue (Fig. 26, step 3). **Label**.

Place the soil measure on the tissue in the sieve. It is important that the soil remains on the tissue paper – spillover results in dirty extractions (Fig. 26, step 4).

Add water to the extraction plates (Fig. 26, step 5). Take care to gently pour water into the plate (dish) and not onto the tissue paper or soil (between the edge of the mesh and the side of the tray). Add a set volume to each dish to wet but not cover the soil or root tissue, ensuring there is sufficient not to dry out. More water is needed for soil samples than root material. Add more later if necessary.

Leave (preferably in the dark) undisturbed for a set period (48 hours if possible) (Fig. 26, step 6) adding more water if it is likely to dry out. Nematodes from the soil or plant tissue will move through the tissue paper into the water below, resting on the tray/plate.

After the extraction period, drain excess water from the sieve and the soil into the extraction (Fig. 26, step 7).

Remove the sieve and dispose of plant tissue/soil.

Pour the water from the plate into a **labeled** beaker (or cup), using a water bottle to rinse the plate (Fig. 26, steps 8 and 9). Leave samples to settle (Fig. 26, step 10).

For counting the nematodes in the extraction, reduce the volume of water by gently pouring off or siphoning the excess (taking care not to lose nematodes and sediment), or by passing the extract through a very small aperture sieve (e.g. 20–30 µm) (Fig. 26, step 11). Wash the nematodes off the small aperture sieve into a beaker (or tube) for counting, or for preserving, if sending away or counting later (Fig. 26, steps 12 and 13).

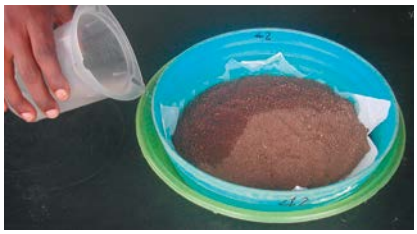
1. Coarsely sieve the sample to remove debris and lumps.

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.

Figure 26 – ***continued opposite***

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.

Figure 26. Extraction tray method for soil samples.

For root samples:
Roots can sometimes be divided into separate categories, such as larger tough roots and finer feeder roots. It is useful to extract nematodes separately from each category, as the root tissue texture varies and the type of nematodes invading may also vary, as well as densities of the same nematode. Extraction efficiencies may also vary, with the nematodes exiting slower from a larger root.

Gently tap soil off the roots/tubers or rinse under a tap and then gently dab dry with tissue paper. Peel tubers carefully with a knife or kitchen peeler to just below the surface.

Chop the roots (or tuber peel) finely with a knife or scissors and place in a **labeled** dish (Fig. 27, step 1). Mix all chopped root material thoroughly.

Remove and weigh a sub-sample (e.g. 5 g) of chopped root material using measuring scales (Fig. 27, step 2).

Place weighed sub-sample on the tissue paper in the **labeled** sieve/basket (Fig. 27, step 3).

Follow the rest of the procedure for soil extraction above (Fig. 26, steps 5–13)

Or:

Use the root maceration method for tough plant tissue (Fig. 28, pages 40–41), then follow the remaining procedure for tray extraction (Fig. 26, steps 5–13)

If sending nematodes away for identification or counting, preserve using the protocol on pages 57–60.

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 5–13 in Figure 26.

Figure 27. Extraction tray method for root samples.

Root maceration method

This method is also known as root maceration followed by incubation in an extraction tray.

Advantage:

- Does not require specialized equipment.

Disadvantage:

- The amount of time spent macerating is critical – it must be sufficient to allow nematodes to easily move out from plant tissue, but damage must be minimized.

Equipment

- Beakers
- Scissors/knife
- Water bottle
- Waterproof pen
- Weighing scales
- Domestic blender

Method

Cut roots or tuber peel into small pieces (Fig. 28, steps 1 and 2). Mix root tissue together.

Weigh a sub-sample (Fig. 28, step 3). Place it in an electric blender with just enough water to cover the blades.

Blend fine roots and tuber peel for two 5 second bursts and tougher roots for two 10 second bursts, waiting for the suspension to settle briefly between the two blendings (Fig. 28, step 4).

Pour the blended suspension of roots and water into a beaker, rinsing out the blender container of all debris, using a water bottle if possible (Fig. 28, step 5).

Gently pour the suspension of roots and water onto tissue paper (Fig. 28, step 6) and then follow the extraction tray method (Fig 26, step 5 onwards).

The roots can be stained before maceration to improve visibility of the nematode after extraction (see page 60).

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.

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 steps 5–13 in Figure 26.

Figure 28. Root maceration method.

Sieving method

Advantages:

- Good extraction of all types of nematodes
- Good for extraction of large and slow moving soil nematodes
- Suitable for extracting nematodes from wet soil
- Useful for cyst extraction from soil.

Disadvantages:

- Nematodes may settle out with soil particles unless soil is well dispersed
- Nematodes can be easily damaged.
- Requires slightly more specialized equipment.

Equipment

For soil motile nematodes:

- Beakers and buckets
- Waterproof pen
- Brass 20 cm diameter (Endecotts® or Retsch®) sieves: 2 mm, 90 µm (or 53 µm) and 38 µm
- Extraction tray equipment

For sedentary cyst (e.g. *Heterodera*) recovery:
As for soil motile nematodes, plus

- Brass 20 cm diameter (Endecotts® or Retsch®) sieves: 2 mm, 250 µm, 150 µm
- Funnels
- Filter paper (or paper towel/tissue)

If it is not known whether sedentary nematodes are present then all equipment should be used.

Method

For soil motile nematodes:

Fill a bucket with about 6 liters of water. Mark a water line on the inside of the bucket with a waterproof pen for consistent water volume between samples (Fig. 29, step 3).

Place a sub-sample of sieved and mixed dry soil, or of wet soil measured by displacing water in a beaker (Fig. 29, steps 1 and 2) into the bucket (Fig. 29, step 4).

Mix the water thoroughly using your hand (Fig. 29, step 5). Allow larger particles to settle for 30 seconds (Fig. 29, step 6).

Slowly pour off the upper ¾ of the water through the nested sieves: use a 2 mm sieve to catch debris for disposal, or just 90 µm and 38 µm ones to catch nematodes if there is no debris (Fig. 29, step 7). This requires two people. Take great care to ensure that water does not escape over the sides of the nested sieves (in between the stacked sieves) when pouring the bucket of water through, as nematodes will be lost in the escaping water. Pour slowly and tap the underside of the bottom sieve gently, if necessary, to help water flow through the the sieves.

Refill the bucket to the marked line (Fig. 29, step 8) and repeat the process once or twice (Fig. 29, steps 5– 7).

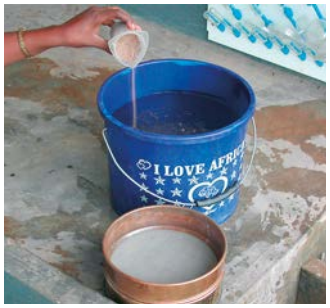
Wash off the debris from the 90 and 38 µm sieves into a **labeled** beaker, ensuring that sieves are properly cleared (Fig. 29, steps 9–11) by washing gently from behind.

Leave beakers for 2–3 hours for nematodes to settle to the bottom. If necessary gently pour off and discard excess water.

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.

Figure 29. Extraction of soil nematodes using the sieving method.

The sievings can then be processed further via the extraction tray method (Fig. 26, steps 3–13, page 37).

For recovery of sedentary cysts:

Air-dry the soil sample before using for extraction (Fig. 30, step 1).

Fill a bucket with about 6 liters of water, and mark the water line on the inside of the bucket with a waterproof pen (Fig. 30, step 2).

Place the measured soil sub-sample in the bucket (Fig. 30, step 3).

Mix the water thoroughly using your hand, then allow soil particles to settle for 60 seconds. Cysts should float (Fig. 30, steps 4 and 5).

Slowly pour off the top ½ of water through the nested sieves: 2 mm to catch debris for disposal, and 250 µm and 150 µm to trap cysts (Fig. 30, step 6).

Wash off the debris from the 250 µm and 150 µm sieves into a labeled beaker (Fig. 30, steps 7 and 8).

Refill the bucket to the marked line and repeat the process (steps 4–8) at least once, collecting all debris for each sample into the same beaker. Repeat this process as much as necessary until you are satisfied that no cysts remain in the bucket.

Prepare and **label** a paper lining (filter paper, milk filter, paper towel etc.) for a funnel (i.e. in a cone shape) held in a stand or beaker (Fig. 30, step 10).

Pour the wash-off (sievings) in the beaker through the filter in the funnel (Fig. 30, steps 11 and 12). Allow water to drain through.

Carefully remove filter papers from the funnel and place in a moistened tray to await direct observation under the microscope (Fig. 30, step 13). Viewing can be done by gently opening the filter paper and spreading the contents across the filter paper, followed by viewing under stereomicroscope.

Or:

Allow filter papers to dry in the funnel, for removal and storage for observation, picking or counting at a later time (Fig. 30, step 14).

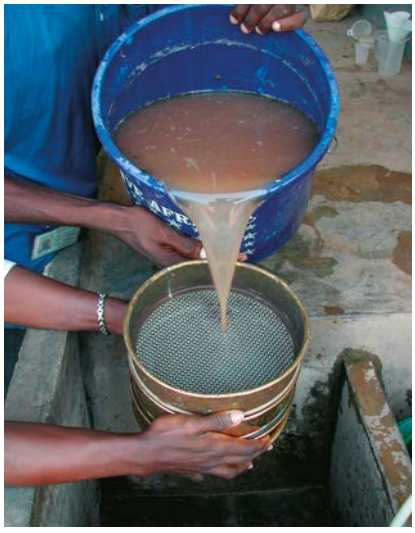
1. Air dry the soil in an open dish.

2. Fill the bucket with water to a marked line.

3. Pour the pre-measured dry soil sub-sample into the water.

4. Mix thoroughly.

5. Allow the soil to settle for 60 seconds.



6. Slowly pour half of the water through the 2 mm, 250 and 150 µm sieves.

7. Rinse down each of the 2 catchment sieves, backwashing where necessary.

Figure 30 – *continued overleaf*

8. Using a wash-bottle, wash the debris from both the 250 and 150 µm sieves into beakers for collection.

9. Refill the bucket with water to the marked line and repeat steps 4–8.

10. Fold a circular piece of filter/tissue paper into quarters, then open it as a cone and place in the funnel which is held in a beaker or stand.

11. Pour the extraction through the filter paper.

12. Leave the liquid to drain through the filter paper.

13. Place filter paper with cyst extract debris into a dish with water in the bottom to keep moist for immediate cyst assessment.

or

14. Leave extracts to dry in the funnel to assess cysts at a later date.

Figure 30. Extraction of cyst nematodes from soil using the sieving method.

Cysts from air-dried samples will float to the surface of the bucket, but if conditions do not allow air drying, cysts can be extracted from fresh soil samples. Many cysts should still float, but fresh, heavier cysts may not, and agitating the water and reducing the settling time may be needed, before decanting the water/suspension through sieves.

Cysts can also be picked from the sieved, dried samples directly under the stereomicroscope, using a fine paintbrush. Determining the cyst density in soil is useful, but it is also necessary to assess the egg number, by first crushing cysts to release eggs (see References and Further Reading).

Incubation method

This method is also called root incubation in plastic bags or jars.

Advantage:

- Does not require specialized equipment.

Disadvantages:

- Extraction efficiency may be relatively poor
- Nematodes are often in poor condition due to lack of oxygen.

Equipment

Plastic bags, jars, conical flask or similar vessel.

Method

Cut/chop tissue finely and mix together (Fig. 31, step 1).

Weigh out a sub-sample/sample.

Place in a closed **labeled** plastic bag or covered container (not metal) holding a small quantity (10–20 ml) of water (Fig. 31, step 2).

Nematodes hatch from eggs or migrate from root tissue into water over a period of 2 to 7 days.

Remove water regularly (e.g. daily) from the container, which will help nematode survival, and bulk in a labeled beaker, using the same beaker for each sample (Fig. 31, step 3).

Replace water after each decanting, cover and leave, repeating the process over 2–7 days (Fig. 31, step 4).

Reduce or concentrate the suspension/extract for each sample and observe directly or store for later use following steps 11–13 in Fig. 26.

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.

Figure 31. Incubation method of extraction.

Direct examination of plant tissue

Infected plant tissue can be examined for nematodes under a dissecting microscope, for example to assess that nematodes are present before sending material for expert identification. The adult sedentary females, which are embedded inside roots (see Figs 10 and 11) can also be teased out of the tissue and used for identification purposes. Where samples are being sent elsewhere for species identification, the (galled) root material itself needs to be sent to the taxonomist, preserved in lactoglycerol solution. Direct observation is also useful for assessing and observing foliar tissue and seed-infecting nematodes (see Fig. 8), and for picking out of individual and specific nematodes to prepare slide collections etc.

For direct observation of plant material:

Wash the plant tissue under a gentle stream of water, or place in a bowl of water for a few minutes, to remove soil and debris, taking care not to dislodge ectoparasitic nematodes feeding or attached on the outside of roots.

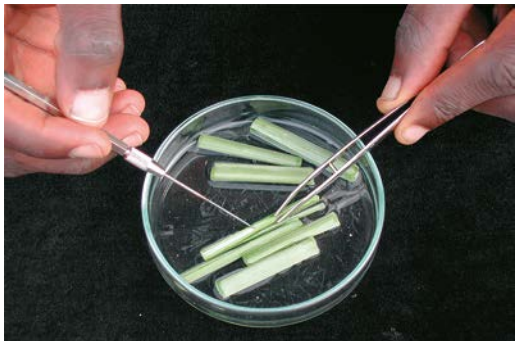
Cut the plant tissue into ~2 cm pieces with a pair of sharp scissors or a knife.

Place the plant tissue into an open Petri dish that has water in the base (Fig. 32).

For immediate observation tease open the tissue with the aid of mounted needles and forceps to release the nematodes from the plant tissue. This is suitable for sedentary endoparasites (Fig. 32).

If the plant tissue contains migratory nematodes it may be useful to leave in a Petri dish overnight or longer even. Nematodes will migrate out of the tissue into the water.

Nematodes can then be picked under the stereomicroscope for identification or preserved (and stained) and/or sent for further identification.



Sheaths teased apart.

Seed coats broken to allow nematodes free movement.

Figure 32. Direct examination of plant material in water.

Handling, fixing and staining nematodes

Various techniques aid the handling and identification of nematodes. These are described in this section.

Handling nematodes

Due to their microscopic size, nematodes can be difficult to handle, particularly for beginners. It is nearly always necessary to handle them in a fluid medium, usually water. Using a dissecting microscope rather than a compound microscope also helps. Individual nematodes need to be selected when establishing pure cultures or preparing identification slides and therefore need to be 'picked'. If handling nematodes from pure cultures, batches of specimens can be transferred with the use of glass pipettes (see Fig. 35). By narrowing the aperture of glass pipettes using heat from a Bunsen burner, even individual nematodes can be handled and transferred.

Picking nematodes

To look at nematodes closely for identification, it is often necessary to individually 'pick' the nematodes from the extraction suspension and place them on a glass slide (Fig. 33). This can be difficult, but gets easier with practice. Nematodes are translucent (see-through) and may be difficult to see; under-stage lighting and a stereoscopic microscope help (Fig. 34). If picking nematodes from plant tissue or root surface, top lighting can also be beneficial.

Figure 33. Picking nematodes using a bamboo splinter on a dissection microscope.

Various instruments can be used for picking, for example a fine insect pin, a bamboo splinter, an eyelash or bristle glued to the end of a mounted needle, a sharpened toothpick, or feather spine (Fig. 35).

Pour or use a pipette to place some of the nematode suspension (or infected plant tissue) into a Petri dish, counting dish or glass block. Keep the suspension shallow.

Place on a stereomicroscope using the lowest convenient magnification.

Gently swirl the suspension to move nematodes to the center of the dish.

Locate a nematode and gently lift the nematode to the surface of the water with the picking tool.

Adjust microscope focus to keep the nematode in view whilst picking the nematode out of the water solution.

Holding the picking instrument under the nematode lift or very gently 'flick' the nematode out of the water. The nematode should be 'hanging' on the tip of the picking instrument.

Gently place the tip of the pick into a drop of water on a slide, in a glass block or other vessel containing some water.

To view nematode(s) on a slide on a compound microscope, it is first useful to 'relax' them by heating briefly on a hotplate (not too hot).

Place glass beads or cover slip splinters at edges of the water droplet and place a cover slip over the droplet.

View under compound microscope.

Using a dissection stereo-microscope with understage lighting.

Using a compound stereo-microscope with understage lighting.

Figure 34. Microscopes used for picking.

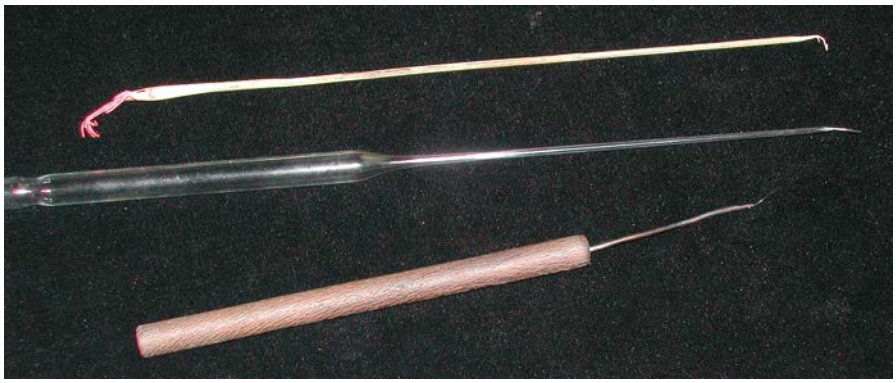


Figure 35. A sample of potential nematode picking tools: a bamboo splint; the tip of a pipette thinned using heat; dissection needle with an eyelash glued to the tip.

Sending nematodes for identification

If, after sampling and extracting, the nematodes can be immediately identified to genus level and counted then this will quickly provide an indication of which parasitic nematode groups are present and whether they are at potentially damaging levels or can be associated with crop damage. However if the expertise to do this is not available, or the species involved need to be established, nematode samples will need to be sent away for identification by a specialist taxonomist.

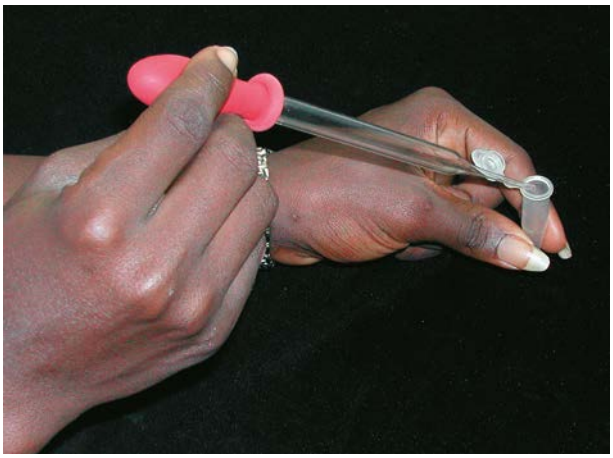
Before sending, samples usually need to be killed (see next section) and preserved, especially if being sent out of the country. Nematodes can be sent live if pre-arranged with the destination lab or if sent within the same country. Sometimes soil and/or plant tissue can also be sent, however it is essential to respect the quarantine regulations for a given country.

Nematodes should be collected in a small vial or tube (Fig. 36) and packed carefully in insulated containers for transportation to the laboratory where they will be identified. The vials should be **clearly labeled** with a code/number. The codes should be recorded on duplicate sheets (one to accompany specimens and one to remain) with all the details of the sample and kept until the results of the identification are returned.

A range of sample bottles for storing or sending samples.

Small microtubes useful for sending samples away for identification.

Using a glass block for preserving nematodes by pipetting in drops of formalin.



Transferring nematodes into a microtube for storage or sending.

Figure 36. Storing nematode samples for transporting.

Nematode identification services

A number of taxonomists based at various centers around the world are able to precisely identify nematodes. However, very few offer a service for routine identification, especially to species level. Some centers that do offer this service are:

Plant Disease and Diagnostic Services
CABI Bioscience UK
Bakeham Lane
Egham
Surrey TW20 9TY, UK
Tel: +44 (0)1784 470111

Biosystematics
ARC-PPRI
P/Bag X134
Pretoria 0001, Republic of South Africa
Tel: +27 (0)12 356 9830

Nematology
Department of Plant Protection
Faculty of Agriculture
University of Jordan
Amman 11942, Jordan
Tel: +962 (0)6 535 5000–3004

Central Science Laboratory
Sand Hutton
York
YO4 1LZ, UK
Tel: +44 (0)1904 462000

Laboratório de Nematologia
(*Meloidogyne* spp., *Bursaphelenchus xylophilus*)
IMAR-CMA
Departamento de Zoologia
Faculdade de Ciências e Tecnologia
Universidade de Coimbra
3004-517 Coimbra, Portugal
Tel: +351 239855760

Embrapa Recursos Genéticos e Biotecnologia
(*Meloidogyne* spp.)
Parque Estação Biológica - PqEB
Av. W5 Norte
Caixa Postal 02372 CEP 70770-900,
Brasília - DF, Brasil
Tel: +55 (61) 3403660/34484930

Laboratório de Nematologia da ESALQ
Setor de Zoologia
Av. Pádua Dias, 11
Caixa Postal 9
13418-900 Piracicaba - SP, Brasil
Tel: +55 (19) 34294338/34294269

Before sending any samples for identification, it is essential to contact the centre and establish their capacity to handle your samples and how best to preserve and transport them.

General advice and training is offered by the nematology sections of both CIMMYT and IITA upon contact. Information can also be sought from the nematology related societies, which are listed on the inside back cover.

Killing nematodes

It is important to kill nematodes quickly, as each species assume a particular 'death shape' when killed quickly which can help in identification. Nematodes are best killed with gentle heat (55–65°C), which retains the nematode body content. If killed at too hot a temperature, body contents are cooked and denatured, causing difficulty for identification. Nematodes can either be killed first and then fixed or killed and fixed in the same process.

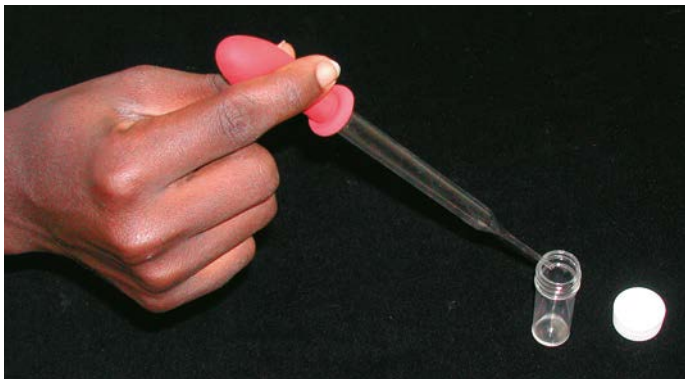
A simple and efficient method for killing nematodes is to add an equal volume of boiling water to the nematode suspension. If the whole extract is being sent, the nematode suspension may need to be reduced in volume so that there is less than half in the sample tube or vial. It may be easier to kill and fix the nematodes in larger tubes then place a reduced volume into smaller tubes (Fig. 36), or remove nematodes from the bottom (Fig. 26, step 13) with a pipette and place in the tubes for transportation.

Nematodes can also be killed by holding the tube containing a small volume of nematode suspension in near-boiling water for 1–2 minutes, but this can take a long time for a large number of samples. It can also be cumbersome and care is needed to ensure sample bottles remain upright and do not topple over into the water, losing the samples.

Fixing nematodes

The simplest method for fixing or preserving samples is to pipette a few drops of formaldehyde (formalin) into recently heat-killed samples. Two or three drops into a 7 ml sample bottle is sufficient (Fig. 37); larger sample bottles will require more. This is a quick and easy method, which will prevent samples from deteriorating during transit and storage before identification, however it does not provide good quality specimens for long-term preservation and can also cause difficulty for identification, especially if not examined immediately.

Take great care with formaldehyde as it is dangerous to health.



Pipetting formalin into a nematode suspension in a 7 ml tube to preserve the sample.

Staining plant tissue with acid fuchsin in lactoglycerol solution on a hot plate.

Fig 37. Fixing and staining nematodes.

Killing and fixing in one step

Heat the fixative to near boiling in a test tube or beaker by immersing in boiling water.

Pour an equal volume of hot fixative to that of the nematode suspension into the nematode suspension (i.e. 2 ml of hot fixative into 2 ml of suspension = 4 ml).

Or:

Collect the nematodes in a glass block in a small drop of water and add 2–3 ml of hot fixative with a pipette.

The most suitable fixatives to use are:

TAF

Triethanol**a**mine	2 ml
Formalin (40% formaldehyde)	7 ml
Distilled water	91 ml

The fixative remains stable for a long time and nematode appearance remains lifelike because the specimens do not dry out.

FA 4:1

Formalin (40% formaldehyde)	10 ml
Glacial **a**cetic acid (proponic acid)	1 ml
Distilled water	89 ml

In FA 4:1 nematodes maintain their structure though they may become discolored after some time.

Formalin glycerol

Formalin (40% formaldeyde)	10 ml
Glycerol	1 ml
Distilled water	89 ml

This has the advantage of keeping the nematode from drying out even if the vials are not properly sealed. Again take great care with all these fixatives as they are dangerous to health.

Preserving sedentary nematodes in root or tuber tissue

The females of sedentary nematodes are required for species identification. Therefore the plant tissue containing the nematodes, such as galled roots, needs to be preserved and sent for examination. Placing a small sub-sample of infected plant tissue into a sample bottle containing lactophenol or lactoglycerol can be sufficient. Staining before preserving can help identification.

Lactophenol can be purchased ready made, or made by mixing equal volumes of glycerol, lactic acid and distilled water (lactoglycerol) and dissolving a small amount (1%) of phenol into it (lactophenol). **Phenol is very toxic** however, so it is usually best to just use lactoglycerol, although this does not preserve samples for long periods.

Staining

Observation of nematodes embedded in plant tissue can be made easier by using appropriate stains, which stain the nematodes while plant tissue remains relatively clear (e.g. see Fig. 5). Thick or bulky roots should be sliced thinly before staining to ensure transmission of sufficient light after clearing.

Stain in lactoglycerol + 0.1% cotton blue or 0.05–0.1% acid fuchsin, then destain in a beaker containing a solution of equal volumes of glycerol and distilled water + a few drops of lactic acid. Destaining is most effective if done over several days.

Gently wash plant material free of soil and other debris, and dry gently by dabbing with paper towels.

Cut or slice thick or wide roots or tuber into small lengths.

Place in muslin cloth, tie up the corners with a piece of cotton string, and **label** clearly with labels attached to each separate muslin 'bag'.

Bring stain solution, using a glass beaker on a hot plate, to near boiling.

Place muslin bags into boiling stain solution and leave for approximately 3 minutes, depending on root thickness. Use a deep beaker, approx. half full of stain solution, as it will froth up when plant tissue is added (Fig. 37).

Remove the muslin bags and rinse in running water.

Place the muslin bags in the clearing solution and leave overnight or longer.

Examine under the microscope. Placing roots side-by-side on a microscope slide and gently squashing them using another slide placed on top enables the stained nematodes to be seen more clearly. Nematodes will be stained red with acid fuchsin or blue with cotton blue.

Meloidogyne egg masses

Phloxine B stains the gelatinous matrix that surrounds *Meloidogyne* eggs, increasing the visibility of egg masses and enabling a rapid count of adult female nematodes/egg masses present. The eggs also remain viable after staining. The solution is made by adding 15 mg (a very small sprinkle) of Phloxine B to 1 liter of water.

Place the rinsed roots in a tray or dish (preferably white) containing Phloxine B solution and leave for 15–20 min. Count stained (pink-red) egg masses.

Estimation of nematode density

Once nematodes have been extracted from soil or plant tissue, they must be first identified and then quantified. This enables assessment of their association with, or their potential to cause, damage.

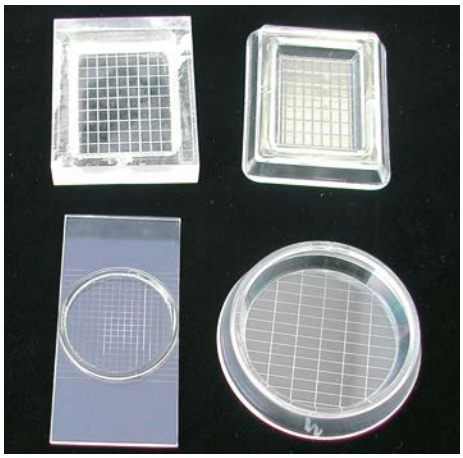
This guide does not provide an identification guide to plant parasitic nematodes; for these see the Further Reading section on page 65. (A very basic description of the most common plant parasitic nematodes is given in Appendix 2.) Nematode density estimation should only be attempted if nematodes have been definitely identified. If the capacity and skills to do this are not available, samples should be sent to the experts (see page 57).

Counting nematodes

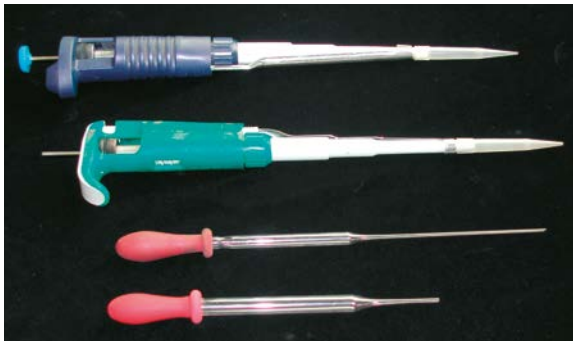
Extracted nematodes can be viewed and counted using a dissecting or a compound microscope (Fig. 34); access to both is ideal. Good quality illumination (understage lighting) is essential. A magnification of about 40× is usually suitable (i.e. a 4× objective combined with a 10× eyepiece), but a compound microscope can also be used (i.e. using the 10× objective), which is useful for nematodes that are in poor condition or are hard to identify. Dissection microscopes allow greater maneuverability and depth of focus, especially for dirty samples. Nematodes that cannot be identified in the counting dish with the counting magnification or at higher magnification using a compound microscope, should be manually picked (Fig. 33) and mounted on a glass slide for identification using higher magnification with a compound microscope.

Various forms of counting dishes exist, but basically a clear plastic dish is needed that has a grid etched on the bottom (Fig. 38). This can easily be prepared by carefully scratching lines on the underside of a small plastic Petri dish. An open rectangular plastic dish with approximately 5 ml capacity is useful for general purposes; it also makes it possible to move nematodes or debris in the dish, and to hand pick for identification at higher magnification (Fig. 33). Sloping sides help minimize optical distortion caused by the meniscus, while raised grids can help reduce the nematodes moving between the grid lines.

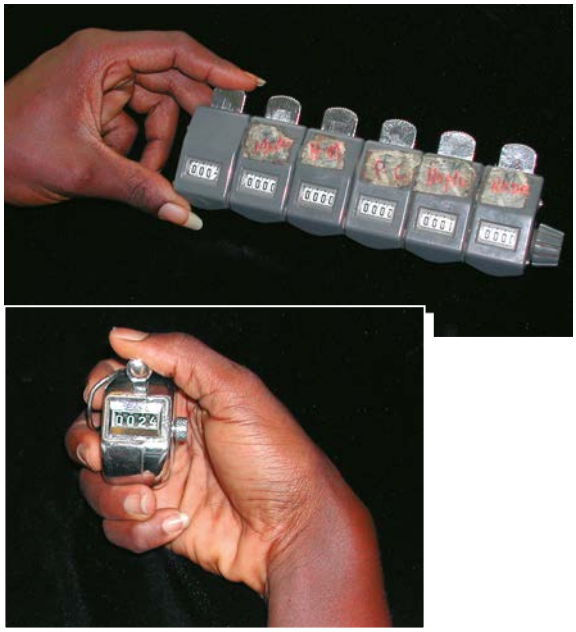
Samples that have been reduced to 5 ml can be counted as a whole sample, but if nematode density is high or the sample is dirty, a proportion (aliquot) can be counted, diluting with water as required. However, care must be taken to ensure that a representative proportion of the total sample is counted which is achieved by thoroughly mixing the sample before taking the aliquout.



A sample of counting dishes.



A sample of pipettes.



Multiple and single tally counter.

Figure 38. Counting tools.

Extract nematodes from a known weight of plant tissue or volume of soil using one of the previously described methods.

Concentrate the extracted suspension to a precise known volume in a measuring cylinder or graduated tube (e.g. 10 ml).

Shake or stir the suspension immediately before removing aliquots.

Use a wide mouth pipette to remove aliquots, to prevent blockage by debris. Pipette tips can be cut if they are too narrow.

Carefully pipette aliquots into the counting dish, avoiding splashing.

If only a few nematodes are present, count them in the total suspension volume.

If nematode density is high, count the nematodes from an aliquot (e.g. 1 or 2 ml). Dilution of the suspension may be necessary to aid counting, for example doubling the volume.

Count all the nematodes in the counting dish in a systematic way following the gridlines on the dish. Sometimes nematodes may float on the surface, but adding a tiny spot of liquid soap overcomes this.

Use a tally counter (ideally a multiple tally counter; Fig. 38) to count the various different nematodes present, or score using the Roman tally system if no tally counter is available.

Return the counted aliquot to the suspension after counting.

Repeat using 2–3 aliquots per sample and then calculate the mean for the combined aliquot score before calculating the total nematode number per sample.

The mean number of nematodes calculated from the aliquots should be multiplied by the total volume of the suspension to calculate the total number in the plant tissue or soil that they were extracted from (e.g. 100 ml soil or 5 g root).

Damage analysis

Scoring of nematode symptoms on plants

Nematode damage can be evaluated at the same time as field sampling for nematodes. The amount of root damage is estimated visually (as a percentage) using a scoring procedure (Appendix 3). Useful damage estimates can be made for root-knot nematode damage in particular, but also for other nematode damage. The damage score usually has a strong relationship with crop yield losses.

Scoring nematode damage provides a rapid indication of the damage at that time. Where basic nematological equipment and expertise are lacking, it may be the only means of assessment. Damage scoring can also be used to help identify resistance or tolerance in varietal screening exercises.

The number of plants assessed can be one or two, up to 25 or more, depending on the crop and area under assessment, and also whether the farmer wants to take a low or a high risk approach to assessment. One person or at least as few people as possible should carry out the scoring, for consistency. The use of score sheets to regularly refer back to is advisable for the same reason.

Some judgment may be needed when assessing nematode damage, for example, plants that have severe root-knot infection may have very few roots left to assess, with galls having rotted away. Galling damage may therefore appear minimal, but in fact the damage due to nematodes is high. It must also be remembered that the response of different crops and crop varieties to a nematode species may vary, in particular to root-knot nematodes. Different nematode species also cause different symptoms, for example, infection by *Meloidogyne hapla* will often result in bead-like galling (as seen on the lettuce in Appendix 3), while *Meloidogyne incognita* may cause more massive galling and fused root flesh (Appendix 3).

The score sheets in Appendix 3 provide examples and a basis upon which to create damage scoring for other crops and nematode damage circumstances. The sheets mostly score damage on a scale from 1 to 5, which balances speed of assessment with accuracy. If more time is available, scoring on a 1–10 scale will provide more accurate damage estimates.

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Appendix 1. Examples of nematode genera and species known to be important crop pests worldwide

Nematodes and damage symptoms	Main crops affected	Distribution
Achlysiella Necrosis of roots *Achlysiella williamsi*	Sugarcane	Australasia
Anguina Seed and leaf galls, distortion of leaves *A. tritici* (ear cockle nematode)	Cereals and grasses Temperate cereals, mainly wheat	Temperate: worldwide Temperate: China, Eastern Europe, India, North Africa, West Asia
Aphasmatylenchus Poor root growth, chlorosis *Aphasmatylenchus straturatus*	Groundnuts	Tropical: West Africa
Aphelenchoides Necrosis and distortion of leaves and seeds, destruction of fungal mycelium *A. arachidis* (groundnut testa nematode) *A. besseyi* (rice white tip nematode) *A. fragariae* (strawberry crimp nematode) *A. ritzemabosi* (leaf nematode) *A. composticola* (mushroom nematode)	 Groundnut Rice Strawberry Chrysanthemum Mushrooms	 Tropical: West Africa Tropical: rice-growing areas worldwide Temperate: Europe, North America, Japan Temperate: Europe, North and South America, East and southern Africa, Australia Mushroom cultivation areas worldwide
Belonolaimus (sting nematodes) Necrosis of roots, chlorosis, wilt *B. longicaudatus*	Sweet corn, vegetables, groundnut, citrus, cotton	Subtropical: southeastern USA
Bursaphelenchus Chlorosis and tree death *B. xylophilus* (pine wilt nematode) *B. cocophilus* (red ring nematode) Necrosis (red ring) of stems and inflorescence, nut fall	 Pine Coconut, oil palm	 Temperate: China, Europe (Portugal), Japan, Korea, North America, Taiwan South and Central America, Caribbean

Nematodes and damage symptoms	Main crops affected	Distribution
Criconemella (ring nematodes) Chlorosis, necrosis of roots and pods, wilting		Temperate and tropical
C. onoensis	Rice	Tropical: USA, West Africa, Central and South America
C. ornata	Groundnut	Subtropical: USA
C. xenoplax	Fruit trees	Subtropical: USA
Ditylenchus (Stem/bulb nematodes) Lesions of stems and leaves, distortion of flowers and foliage, bulb and tuber rot		
D. africanus (groundnut pod nematode)	Groundnut	Subtropical: southern Africa
D. angustus (ufra nematode)	Rice	Tropical: Bangladesh, India, Burma, Vietnam
D. dipsaci	Field beans, onions daffodils and other bulb crops, cereals	Europe, North and South America, Eastern Australia
D. myceliophagus	Mushrooms	Temperate: mushroom cultivating areas worldwide
Helicotylenchus (spiral nematodes) Necrosis of roots	Widespread on many crops but damage largely unknown	Temperate and tropical: worldwide
H. multicinctus	Bananas and plantains	Tropical/subtropical: banana growing areas worldwide
Hemicriconemoides Root destruction, chlorosis, twig dieback		
H. mangiferae	Fruit trees	Subtropical: South Asia, Africa, South and Central America, Caribbean
Heterodera (cyst nematodes) Cysts on roots, poor root growth, chlorosis, wilting		
H. avenae (cereal cyst nematode)	Small grain cereals (wheat, barley, oats)	Global: Central West Asia and North Africa, Northern Europe, China, India, Australia, Pacific North West USA
H. cajani (pigeon pea cyst nematode)	Pigeon pea	India
H. ciceri (chickpea cyst nematode)	Chickpea, lentil	Mediterranean
H. filipjevi (cereal cyst nematode)	Small grain cereals (wheat, barley, oats)	Central West Asia, India, China, Northern Europe
H. glycines (soybean cyst nematode)	Soybean, beans	Subtropical: North and South America, Japan, China
H. latipons (cereal cyst nematode)	Small grain cereals (wheat, barley, oats)	West Asia
H. mani	Small grain cereals (wheat, barley, oats)	West Asia
H. oryzae (rice cyst nematode)	Rice	Tropical: India, Bangladesh
H. sacchari (sugarcane cyst nematode)	Sugarcane, rice	Tropical: West Africa, India
H. schachtii (sugarbeet cyst nematode)	Beets, swedes and other brassicas	Temperate/subtropics Europe, North America, West and southern Africa, Australia
H. zeae (maize cyst nematode)	Maize	Tropical: India

Nematodes and damage symptoms	Main crops affected	Distribution
Hirschmanniella (Rice root nematodes and mitimiti nematode) Root lesions and corm rot (mitimiti disease)		
H. gracilis	Rice	Tropical
H. imamuri	Rice	Tropical
H. oryzae	Rice	Tropical: West Africa, North and South America, South and Southeast Asia
H. spinicaudata	Rice	Tropical: Africa, North and South America
Hoplolaimus (lance nematodes) Necrosis of roots		
H. columbus	Cotton	Tropical: USA, Egypt
H. seinhorsti	Cotton, vegetables	Tropical: Africa, South Asia, South America
Longidorus (needle nematodes) Root tip galling. Transmit viruses		
L. elongatus	Strawberry, sugarbeet	Temperate: Europe, Canada
Meloidogyne (root-knot nematodes) Galling of roots and tubers, chlorosis, wilting		
M. acronea	Cotton, sorghum	Tropical: southern Africa
M. africana	Coffee	Tropical: Africa
M. arenaria	Groundnut	Tropical: worldwide
M. artiellia	Wheat, barley and legumes	Mediterranean countries including Italy, France, Greece and Spain, West Asia, Israel and Western Siberia
M. chitwoodi	Potatoes, sugarbeet, cereals	Temperate: North America, Mexico, South Africa, Europe
M. coffeicola	Coffee	Tropical: South America
M. exigua	Coffee	Tropical: South America
M. graminicola	Rice	Tropical: South and Southeast Asia
M. hapla	Pyrethrum, vegetables, clover	Temperate/subtropical: worldwide
M. incognita	Vegetables, cotton, tobacco, very wide host range	Tropical: worldwide
M. javanica	Vegetables, cotton, tobacco, very wide host range	Tropical: worldwide
M. oryzae	Rice	Tropical: South Asia
M. mayaguensis	Vegetables, papaya, wide host range	Tropical: worldwide
M. naasi	Wheat, barley	Northern Europe, New Zealand, Chile, USA, Iran and former USSR

Nematodes and damage symptoms	Main crops affected	Distribution
Nacobbus (false root-knot nematodes) Root galling *N. aberrans*	Vegetables, potato, sugar beet	Temperate/subtropical: South, Central and North America, Europe (glasshouses)
Paralongidorus (needle nematodes) Root tip galling. Transmit viruses *P. australis*	Rice	Subtropical: Australia
Paratrichodorus (stubby root nematode) Shortened (stubby) blackened roots. Transmit viruses *P. minor*	Vegetables	Temperate/subtropical: Europe, USA
Pratylenchus (lesion nematodes) Necrosis of roots, corms and tubers		
P. brachyurus	Groundnuts, pineapple, cassava	Tropical: worldwide
P. coffeae	Bananas, yams, coffee, citrus, spices, very wide host range	Tropical: worldwide
P. goodeyi	Bananas	Subtropical: East and West Africa, Canaries
P. loosi	Tea	Subtropical: South Asia
P. neglectus	Potatoes, vegetables, small grained cereals (wheat, barley, oats)	Australia, West Asia, North Africa, USA, Canada
P. penetrans	Fruit and nut trees, vegetables, soft fruits, flower crops	Temperate: worldwide
P. thornei	Small grained cereals (wheat, barley, oats)	Australia, West Asia, North Africa, Israel, Mexico, USA
P. zeae	Maize, upland rice	Tropical: South and Southeast Asia; Africa
Radopholus (burrowing nematodes) Necrosis of roots and tubers, rots, root breakage, toppling		
R. citri	Citrus	Tropical: Indonesia
R. similis	Bananas, citrus, root and tubers, coconut, tea, black pepper and other spices	Tropical: worldwide
R. nativus	Cereal and grain legumes	Temperate: Australia

Nematodes and damage symptoms	Main crops affected	Distribution
Rotylenchulus (reniform nematodes) Poor root growth, chlorosis, stunting *R. parvus* *R. reniformis* *R. variabilis*	 Pigeon pea, sweet potato Pineapple, vegetables Sweet potato	 Tropical: Africa Tropical: worldwide Tropical: Africa
Rotylenchus (spiral nematodes) Unthrifty growth *R. robustus*	 Vegetables, tree seedlings	 Temperate: Europe, North America, India
Scutellonema (spiral nematodes) Dry rot of tubers, poor root growth *S. bradys* (yam nematode) *S. cavenessi*	 Yams, cassava Groundnuts	Mainly in the tropics and Africa Tropical: West Africa, Caribbean Tropical: West Africa
Trichodorus (stubby root nematodes) Shortened (stubby) blackened roots. Transmit viruses *T. primitivus* *T. viruliferus*	 Sugarbeet, potato	 Temperate/subtropical: Europe, North America
Trophotylenchulus Reduced root growth *T. obscurus*	 Coffee	 Tropical: Africa
Tylenchorhynchus (stunt nematodes) Stunting of roots	 Cereals, vegetables	 Temperate and tropical
Tylenchulus Poor root growth, slow decline of trees *T. semipenetrans* (citrus nematode)	 Citrus	 Subtropical/tropical: citrus-growing areas worldwide
Xiphinema (Dagger nematodes) Root tip galling. Transmit viruses *X. americanum* *X. diversicaudatum* *X. index*	 Trees, grape, Roses, grape, soft fruits Grape, fruit trees, rose	 Temperate/subtropical: worldwide Temperate: Europe, North America, Australia, New Zealand Temperate: Europe, South America, Mediterranean, southern Africa, Eastern Australia

Adapted from Table 13.1 in J. Bridge and T.D. Williams, Plant parasitic nematodes, pp.140–162, *in* Waller et al. (2002) (courtesy of CABI Publishing).

Appendix 2.
Basic identification of nematodes

This guide does not provide a taxonomic guide to plant parasitic nematodes; for this see the References and Further Reading section. A very basic description of the most common plant parasitic nematodes is however given below. Figure 39 shows the visual differences between plant parasitic nematodes (head regions) and non-plant parasitic nematodes, to aid differentiation.

Meloidogyne – root-knot nematode
Adult female is pear shaped or spheroid with an elongated neck while males are worm-like (vermiform).
Juveniles and females are endoparasitic causing galls.
Stylet is slender with basal knobs.
The eggs are laid in a gelatinous matrix.

Pratylenchus – lesion nematode
Vermiform nematode.
Females with one ovary.
Relatively broad, flattened head and rounded tail.
Lip region is flat and the stylet is stout 14–19 µm long with massive basal knobs.
Causes lesions in plant roots.
All stages are infective and are migratory endoparasites.

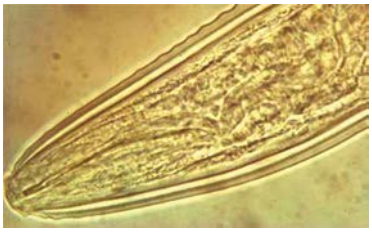
Heterodera – cyst nematode
Adult female lemon shaped forming cyst on maturity.
Female is semi-endoparasitic with only anterior portion inside the plant material.
Eggs are retained in the cyst but can additionally possess an egg mass.
Juvenile stylet is strong with prominent basal knobs.

Helicotylenchus – spiral nematode
Lip region is high, rounded conical.
Nematode is usually coiled into a loose spiral or C shape.
Female with 2 ovaries with dorsally curved tail.
They are semi-endoparasites or ectoparasite and usually found in soil.

Scutellonema – false spiral nematode
Basically the same description as *Helicotylenchus*, but:
The stylet is shorter and basal knobs are more pronounced.
The relaxed shape is straight or a slight C shape.
They are mostly ectoparasitic.

Xiphinema and ***Longidorus*** – spear nematodes
Very long nematodes
The stylet is a long needle shaped structure without pronounced basal knobs.
The relaxed shape is usually straight.
They are ectoparasitic.

Plant parasitic nematodes:

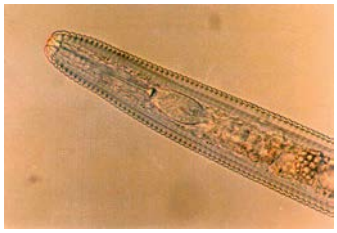


Paratrichodorus [JB]

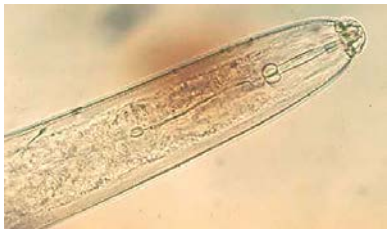
Heterodera [JB]

Scutellonema [JB]

Hemicriconemoides [JB]



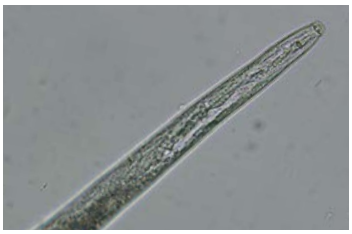
Hemicycliophora [JB]



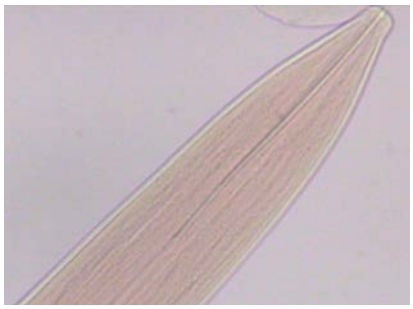
Hoplolaimus [JB]

Tylenchorhynchus [JB]

Plectus [JB]



Meloidogyne J2 head [SM]



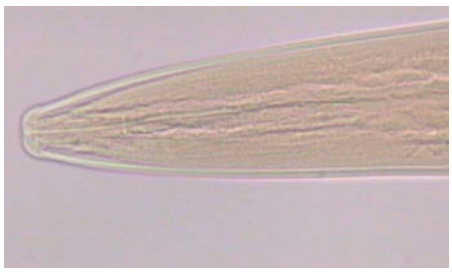
Xiphinema [LA-B]

Filenchus [LA-B]

Meloidogyne female head [LA-B]

Xiphinema [SM]

Helicotylenchus [SM]

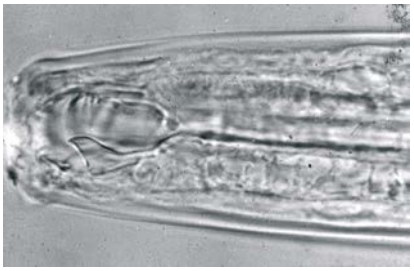


Longidorus anterior female head [LA-B]

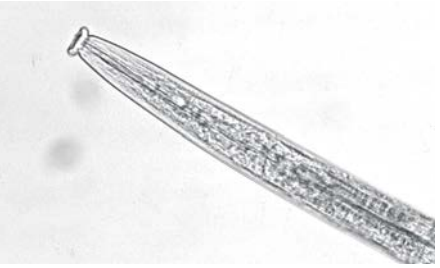
Pratylenchus [SK]

Figure 39 – *continued overleaf*

Non-plant parasitic nematodes:



Mononchus [JB]



Discolaimus [JB]

Figure 39. Comparison of various plant and non-plant parasitic nematode head regions.
(Photographs by J. Bridge [JB], S. Mack [SM], L. Al-Banna [LA-B] and S. Kelly [SK].)

Appendix 3.
Score sheets for measuring nematode damage

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.

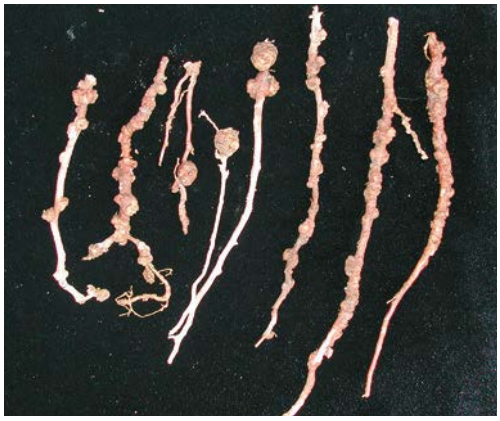
Cassava roots

1. No galls observed, feeder roots intact.

2. At least one gall observed.

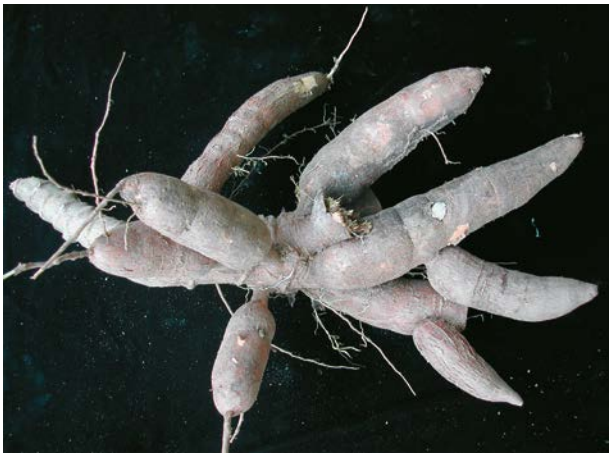
3. Numerous galls, about 50% of roots affected.

4. Numerous galls, most roots affected.

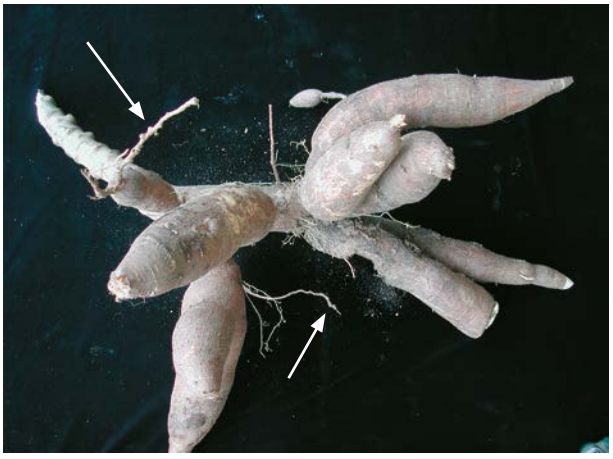


5. Heavy galling on most roots, with necrosis, and feeder roots heavily affected or absent.

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.

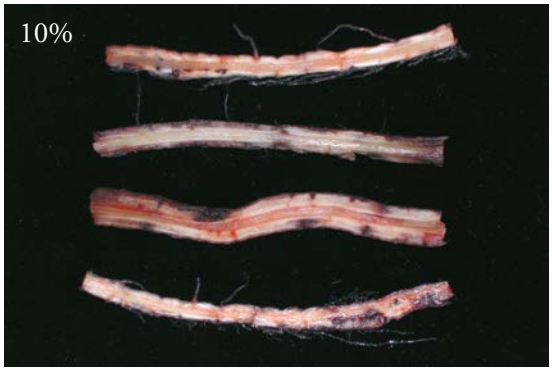
3. Mild galling.

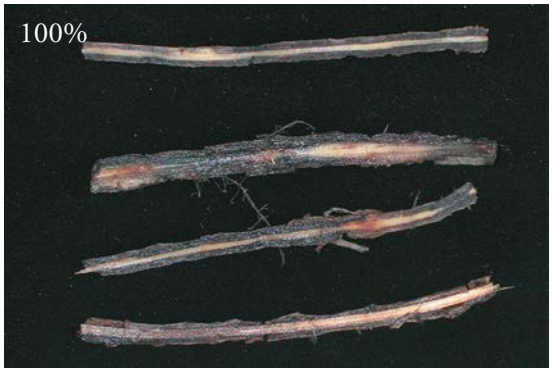
4. Moderate galling.

5. Severe galling.

Lesion scoring for banana roots

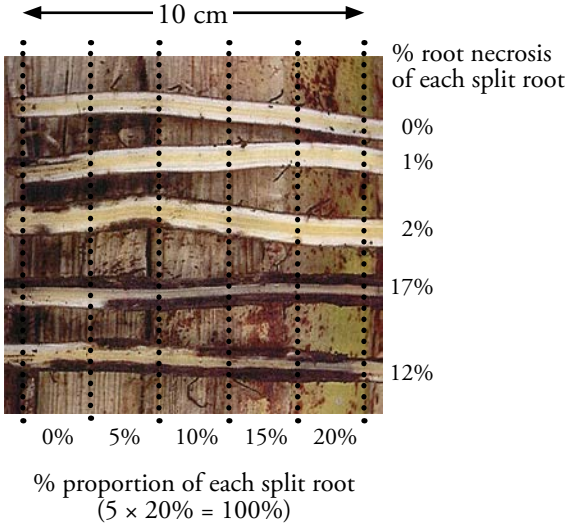
Adapted from Paul Speijer and Dirk De Waele (1997).





Lesion scoring for *Musa*

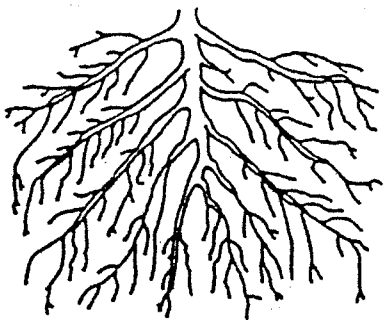
Example of scoring five lengthwise sliced banana roots for root necrosis (%) of root cortex surface showing necrosis caused by migratory lesion endoparasites (courtesy of Paul Speijer and Dirk De Waele, 1997).



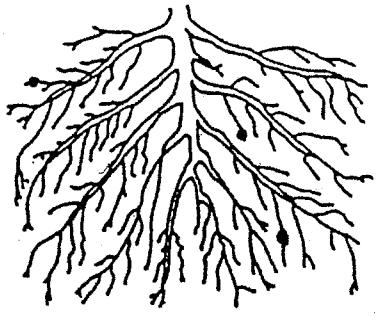
Randomly select five funtional roots per sample (plant). Each one should be at least 10cm long. Slice each root lengthwise and discard one half. Score the other half of the root for the percentage of root cortex showing necrosis. Each root contributes 20% of the whole sample so that when added up you get 100% for the 5 roots. So if half the root exhibits necrosis then score it at 10%. If the root shows no necrosis score it at 0% (see figure above). Once you've scored each root out of 20 then add up the 5 scores to get the total percentage of necrosis across the whole sample.

Diagrammatic root-knot scoring chart

Courtesy of John Bridge and Sam Page (1980).

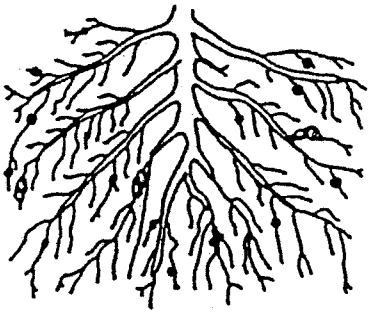


0 – No knots on roots.

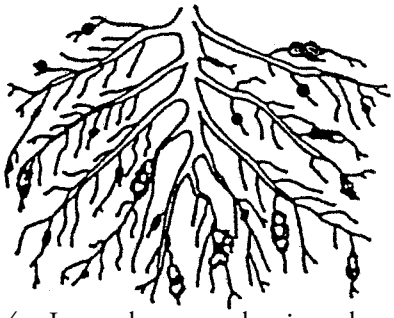


1 – Few small knots, difficult to find.

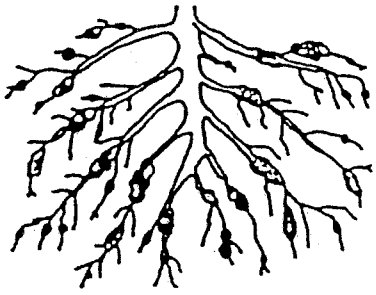
2 – Small knots only but clearly visible. Main roots clean.



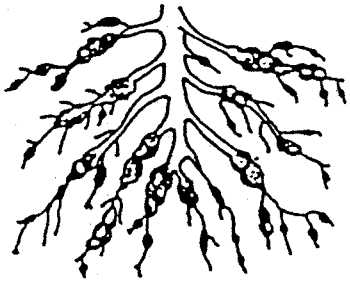
3 – Some larger knots visible. Main roots clean.



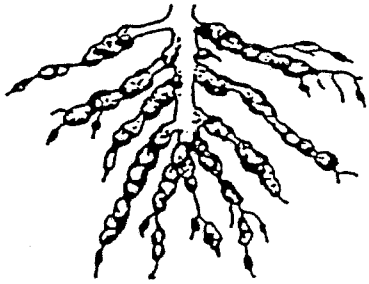
4 – Larger knots predominate but main roots clean.



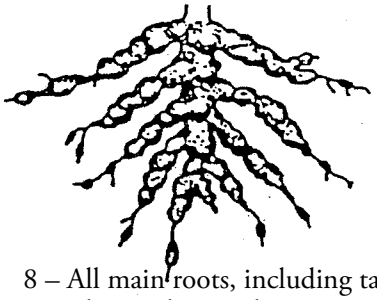
5 – 50% of roots affected. Knotting on some main roots. Reduced root system.



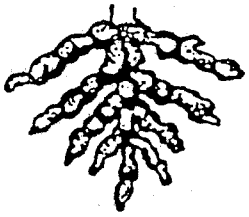
6 – Knotting on main roots.



7 – Majority of main roots knotted.



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



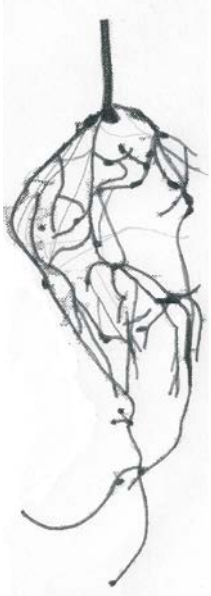
9 – All roots severely knotted. Plant usually dying.

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

Cyst damage scoring sheet for wheat

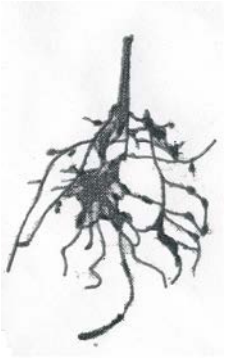
Adapted from A.D. Rovira in Brown and Kerry (1987).

1 – No damage, clean.



2 – Slight damage.

3 – Mild damage.



4 – Moderate damage.

5 – Severe damage.

Useful networks and organizations

Afro-Asian Society of Nematologists
(http://www.ifns.org/membership/aasn.html)

Australasian Association of Nematologists
(http://nematologists.org.au)

Brazilian Nematological Society
(http://www.ciagri.usp.br/~sbn/sbn_i.htm)

Cereal Nematode Network

Chinese Society of Plant Nematologists
(http://www.ifns.org/membership/cspn.html)

Nematology Initiative in East and Southern Africa

Egyptian Society of Agricultural Nematology
(http://www.ifns.org/membership/esan.html)

European Society of Nematologists
(http://esn.boku.ac.a)

International Federation of Nematology Societies
(http://www.ifns.org/)

Japanese Nematological Society
(http://www.ifns.org/membership/jns.html)

Nematological Society of India
(http://www.ifns.org/membership/nsi.html)

Nematological Society of Southern Africa
(http://www.ifns.org/membership/nssa.html)

Organization of Nematologists for Tropical America
(http://www.ontaweb.org)

Society for Invertebrate Pathology
(http://www.sipweb.org/)

Society of Nematologists
(http://www.nematologists.org)

West and Central African Nematology Network

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Plant parasitic nematodes are ever-present in farmers' fields, but the damage they cause is often attributed to other pests and diseases or other crop problems. In developing countries in particular, where resources and facilities are scarce, it is difficult to accurately identify and quantify the nematode problem.

This guide aims to help overcome this limitation by providing an easy-to-follow reference for assessing plant parasitic nematode problems. It provides clear instructions, with many illustrations, on procedures for collecting and processing samples for nematode assessment, as well as information on accessing further identification and diagnosis support. The manual is aimed at technicians, field workers, extension agents and others with an interest in crop production and crop protection, particularly in those parts of the world where access to expert help and advanced facilities is limited. This guide will hopefully simplify some aspects of nematology, and help to lessen the mystery surrounding this crop production problem.

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