RURAL INDUSTRIES RESEARCH
& DEVELOPMENT CORPORATION

Advisory Services for Nematode Pests

Operational Guidelines

A report for the Rural Industries Research and Development Corporation

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Foreword

Plant-parasitic species attack the roots or above-ground parts of most crops and the damage they cause is often so serious that it is impossible to maintain economically viable agriculture without some form of nematode control. Nematicides are used routinely in many horticultural, vegetable and ornamental crops, while integrated management programs involving crop rotation and nematode-resistant varieties are used to control nematodes in some field and pasture crops.

Because most farmers, agronomists and crop management consultants have only a basic knowledge of nematodes, they may seek professional help when making decisions about nematode management. Specifically, they will have soil or root samples assayed for nematodes, they will seek advice on whether the nematodes present are likely to cause economic damage and they will require a recommendation on control. Such services are provided by government and commercial laboratories. However, in the absence of an accreditation system for nematological laboratories, the quality of the information available to clients cannot be guaranteed.

This book, which has been compiled by members of the Australasian Association of Nematologists, provides general guidelines on how an advisory service for nematodes should operate. It is hoped that these guidelines will help improve the quality of advice on nematode pests available to farmers in Australia and New Zealand. Although the book is mainly written for those who are operating nematode diagnostic services, it will be useful to their clients. It gives some indication of the procedures involved and the issues that should be addressed by those providing professional advice on nematode problems.

This report, a new addition to RIRDCs diverse range of over 700 research publications, forms part of our Resilient Agricultural Systems R&D program, which aims to enable agricultural production systems that have sufficient diversity, flexibility and robustness to be resilient and respond to challenges and opportunities.

Most of our publications are available for viewing, downloading or purchasing online through our website at www.rirdc.gov.au/eshop

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Acknowledgements

About the authors

Dr Graham Stirling has a Master's degree in nematology from the University of Adelaide and a Ph.D in nematology/plant pathology from the University of California at Riverside. He has 28 years experience as a nematologist, having worked with nematode problems on a wide range of crops, including wheat, rice, sugarcane, grapevines, citrus, pineapple, ginger, apple, stonefruit and vegetables. During his career, Dr. Stirling has operated nematode diagnostic services for both the South Australian Department of Agriculture and the Queensland Department of Primary Industries. He also has broad experience in integrated nematode management and expertise in the use of nematode monitoring as a management tool. Currently he is Principal Scientist, Biological Crop Protection Pty Ltd, Brisbane.

Dr. Julie Nicol was awarded her Ph.D in nematology by the University of Adelaide in 1997. She has worked mainly with nematodes in field crops, where she has experience with sampling and extraction procedures, field population modeling and resistance assaying. She has also been involved in work on plant nematodes in grapevines. Dr. Nicol previously held a lectureship position in the Faculty of Agriculture and Natural-Resource Management at the University of Adelaide and is currently a post-doctoral fellow with CIMMYT in Mexico, where she is working as a root pathologist with the cereal breeding team.

Mrs Frances Reay recently retired after working with nematodes for more than 25 years. She graduated from Imperial College in the United Kingdom and was employed as a nematode taxonomist by the University of Adelaide from 1971 to 1993. In the last few years of her career, she undertook taxonomic studies on Antarctic nematodes for North Carolina State University and provided taxonomic expertise to the nematode diagnostic services of the South Australian Research and Development Institute.

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Executive Summary

Nematodes are unsegmented worms. They are the most abundant group of multicellular animals on earth, thriving in most habitats, and are important pests but, because they live in soil, they rank amongst the most difficult pests to diagnose, identify and control. Their effects are often underestimated by farmers, agronomists and pest management consultants. Estimates from several independent studies suggest that plant-parasitic nematodes reduce global agricultural production by about 12%. This means that crop losses from nematodes may be as high as $400 million per annum in Australia alone.

Chapter 1 gives an overview of nematodes and the extent of problems they cause. It lists the nematode species which are economically important on various crops grown in Australia and indicates that most field crops, horticultural crops and pastures are affected by at least one major nematode pest.

Chapter 2 looks at integrated nematode management, including nematode monitoring, diagnostic and advisory services for nematodes, and diagnostic and predictive assays. It explains how in recent years chemical control options have diminished because nematicides are being withdrawn for health and environmental reasons. Horticultural industries are therefore moving towards a more integrated approach to nematode control. Thus in all industries where nematodes cause problems, there is a trend toward integrated pest management (IPM), with all available control options now being used in a compatible manner to reduce nematode populations to levels which cause tolerable economic loss.

Chapter 3 looks at sampling methodology for nematodes. Unlike some pests and diseases, nematodes cannot be monitored by observation in the field. Nematodes must be extracted for microscopic examination in the laboratory and so an additional, relatively expensive processing step is involved. The laborious nature of nematode processing procedures means that the number of samples must be kept to a minimum. Sampling methodology is therefore crucial, as the few samples that are collected must be representative of the area involved. Even with the best laboratory procedures, data obtained from a nematode assay are of little value if the field sample is inadequate.

Chapter 4 briefly discusses handling and storage procedures for nematode samples.

Chapter 5 provides details for basic nematode extraction procedures both from soil and plant material and lists the advantages and disadvantages of each. It also discusses bioassay procedures for detecting and quantifying nematodes. Several procedures are often used together to achieve a desired result. Thus, the bulk of the soil may be removed by sieving or elutriation, but the final separation of nematodes from debris may be done on an extraction tray or by centrifugal flotation. No single extraction method is suitable for use in all situations. The size, mobility and life cycle stage of the nematodes being extracted determine the appropriate technique. Soil texture is also important, as some methods work well only for sandy soils.

After nematodes have been extracted from soil or roots, they must be identified and counted under a microscope. Chapter 6 provides an in-depth description of these procedures, and in fact takes up more than half this whole report on this issue, detailing nematode biology and morphology, characteristics of major nematode groups, and keys to identification.

Chapter 7 looks at interpreting results of nematode assays and points out that although a nematode count using appropriate procedures is important for diagnostic purposes, it should not be viewed as the only information needed to make a management decision. Knowledge of the agronomy of the crop, historical information on nematode population densities, details of responses to nematicides in previous crops, observations of symptoms and their distribution in the current crop and knowledge of the root diseases and other limiting factors that are present should all be used in interpreting the result. A certain number of nematodes may cause problems in one situation, but may be relatively unimportant in another environment or under a different crop management system.

Finally, Chapter 8 looks at the quarantine importance of diagnosing nematodes. The isolation of Australia and New Zealand from the rest of the world and the relatively strict quarantine regulations in both countries means that we are not plagued by many of the nematode pests that are important elsewhere. However, since new nematode pests are most likely to be detected in diagnostic samples, people involved in diagnostic services should be aware of the range of nematodes that could be introduced and need to be continually vigilant with regard to quarantine issues.

In addition to detecting newly introduced nematodes, diagnostic services may become involved in quarantine issues through their role in assessing samples of produce destined for overseas export markets. As international trade increases, there will be an increasing demand from overseas quarantine authorities to ensure that agricultural products (e.g. tubers, rhizomes, rooted plants), are free of specific nematodes. If protocols for collecting and processing samples are set by the importing country, these must be followed by the diagnostic service. In the absence of specific protocols, the level of risk should be assessed and appropriate assessment procedures developed.

1. Nematodes: Important Pests of Crop Plants

Nematodes are unsegmented worms. They are the most abundant group of multicellular animals on earth, thriving in most habitats. Parasitic forms affect the health and well-being of most plants and animals and cost the world's crop and livestock industries tens of billions of dollars per annum in lost production. In addition, more than two billion people currently suffer poor health from nematode infections. Nevertheless, not all nematodes are pests. Beneficial species are common and they contribute to the health of ecosystems through their role in nutrient cycling, by feeding on insects, weeds or plant pathogenic fungi or by preying on plant-parasitic nematodes.

Of the multitude of nematode species known, about 2500 species are able to feed on plants. Most attack roots and underground parts of plants (e.g. rhizomes, tubers). Some are able to feed on leaves and flowers. Nematodes are found in all climates and every type of soil, while every agricultural crop will host some species.

Plant-parasitic nematodes obtain their nutrients exclusively from plants. They cannot feed on dead plant tissue and must have access to living plants if they are to complete their life cycles. While feeding, nematodes may damage plants and cause disease. Some of the more important nematodes and the symptoms they produce are described in Table 1 and depicted in Figure 1.

Plant-parasitic nematodes are important pests but, because they live in soil, they rank amongst the most difficult pests to diagnose, identify and control. Their effects are often underestimated by farmers, agronomists and pest management consultants. Estimates from several independent studies suggest that plant-parasitic nematodes reduce global agricultural production by about 12%. This means that crop losses from nematodes may be as high as $400 million per annum in Australia alone. The extent of the problems caused by nematodes is illustrated by the information presented in Tables 2 and 3. These tables list the nematode species which are economically important on various crops grown in Australia and indicate that most field crops, horticultural crops and pastures are affected by at least one major nematode pest. Nematodes which cause indirect damage (e.g. by vectoring viruses or exacerbating damage caused by fungal pathogens) are not listed.
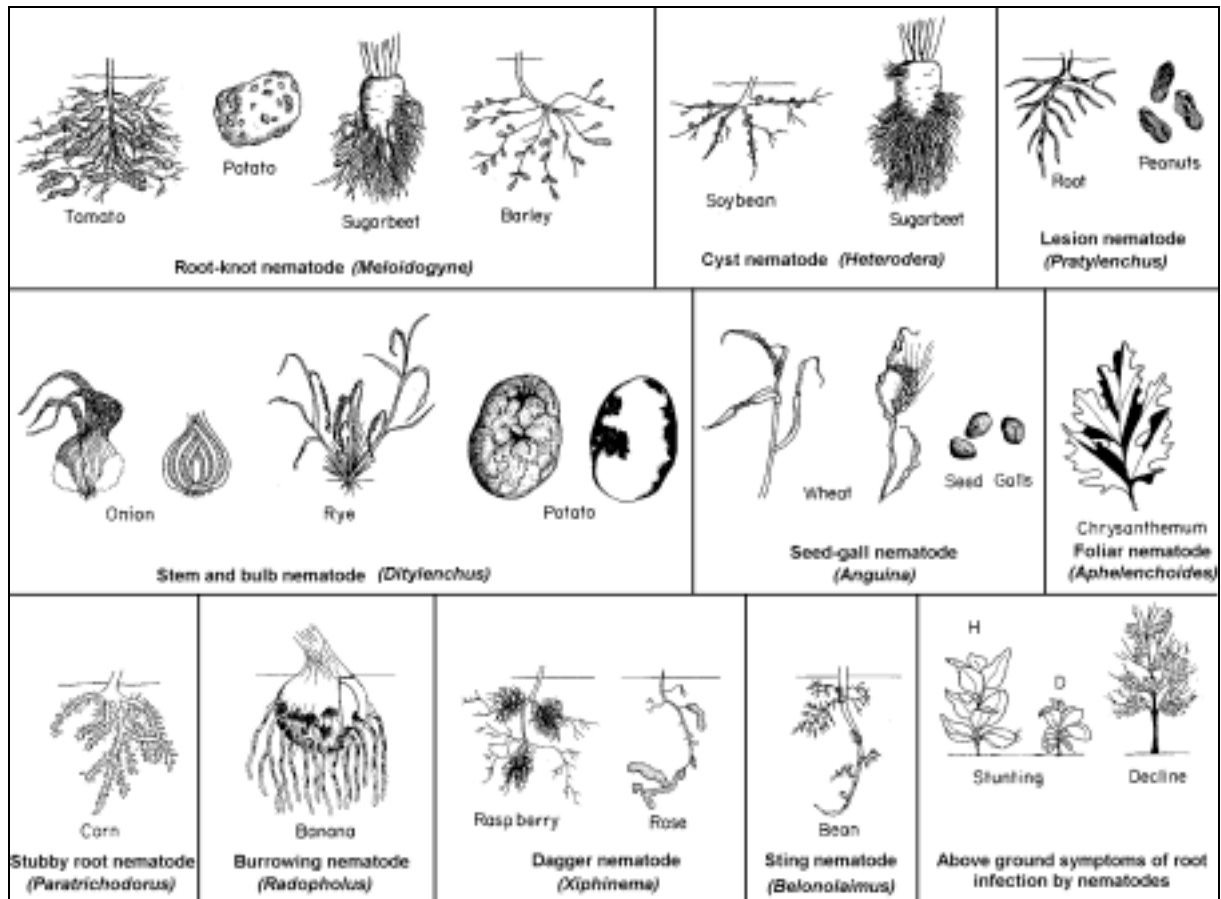
Figure 1. Types of symptoms caused by some of the most important plant-parasitic nematodes. From "Plant Diseases caused by Nematodes" in Plant Pathology, 3rd. edition by George N. Agrios (1988). Reprinted by permission of Academic Press Inc.

Table 1. **Symptoms produced by plant-parasitic nematodes on crops of economic importance in Australia and New Zealand.**

Common Name	Genera	Symptoms	Crops
Root knot	*Meloidogyne*	Stunting and galling of roots	Hundreds of horticultural, ornamental and field crops and many pasture species
Cyst	*Heterodera* *Globodera*	Malformation and stunting and of roots	Cereals, brassicas, potato, clovers, turf
Lesion	*Pratylenchus*	Root lesions, destruction of feeder roots	Numerous crops including cereals, sugarcane, grape, stone fruit, pome fruit, peanut, pineapple, turf
Burrowing	*Radopholus*	Girdling and rotting of roots	Banana
Citrus	*Tylenchulus*	Stunting and general deterioration of roots	Citrus, grape
Reniform	*Rotylenchulus*	Destruction of feeder roots	Pineapple
Stubby root	*Paratrichodorus*	Stunting, debilitation and swelling of root tips	Numerous crops, but particularly sugarcane and turf
Dagger	*Xiphinema*	Stunting, galling and malfunction of root tips	Numerous crops, particularly perennial horticultural crops
Needle	*Paralongidorus*	Stunting and necrosis of root tips	Rice
Spiral	Several genera, but particularly *Helicotylenchus* and *Rotylenchus*	General debilitation and loss of feeder roots	Generally of limited importance. One species damaging to banana
Pin	*Paratylenchus*	General debilitation and loss of feeder roots	Most crops. Can be damaging in specific situations, but generally unimportant
Stunt	*Tylenchorhynchus*	”	”
Sheath	*Hemicycliophora*	”	”
Ring	*Criconemella, Criconema*	”	”
Stem and bulb	*Ditylenchus*	Leaf distortion, swelling of crown tissues, discoloration of bulbs	Ornamental bulb crops, onions, oats, rye
Seed and leaf gall	*Anguina*	Stem, leaf and seed galls	Grasses
Leaf	*Aphelenchoides*	Necrotic lesions on leaves, leaf distortion, malformation of plant	Chrysanthemums, ferns, lilies and other ornamentals, strawberry

Table 2. Nematodes of economic importance[*] on field crops and pastures in Australia and New Zealand.

Crop	Nematode	Comments on importance
Barley	cereal cyst nematode (*Heterodera avenae*)	Can cause heavy losses in southern cereal-growing areas.
	lesion nematode (*Pratylenchus neglectus*)	Widespread and causes some yield loss.
Canola	lesion nematode (*Pratylenchus neglectus*)	A good host but yield is not affected.
Chickpea	lesion nematode (*Pratylenchus thornei, P. neglectus*)	Both species can increase to high populations and cause significant losses.
Clover (subterranean)	root-knot nematode (*Meloidogyne hapla*)	Severe root damage can occur but distribution and importance unknown.
(white)	root-knot nematode (*Meloidogyne* spp.)	Widely distributed and causes severe root damage, particularly in warm climates.
	clover cyst nematode (*Heterodera trifolii*)	Widely distributed and an important contributor to poor productivity and lack of persistence.
	stem nematode (*Ditylenchus dipsaci*)	Occurs in high rainfall areas of southern Australia and in New Zealand. Can cause heavy losses.
Cotton	no records	Nematodes can be important overseas but no studies have been done in Australia.
Cowpea	root-knot nematode (*Meloidogyne* spp.)	May cause problems to root-knot susceptible crops when grown in rotation as a green manure crop. Some cultivars resistant.
Faba bean	Stem nematode (*Ditylenchus dipsaci*-oat race)	Excellent host but very tolerant.
Kikuyu	lesion nematode (*Pratylenchus zeae*)	Present in high numbers in some areas of pasture decline but importance has not been confirmed.
Lucerne	root-knot nematode (*Meloidogyne* spp.)	Crop growth can be affected, particularly in light soils.
	lesion nematode (*Pratylenchus brachyurus, P. neglectus, P. coffeae*)	High populations can cause poor growth but importance not evaluated.
	stem nematode (*Ditylenchus dipsaci*-lucerne race)	Causes patchiness and reduced persistence.
Lupin	root-knot nematode (*Meloidogyne* spp.)	May cause losses on light soil.
Maize	lesion nematode (*Pratylenchus zeae*)	High populations have been associated with poor early growth, but importance not evaluated.
Mung bean	root-knot nematode (*Meloidogyne* spp.)	Severe galling has been observed on crops grown in light soil.
	lesion nematode (*Pratylenchus thornei*)	High populations associated with poor growth in heavy soil on the Darling Downs.
Mustard	lesion nematode (*Pratylenchus neglectus*)	Excellent host. Effect on yield not known.
Navy bean	root-knot nematode (*Meloidogyne* spp.)	Occasionally severe when crop grown on light soil.
	lesion nematode (*Pratylenchus brachyurus*)	Common and may cause yield reductions in early sown crops.

Table 2.   continued

Crop	Nematode	Comments on importance
Oats	cyst nematode (*Heterodera avenae*)	Resistant but intolerant to the nematode. Crop losses occur when oats are sown in fields where high populations have developed on other cereals.
	lesion nematode (*Pratylenchus neglectus*)	Susceptible but intolerant to the nematode.
	stem nematode (*Ditylenchus dipsaci*-oat race)	Widespread in high rainfall areas with clay soils. Susceptible varieties are totally destroyed, but resistant varieties are available.
Pea	stem nematode (*Ditylenchus dipsaci*-oat race)	Susceptible. Seedlings are very intolerant.
Peanuts	root-knot nematode (*Meloidogyne hapla*)	Problem on some crops in Queensland but usually controlled by crop rotation.
	lesion nematode (*Pratylenchus brachyurus*)	Widespread and can cause economic damage.
Rice	needle nematode (*Paralongidorus australis*)	Causes severe root damage and heavy crop losses but restricted to the Burdekin area of Queensland.
Sorghum	lesion nematode (*Pratylenchus thornei, P. brachyurus, P. zeae*)	Some stunting of crops associated with high nematode populations.
Soybean	root-knot nematode (*Meloidogyne* spp.)	Can be severe when present on a susceptible cultivar.
	lesion nematode (*Pratylenchus brachyurus, P. thornei, P. zeae, P. penetrans*)	May reduce yield but importance not investigated.
Stylosanthes	root-knot nematode (*Meloidogyne* spp.)	Severe galling seen on some plantings.
Sunflower	root-knot nematode (*Meloidogyne* spp.)	Considered unimportant but damage has been observed on crops in light soil.
Tobacco	root-knot nematode (*Meloidogyne* spp.)	Major problem. Nematicides are widely used and usually give good control.
	lesion nematode (*Pratylenchus brachyurus*)	Common on tobacco following pastures but importance unknown.
Triticale		Resistant to nematodes that are important in cereal-growing areas.
Vetch	lesion nematodes (*Pratylenchus neglectus and P. thornei*)	Resistant to *P. neglectus*, susceptible to *P. thornei*.
Wheat	cyst nematode (*Heterodera avenae*)	Reduces yield throughout the southern wheat belt.
	lesion nematode (*Pratylenchus thornei* and *P. neglectus*)	Widespread and of economic importance.

[*]Nematodes are listed when there is good evidence that they cause economic losses. Many other species usually are associated with each crop but, in many cases, their importance has not been assessed.

Table 3.   Nematodes of economic importance* on horticultural crops in Australia and New Zealand.

Crop	Nematode	Comments on importance
Almond	root-knot nematode (*Meloidogyne* spp.)	Widespread and can cause serious damage on some rootstocks in light soils.
Aloe vera	root-knot nematode (*Meloidogyne* spp.)	Common and can cause heavy losses.
Apple	lesion nematodes (*Pratylenchus penetrans* and *P. jordanensis*)	A major problem, particularly in replant situations.
	dagger nematode (*Xiphinema* sp.)	Probably important when present in high populations.
Avocado	few records	Nematodes probably not of major importance.
Banana	burrowing nematode (*Radopholus similis*)	A major problem in all banana growing areas.
	spiral nematodes (*Helicotylenchus multicinctus, H. dihystera*)	*H. dihystera* widespread but not important. *H. multicinctus* less common and can cause damage.
	root-knot nematode (*Meloidogyne* spp.)	Common but considered unimportant. Can cause damage to tissue cultured plants.
	lesion nematodes (*Pratylenchus coffeae, P. goodeyi*)	Widespread and important. Symptoms can be confused with burrowing nematode.
Bean (French)	root-knot nematode (*Meloidogyne* spp.)	Occasional problem.
Capsicum	root-knot nematode (Meloidogyne spp.)	Can be a problem but not common because pre-plant nematicides are usually used.
Carrot	root-knot nematode (*Meloidogyne* spp.)	Major problem on light soils.
Celery	root-knot nematode (*Meloidogyne* spp.)	Can be damaging in some areas.
Citrus	citrus nematode (*Tylenchulus semipenetrans*)	Widespread. Causes a slow decline of established trees.
	lesion nematode (*Pratylenchus brachyurus*)	Occasionally present in high numbers.
Brassicas (broccoli, cabbage, cauliflower, beet, turnip)	beet cyst nematode (*Heterodera schachtii*)	Can be a serious problem on farms where continuous cropping is practiced.
	root-knot nematode (*Meloidogyne* spp.)	Occasionally a problem on light soils.
Cucurbits (cucumber, squash, gherkin, zucchini, melons)	root-knot nematode (*Meloidogyne* spp.)	Occasional problem. Can be serious on pumpkins and melons grown under high temperatures in light soils.
Custard apple	burrowing nematode (*Radopholus similis*)	Associated with decline of trees in Queensland. Distribution and importance unknown.
Duboisia	root-knot nematode (*Meloidogyne* spp.)	A serious problem in Queensland.
Eggplant	root-knot nematode (*Meloidogyne* spp.)	Common on light soils and can cause heavy losses.
Ginger	root-knot nematode (*Meloidogyne* spp.)	Major problem but usually controlled with nematicides.
	burrowing nematode (*Radopholus similis*)	Causes rotting of rhizomes but not widely distributed.

Table 3.   continued

Crop	Nematode	Comments on importance
Grape	root-knot nematode (*Meloidogyne* spp.)	Widespread. A serious problem on own-rooted vines.
	lesion nematode (*Pratylenchus* spp.)	High populations in some vineyards will cause economic losses.
	citrus nematode (*Tylenchulus semipenetrans*)	Common and damaging in medium-textured soils where grapes are grown in association with citrus.
	dagger nematode (*Xiphinema* spp.)	Common, but importance masked by other nematodes.
Kiwi fruit	root-knot nematode (*Meloidogyne* spp.)	Common but a minor problem with good vine and water management.
Lettuce	root-knot nematode (*Meloidogyne* spp.)	Major problem in areas of monoculture.
Lychee	dagger nematode (*Xiphinema* spp.)	Present in some plantings but significance not evaluated.
Macadamia	dagger nematode (*Xiphinema* spp.)	Present in some plantings but significance not evaluated.
	lesion nematode (*Pratylenchus* spp.)	Has been associated with poor seedling growth but effects not known.
Mango	few records	Nematodes not a major problem, but their importance not evaluated.
Onion and garlic	stem and bulb (*Ditylenchus dipsaci*)	Is an occasional problem in cool climates.
	root-knot nematode (*Meloidogyne* spp.)	Rarely observed.
Ornamental crops		
• carnations	root-knot nematode (*Meloidogyne* spp.)	A major problem if not controlled with pre-plant nematicides.
• chrysanthemum	leaf nematode (*Aphelenchoides ritzemabosi*)	Severe problem in some seasons.
• various ferns	leaf nematode (*Aphelenchoides fragariae*)	Can cause heavy losses when environmental conditions are conducive.
• protea	root-knot nematode (*Meloidogyne* spp.)	Sometimes cause severe root damage.
• rose	root-knot nematode (*Meloidogyne* spp.)	Can be a major problem in commercial plantings.
• riceflower	root-knot nematode (*Meloidogyne* spp.)	One of the main factors limiting riceflower production.
Papaya	root-knot nematode (*Meloidogyne* spp.)	Not a general problem, but can be severe in some old plantings and in replant situations.
Passionfruit	root-knot nematode (*Meloidogyne* spp.)	Severe damage on *Passiflora edulis* but not on other rootstocks.
Pear	lesion nematode (*Pratylenchus penetrans*)	Distribution unknown but merits investigation, particularly in replant situations.
Pineapple	reniform nematode (*Rotylenchulus reniformis*)	Can cause heavy losses but restricted mainly to north Queensland.
	root-knot nematode (*Meloidogyne javanica*)	Major problem. Nematicide treatment is essential for control.
	lesion nematode (*Pratylenchus brachyurus*)	Widely distributed and sometimes increases to levels which cause economic damage.

Table 3. continued

Crop	Nematode	Comments on importance
Potato	root-knot nematode (*Meloidogyne* spp.)	May cause severe damage but importance depends on planting time, the level of infestation in seed and the susceptibility of the previous crop.
	lesion nematode (*Pratylenchus* spp.)	Heavy losses from 'pimple' have been seen in Queensland on potatoes grown after pasture. May increase susceptibility of potatoes to *Verticillium* wilt.
Rhubarb	root-knot nematode (*Meloidogyne* spp.)	Occasional problem.
	beet cyst nematode (*Heterodera schachtii*)	Occasional problem.
Peach	root-knot nematode (*Meloidogyne* spp.)	Serious on some rootstocks. Nemaguard and Okinawa are resistant.
	lesion nematode (*Pratylenchus* spp.)	Can cause significant root damage but importance tends to be underestimated.
	dagger nematode (*Xiphinema* spp.)	Common but significance not fully evaluated.
Strawberry	bud nematode (*Aphelenchoides besseyi*)	Rarely a problem as nematode-free planting material will prevent its introduction.
	root-knot nematode (*Meloidogyne hapla*)	Once a serious problem but now controlled with clean planting material.
	lesion nematode (*Pratylenchus vulnus*)	Previously a problem, but now rarely seen.
Sweet corn	root-knot nematode (*Meloidogyne* spp.)	Minor problem. High populations may occur on some varieties when grown in rotation with susceptible crops.
Tea	root-knot nematode (*Meloidogyne* spp.)	Severe galling can occur on seedlings but not a problem on mature plants.
Tomato	root-knot nematode (*Meloidogyne* spp.)	Widespread problem. Pre-plant nematicides or appropriate rotations essential for adequate control.
	reniform nematode (*Rotylenchulus reniformis*)	Reduces yield in north Queensland but uneconomic to control.
Turf	lesion nematode (*Pratylenchus zeae*)	Widespread. Associated with poor growth of several turf grasses.
	stubby root nematode (*Paratrichodorus minor*)	Widespread and economically important.
	sting nematode (*Belonolaimus lolii*)	Serious damage, but distribution restricted.
	cyst nematode (*Heterodera graminis*)	Causes poor growth, but distribution limited to certain areas of NSW.
	dagger nematode (*Xiphinema* spp.)	Common and probably important. Tends to be underestimated because many other nematodes are usually present.

[*]Nematodes are listed when there is good evidence that they cause economic losses. Many other species usually are associated with each crop, but in many cases, their importance has not been assessed.

2. Integrated Nematode Management

In the past, the options available for nematode control tended to depend on the intensity of cropping and the value of the crop. Thus for field crops, where nematicides were too expensive to use on more than a limited scale, crop rotation and the use of cultivar resistance were the dominant control measures. In intensively managed horticultural, vegetable and ornamental crops, nematicides and broad-spectrum soil fumigants were used routinely for nematode control. Recently, however, chemical control options have diminished because nematicides are being withdrawn for health and environmental reasons. Horticultural industries are therefore moving towards a more integrated approach to nematode control. Thus in all industries where nematodes cause problems, there is a trend toward integrated pest management (IPM), with all available control options now being used in a compatible manner to reduce nematode populations to levels which cause tolerable economic loss.

With the development of the concept of IPM, monitoring for above-ground pests has become an increasingly important component of modern agriculture. Samples are collected to determine pest infestation levels in an established crop or the pattern of pest distribution in a field. This information is used to determine whether or not action needs to be taken against the pest. Such procedures are now used for many insect pests, but their success is only as good as the quality of the sample upon which population measurements are based. Equally important are the reliability of the methods used to estimate the population density of the pest and the quality of the information available on the relationship between pest density and yield. The same conditions apply when monitoring for nematode pests, with the procedures involved being outlined in Table 4.

Table 4.   **Nematode monitoring: a useful component of integrated pest management programs for nematodes.**

THE CONCEPT
"Nematode infestation levels are monitored, a management decision is made and appropriate strategies are used to maintain nematode population densities at levels which cause tolerable crop losses".

THE PROCESS

Sample collection
Soil (or root) samples are collected from a field.

↓

Nematode extraction

Nematodes are extracted from samples using methods which are appropriate for the situation and the nematode species expected. Quality control procedures are employed to ensure repeatable results.

↓

Nematode identification

Nematodes are identified to at least genus level and counted by examining nematode suspensions under a microscope in the laboratory. An appropriate molecular test may also be used to identify and quantify nematodes.

↓

Nematode quantification

Actual nematode population density is estimated by correcting counts for extraction efficiency, or by extrapolating from the relationship between molecular measurements and nematode density.

↓

Relationship between nematode numbers and damage threshold

Estimates of nematode population density are compared with the damage threshold for the particular nematode and crop. Factors which affect the relationship between nematode numbers and yield (e.g. soil texture, season of year, presence of other root pathogens, moisture availability and nutrient management) are also considered.

↓

Management decision

A decision is made on whether nematodes should be controlled. No action may be required and monitoring may continue, or one or more of the following control procedures may be employed:
- crop rotation, fallowing
- resistant/tolerant cultivar
- green manuring/organic amendments
- various cultural controls
- improved crop husbandry
- biological controls
- nematicides

As growers become aware that the principles of IPM apply just as much to nematodes as they do to other pests, there will be a trend away from the use of nematicides and other single control tactics. Non-chemical control measures such as cultivar resistance and biological control will be used more commonly. Since such control measures are specific to certain nematode species, growers will need accurate species identification if they are to use them successfully. In situations where chemical control remains an option, economic and environmental considerations will mean that growers will be obliged to determine whether nematode population densities are high enough to warrant treatment.

The difficulty of making appropriate management decisions about nematode control is compounded by the fact that nematodes are only one of many possible causes of root disease problems and sub-optimal crop yields. Other factors that are often involved with nematodes in causing such problems include:

- fungal pathogens (e.g. *Pythium*, *Phytophthora*, *Fusarium*, *Verticillium*, *Rhizoctonia* and *Sclerotinia*), bacterial pathogens (e.g. *Ralstonia*) and root-feeding arthropods (e.g. white grubs, symphylids) .
- compacted, poorly aerated soils
- moisture stress
- nutritional deficiencies, excesses or imbalances
- salinity
- poor drainage, waterlogging
- herbicide damage

The difficulties involved in diagnosing nematode problems and the growing complexity of integrated management programs for nematodes mean that there will be an increasing demand from farmers for professional help in making decisions on nematode management. That help is likely to come initially from agricultural advisers and pest management consultants who work at the farm level and have an intimate knowledge of the cropping system involved. By sending samples to a diagnostic laboratory and liaising with a nematologist, they should be able to make appropriate recommendations. The issues which must be considered if this process is to provide farmers with the best available nematological advice are described in the remainder of this book

2.1   Diagnostic and advisory services for nematodes

Before a nematode problem can be diagnosed with certainty, nematodes must be separated from soil samples or plant material and then identified and counted. Since there are costs involved in such analyses, the first step in providing advice on nematodes is to consider whether a sample for nematodes is really necessary. The following guidelines give some indication of the situations where nematode samples are most likely to be required.

- Nematodes are especially problematic when soils and irrigation practices are marginal and management is poor. Marginal soils generally include sands and heavy clays, shallow soils and highly stratified soils. Irrigation practices are marginal if they fail to provide water at the required frequency or quantity. Poor management involves inadequate crop rotation or cropping sequences (e.g. several nematode susceptible crops in succession). Growers with medium textured soils of adequate rooting depth

who use good irrigation management and reasonable rotation practices are not as likely to experience nematode problems.

- Nematode problems often appear as irregular patches of poor growth in a field. Unthrifty plants are often associated with sandy patches or areas of lighter soil texture.
- Certain crops are highly susceptible to specific nematodes (see Table 1). Also, there are certain regions where nematode problems are endemic (e.g. the sandy soils of coastal Queensland, the inland irrigation areas of New South Wales, Victoria and South Australia, and the sandy soils of Western Australia).
- History of nematicide use is a useful guide to whether nematodes are likely to be a problem. Generally, when crop yields increase after nematicide application, nematodes are important. However, one must be cautious in interpreting the causes of such responses, as some pesticides have a wide spectrum of activity and may affect organisms other than nematodes. Also, it is possible to underestimate the importance of nematodes if the application conditions are sub-optimal for the nematicide.

2.2   Diagnostic and predictive assays

Once the decision has been made to sample for nematodes, some consideration should be given to the type of sample that is required. If a crop is already established, root and soil samples may be collected to determine whether nematodes are causing the poor growth observed. Root diseases can also be caused by fungi, bacteria, root-feeding insects and a range of soil physical and chemical factors. It is therefore important to identify the cause so that appropriate control measures are used. Such assays are sometimes referred to as diagnostic assays.

When samples are collected before planting, a nematode assay can indicate whether the nematode population density is high enough to cause damage in the following crop. Since most nematode management decisions are made before planting, a pre-plant nematode assessment can be used to decide whether nematode control measures are warranted. Such assays are sometimes referred to as predictive assays.

Predictive samples can also be taken from established crops. They are particularly useful in perennial crops where nematicides are registered for application after planting (e.g. citrus, grape, banana and pineapple). Nematode populations are monitored on a regular basis and the data are used to decide when a nematicide should be applied.

When a nematode problem is being diagnosed, diagnostic assays provide important information. However, such assays are always done after damage has occurred, when it is often too late to apply appropriate control measures. In future, there should be more emphasis on predictive assays, so that information on potentially damaging nematode populations is available before damage is done. It is then possible to implement control measures which will avoid or minimise damage.

The protocols outlined in Tables 5 and 6 can be used as a general guide for collecting diagnostic and predictive samples.

Table 5.   Protocol for diagnosing nematode problems in an established crop.

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Objective:
- To determine whether nematodes are involved in a decline or poor growth problem in an established crop.

What to sample?

- Assess the symptoms and note their distribution (random, regular, etc).
- Look for a gradation in symptoms (i.e. slight, moderate and severe) and sample plants with different symptom severity separately. Also collect samples from healthy plants. Do not sample plants that are in very poor health or dead.
- Collect both roots and soil. Some plant-parasitic nematodes are found mainly in soil but others remain in roots for most of their life cycle.
- When nematodes that affect flowers, leaves, and stems are suspected, collect diseased tissue from affected plants

Where to sample?

- Sample the root zone of affected plants (e.g. at the outer edge of the foliage for tree crops and the wetted soil area in crops where roots are concentrated by trickle irrigation).
- Sampling depth varies with the host crop.
  Turf: 0-10 cm
  Field crops, pastures, vegetables: 0-20 cm
  Trees, vines, perennial ornamentals: 10-30 cm (discard the surface soil to minimise the influence of dried topsoil, weeds and cover crops)

How to sample

- Use a spade, auger or sampling tube to collect roots and a handful of soil and place in a bucket. Repeat this process at least five times to obtain a representative sample. Mix gently and retain 500g soil and 100g roots for laboratory analysis.

Care of samples

Samples should be sealed in a plastic bag to retain moisture. Do not expose to excessive heat. Temperatures above 40°C for more than a few minutes (e.g. by leaving samples in a closed vehicle in the sun during summer) can kill nematodes. Refrigeration should be avoided as it is detrimental to some warm-climate species. Temperatures of 10-25°C are ideal and can be maintained in an insulated picnic cooler, in an air conditioned building or vehicle, or in a cool, shady area. Dispatch to the laboratory as soon as possible.

Information required

Diagnoses are improved if adequate background information is provided. The following details are particularly important:
  Crop and cultivar
  Previous crop
  Area involved
  Description of symptoms and their distribution
  Soil texture, soil depth and variability of soil
  Frequency of irrigation and/or rainfall
  Previous nematicide use and details of responses obtained

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Table 6.   Protocol for collecting predictive samples for nematodes.

<u>**Objective**</u>
To estimate nematode population density and use the estimate to predict whether nematodes are likely to cause problems later in the current crop or in the next crop.

<u>**What to sample?**</u>
- Roots or soil, depending on the method of analysis for the particular crop. For example:
        citrus - roots or soil
        grape - roots and soil
        pineapple - soil
        banana - roots
        fallow fields - soil

<u>**How to sample?**</u>
- Subdivide the field into areas that have similar cropping history, soil texture or soil depth, or that are homogeneous with regard to other possible variables.
- Collect a composite sample of roots or soil that is representative of the sampling area. Composite samples should be made up of at least 20 and preferably 50 sub-samples. These sub-samples should be mixed and about 500g soil or 100g roots retained for analysis. Larger volumes of soil are needed for bioassays.

<u>**When to sample**</u>
- This will vary for each crop but is generally a defined period that is related to the growth stage of the crop. Pre-plant samples must be collected 4-6 weeks before planting to allow time to process the sample and apply control measures. Sometimes a longer time-frame may be needed (e.g. if nematode-resistant planting material must be ordered).

<u>**Care of samples**</u>
See details for diagnostic samples (Table 5)

<u>**Information required**</u>
- Current crop and cultivar (or the crop and cultivar to be planted in the case of pre-plant samples)
- Details of the site (e.g. area, soil type, variability of soil, previous cropping history).
- Standard of crop management, particularly with regard to irrigation and nutrition.
- Likely importance of other pests and pathogens of roots.
- Details of previous nematicide use and the responses that were obtained.

2.3 Interpreting the results of nematode assays

Assays for nematodes are an indispensable aid to making rational decisions about nematode management. However, such assays are of limited value when samples are of poor quality. It is particularly important that samples are representative of the sampling area. Samples should also reach the laboratory in good condition because the efficiency of some extraction techniques is influenced by the viability of the nematodes in the sample.

Since IPM practices for nematodes cannot be effective unless estimates of nematode population density are accurate, strict attention must be given to standardising laboratory procedures. Appropriate extraction methods must be employed and nematodes must be identified correctly. To ensure that estimates reflect the actual number of nematodes present, nematode counts should be corrected for extraction efficiency. Nematode counts reported in this way can be compared with results from other laboratories or related to published data on damage thresholds.

One major limitation to interpreting results of nematode assays is the availability of reliable data on the relationship between nematode numbers and yield for the crop or nematode of interest. A typical relationship between nematode numbers and yield is shown in Figure 2A, with yield losses increasing as nematode population density increases. In this hypothetical example, there is little yield loss at population densities less than 100 nematodes per unit of soil (part X of the graph) and a steady decline in yield as nematode populations increase from 100 to 1000 nematodes/unit of soil (part Y). Once such a relationship has been established, making recommendations on nematode control is relatively straightforward. At population densities less than the tolerance level (T) of 100 nematodes/unit of soil, the crop is not damaged by nematodes and no action is necessary. Some damage occurs at populations of 100-200 nematodes/unit of soil, but yield losses are insufficient to justify the cost of treatment. It is only when the population is above the economic threshold (E) of 200 nematodes/unit of soil that control is warranted.

In practice, the situation is not so simple. There is a much broader relationship between nematode numbers and yield, with the relationship varying with prevailing field conditions, including soil texture, cultivar, nutritional status and available moisture. Thus the real situation may be closer to that depicted in Figure 2B. In a sandy soil, with an intolerant cultivar or where moisture or nutrients are limiting, curve number 1 may apply, with the economic threshold being reached when the nematode population approaches 200 nematodes/unit of soil (E_1). In another scenario (e.g. in a clay loam soil, with a tolerant cultivar or where moisture or nutrients are not limiting), the population would have to reach 400 nematodes/unit of soil (E_2) before control measures are considered.

Because environmental factors and the standard of crop husbandry influence the relationship between nematode density and yield, it is important that they are considered when results of nematode assays are being interpreted. Assay results may indicate the potential for nematode damage, but with a favourable environment and good crop management, losses due to nematodes may be minimal.
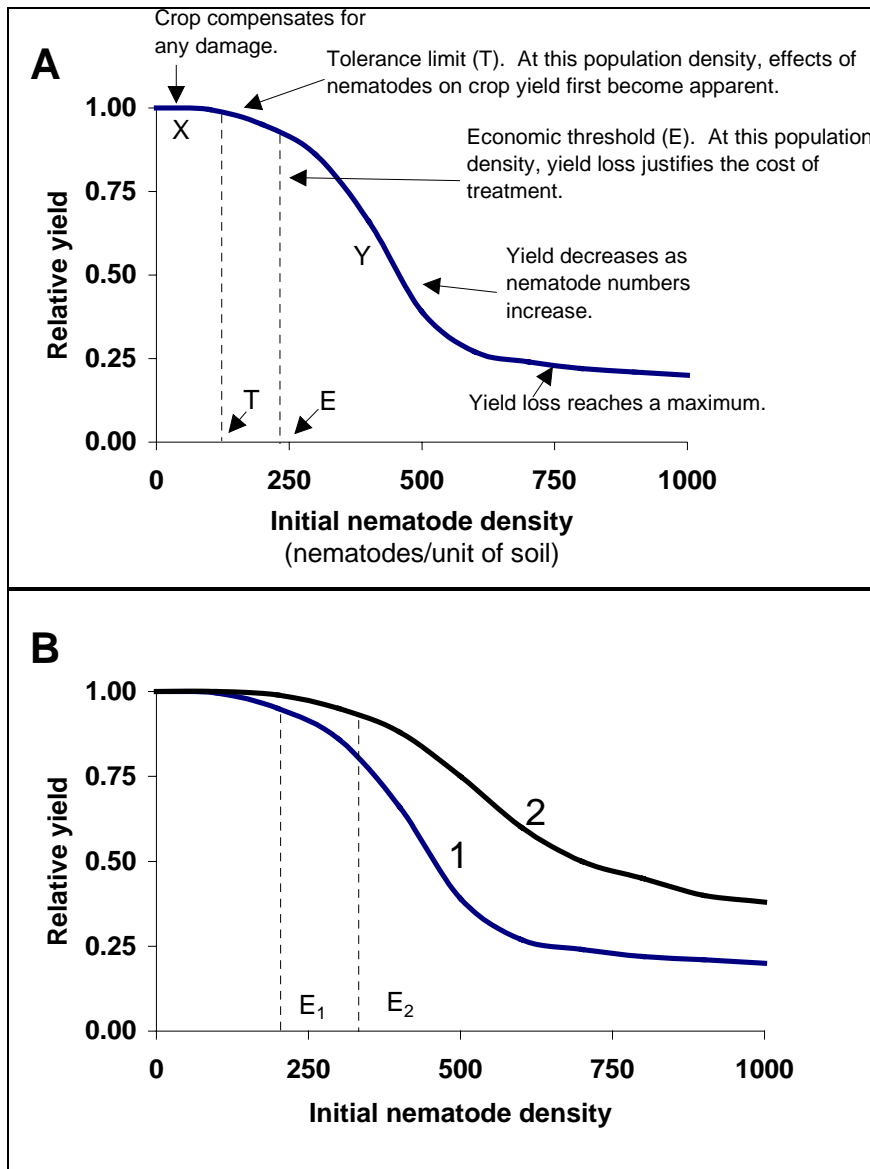
Figure 2. **Hypothetical relationship between nematode population density and yield. A, typical relationship; B, Relationship as influenced by other factors (e.g. soil texture).**

Since damage thresholds can differ from one management system to another, successful predictive systems at a regional or farm level may depend to some extent on the availability of local data on losses caused by nematodes. Well-monitored nematicide test strips can provide such data, and can be used to indicate the nematode population densities that cause yield losses in local soils and local crop management systems.

3. Sampling for Nematodes

Unlike some pests and diseases, nematodes cannot be monitored by observation in the field. Nematodes must be extracted for microscopic examination in the laboratory and so an additional, relatively expensive processing step is involved. The laborious nature of nematode processing procedures means that the number of samples must be kept to a minimum. Sampling methodology is therefore crucial, as the few samples that are collected must be representative of the area involved. Even with the best laboratory procedures, data obtained from a nematode assay are of little value if the field sample is inadequate.

3.1 Sampling considerations

When sampling for nematodes, the following points should be taken into consideration.

Aggregated distribution of nematodes. Since plant-parasitic nematodes feed on plant tissues, they aggregate around plants. Their distribution is therefore related to the distribution of their hosts. In the case of species that feed on roots, depth and lateral distribution in soil is influenced by the degree of proliferation of the root system.

Effect of soil parameters on nematode distribution. Nematodes are rarely distributed evenly across a field. Some species are favoured by certain soils and their distribution may change with subtle changes in soil texture.

Changes in nematode population with time. Nematode populations fluctuate over time. Numbers will increase in the presence of a host crop, the rate of increase being greatest when environmental conditions are favourable. When a field is fallow or a non-host crop is present, nematodes die of starvation and populations decline. The number of nematodes in a sample will therefore be affected by sampling time.

Soil moisture. Many plant-parasitic nematodes have the capacity to survive periods of dryness through a behavioural adaptation in which their surface area is reduced by a process known as coiling. Since nematodes are very susceptible to mechanical damage in this desiccated state, the process of collecting samples from dry soils may damage nematodes. Nematode population densities are therefore likely to be underestimated in dry soil and it is preferable to wait until soil moisture is adequate before collecting samples.

Vertical distribution. Nematodes are mobile animals that tend to migrate to a favourable environment in soil. When a host crop is present, their vertical distribution generally mirrors that of the crop root system (particularly the fine roots on which nematodes feed). When that field is fallowed, the existing distribution will remain, except that the bulk of the population may be slightly deeper in soil (largely due to desiccation of nematodes near the surface).

3.2 Sampling procedures

The precision of an estimate of nematode population density will improve as the number of samples taken is increased. However, to minimise cost and time, it is important to collect a minimum number of representative samples. In the case of soil, this is done by collecting a composite of a large number of cores from different points in the field. Thus, the main sampling decision that must be made is the number of cores to be included in a single composite sample. As a general guide, a single composite sample of about 50 cores from an area of 0.5 to 1 ha will give a population estimate within 25% of the true mean.

The size of the sampling unit varies from crop to crop and is largely determined by the value of the crop. Thus, economics dictates that sampling can be more intensive on high value horticultural and ornamental crops than on low value field crops. As a rule of thumb, it is not unreasonable for a grower to protect an investment by spending 1% of the value of a crop on nematode sampling. Assuming a cost of $50/sample, this means that the sampling unit may be less than 0.5 ha in many horticultural crops and greater than 5 ha in cereal crops and pastures (Table 7).

Another way to make decisions about sampling intensity is to consider the likelihood of nematode damage, the likely extent of crop losses and the cost of control measures. When heavy losses are probable and the cost of control is relatively low, there is little point in sampling for nematodes, as control measures are likely to be implemented as a routine measure. Nematode assays are most useful when a nematode problem is potentially serious but sporadic in nature and the cost of control is relatively high. In such circumstances, sampling units can be relatively small because expenditure of a few hundred dollars per hectare on nematode assays is an acceptable risk minimisation strategy.

Table 7.   Relationship between crop value and size of sampling unit for nematode samples.

Value of crop ($/ha)	Sampling unit (ha)*
1000	5
2000	2.5
5000	1
10000	0.5
20000	0.25

* Area from which one representative sample should be collected if 1% of the crop value is spent on nematode sampling.

Once the size of the sampling unit has been established, fields should be subdivided into sections no greater than this area. Each sampling unit should be relatively uniform. This means that parts of the field which show different patterns of crop growth or have different soil moisture, soil texture or drainage characteristics, are sampled separately. A sample consisting of at least 50 cores is then taken for each sampling unit. A useful approach is to collect the soil cores in a clean bucket, mix the soil thoroughly and take a 500g sub-sample for processing. Be careful to mix the soil gently as nematodes can be destroyed by abrasion,

particularly if soil is dry. However, thorough mixing is essential, as poor sub-sampling procedures can introduce extra errors into the estimate.

Additional precision will be obtained by collecting more than 50 cores from each sampling unit. In seeking additional precision, it is important to recognise that the laboratory component of the analytical process is more expensive than field sampling. It therefore costs less to increase the precision of an estimate by increasing the number of cores per sample than by increasing the number of samples.

The principles which apply to sampling procedures for soil also apply to collecting roots or above-ground samples from plants. With regard to roots, few sampling protocols have been published. Duncan *et al.* (1994) discussed some of the issues involved in sampling for citrus nematode and estimated citrus fibrous root density to within 25% of the mean by sieving roots from two composite samples of 15 soil cores. Since nematode population density is regulated in part by fibrous root availability, such a sampling scheme is likely to provide a representative sample and should provide a reasonable estimate of nematode population density. Since the density of fibrous roots decreases with the distance from the trunk of the tree, it is more efficient to sample systematically at a defined distance from the tree trunk, rather than in a random manner.

For burrowing nematode on banana, sampling strategies developed in north Queensland by Pattison *et al.* (1997) involve collecting roots from 20 sampling points in each 2 ha section of the plantation. At each sampling point, a 25 x 25 x 25 cm block of soil is removed from near the stool with a spade and five roots a least 15 cm long are selected at random. Thus a total sample of 100 root pieces is available to index for root damage and for extraction of nematodes.

When nematodes produce above-ground symptoms, it is often more important to determine the proportion of plants infested than to estimate nematode population density. Since most above-ground parasites produce specific symptoms (e.g. crinkling and distortion of leaves, seed galls, or necrotic leaf lesions), an area can be surveyed by selecting plants at random and counting the proportion of infected plants.

Although the principles of sampling for nematodes are similar for all crops, the protocol used in each situation will depend on the nematode species, the value of the crop, the size of the field and the degree of accuracy required. The information provided in Table 8 indicates some of the issues that should be considered for a number of common situations.

Table 8.   Issues to be considered when collecting predictive samples for nematodes in different field situations.

Previously uncropped sites *-detection of nematodes that might affect the crop to be planted*	Annual broadacre field crops *-preplant sampling to determine whether nematodes are likely to cause problems in the next crop*	Perennial broadacre field crops and pastures *-sampling established crops to determine whether nematodes should be controlled*	Vegetables and annual ornamentals *-preplant sampling to determine whether control measures are needed in the next crop*	Perennial horticultural crops *-preplant sampling to determine whether nematodes are likely to cause problems after replanting*	Perennial horticultural crops *-sampling an established crop to determine whether nematodes are causing economic damage and nematicide treatment is warranted*	Turf *-sampling to determine if nematodes are causing economic damage*
•Sample intensively and use appropriate extraction procedures as nematode populations are likely to be low and difficult to detect. •Sample near vegetation rather than in bare soil. •Ensure that the sample is representative of the plant species present (i.e. sample all weeds, grasses, shrubs and trees). •If trees are present which might host economically important nematodes (e.g. *Meloidogyne* on *Acacia*), ensure some cores are taken in the root zone. •Collect samples at least two months before planting to allow time to bioassay soil for specific nematodes.	•Are economically important nematodes likely to be present? The host status of the previous crop is a useful guide. •Sample well in advance of planting to allow time for bioassays (e.g. 3-4 months for cereal cyst nematode). •Since large areas are involved, cost is an important consideration. Consider whether it is possible to sample only areas where nematode problems are most likely	•If there is variability in crop growth, sample poor patches separately from healthier areas. •Collect both roots and soil. •Observe roots for symptoms of nematode damage. •For deep rooted crops (e.g. lucerne) ensure that some samples are taken at depths where most of the roots occur. •Collect a composite sample of no fewer than 20 cores	•Consider making observations at the end of the previous crop. For nematodes such as root knot nematode, the level of galling provides an indication of the distribution or density of nematodes and their likely impact on the next crop. •Recognise that nematode populations decline in fallow soil. Since pre-plant nematode densities may be low, sampling and extraction procedures must be adequate to detect low numbers of potentially important nematodes at this time. •Collect samples early to allow time for bioassays (e.g. two months before planting for root-knot nematode). •Limit sampling units to about 0.5 ha and collect no less than 20 cores/sample.	•Previous cropping history will provide a good indication of the nematodes likely to be present in a replant situation. •If the previous crop was trees or vines, collect samples of old roots that may still be harbouring parasitic nematodes. •Where crops are to be planted in virgin soil, there may be a greater chance of introducing nematodes on machinery or planting material than having them in virgin soil. There is no point sampling for nematodes unless such issues are addressed.	•Samples should be collected in the root zone of the crop. •For some crops, roots rather than soil may give a more appropriate sample. •Limit sampling units to no more than 0.5 ha and ensure samples consist of a minimum of 20 sub-samples. •For burrowing nematode on banana, a root disease index may be more useful than a nematode count. •In some crops, samples should be collected at specific growth stages. On pineapple, for example, samples should be collected 12 months after planting and at plant crop harvest.	•Areas of poor or uneven growth should be sampled separately from healthy areas. •Collect samples from within the root zone (0-15 cm depth). •Use a thin corer (less than 10 mm diameter) and collect many small cores rather than a few large cores (e.g. at least 20 small cores per golf or bowling green).

4. Handling and Storage of Samples

4.1 Handling and transport

Soil and root samples for nematode assays are perishable and should be cared for accordingly. Samples should be placed in plastic bags and sealed tightly to keep the soil moist. Since roots are particularly vulnerable to drying out, a handful of moist soil should always be included with a root sample. Many nematodes are killed by exposure to temperatures above 40°C for as little as one hour and such overheating can readily occur if samples are placed in direct sunlight or in a closed vehicle. When conditions are hot, samples should be transported in insulated containers. Since some nematodes can be killed by refrigeration, samples are best stored at about 10°C.

Nematodes can also be damaged if packages are handled roughly while in transit to the laboratory. Samples should therefore be forwarded in a sturdy cardboard box that is tightly packed to minimise movement of samples. Details of samples are best recorded in pencil on a label and attached to the outside of each bag. Information written with a felt pen on the bag may be erased during transit and labels placed inside bags can deteriorate and become unreadable. When samples are posted to a laboratory, it is important to be aware of quarantine restrictions which may prohibit samples being transported from one area to another, from state to state or country to country.

4.2 Receipt of samples by the laboratory and storage

Upon receipt of samples, information supplied by the client must be matched to each sample and details recorded. Samples should be inspected and soils that are very dry should be noted, as the nematodes are likely to have been damaged during collection and in transit. Such samples should be moistened without being disturbed and then left for 24 hours before nematodes are extracted. Roots can deteriorate quickly and should be processed immediately.

When soil is stored in plastic bags at 10°C, most plant-parasitic nematodes will survive for a few weeks with only minor changes in nematode numbers. Storage of moist soil at temperatures which are sufficient for nematode activity (i.e. 15-25°C) may cause eggs to hatch and nematodes to emerge from root fragments, and can result in greater recovery of nematodes than from non-stored samples. Refrigeration is not always recommended because chilling injury can cause the death of some warm-climate species. At high temperatures, nematodes will quickly lose vitality because metabolic activity increases and their food reserves dwindle rapidly.

Conditions under which soil samples should be stored depend to some extent on the extraction procedure to be used. For methods which depend on nematode mobility (e.g. Baermann funnel, Whitehead tray), nematodes must be maintained in a physiologically active state and this is best achieved in moist soil at 10°C. However, where sugar flotation methods of extraction are used, soil samples can be stored at minus 15°C. This treatment will kill most species but they can be readily recovered by flotation. However, identification is often more difficult for dead nematodes than for living nematodes. When cyst nematodes are to be extracted using flotation methods, samples should be air-dried before storage.

5. Extraction Procedures

5.1 Extraction of worm-like nematodes from soil

A wide range of techniques are used to extract nematodes from soil, but many of these methods vary only in minor details. Most are variations on a few basic techniques that have been used by nematologists for many years. The main procedures and their advantages and disadvantages are listed in Table 9.

It should be apparent from Table 9 that several procedures are often used together to achieve a desired result. Thus, the bulk of the soil may be removed by sieving or elutriation, but the final separation of nematodes from debris may be done on an extraction tray or by centrifugal flotation. No single extraction method is suitable for use in all situations. The size, mobility and life cycle stage of the nematodes being extracted determine the appropriate technique. Soil texture is also important, as some methods work well only for sandy soils.

Specific details for basic nematode extraction techniques from soil are given below. It is not practicable to provide details of all techniques in a book of this type and readers are referred to other references (see further reading on extraction procedures in section 9.5) for more information. The techniques covered here have been chosen because they are relatively practical, cost-efficient methods that are suitable for use in a diagnostic laboratory.

5.1.1 Extraction trays

This procedure (illustrated in Figure 3A) is a modification of the Baermann funnel which has been widely used in nematology for more than 80 years. It overcomes the problems of nematodes lodging on the sloping sides of funnels or dying in the collection chamber through lack of oxygen and allows larger samples to be processed. Because it is based on a technique described by Whitehead and Hemming (1965), it is sometimes referred to as a Whitehead tray. A basket made of coarse mesh is placed inside a suitable tray (approximate size 20 cm x 30 cm). The mesh is covered with paper tissue and 200 ml finely crumbed soil is spread evenly in a thin layer over the tissue. Trays are then placed in a suitably covered rack to reduce evaporation and water is carefully added down the inside edge of the tray until the soil is wet but not flooded. Trays are then incubated for a standard time (usually 1-4 days). Nematodes which collect in the water at the bottom of the tray can be concentrated by allowing them to settle in a narrow cylinder for several hours and siphoning off the supernatant water. Alternatively, the suspension can be sieved twice through a 38 μm aperture sieve with the nematodes being washed off the sieve each time and collected in a small container.

Table 9.   Basic procedures for extracting nematodes from soil.

Technique	Basic methods	Advantages	Disadvantages
Baermann funnel (or Whitehead tray)	Soil is placed on a mesh support covered by tissue, in a funnel or tray. Water is added to saturate but not cover the soil. Nematodes in soil or hatching from eggs move through the tissue into the water below.	Specialised equipment is not required. Juveniles hatching from eggs may be extracted. Useful for mobile nematodes (e.g. juveniles of *Meloidogyne*, *Pratylenchus* and *Radopholus*).	Poor extraction of larger nematodes (e.g. *Xiphinema*) or immobile nematodes (e.g. criconematids). In some soils (e.g. fine clay) samples are excessively dirty. Good temperature control is important. Maximum recovery takes 3-14 days.
Centrifugal flotation	Nematodes are floated out of soil or organic debris in a solution with a specific gravity greater than that of nematodes. Sucrose or Mg SO$_4$ solutions with specific gravities of 1.15 - 1.18 are usually used.	Nematodes are immediately available for study. Good for slow moving species as it does not rely on mobility. Eggs may be recovered, but specific identification of eggs is almost impossible.	Nematodes may be distorted or killed by osmotic stress. The chemicals required for the extraction solution add to the cost of this procedure. Limited to small volumes of soil (20 - 50 ml).
Sieving or elutriation followed by Whitehead tray or centrifugal flotation.	Soil is mixed with water and decanted through sieves with aperture sizes ranging from 2 mm to 38 μm. Alternatively, elutriation techniques use a rising flow of water to separate nematodes and organic debris from soil particles. The suspension flows onto a sieve and nematodes are then separated from debris with a Whitehead tray or by centrifugal flotation.	Good extraction of all types of nematodes. Large soil samples can be processed (200 ml-1 L). When used alone, Whitehead tray and centrifugal flotation techniques tend to be limited to a sample size of 200 ml or less.	Nematodes may settle out with soil particles unless soil is well dispersed. A considerable labour component is involved in the sieving or elutriation phase. The elutriation process requires specific equipment thereby increasing costs.
Semi-automatic elutriation	A partly mechanised procedure which combines elutriation with final separation by centrifugal flotation.	Can process large samples and is possibly more consistent than some methods. Semi-automation reduces labour costs.	Equipment is expensive. Regular calibration is required for consistency. Clean up between samples is time consuming.
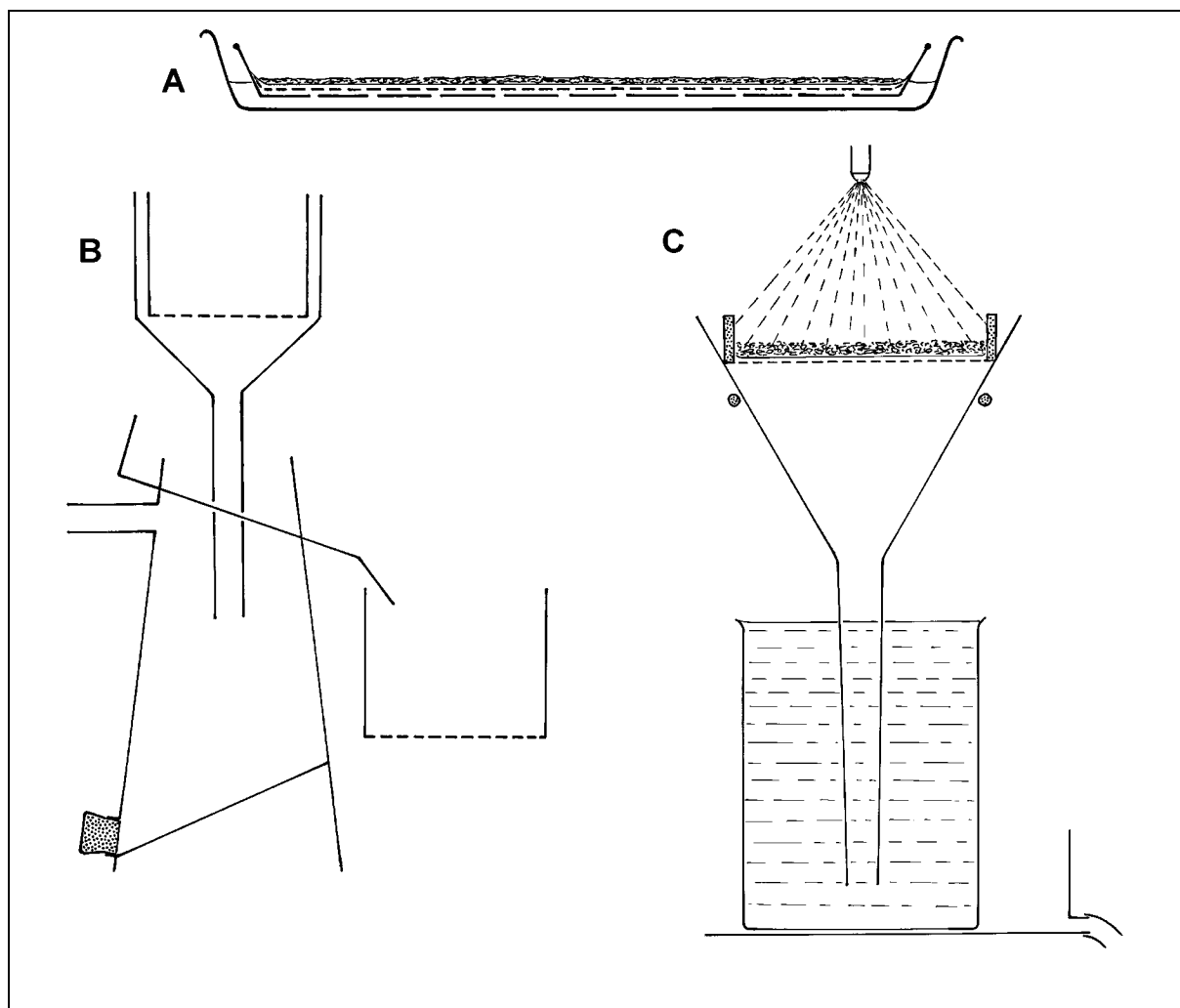
Figure 3.　　**Extraction of nematodes from soil and roots. A, Whitehead tray; B, Fenwick can; C, mist extraction.**

Several factors can affect the extraction efficiency of Baermann methods. It is therefore important that these factors are standardised when these methods are used to quantify nematode populations.

Incubation time. An extraction period of 1-4 days allows only a percentage of the nematodes that are present to emerge. Some nematodes (e.g. *Meloidogyne* juveniles from egg masses and endoparasitic nematodes in root fragments) continue to emerge for 7 - 14 days. When using this technique, it is therefore important to collect data on the proportion of the total population that emerges each day over a period of 1 - 2 weeks. A standard incubation time is then chosen which gives a satisfactory extraction efficiency for the nematode species of interest.

Incubation temperature. Baermann methods of extraction rely on nematodes moving out of roots or soil and migrating through tissue. Since nematode mobility is temperature dependent, temperature has a marked effect on extraction efficiency. Trays should be

incubated at a temperature which is optimum for the nematodes being extracted. Temperatures of 25-27°C are ideal for most situations.

Water level. If soil in baskets is covered with water, extraction efficiencies may be reduced due to lack of oxygen. Since nematodes move best in moist, aerated soil, care should be taken to ensure that the amount of water added is appropriate, and consistent from sample to sample. Evaporation must also be minimised so that the soil remains saturated throughout the extraction period.

Filters. A range of materials can be used as filters to cover the coarse mesh basket (e.g. paper tissues, muslin cloth, milk filters). It is important to use a filter which prevents the water from becoming too dirty and allows nematodes to move readily through. Changes in the type of filter or the number of layers used will alter the extraction efficiency. For instance, two layers of facial tissue will improve the clarity of suspensions obtained from fine particled soils, but recovery of nematodes, particularly large nematodes such as *Xiphinema*, will be reduced.

Sieving procedure. If nematodes are concentrated by sieving, the technique used must be standardised. The diameter of most nematodes is usually less than the aperture of the sieves used (25-45 μm). Therefore, sieving must be done quickly to prevent nematodes wriggling through sieves and should be repeated at least once to improve recovery.

Baermann methods of extraction rely on nematode mobility, and are unsatisfactory for large nematodes (e.g. *Xiphinema, Longidorus* and *Paralongidorus).* Recovery of small but relatively immobile nematodes (e.g. criconematids) is also poor. These problems can be overcome to some extent by using a decanting or elutriation procedure to remove most of the soil particles before placing the sample residue on a tray. Flegg (1967) described such a method for *Xiphinema* and *Longidorus*.

5.1.2 Sieving or elutriation followed by centrifugal flotation

Diagnostic laboratories often require an extraction technique which is not dependent on nematode mobility. In such a situation, centrifugal flotation methods are the main option. They are based on the principle that nematodes float out of soil or organic debris in a solution with a specific gravity greater than their own. Most nematodes are retrieved with relatively high extraction efficiencies and they are particularly useful for extracting sluggish or dead nematodes and nematode eggs. They are sometimes more efficient than Baermann, sieving or elutriation methods but have the disadvantage that nematodes may be distorted or killed by the osmotic stress that is imposed during the extraction procedure.

Centrifugal flotation methods can be used to recover nematodes directly from soil but such methods are limited to relatively small samples (e.g. soil volumes of 20-50 ml). It is more common to use elutriation, or decanting and sieving, to separate nematodes, organic debris and fine sand particles from the larger soil particles, and then use centrifugal flotation for the final separation. Two commonly used methods are sieving/centrifugation and semi-automatic elutriation.

Sieving/centrifugation

Samples (100-150 ml) are washed through a 0.8-1.2 mm aperture sieve into a bucket and made up to about 6 L with water. After stirring, heavy particles are allowed to settle for 30 seconds before the supernatant is decanted through a 53μm aperture (or finer) sieve. The bucket is refilled and the process repeated. The sievings are collected in two 50 ml centrifuge tubes which are balanced before spinning at 1750 rpm for 4 min. The supernatant is decanted and replaced with sucrose solution (specific gravity 1.18, or 484g sucrose made up to 1L with water), into which the pellet is thoroughly dispersed. The tubes are balanced and spun for 30 sec. The supernatant is poured through 53 μm aperture sieves (or less) and the sievings are washed into a container for examination. Specific details are provided by Jenkins (1964)

Semi-automatic elutriation

An elutriator developed by Byrd *et al.* (1976) can be used to process four samples at once. Soil samples (500 ml) are added to the elutriator, with air and water flowing at desired rates. The elutriator is run for 3-4 minutes, with roots being collected on a 500 μm sieve and nematodes on a 38 μm sieve. Both sieves are attached to a motorised shaker. A 1 mm sieve should be used in lieu of the 500 μm sieve if large nematodes that may be trapped on the smaller sieve (e.g. *Xiphinema*), are likely to be present.

Material collected on the 38 μm sieve is washed into a beaker and cleaned by flotation and sieving. A sucrose solution is added to the beaker so that the specific gravity is increased to 1.18. The solution is then stirred with a motorised stirrer, allowed to settle for a short period and then decanted onto a 500μm sieve over a 38 μm sieve. The nematodes collected are washed into a container for counting.

When using sieving and flotation methods to quantify nematode populations, the following issues must be considered:

Sample size. This will affect the efficiency of nematode recovery and must be pre-determined for the range of soils that are washed.

Pre-treatment of soil. Soaking soils for 12 hours before extraction or dispersing clays with materials such as Calgon (sodium hexametaphosphate), at a concentration of 35g/litre of water, often improves the efficiency of sieving/centrifugation extraction procedures.

Sieve aperture size. Choice of sieve aperture is usually a compromise between the need to retain small nematodes and the need to alleviate clogging problems. The sieve sizes commonly used for nematodes are 53 μm, 45 μm, and 38 μm, with the latter being preferred for small nematodes such as *Meloidogyne* and *Tylenchulus* juveniles.

Choice of flotation solution. Sucrose solutions are used extensively because sugar is readily available and relatively cheap. Molasses can be used in lieu of sugar. $MgSO_4$ is an alternative that does not have the stickiness of sucrose and can be recovered.

Density of solution. Most methods described use solutions of specific gravity 1.15-1.18. Increasing the specific gravity does not necessarily increase the yield of nematodes and may damage them or result in dirtier samples. It is critical that the solution is made up to the correct density. A solution of specific gravity 1.18 is made by dissolving 484 g of sucrose or 409 g of $MgSO_4.7H_2O$ in water and making the volume up to 1 L of solution.

Speed of centrifugation. Speeds used for nematode extraction are equivalent to a relative centrifugal force (RCF) of 700-2900, with 1800 most often being used.

5.2   Extraction of nematode cysts from soil

Most extraction methods for *Heterodera* and *Globodera* cysts rely on the capacity of cysts to float or remain suspended in an upcurrent of water.

5.2.1 Elutriation followed by sieving

Fenwick can
This widely used apparatus, which is described by Southey (1986) and depicted in Figure 3B, is suitable for samples up to 300g. The rising stream of water in this apparatus causes organic material and soil to overflow onto a collar and pass down into collecting sieves. About 70% of cysts are generally recovered.

Fluidising column
This procedure was developed by Trudgill *et al*. (1973) and is a refinement of various elutriation techniques. It can be used for extracting nematodes of any type or size but its principal value is for recovering cysts from root washings and soil. The rate of flow of water in a cylindrical column is adjusted so that the upward flow is sufficient to carry cysts to the top of the column. The overflow carries nematodes and debris into a perspex collar and then into collecting sieves.

Both the above techniques give good recovery of cysts from soil, with extraction efficiencies of 90% being achievable. They can be used for both wet and dry soils. Since cysts which are full of eggs do not float as well as relatively empty cysts, it is important that flow rates are sufficient to recover full cysts.

5.3   Extraction of nematodes from plant material

Many techniques can be used to extract nematodes from plant material, with the most commonly used procedures being listed in Table 10. In practice, however, usually only one technique is used in any one laboratory. The choice of method depends largely on the type of plant tissue involved, the nematode species present and the facilities available. Three useful methods which do not require specialised equipment are the Baermann funnel, root incubation in plastic bags and root maceration followed by incubation in an extraction tray. The more sophisticated mister extraction technique is used in most nematology laboratories and is the only method to be discussed in detail here

Table 10.   **Basic procedures for extracting nematodes from roots and other plant tissues.**

Technique	Basic methods	Advantages	Disadvantages
Baermann funnel (or Whitehead tray)	Roots are chopped into small pieces and placed on a tray as for soil (see Table 9).	See Table 9.	See Table 9.
Root incubation in plastic bags	Tissue is cut into small pieces and incubated in a closed plastic bag containing a small quantity of water. Nematodes hatch from eggs or migrate from root tissue into water.	Does not require specialised equipment.	Extraction efficiency may be relatively poor. Nematodes are often in poor condition due to lack of oxygen.
Root maceration and incubation in an extraction tray	Roots are macerated in water with an electric blender. The resulting suspension is then placed on a Whitehead tray.	Does not require specialised equipment.	Maceration time is critical. It must be sufficient to allow nematodes easy egress from plant tissue, but damage must be minimised.
Misting technique	Plant tissue is chopped into lengths <1cm long and placed on mesh in a funnel. The tissue is sprayed with an intermittent mist of water and nematodes are collected in a container at the base of the funnel.	Nematodes are usually in good condition if removed every 24 hours. The technique is suitable for most endo-parasitic nematodes.	Temperature control is important as it affects nematode mobility. Misting equipment is required and mist nozzles must be well maintained.

Misting technique

Roots are chopped into pieces less than 1 cm long and placed on a mesh support in a funnel (Figure 3C). Funnels are placed in a closed chamber and subjected to an intermittent mist of water (usually one minute of misting every ten minutes). Tubing is attached to the base of the funnel and this is directed into the bottom of a collecting beaker or test-tube. In the humid environment at the top of the funnel, nematodes, being obligate parasites, will move out of the dead roots and juveniles will hatch from eggs on the root surface. They are then washed by the mist into the bottom of the beaker. The beaker gradually fills, but the accumulating water does not create enough turbulence to carry nematodes with it.

Nematodes extracted by the misting technique are usually in good condition because the overflow of water from the collecting vessel ensures good oxygen exchange. Sap and toxic decomposition products are also washed away. In an alternative collection system, nematodes can be retained in a small vial attached to the tubing, or the tubing can be sealed with a clip, with nematodes being recovered by opening the clip. Specimens obtained in this manner are often in poor condition because oxygen exchange is limited.

A number of factors must be given attention if high extraction efficiencies are to be obtained by misting or, perhaps more importantly, if results are to be consistent from sample to sample.

Temperature.   Misted water may be relatively cool, which means that under some conditions, the temperature of plant material in the funnels is too low for good nematode mobility and egg hatch. Extraction efficiency is improved by heating the water supply to an appropriate temperature so that the temperature under the mist is optimum for nematodes (usually 22-27°C).

Extraction time. Nematodes take some time to move out of plant tissue or hatch from eggs. Extraction time will vary with the thickness and type of plant tissue, and must therefore be standardised (usually 2-4 days).

Nematode species.   Although suitable for the recovery of most nematodes, misting techniques are not suitable for *Aphelenchoides*. Unlike other plant nematodes, which do not propel themselves through water, this nematode is an active swimmer and is lost in the overflow water.

Variation in mist volumes. Mist nozzles should be placed so that all samples in the misting cabinet receive approximately the same volume of water. Good design and maintenance are important, as excessive amounts of water dripping from nozzles or pipes may cause turbulence in the collecting vessel. This may result in nematodes being lost in the overflow water.

5.4   Bioassay procedures

Bioassays are the most reliable method of detecting and quantifying certain sedentary endoparasitic nematodes. Prot and Netscher (1978), pointed out that *Meloidogyne* juveniles can move more than 50 cm in 10 days to infect roots. Thus, the potential inoculum available to a single susceptible plant represents all juveniles in an hemisphere of soil of radius 50 cm (i.e. 262 L of soil). If there are 130 nematodes in this hemisphere, there will be enough nematodes to infect the plant and increase to damaging levels. However, the chance of detecting a population of this magnitude (1 juvenile per 2 L soil) is relatively low if volumes of only 200 ml soil are processed. The fact that root-knot nematodes will be present as clumps of eggs in one or a few egg masses makes detection even less likely. If many small samples were processed, most would be negative but a few would have high numbers of nematodes. Difficulties in detecting low but economically important populations of some nematodes can be overcome to a certain extent with bioassay procedures. The principles behind these techniques and their advantages and disadvantages are listed in Table 11. Their main limitation is that they can be used only for nematodes that produce specific, easily identifiable symptoms.

Table 11.   **Bioassays for detecting and quantifying nematodes.**

Technique	Basic methods	Advantages	Disadvantages
Plant bioassay of field soil or soil to which roots have been added.	• A host that is susceptible to the nematode of interest is grown in soil. • The presence of specific symptoms confirms its presence (e.g. galls on tomato indicate root-knot nematode). • For nematodes that do not produce such specific symptoms, nematodes can be extracted from roots of the bioassay plant.	• Specialised equipment or technical training is not required. • Visual rather than microscopic assessment can be used if symptoms are specific. • Useful for detecting low populations because large soil volumes can be used. Unlike most other methods, most life stages, including eggs, are detected. • Can sometimes be used to give quantitative data.	• Lag time for results is usually 6-10 weeks. • Most useful for nematodes that produce specific and identifiable symptoms. • Requires larger soil volumes than other techniques. • Requires a knowledge of nematode biology and host range, and availability of suitable temperatures for growth of plants and nematodes.

Bioassays for root-knot nematode.   Samples are collected as described previously but at least 1L of soil must be retained. Samples of 1-5 L of soil are added to pots, a susceptible tomato seedling is planted and the plants are grown in a warm environment (ideally 22-30°C). If information on the presence or absence of the nematode is all that is required, plants are carefully washed from soil after 4-8 weeks and roots observed for signs of galling. High gall ratings indicate a high population of root-knot nematode (Figure 4A). Bioassays can also be used to quantify the root-knot nematode population, provided plants are harvested before a second generation of juveniles invade roots. Since reproduction can begin in 4-5 weeks under ideal conditions (i.e. temperatures of 25-28°C), plants are best removed from soil 3-4 weeks after planting. Provided nematode populations are low, (i.e. less than about 100 root-knot nematodes/litre), a single gall will generally represent a single nematode. Thus, counting the number of galls on the root system will give an indication of the number of root-knot nematodes in the potted soil volume. When counting galls, roots should be floated in water in a tray with a dark coloured base, as this enables small galls to be seen more easily with the naked eye. Issues related to the use of bioassays for diagnostic purposes are discussed by McSorley and Pohronezny (1981), while specific details of the technique are listed by Barker (1985).

Bioassays for cereal cyst nematode (CCN). Bioassays to determine the level of nematode infestation in cereal fields prior to sowing are based on a technique developed by Simon (1980). Soil is collected in January or February and incubated for 4 weeks at 10-15°C to allow CCN eggs to hatch. Seedlings of a susceptible wheat cultivar are then grown in soil for 4 weeks under artificial light and under temperatures which simulate winter conditions. The seedlings are removed from the soil and the amount of CCN damage is assessed on a scale of 0-5 according to the number of knots present. A zero rating indicates no infestation while roots with a rating of 5 are stunted and heavily knotted (Figure 4B). Results of the bioassay therefore indicate the potential for CCN damage to the next crop if the season is favourable for the nematode.
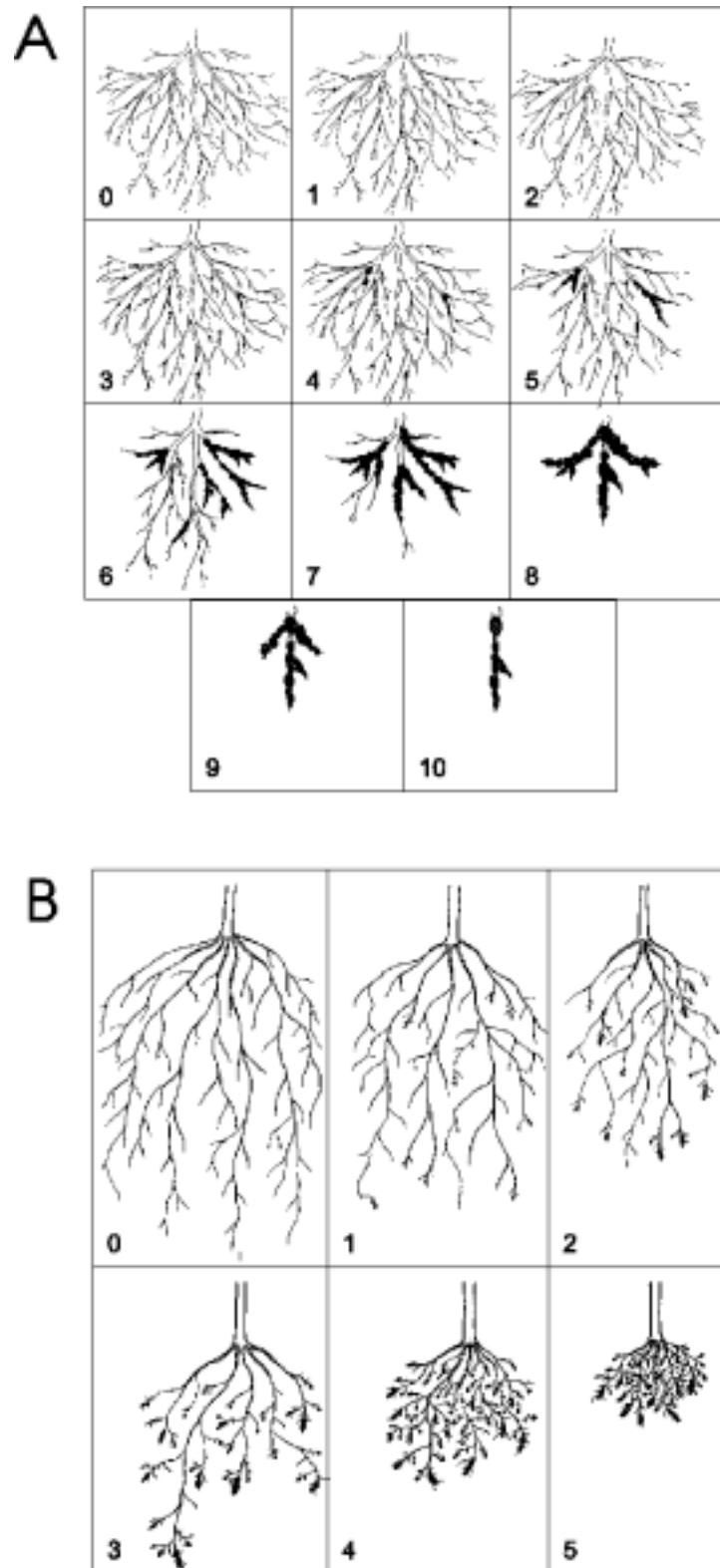
Figure 4. Bioassays for root-knot and cereal cyst nematode. A, Root-knot ratings on a 0-10 scale (From Zeck, 1971, Pflanzenschutz Nachtrichten Bayer, 24, 141-144.); B, Knotting (0-5 scale) caused by cereal cyst nematode (After Simon, 1980).

Advantages and disadvantages of bioassays. One of the most useful features of bioassays is that technical training and specialised equipment is not required. Thus growers could run their own bioassays for root-knot nematode, provided they can grow plants at a temperature which is suitable for the nematode. Bioassays have two further advantages over other methods of quantifying nematodes. Much larger samples can be processed, which increases the chances of detecting low populations of nematodes. Nematodes in the egg stage can be detected because there is also enough time for juveniles to hatch and invade roots.

Bioassay procedures are also useful when diagnosis of the species of root-knot nematode or information on host range is required. *Meloidogyne* juveniles extracted from soil or roots cannot be easily identified to species, whereas females taken from bioassay plants can be identified by perineal patterns or molecular techniques (see sections 6.2.7 and 6.2.8). Bioassays can also indicate whether the root-knot nematodes present in a soil sample will attack a particular crop. For example, only one of the four common root-knot nematode species (*Meloidogyne hapla*) will attack strawberry. It is not possible to predict whether root-knot juveniles extracted from a soil sample will multiply on strawberry. However, when strawberry runners are planted as a bioassay in potted soil, the presence of galls 6-8 weeks later will indicate *M. hapla*.

Despite the many advantages of bioassay procedures, their main disadvantage is the time taken for a result. Since bioassays are normally done on pre-plant samples, samples must be collected 6-10 weeks before planting so that there is sufficient time for processing. Because bioassays are specific for certain nematodes, they also do not provide information on other parasitic nematodes that may be present. This is an important limitation in crops where several nematode species are economically important.

5.5   *Determining extraction efficiency*

The term 'extraction efficiency' indicates the proportion of a nematode population that is removed from a sample by a particular extraction procedure. No nematode extraction procedure will recover all of the nematodes in a sample and extraction efficiencies for many commonly used techniques range from about 10% to 50%. If several laboratories were to process aliquots of the same nematode sample, they would obtain different numbers of nematodes, depending on the extraction efficiency of the technique used.

Because strategic decision making processes for nematodes depend on estimating nematode population densities and comparing them to experimentally derived damage thresholds, it is important that data derived from different laboratories are standardised. An estimate of nematode density obtained in one laboratory can then be compared in a meaningful way with another estimate from another laboratory. Such standardisation can be achieved if estimates of nematode population density are corrected for extraction efficiency. Thus, a laboratory extracting 100 nematodes from a sample using a method which is 33% efficient and another extracting 150 nematodes with 50% efficiency would both report an actual nematode population density of 300.

If a diagnostic laboratory wishes to ensure that its estimates of nematode population density are meaningful, it must obtain data on the efficiency of its extraction methods. Extraction efficiencies should also be checked regularly as a standard quality control procedure to ensure that they do not change over time. The following procedures are useful for this purpose.

Adding nematodes to soil samples. The extraction efficiency for a particular nematode species or life cycle stage can be determined by inoculating soil that is free of the target nematode with a known number of specimens of the nematode. The nematodes recovered are counted. This method is useful, but has the limitation that inoculated nematodes may not be dispersed in the same way and may not be associated with soil particles in the same manner as they are under natural conditions. Also, some nematodes may die during the inoculation process.

Repeated extractions. Nematodes are extracted repeatedly from the same soil sample over a period of time and the number of nematodes recovered with each extraction is determined. The cumulative number of nematodes is then plotted against the number of extractions, and the total number of extractable nematodes in the sample is estimated by extrapolation. The proportion of extractable nematodes recovered during the first separation can then be calculated. This proportion will represent the extraction efficiency when the number of extractable nematodes and the total number of nematodes in the sample are similar. This latter point can be confirmed by extracting the nematodes which remain after a series of extractions, using a procedure with a known extraction efficiency.

If sieving is involved in the extraction procedure, the efficiency of the sieving process may need to be determined so that losses due to sieving can be accounted for. These losses can be measured by sieving a nematode suspension and counting the number retained. The water passing through the sieve is collected and the nematodes present are concentrated by settling, filtering or centrifugation and counted. The efficiency of the sieving technique can then be calculated.

Comparisons with bioassays. A soil sample is divided so that one half is used to extract nematodes with the procedure under test. The remainder of the sample is used for a bioassay in which a suitable host is grown for a period of usually a few weeks (long enough to allow nematodes to enter roots, but not so long that reproduction occurs). The roots are then stained (see Table 12) to enable the nematodes in roots to be visualised, and endoparasitic nematodes are counted microscopically. There are several problems with this method, the main ones being that it is limited to endoparasitic species and there may be some host status effect on the rate of penetration into the root system.

Use of different extraction techniques. A soil sample is divided and one part is subjected to the separation process under investigation. The other part is processed using a procedure with a known extraction efficiency to enable the absolute number of nematodes in the sample to be calculated. The efficiency of the technique under investigation can then be calculated.

Table 12.   Procedures for staining nematodes in plant tissue.

This method, developed by Byrd *et al.* (1983), eliminates exposure of personnel to toxic substances (e.g. phenol) which are utilised in some other methods. Since root tissue is cleared with NaOCl prior to staining with acid fuchsin, roots do not become heavily stained. As a result, the time for destaining is short and frequently destaining is unnecessary.

Procedure

1. Cut infected roots into pieces and clear roots by immersing them for 4 minutes in household bleach (NaOCl) diluted to a concentration of 0.8-2% available chlorine (use higher concentrations for older or thicker roots).

2.   Rinse roots for 45 seconds in running tap water and then soak roots in tap water for 15 minutes to remove residual NaOCl.

3.   Drain the water and transfer roots to a beaker with 30-50 ml of tap water.

4. Add 1 ml of a stock solution of acid fuchsin, prepared by dissolving 3.5g acid fuchsin in 250 ml acetic acid and 750 ml distilled water.

5.   Boil the solution for about 30 seconds on a hot plate or in a microwave oven, then allow it to cool to room temperature.

6.   Place the roots in 20-30 ml of glycerin, acidified with a few drops of 5N HCl and heat to boiling for destaining.

7.   Spread roots between two glass plates or slides with a small amount of glycerin and apply light pressure before examining nematodes under a stereomicroscope.

Other useful staining methods can be found in Southey (1986).

Quantitative studies and published data on extraction techniques (McSorley and Parrado 1982; Viglierchio and Schmitt 1983 a,b; Viglierchio and Yamashita 1983; Barker 1985) indicate that current nematode extraction methods are exceedingly poor diagnostic tools. Such studies, and years of practical experience by researchers have shown that:

- Most commonly used extraction procedures recover less than 30% of the nematodes present.
- There is often considerable variability between samples. Consistent results with some methods may be achievable when they are used by one operator. However, other methods may be too variable for determining disease thresholds.
- Extraction efficiencies are poorer in heavy soils than in sands.
- Major losses of nematodes can occur during the sieving process. For example, 40-50% of small nematodes (e.g. *Pratylenchus* and juveniles of *Meloidogyne*) may be lost when suspensions of nematodes in water are poured over a 38μm sieve. When soil is present (i.e. with extraction methods involving decanting and sieving), the proportion of nematodes

recovered will be much lower. Repeated sieving of suspensions will increase extraction efficiency.

- In Baermann tray/funnel procedures, the type of tissue which is used is important. Substantial numbers of nematodes are retained on some tissues.
- The main limitation of density flotation techniques is that a large proportion of nematodes are bound to soil particles and are therefore unavailable for extraction with such techniques.

Because extraction efficiencies are influenced by many factors and extraction techniques may be used for a variety of purposes, there is little point in making recommendations regarding the best extraction methods for diagnostic services. It is the responsibility of each diagnostic laboratory to compare various extraction techniques and demonstrate that those chosen are reliable and provide the highest extraction efficiency achievable. Once standard extraction protocols have been established, quality control programs should be initiated so that extraction efficiencies are checked on a routine basis.

6. Identification and Counting

6.1 Counting procedures

After nematodes have been extracted from soil or roots, they must be identified and counted under a microscope. Counting can be done with either a compound or dissecting microscope, and a magnification of about 40 x is usually suitable (i.e. a 4x objective combined with a 10x eyepiece). A compound microscope is preferred as additional magnification (i.e. the 10x objective) is then available for nematodes that are in poor condition or are hard to identify. Good quality transmitted illumination is essential.

The proportion of the extracted suspension that is counted depends on nematode density. When many nematodes are present, it may be sufficient to count a small proportion of the sample. However, in situations where it is important to detect or quantify low nematode populations, the suspension should be concentrated and all of it counted. When a subsample is taken for counting, the suspension must be agitated while the sample is being removed to ensure a representative subsample. A wide-mouthed pipette should be used to prevent clogging and to ensure that large nematodes are included in the subsample. If disposable pipette tips are used, the tips should be cut back to provide a larger opening. Figure 5 illustrates some of the equipment required for counting nematodes.

For counting, a variety of dishes can be used, varying in sophistication from plastic petri dishes with a grid etched on the bottom of the dish to specially designed nematode counting dishes. One of the most useful dishes for general purposes is an open rectangular plastic dish of about 5ml capacity with sloping sides to minimise the optical distortion caused by the meniscus, and a base marked with equidistant lines. Such a dish will hold the whole sample but a proportion of the suspension can be counted if nematode density is high. Because it is open, access is available if nematodes have to be hand picked for identification at higher magnifications. Whatever counting dish is used, care must be taken to ensure that a representative proportion of the total sample is counted. Sampling errors which occur during counting must be minimised if the gains made through quality control in other parts of the extraction process are to be retained. Since nematodes are never spread evenly over a dish, counting errors can only be minimised by counting a large proportion of the sample.

6.2 Nematode identification

A capacity to identify nematodes is one of the most important skills required by a diagnostic nematologist. To the untrained eye, most nematodes appear similar and it is only through close examination that the differences between species can be appreciated. Such differences cannot be recognised without some knowledge of nematode biology and morphology.

Figure 5. Equipment required for counting nematodes. In the background is a rack with test tubes containing nematodes in water. Alternatively on the far left, a small sample vial can be used for storage of nematodes with minimal water. Far right is a measuring cylinder used to quantify the volume of the sample to be counted. Front centre is a pipette which is used to measure accurately an aliquot of the total sample. This aliquot is placed in the modified Doncaster dishes, three of which can been seen in front of the pipette. These each contain concentric circles and usually hold a 1 ml aliquot of the sample.

6.2.1 Nematode biology and morphology

Nematodes are unsegmented worms with slender, cylindrical, bodies. However, certain species change their shape during development and lose their worm-like appearance. Most nematodes which attack plants are microscopic (0.5 - 2.0 mm long), but considerable variation in both size and shape can occur (Figure 6).

The body is covered by a non-living, flexible and transparent cuticle which has a protective function. Beneath the cuticle, the nematode has digestive, nervous, excretory and reproductive systems, but no circulatory or respiratory system (Figure 7).

Typically there are six stages within a nematode life cycle: an egg, four juvenile stages and an adult (Figure 8). The juvenile and adult stages are separated by moults. In plant-parasitic nematodes, the first moult occurs in the egg and eggs hatch to produce second-stage juveniles (J2).

The adult can be easily identified by the presence of a reproductive system (Figure 7). The female contains one or two ovaries, a uterus, vagina and vulva, and usually one or two spermathecae where sperm are stored. The males can be readily distinguished from females by the presence of copulatory apparatus, namely spicules. The reproductive system of the male consists of one or two testes, a seminal vesicle and a vas deferens which leads into the cloaca.
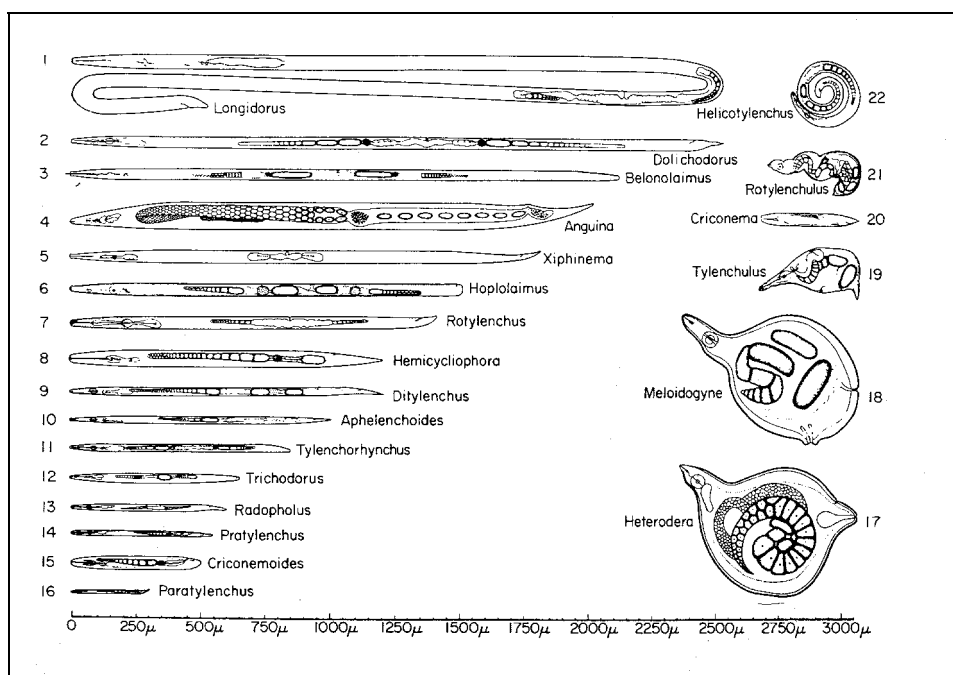
Figure 6. **Morphology and relative size of some of the most important plant-parasitic nematodes. From "Plant Diseases caused by Nematodes" in *Plant Pathology*, 3rd. edition by George N. Agrios (1987). Reprinted by permission of Academic Press Inc.**
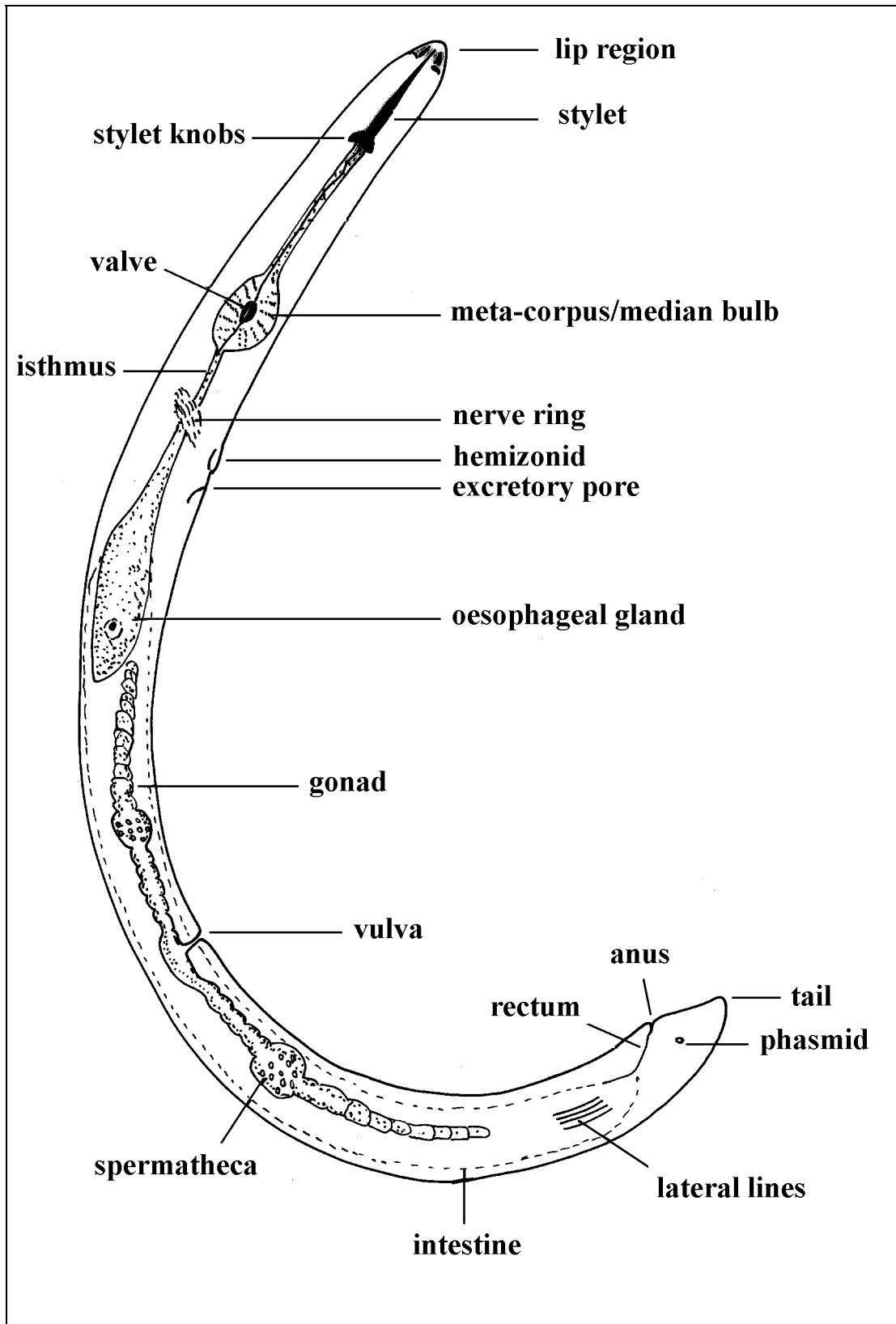
Figure 7. The important morphological features of a plant-parasitic nematode.
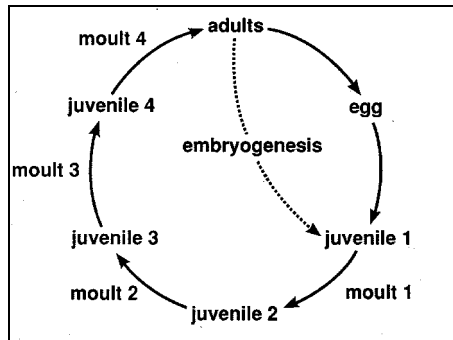
Figure 8.   **Generalised life cycle of a nematode.**
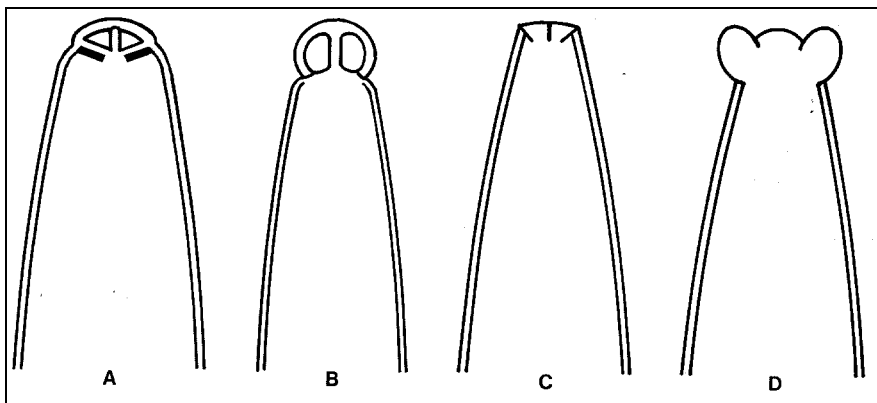
Figure 9.   **Variations in head structure. A, flat; B, raised; C, continuous; D, offset.**

Since many important taxonomic characters can only be seen at a magnification of 40-100x, the range of characters which can be used at the low magnifications used for counting is relatively limited. Some of the most useful morphological features are:

Head structure. The lip region at the head of the nematode is a useful diagnostic character. The lips may be flattened, raised, continuous or offset, as indicated in Figure 9.

Stylet. Most plant parasitic-nematodes have a stylet, which is a relatively long and slender feeding structure often referred to as a spear. It is hollow and needle-like, like a hypodermic syringe, and is inserted into plant cells by the nematode. The structure of the spear is important in identification. Two distinct types can occur, one being known as a stylet and the other as an odontostylet (Figure 10). At low magnifications, the former appears as a thin line with two basal knobs (Figure 10A). The odontostylet has two parts: the visible part or odontostylet and the posterior half or odontophore, which may have flanges and is much less distinct (Figure 10B). Since the flanges of the odontostylet are often not visible at low magnifications, the stylet appears as a thin line without any obvious structure at the base.
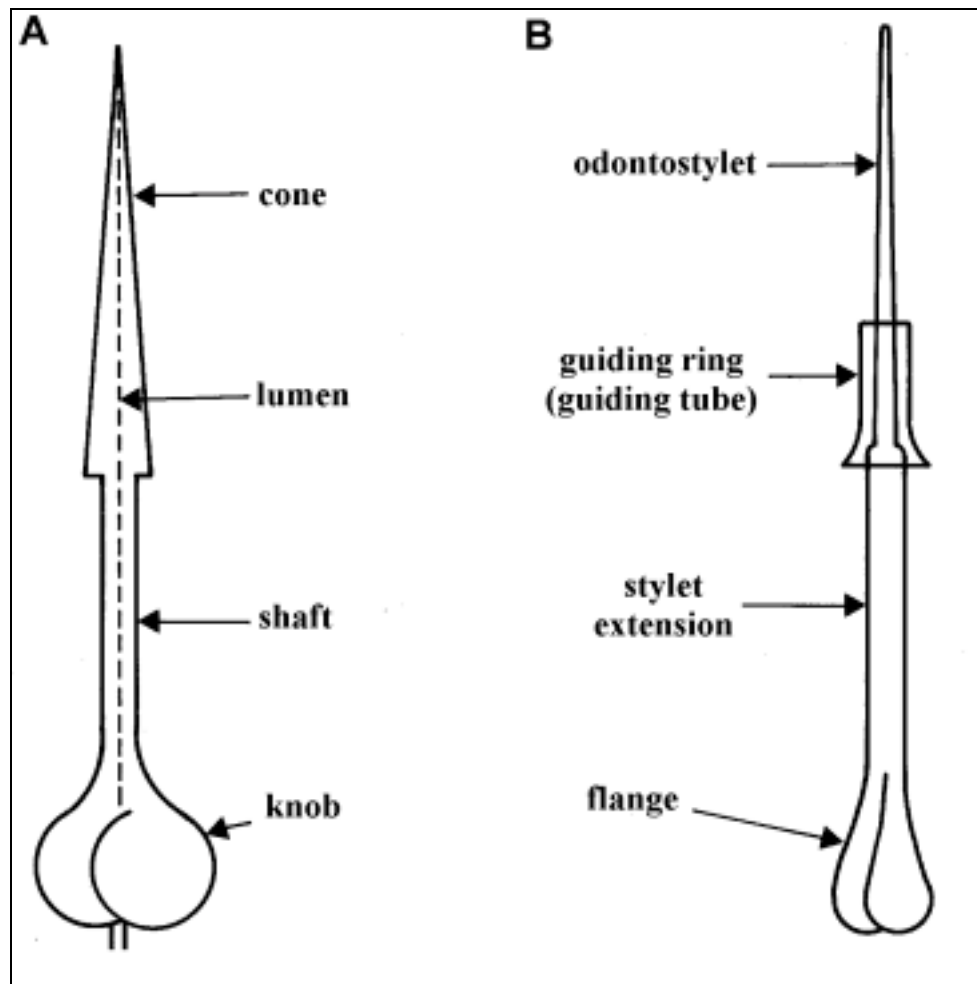
Figure 10. **Generalised structure of the two types of stylet associated with plant parasitic nematodes. A, stylet; B, odontostylet.**

Position of the vulva. At low magnifications, the vulva usually appears as a thin line perpendicular to the ventral side of the nematode. Female nematodes have one or two ovaries and therefore the vulva can be located in the middle of the body or towards the posterior end. Vulval position is usually designated by the letter V and is determined by the distance from the anterior end as a percentage of the total length of the nematode. In nematodes with a vulva in the middle of the body, V=50%, whereas a vulval position three-quarters of the way towards the posterior end of the body would be designated as V=75%. Vulval position can be a useful taxonomic character at both generic and species levels.

**Tail shape**. The tail is the part of the body posterior to the anus. Tail shape is a useful taxonomic character, particularly at the species level. A variety of common tail shapes are illustrated in Figure 12.

Oesophagus. The morphology of the oesophagus can be very diverse, which makes it an important diagnostic tool. Two main types of oesophagus occur among plant parasitic nematodes:

- <u>Tylenchid</u> oesophagus with a median bulb and glandular lobe or terminal bulb (Figure 11D).
- <u>Dorylaimid</u> oesophagus with a muscular terminal bulb but no median bulb (Figure 11A).

Although most plant parasitic nematodes have a tylenchid oesophagus, the structure of this type of oesophagus can vary greatly. There is also variation in the position of the oesophageal glands, which may overlap or abut the intestine. At low magnifications, this difference can be detected by observing the junction between the oesophagus (which usually has little colour) and the darker, food-filled intestine.

Nematodes with an aphelenchid oesophagus (Figure 11 E) are usually associated with the parasitism of foliage and comprise only a few plant parasitic nematodes. Apart from the position of the dorsal oesophageal opening (which cannot be seen at low magnification), the oesophagus is similar to the tylenchids. However, the median bulb tends to be more conspicuous and squarish in shape, and can cover up to 80% of the nematode's body width in some species. Nematodes with a dorylaimid oesophagus (Figure 11A) are common in soil but relatively few are plant parasites. Those with a mononchid oesophagus (Figure 11B) are predators.
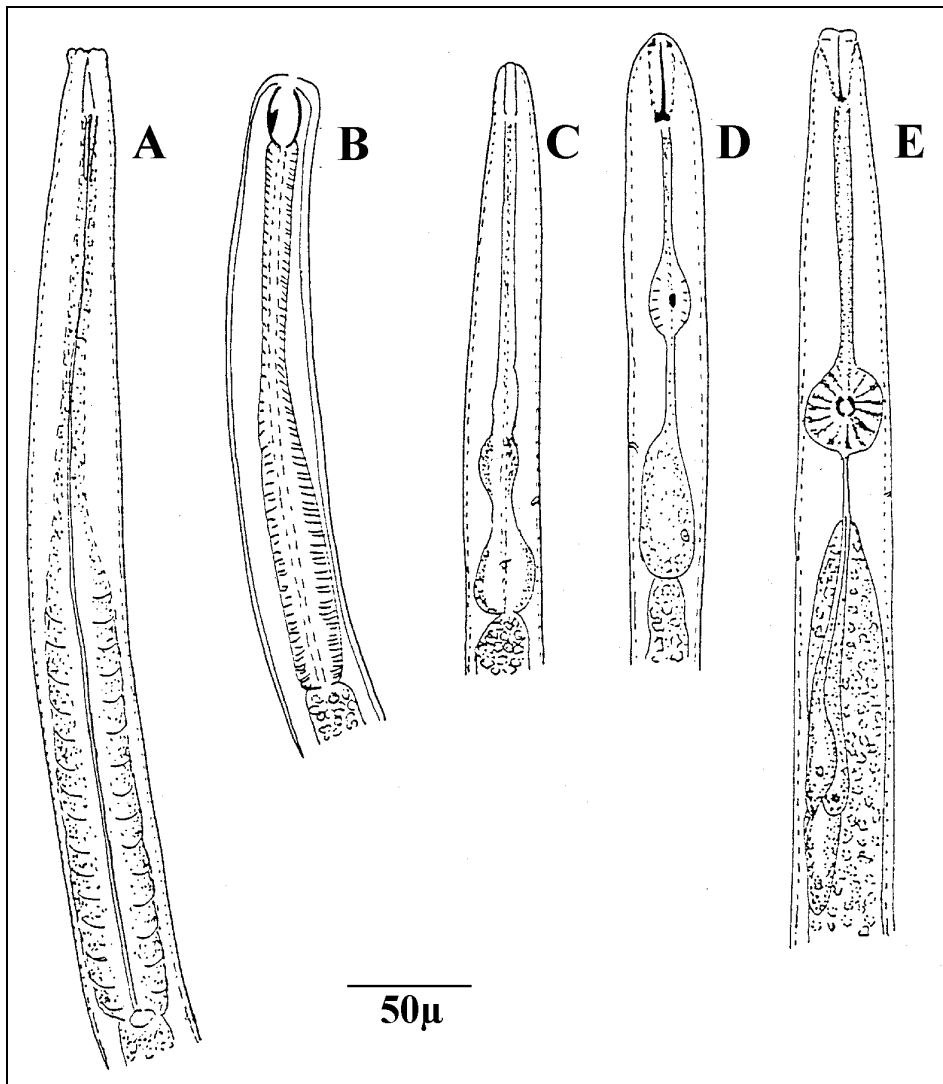
Figure 11. Variations in the type of oesophagus associated with plant and soil nematodes. A, dorylaimid; B, mononchid; C, rhabditid; D, tylenchid; E, aphelenchid.
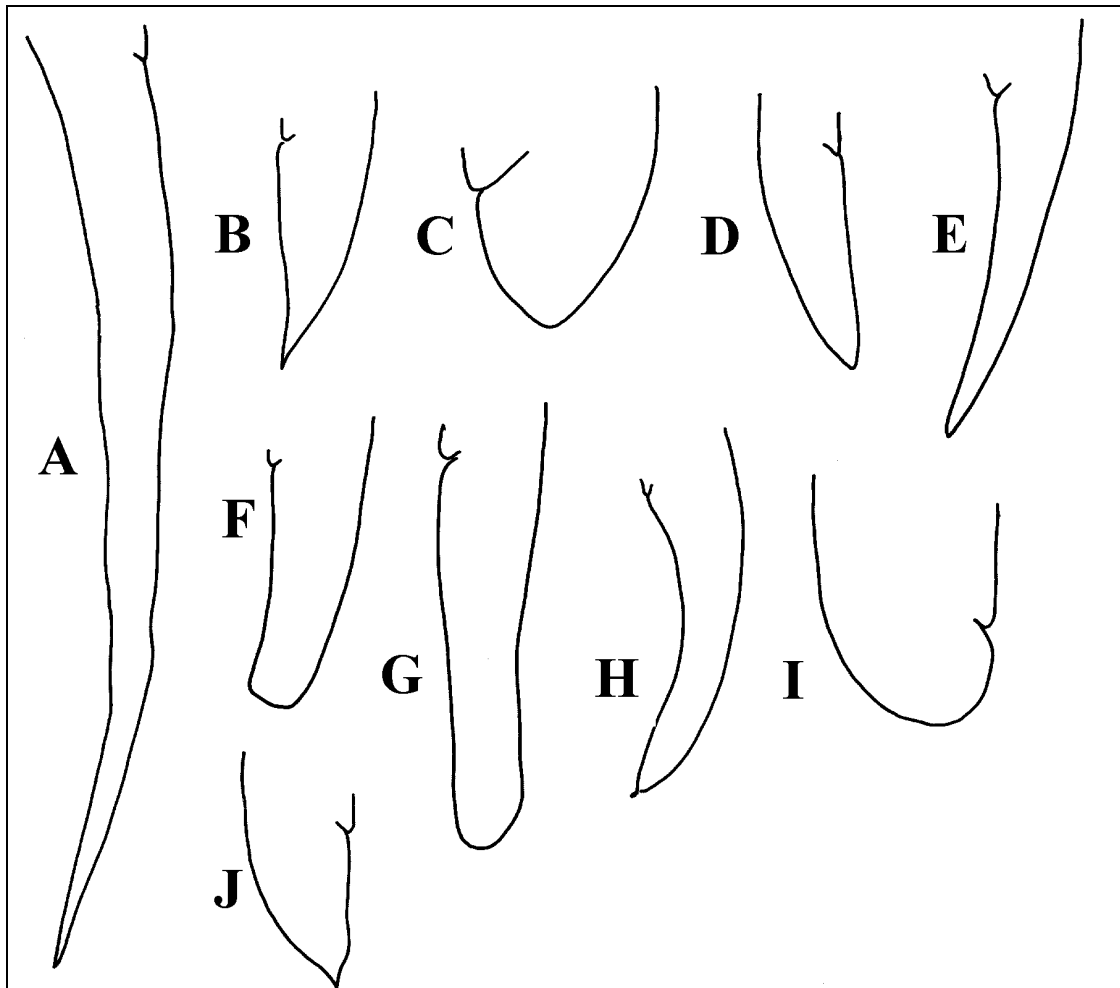
Figure 12. Variations in tail shape. A, filiform, very long; B, conoid, pointed terminus; C, conoid, short; D, conoid; E, moderately long, evenly tapered; F, truncate; G, cylindrical, terminus slightly bulbous; H, conoid with terminal mucro; I, hemispherical; J, conoid with digitate terminus.

6.2.2 Characteristics of major nematode groups

Most soil samples contain at least five species of plant-parasitic nematodes, a wide range of free-living species that feed on bacteria or fungi, and several types of predacious and omnivorous nematodes. Although laboratories involved in nematode diagnosis are mainly interested in nematodes that feed on plants, it is useful to be aware of the other nematodes that always occur in samples. Their presence or number often indicates something important about the habitat from which they were extracted. For example, high numbers of free-living nematodes indicate that soil is microbially active or that decomposing organic matter is present.

Five major groups of nematodes are found in soil:

Tylenchids Most plant-parasitic nematodes belong to the sub-order Tylenchida and are therefore referred to as tylenchids. Due to their feeding habits, nearly all nematodes in this group have stylets. The only exceptions are some stages of a few species, where the stylet may be barely visible under low power, or absent. The majority of tylenchids have knobs at the base of the stylet, to which muscles are attached to extend or retract the stylet. A muscular median bulb is also present and ingestion occurs when the bulb contracts and the contents of plant cells are sucked out by the nematode.

Although the stylet and median bulb are important features of most plant-parasitic nematodes, the presence of a stylet does not always indicate that a nematode is parasitic on plants. Within the tylenchids, for example, there is a group of small nematodes with short, slender stylets and pointed tails that are common in soil. They are often referred to by the older generic name *"Tylenchus"* but really consist of several genera. They are not economically important and little is known of their feeding habits. They probably feed on fungi, but may also feed on the feeder roots and root hairs of plants.

Aphelenchids. Some common stylet-bearing nematodes belong to the sub-order Aphelenchina (often referred to as the aphelenchids). These nematodes have a stylet with small knobs, except that in some species the base of the shaft is swollen or without knobs. All have a large median bulb, which is a useful diagnostic character. Some genera are parasitic on plants (e.g. *Aphelenchoides*) but most feed on fungi (e.g. *Aphelenchus, Paraphelenchus*). They are found in most soil samples. The plant parasites, such as *Aphelenchoides*, are mostly found in above-ground parts of plants.

Dorylaimids. Dorylaimids are mainly omnivorous nematodes that are relatively common in soil samples. They usually have a stylet without knobs and they use it to feed on fungi, algae and small invertebrates such as protozoa, rotifers, enchytraeids and nematodes. There is no median bulb, but instead, dorylaimids have a muscular pharynx, which is usually cylindrical. They are amongst the largest soil nematodes (often greater than 2 mm long) and, when viewed under the dissecting microscope, their body contents often have a dark colour. A few genera are plant parasites.

Mononchids. Mononchids are always predators. Their mouth is cup or goblet-shaped and contains one or more teeth on the inner surface with which the nematode seizes its prey. Some species consume their prey whole, whereas others suck out the body contents. The

large, toothed mouth is readily visible at low magnifications. As with dorylaimids, there is a muscular, cylindrical pharynx but no median bulb (Figure 11B).

Rhabditids. Rhabditids feed on bacteria. They are common in soil and can be present in high numbers, particularly when decaying organic matter is present. Their cylindrical or cup-shaped mouth allows ingestion of bacteria and they always have a more or less rounded basal bulb abutting the intestine (Figure 11C). There may also be a median bulb which may seem similar to plant parasitic nematodes. However, they can be distinguished from plant parasites by the lack of a stylet.

6.2.3 Identification at low magnification

Because it is impractical for staff in diagnostic laboratories to observe nematodes at magnifications of more than 100x, they face the difficult task of identifying nematodes at the low magnifications which are used for counting. Since there are few morphological features that can be seen at these magnifications, identification is based on characters such as general size and body shape, rate of movement, tail shape, length and thickness of the stylet, vulval position and the type of junction between the oesophagus and intestine. The skills involved in differentiating nematodes using such a limited range of characters cannot be learnt from textbooks, and it is only after many months of observing nematodes that one can become familiar with the range of nematodes that occur in soil samples. With experience, it is possible to reach the point where most of the common plant-parasitic nematodes can be identified to genus level. Those working in a defined region or on a single crop, where there is a good knowledge of the local nematode species, or where particular species have readily observable characteristics, may eventually be able to identify common species at low magnification.

There are often situations where it is impossible to identify a nematode at low magnification. Those involved in counting nematodes must be able to decide whether such a nematode should be mounted on a slide and identified at a higher magnification, or whether it should be sent to a taxonomic expert for identification. Staff must also be able to differentiate economically important plant-parasitic species from other stylet-bearing nematodes that are superficially similar at low magnification. One frequently encountered problem is the inability to differentiate *Meloidogyne* juveniles in soil samples from a number of common fungal feeding species. Both *Meloidogyne* juveniles and many fungal feeding nematodes are relatively small, and have a pointed tail and a short thin stylet that is just visible at a magnification of 40x. With experience, it is possible to differentiate these nematodes, but this issue illustrates the importance of using professional staff who are adequately trained in nematological techniques.

There are no easy ways of learning to identify nematodes at low magnification, and the best way to develop the skill is to train under an experienced nematologist. However, the information presented in Table 13 may be useful, as it has been prepared with the beginner in mind. Characters that are readily observable at low magnification (e.g. size, presence and type of stylet, vulval position) and information on distribution are presented, and can be used to allocate unknown nematodes to one of the major nematode groups or genera.

Table 13. Characteristics of common nematode groups and diagnostic features that are useful at magnifications of 25-100x.

Group 1: Small nematodes (generally less than 0.7 mm in length), with sedentary endoparasitic adults.

Common name	Root-knot	Cyst	Citrus	Reniform
Examples of genera	*Meloidogyne*	*Heterodera, Globodera*	*Tylenchulus*	*Rotylenchulus*
Key characteristics	• J2 the only stage common in soil. Males sometimes present, much greater in length than J2. • Spherical-shaped females embedded in roots, with egg mass usually on the root surface. • J2 stylet about 10µm long with knobs. Just visible in living nematodes at 40x magnification. • Oesophagus overlaps intestine ventrally. • J2 with conoid tail, tip irregular in outline, internally clear.	• J2 and cysts the only two stages usually found in soil. • Lemon-shaped cysts (*Heterodera*) or sphere-shaped (*Globodera*). The female's neck is embedded in roots but the body remains exposed. Eggs mainly retained within the body of the female. • J2 stylet 20-30µm long, with distinct knobs. • J2 oesophagus overlaps intestine ventrally. • J2 with conoid tail, tip clear.	• J2 and males similar in size and both common in soil. • Saccate females partly embedded in roots. Eggs on root surface, but no obvious egg mass. • J2 stylet about 13µm long, with knobs. • Oesophagus abuts intestine. • Conoid tail.	• J2 and immature females both occur in soil. • Kidney-shaped females are partly embedded in roots and are covered with a distinct egg mass. • Males with a reduced spear occur in some species. • Position of vulva (V) at 65-70%. • J2 and female stylets are 12-15µm long, with knobs. • Oesophagus overlaps intestine ventrally. • Conoid, bluntly rounded tail.
Distribution	Hosted by many weeds and most vegetable, horticultural and ornamental crops. Common in field crops in warm climates when soil moisture is not limiting.	Common in temperate climates: Cereal cyst- wheat and barley. Clover cyst- clover. Potato cyst- Solanaceae and Compositae. Beet cyst- brassicas and cruciferous crops.	Common on citrus. Often occurs on grapes when they are grown in association with citrus.	Confined to the tropics and sub-tropics. *R. reniformis* has a wide host range and *R. parvus* is common on grasses.
Comments	J2 can be differentiated from fungal feeding tylenchids by their longer, slightly more robust spear, overlapping oesophagus, less pointed tail and absence of vulva or spicules.	J2 similar to *Meloidogyne*, but a longer, more robust nematode, with much heavier stylet and knobs.	Can occur with *Meloidogyne* on grape. J2's differentiated from *Meloidogyne* by the stronger stylet, abutting oesophagus and more pointed tail.	Dead, free-living stages of the nematode assume an open C shape.

Table 13.   (continued)
Group 2. Small nematodes (less than 0.7 mm long), with worm-like adult stages.

Common name	Fungal feeding tylenchids	Pin	Ring
Examples of genera	*Cephalenchus, Psilenchus, Aglenchus, Boleodorus, Coslenchus, Filenchus, Basiria, Malenchus*	*Paratylenchus*	*Criconemella, Criconema, Ogma, Hemicriconemoides*
Key characteristics	• Short, delicate, knobbed stylet about 10-15µm long. • Oesophagus abuts intestine. • Long (filiform) tail, no clear area at terminus.	• Nematodes less than 0.5 mm long. • Females with knobbed stylet 12-40 µm long. *Gracilacus*, a closely related genus recognised by some authorities, has a stylet 40-120µm long. Males with degenerate oesophagus and stylet reduced or absent. • V = 80. • Vulva often at an angle, with vulval lips slightly protruding. Body narrows distinctly after the vulva.	• Small, stout nematodes (< 1 mm long but thick relative to length). • Annules prominent, giving nematodes a distinctly serrated appearance. • Prominent stylet (>50µm in length), with obvious knobs. • Basal bulb of oesophagus abuts intestine and is smaller than median bulb. • Posterior vulva. • Males slender, with no stylet. • Slow moving or immobile.
Distribution	Present in most soils.	Widely distributed. When present, the nematode usually occurs in high numbers.	Common.
Comments	Delicate stylet is barely visible at 40x magnification but can be easily seen at 100x.	Dead nematodes often C-shaped. Live nematodes make alternate 'S' and closed 'C' movements.	Because of their slow moving behaviour, populations are underestimated with extraction methods which are reliant on nematode motility.

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Table 13.   (continued)

Group 3. Medium sized nematodes (generally 0.7-1.2 mm long) with a prominent, knobbed stylet.

Common name	Spiral	Stunt	Lesion	Burrowing	Sheath
Examples of genera	*Rotylenchus, Hoplolaimus Helicotylenchus, Scutellonema,*	*Tylenchorhynchus Merlinius*	*Pratylenchus*	*Radopholus*	*Hemicycliophora*
Key characteristics	• Large, robust, well-developed stylet (20-50μm long). • Oesophagus overlapping intestine. • V = 60. • Tail usually broadly rounded, but varies with genus and species. • Dead nematodes coil in a spiral form, or C shape.	• Short spear, (15-30 μm long) of medium thickness with slightly flattened knobs. • Abutting oesophagus. • V=50. • Broadly rounded tail.	• Short (15-19μm), strong stylet. • Lip region broad and flat, and continuous with body contour. Distinct head skeleton. • Oesophagus overlaps intestine ventrally. • V=67-85. • Tail shape varies, depending on species.	• Female with short (17-20μm) strong spear. Male has a high, rounded lip region and a reduced spear. • Oesophagus overlaps intestine dorsally. • V=55 (*R. similis*).	• Long (about 90μm), well developed stylet. • Adult female and juveniles enclosed in a double cuticle (sometimes known as a sheath). • Stylet and double cuticle lacking in males. • Oesophagus abuts intestine, basal bulbs smaller than median bulb. • V=90.
Distribution	Common. Wide host range. *Helicotylenchus dihystera* and *Rotylenchus brevicaudatus* are widely distributed.	Common on cereals and grasses.	Common. Present in most soil and root samples, but species will vary according to the crop.	*R. similis* is widespread in tropical regions. It has a broad host range but is common and economically important on banana.	Uncommon.
Comments	Oesophageal overlap (dorsal v. ventral) is useful for separating some genera. Size and position of phasmids separates several genera, but can only be seen under oil immersion objective.	These genera are separated under oil immersion by the number of lateral lines.	Males are present in about half the species. Similar in morphology to females. The oesophageal-intestinal junction is useful for differentiating juvenile lesion and stunt nematodes.	All species have marked sexual dimorphism.	

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Table 13.   (continued)

Group 4. Medium sized nematodes (generally 0.7-1.2 mm long) with a small, thin or insignificant stylet, or no stylet.

Common name	Rhabditids	Stubby	Leaf and bud nematodes and fungal feeding Aphelenchids		Stem and bulb	Seed and leaf gall
Examples of genera	Numerous genera	*Paratrichodorus*	*Aphelenchoides*	*Aphelenchus*	*Ditylenchus*	*Anguina*
Key characteristics	- Relatively thick, stout nematodes, 0.5-1.5 mm long. - Mouth is an open, cylindrical chamber which may be difficult to visualise at low magnification. - Rounded basal bulb abutting the intestine. - Tails generally pointed. - Move relatively quickly.	- Relatively thick, stout nematodes. - Thin stylet with an obvious curvature. No knobs. - Median bulb absent. - V=50, vulva inconspicuous. - Broadly rounded tail region. - Cuticle loose and sometimes swollen.	- Nematodes slender. - Short, thin stylet (<20μm) without obvious knobs. - Conspicuous, rounded median bulb. - V=65-70. - Tail conoid with sharply pointed terminus. Tail tip with one to many mucro. - Males with rose thorn-shaped spicules. - Fast moving.	- Short, thin stylet without obvious knobs. - Conspicuous, well developed, rounded median bulb, easily visible at low magnification. - V=80. - Rounded tail.	- Short, thin slender spear (10-12μm) with knobs. - V=75-80. - Conoid tail with pointed terminus. - Fast moving.	- J2, the only stage in soil, is 0.8-1 mm long, with a short (10μm) spear. - Females (2.5-4 mm long) are found in stem and seed galls. - Female body is distended. - Females ventrally coiled and almost immobile.
Distribution	Common. Occur in most soils and populations increase when decomposing organic matter is present.	Common, particularly in sandy soils. *P. minor* is widespread on grasses.	Common. Many species are fungal feeders (e.g. mushroom parasites), but some are important plant parasites.	Common. *A. avenae*, the most common species, occurs in most soils.	Occurs on lucerne, onions, clovers and other hosts in cool climates.	*A. funesta*, a causal factor in annual ryegrass toxicity, occurs on ryegrass in the southern states of Australia.
Comments	Bacterial feeding nematodes.	The only plant-parasitic nematode with a curved spear.	Plant parasitic species are found above-ground in leaves and buds.	Fungal feeding nematodes.	Nematodes are found in large numbers in infested tubers, bulbs, leaves and stems.	Nematodes are found in galls which develop in the flowers of infested plants.

Table 13.   (continued)

Group 5. Large nematodes (greater than 1.2 mm long).

Common name	Dagger	Needle	Mononchids	Dorylaimids
Examples of genera	*Xiphinema*	*Paralongidorus*	Many genera	Many genera
Key characteristics	• Body long (1.5-5 mm) and thin. • Long, thin stylet with basal flanges. Total length of spear plus flanges >100μm. Juveniles with two stylets. • Guiding ring around posterior end of stylet. • Bottle-shaped oesophagus. • Median bulb absent. • Assume a wide C shape when dead.	• Body long (2-11 mm) and thin. • Long, thin stylet without basal flanges. • Guiding ring around anterior end of stylet. • Bottle-shaped oesophagus • Median bulb absent. • Assume a wide C shape when dead.	• Large, open mouth armed with one or more teeth. • Cylindrical oesophagus. • Median bulb absent. • Fast moving.	• Usually large, dark coloured nematodes. • Relatively small spear usually without knobs or flanges. • Bottle-shaped oesophagus. • Median bulb absent.
Distribution	More common under perennial crops.	Uncommon.	Quite common.	Present in most soil samples.
Comments	Some species virus vectors. Extraction techniques that depend on motility may underestimate numbers. Sometimes nematodes are not recovered from upper soil layers as they tend to occur deep in soil.	Because of their large size, they are not readily recovered with extraction methods that depend on motility.	Predatory on nematodes and other invertebrates.	Omnivorous nematodes that feed mainly on other soil organisms (e.g. nematodes, protozoans, rotifers and algae).

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6.2.4 Identification of important nematode genera

With experience, it is possible to identify nematodes to genus level with a dissecting microscope or under the low magnifications of a compound microscope. However, there are certain groups of nematodes (e.g. spiral nematodes) where generic status cannot be determined without mounting specimens on a slide and examining them at a high magnification. One simple method suitable for temporary mounts is to hand-pick nematodes into a drop of water on a slide. Nematodes are then killed by heating the slide briefly over a gas burner until it feels hot. A coverslip is placed over the specimens and they are examined at a magnification of 400x-1000x.

Plant nematodes found in Australia can be identified to genus level using the following key. The key does not include every genus that has been recorded, as many nematodes are little known or rarely encountered in cultivated soils. Nevertheless, all common genera are included. Once an unknown nematode has been tentatively identified using this key, its identity should be confirmed using the description and figures provided in section 6.2.5, and by reference to the taxonomic literature cited in section 9.

Table 14   Key to identifying important plant nematode genera in Australia.

This key does not include every genus of plant nematode recorded in Australia. Not included are genera that are little known or are rarely encountered in cultivated soils. Since the key is based on adults, it does not include infective stages, such as J2 of sedentary endoparasitic genera.

< = less than, > = more than, ± = more or less.

| 1a. | Nematodes in plant foliage, seeds or bulbs ....2 |
| 1b. | Nematodes in soil and/or roots..4 |

| 2a. | Female body stout, spiral when dead ....*Anguina* |
| 2b. | Female slender, straight to slightly curved when dead.......................................3 |

3a.   Median bulb large, almost filling body width; tail with mucro; males with
thorn shaped spicules .... *Aphelenchoides*
3b.   Median bulb not filling body width; tail without mucro; males with slender,
curved spicules ....*Ditylenchus*

| 4a. | Nematodes found in roots ....5 |
| 4b. | Nematodes found in soil...13 |

| 5a | Mature females swollen and sedentary in roots ....6 |
| 5b. | Mature females vermiform, migratory in roots ....10 |

6a.   Mature white females very swollen, pear to lemon shaped, sometimes spherical, tail absent 7
6b.   Mature females partially swollen, kidney to irregularly saccate-shaped, tail present 9
6c   Mature females ± sausage shaped, 0.55-0.9 mm long; neck region thin; vulval lips protruding;
V = 51-61%; usually one large (90x60 µm) egg in uterus; gelatinous matrix present;
pointed tail ....*Achlysiella*

7a.   Females remaining whitish, with terminal perineal pattern and opaque to yellow
or orange-brown gelatinous egg masses containing eggs...................................*Meloidogyne*
7b.   Females white at first, darkening to yellow, then dark brown when dead, forming cysts
containing eggs; no egg mass outside female; cyst surface with lace-like or zig-zag pattern..............8

8a   Female lemon shaped, with terminal cone; vulval slit up to 60 µm; long underbridge
& bullae usually present.. *Heterodera*
8b   Female spherical, terminal cone absent; vulval slit < 15 µm; tuberculate area near
vulva; under-bridge and bullae usually absent.. *Globodera*

9a.   Mature females kidney shaped; eggs in gelatinous matrix; oesophageal glands
overlap intestine; excretory pore anterior, ± opposite glands; two ovaries; tail short,
pointed; mainly occurs in tropics & sub-tropics...*Rotylenchulus*
9b.   Mature female very ventrally curved; eggs in gelatinous matrix; basal bulb
abutting intestine; excretory pore very posterior, just anterior to vulva, 65 - 85%
of body length; one ovary, post-vulval sac absent; tail terminus conoid-rounded ....*Tylenchulus*

| 10a. | Females with two opposing ovaries ....11 |
| 10b. | Females with one ovary...12 |

11a.   At least one oesophageal gland nucleus anterior to, or opposite well-developed
oesophago-intestinal valve; deirids present; male tail with ventral contraction just
anterior to hyaline terminus; bursa enveloping tail terminus ....*Pratylenchoides*
11b.   All oesophageal gland nuclei posterior to oesophago-intestinal junction; deirids absent;
male tail without ventral contraction; bursa usually not quite extending to tail terminus..*Radopholus*

Table 14   continued.

12a.   Longest oesophageal gland overlaps intestine ventrally ....*Pratylenchus*
12b.   Longest oesophageal gland overlaps intestine dorsally..*Radopholoides*

13a.   Dorylaimid oesophagus, no median bulb ; stylet without prominent knobs; juveniles
      with functioning and replacement stylets 14
13b.   Tylenchid oesophagus, median bulb present; stylet usually with distinct knobs,
      replacement stylet not present in juveniles...16

14a.   Nematodes < 1 mm long, cigar shaped; stylet curved; anus sub-terminal ....*Paratrichodorus*
14b.   Nematodes over 1.3 mm long, slender relative to their width; stylet straight,
      very long; anus not terminal..15

15a.   Guiding ring posteriorly situated; stylet base (odontophore) with flanges ....*Xiphinema*
15b.   Guiding ring anteriorly situated; stylet base without flanges or knobs ....*Paralongidorus*

16a.   Females with two opposing ovaries 17
16b.   Females with one ovary...34

17a.   Stylet > 30µm long...18
17b.   Stylet < 30µm long...20

18a.   Adult nematodes < 2 mm long; lip region not grooved; lateral canals faint or absent.......*Morulaimus*
18b.   Adult nematodes > 2 mm long; lip region with 4 longitudinal grooves;
      lateral canals thick & conspicuous 19

19a.   Lateral field with only 1 groove-like line; rare in Australia...*Belonolaimus*
19b.   Lateral field with 4 lines; uncommon..*Ibipora*

20a.   Nematodes spiraled to C shaped when dead; < 1.2 mm long 21
20b.   Nematodes straight to slightly ventrally curved or sinuate when dead 25

21a.   Oesophageal glands overlapping intestine in 3 lobes..22
21b.   Oesophageal glands in pyriform or rounded basal bulb, abutting intestine 30

22a   Longest oesophageal gland overlaps ventrally...*Helicotylenchus*
22b.   Longest oesophageal gland overlaps dorsally 23

23a.   Phasmids very small...*Rotylenchus*
23b.   Phasmids large, lens-like, often slightly yellowish 24

24a.   Phasmids at or near anus, close to or opposite each other; stylet knobs rounded ....*Scutellonema*
24b.   Phasmids near vulva, 1 anterior, 1 posterior or, if both posterior to vulva, then
      much anterior to anus, not opposite each other; stylet knobs tulip shaped, with
      anterior projections...*Hoplolaimus*

25a.   Oesophageal glands in pyriform or rounded basal bulb, abutting intestine 26
25b.   Oesophageal glands overlapping intestine 27

26a.   Lateral field with 2-5 lines ....*Tylenchorhynchus*
26b.   Lateral field with 6-8 lines ....*Merlinius*

27a.   Lip region rounded, set off..*Telotylenchus*
27b.   Lip region more or less continuous with the body contour...28

Table 14   continued.

28a.   Longest oesophageal gland overlaps ventrally...*Zygotylenchus*
28b.   Longest oesophageal gland overlaps dorsally 29

29a.   At least one oesophageal gland nucleus anterior to, or opposite well-developed
        oesophago-intestinal valve; deirids present; Male tail with ventral contraction
        just anterior to terminus; bursa enveloping hyaline tail terminus................................*Pratylenchoides*
29b.   All oesophageal gland nuclei posterior to oesophago-intestinal junction;
        deirids absent; male tail without ventral contraction; bursa usually not quite
        extending to tail terminus ………………………………………………*Radopholus/Achlysiella**

30a.   Excretory pore posteriorly situated, 65-85% of body length .... *Tylenchulus*
30b.   Excretory pore anteriorly situated, ± opposite isthmus or oesophageal glands................................31

31a.   Tail conoid or pointed, not filiform...32
31b.   Tail pointed, long and filiform 33

32a.   Stylet > 30 µm long; nematodes small, C shaped when dead, body usually
        narrower after vulva ....*Gracilacus*
32b.   Stylet 13- 30 µm long; nematodes small, C shaped when dead, body usually
        narrower after vulva ....*Paratylenchus*
32c.   Stylet < 13 µm long; nematodes medium sized, straight to slightly curved when dead......*Ditylenchus*

33a   Nematodes < 1.5 mm long, stylet < 40µm long ………………………………………………*Filenchus*
        (also related forms: *Psilenchus, Coslenchus, Cephalenchus*, etc.)
33b   Nematodes > 1.3 mm long, stylet > 65 µm long........................ ....*Tylodorus*

34a.   Female with double cuticle (most noticeable in tail region)...35
34b.   Female with single cuticle (or double cuticle not observed) 36

35a.   Sheath often loose; lip region often dome-like; stylet knobs rounded; outer
        cuticle with lateral field; juveniles with double cuticle...*Hemicycliophora*
35b.   Sheath close fitting; lip region not usually dome-like; stylet knobs anchor
        shaped; lateral field absent in female; juveniles with scaled single cuticle............*Hemicriconemoides*

36a   Oesophageal glands in pyriform or rounded basal bulb, abutting intestine 37
36b.   Oesophageal glands overlapping intestine 43

37a.   Cuticle thick, annulation conspicuous, ring-like; isthmus very short, wide, visible
        only as narrowing between large median bulb and smaller basal bulb 38
37b.   Cuticle and annulation not as above; isthmus distinct, narrow 42

38a.   Female with double cuticle; juvenile scaled single cuticle....................................*Hemicriconemoides*
38b.   Female with single cuticle; juveniles with or without scales 39

39a   Female with scales, in rows, alternate or as fringe; juveniles also scaled,
        but scales different from female...*Ogma/Pateracephalanema*
39b.   Female without scales; juveniles with or without scales..40

*Achlysiella is separated from *Radopholus* mainly because its mature female is swollen and sedentary (see 6c).

Table 14   continued.

40a.   Lip annules narrower than following body annules; small submedian lobes project
        from anterior surface of lip region; juveniles with smooth annules ..... *Criconemella/Macroposthonia*
40b.   Lip region with 1 or 2 annules, expanded, wider than and offset from
        following body annules; submedian lobes absent 41

41a.   Nematodes very small, usually C shaped; first annules disc-like; juveniles
        not scaled.. *Discocriconemella*
41b.   Nematodes slightly curved; first annule expanded, offset from
        body; anterior vulval lip usually overhanging vulva; juveniles scaled .... *Criconema/Nothocriconema*

42a.   Tail conoid or pointed, not filiform...30
42b.   Tail pointed, long and filiform 33

43a   Tail tapering, with mucro or sharply pointed terminus 44
43b   Tail conoid, terminus conoid or rounded 45

44a.   Median bulb large, almost filling body width; tail with mucro; male with
        thorn shaped spicules .... *Aphelenchoides*
44b.   Median bulb not filling body width; tail without mucro; spicules slender, arcuate ....*Ditylenchus*

45a.   Longest oesophageal gland overlaps ventrally...*Pratylenchus*
45b.   Longest oesophageal gland overlaps dorsally 46

46a   Stylet knobs tulip-shaped, with anterior projections; stylet > 20µm; male lip
        region asymmetrical .... *Hoplotylus*
46b.   Stylet knobs rounded, without anterior projections; stylet < 20µm; male lip
        region symmetrical ....*Radopholoides*

6.2.5 Characteristics of important nematode genera

SEDENTARY ENDOPARASITES

Root-knot nematodes (Figures 13 and 14).
Meloidogyne   Second-stage juveniles (J2) enter the root, modify the root cells near their heads and begin to feed. Galls form in response to the presence of the nematode. Juveniles develop into pear shaped females which are partially or completely buried in root tissue. Eggs are laid into a gelatinous egg sac which is either buried in the root (in large galls) or protrudes from the surface of small galls. Males generally uncommon, but most often present when plants are heavily galled and nematode populations are high.

Female: Stylet short, usually about 15 μm long. Oesophageal gland overlaps ventrally, excretory pore anterior to median bulb. Vulva terminal. Anus dorsal, at base of dorsal vulval lip. Didelphic, ovaries coiled. Perineum with finger print pattern. Tail absent.
Male: Vermiform, 1-2 mm long. Stylet longer and more robust than female. Excretory pore posterior to median bulb. Posterior region of body twisted, tail rounded, without bursa.
Juvenile (J2): Straight to slightly curved when relaxed. Lip framework lightly sclerotised. Excretory pore posterior to hemizonid. Tail pointed with terminal hyaline region.

Widespread, economically important nematode. M. *javanica, M. incognita* and *M.arenaria* are widespread on crop plants, particularly in warm climates. *M. hapla* is also common, but is confined mainly to regions with a cool climate.

Cyst nematodes (Figure 15)
Life history is similar to *Meloidogyne*, but the female is only partly embedded in root tissue and eggs are produced within the body. As the female ages, the cuticle gradually hardens and darkens to form cysts containing eggs. The cuticle is strongly annulated and the annules are modified to form a lace-like pattern on swollen females and cysts.

Female. Stylet 25-30μm long. Excretory pore at the level of, or posterior to, the median bulb. Vulva terminal.
Male. Vermiform with annulated cuticle. Three or four lateral lines. Lip region strongly sclerotised. Tail short, rounded, bursa absent.
Juvenile (J2). Stylet robust, oesophageal glands overlapping ventrally. Lip framework sclerotised, more than in *Meloidogyne*. Tail pointed, with terminal hyaline region. Body almost straight when relaxed

Widespread and damaging nematodes in agricultural and horticultural crops.

Heterodera   Female lemon-shaped, with short neck and terminal cone. Vulval slit 10-60 μm long. Underbridge and bullae usually present
H. avenae (cereal cyst nematode) is widespread on cereals, particularly wheat and barley. *H. schachtii* damages brassicas and other cruciferous crops while *H. trifolii* is common on clovers.
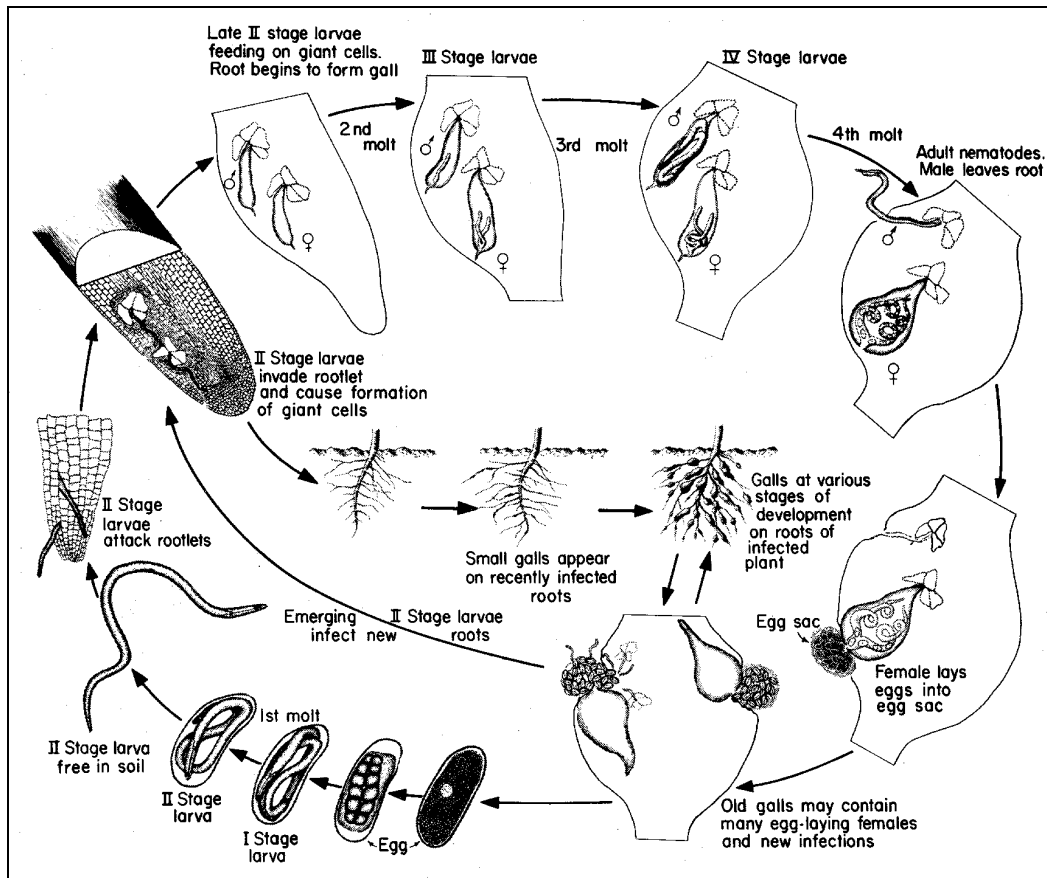
Figure 13. Disease life cycle of a root-knot nematode, *Meloidogyne* sp. From "Plant Diseases caused by Nematodes" in *Plant Pathology*, 3rd. edition by George N. Agrios (1987). Reprinted by permission of Academic Press Inc.
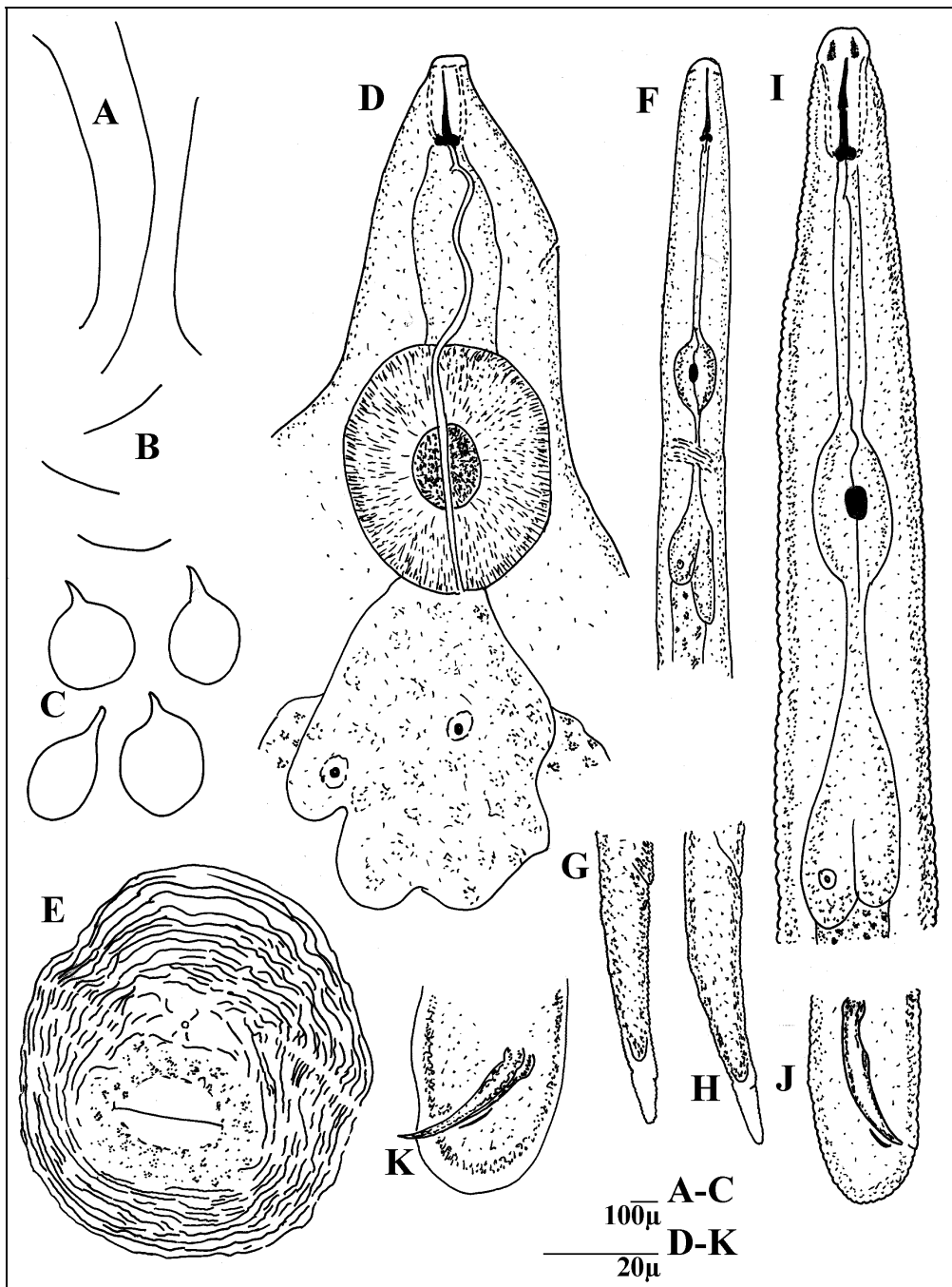
Figure 14. *Meloidogyne* **A, male shape when relaxed; B, J2 shape when relaxed; C, female shape when relaxed; D, oesophageal region of a female; E, perineal end of a female; F, anterior end of a J2, G & H, J2 tails; I, anterior end of male; J & K, male tails.**
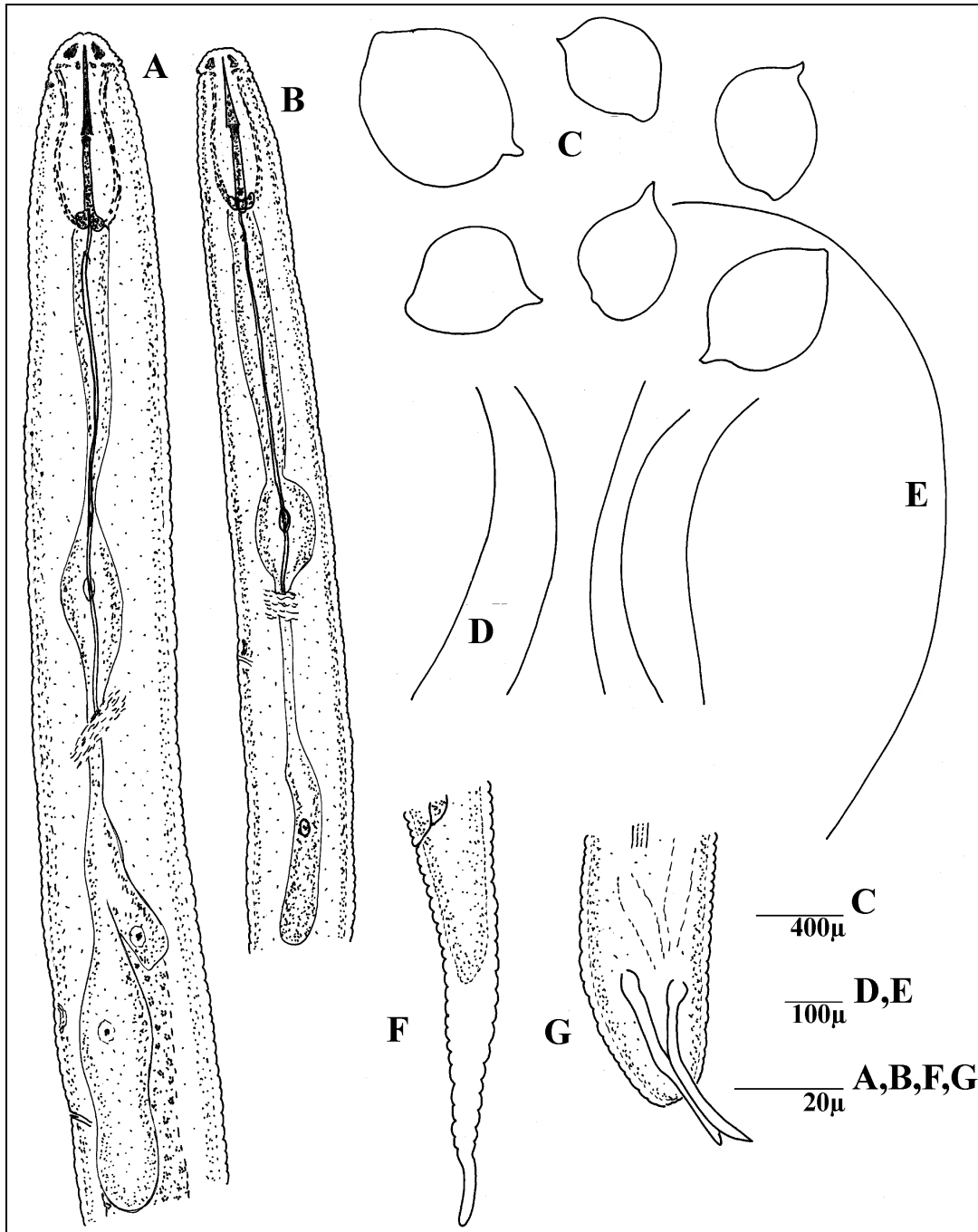
Figure 15. *Heterodera avenae* **A, anterior end of male; B, anterior end of a juvenile; C, shape of female cysts; D, shape of juveniles when relaxed; E, shape of male when relaxed; F, juvenile tail; G, male tail.**

Globodera Female spherical, with short projecting neck, but without terminal cone. Vulval slit is less than 15 μm. Tuberculate area near vulva present. Underbridge and bullae usually absent. Parasites of Solanaceae and Compositae, usually occurring in cooler climates than *Heterodera*. *G. rostochiensis* and *G. pallida* are found in most regions of the world where potatoes are grown. *G. rostochiensis* occurs in a quarantine area in Victoria.

Reniform nematode (Figure 16)
Rotylenchulus Capable of developing from egg to adult without feeding, producing equal numbers of males and females. Vermiform adults are about the same size as newly hatched juveniles. Juveniles develop to immature females in soil, where they penetrate the root, causing development of giant cells. Posterior region of female swells to become kidney-shaped. Eggs are laid in a gelatinous matrix. Nematodes can survive for several months in dry soil.

Mature female: Kidney-shaped, didelphic, ovaries reflexed or coiled. Tail persisting.
Immature female: Vermiform, cephalic region high, continuous, sclerotised. Dorsal oesophageal gland opening one stylet length posterior to stylet knobs. Oesophageal glands overlap ventrally. Tail elongate-conoid, with long hyaline terminal region.
Male: Stylet weaker than in females, oesophagus reduced, sclerotisation weaker. Bursa low, not enclosing tail tip. Tail elongate-conoid, terminal portion hyaline.
Juvenile: Oesophageal glands elongate, overlapping intestine ventrally and laterally. Tail tapering to rounded tip, hyaline region shorter than adults.

Rotylenchulus reniformis is confined to the tropics and sub-tropics where it is widespread. Economically important on many horticultural and field crops, particularly pineapple. *R. parvus* is common on grasses.

Citrus nematode (Figure 17).
Tylenchulus The J2 hatches from eggs and develops without feeding to an immature, worm-like female in about 7 days. The nematode then partially penetrates root cortical cells and establishes a feeding site with nurse cells. The posterior, exposed part of the female swells mostly dorsally, to become ventrally curved. Eggs are laid into a gelatinous matrix which covers the female.

Mature female: Dorsal oesophageal gland opening is 1/3 to 1/2 stylet length behind the stylet knobs. The basal bulb is set off from the intestine. Excretory pore is posteriorly situated, position varies between species. Post-vulval region elongate and tapering. Vulval lips bulging, post-vulval sac absent. Females removed from roots are frequently broken at the neck.
Immature female: Straight to ventrally curved, ovary immature. Excretory pore posterior. Vulval lips thick, bulging. Anus obscure.
Male: Less than 0.5 mm long, stylet and oesophagus are partially degenerate. Excretory pore posterior at 50-60%. Bursa absent.
Juvenile (J2): Straight to slightly curved. Two lateral lines, narrow lateral field. Excretory pore posterior at 50-60%. Lip framework moderately sclerotised. Basal bulb set off from intestine. Anus obscure.

In Australia, *Tylenchulus semipenetrans* occurs in high numbers in most citrus growing areas. It also occurs on grapevines when they are grown in association with citrus.
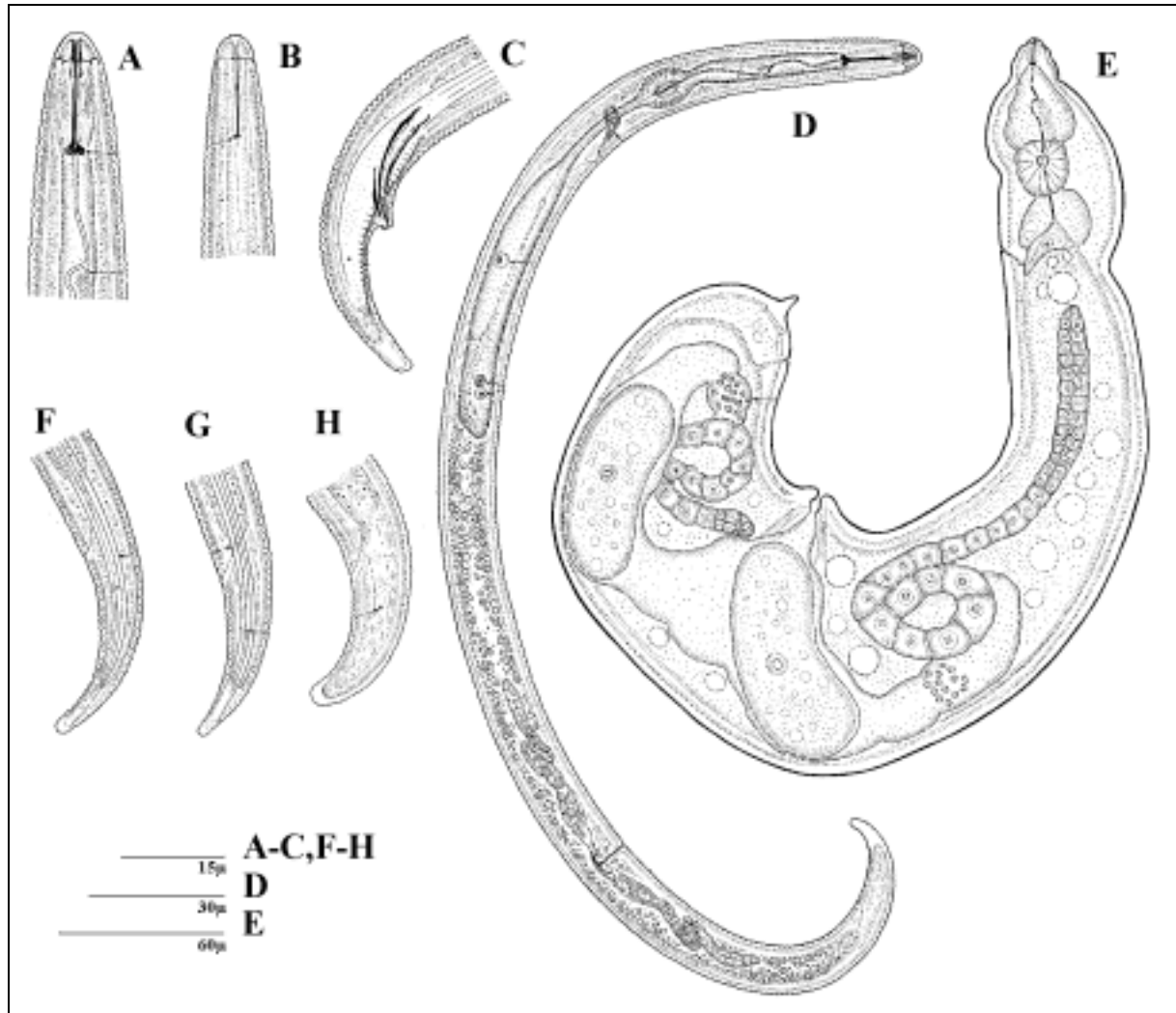
Figure 16. *Rotylenchulus reniformis* **A, head of immature female; B, male head; C, male tail; D, immature female; E, mature female; F,G, tails of immature females; H, juvenile tail. (From CIH Descriptions of Plant-parasitic Nematodes, Set 1, No. 5. Reprinted with permission of the International Institute of Paraasitology**)
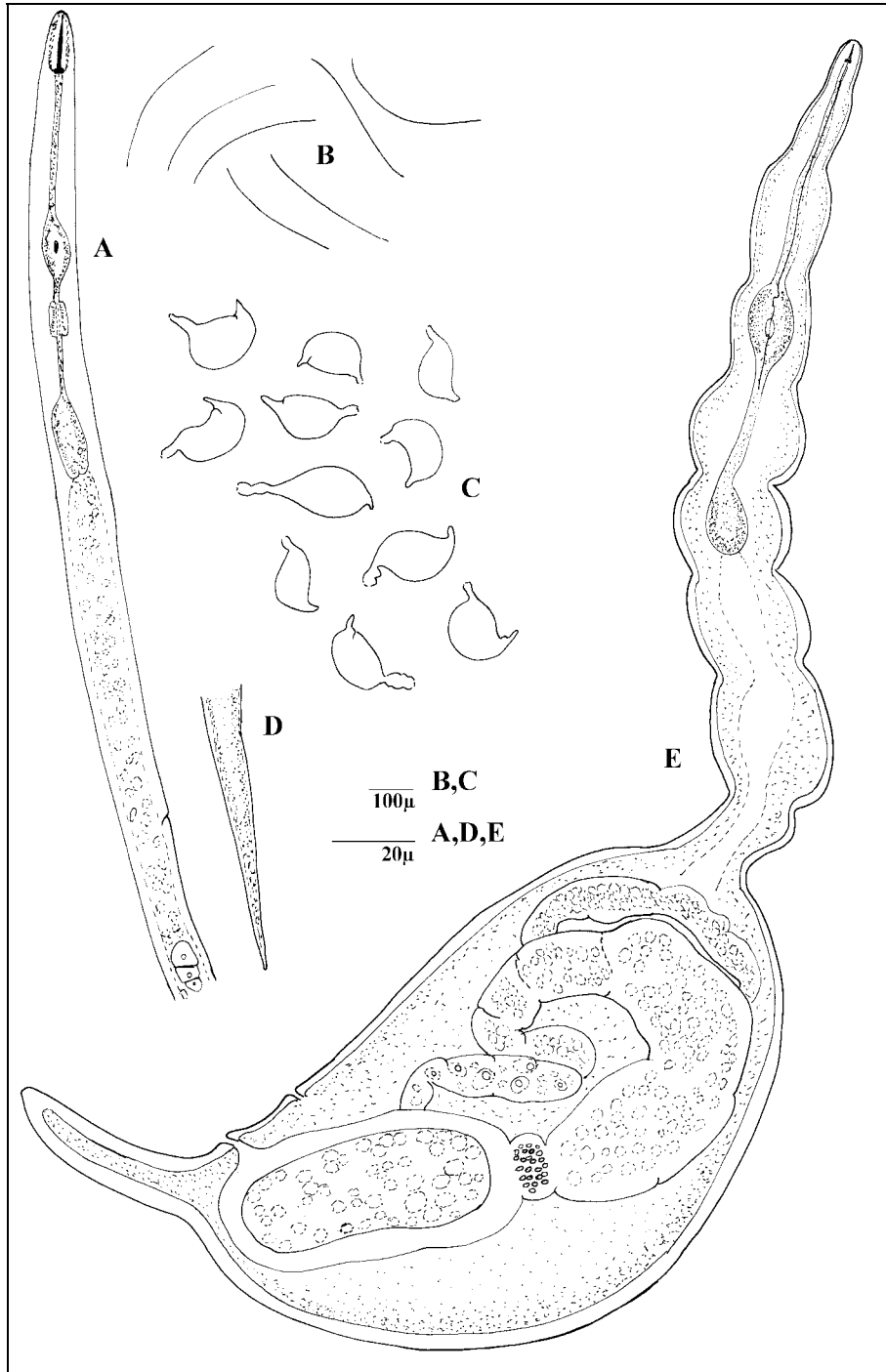
Figure 17. **Tylenchulus semipenetrans A, juvenile anterior region: B, juvenile shape when relaxed; C, saccate female shape when relaxed; D, juvenile tail; E, whole, mature (gravid) female.**

MIGRATORY ENDOPARASITES

Root lesion nematodes (Figures 18 and 20)
Pratylenchus   Head truncate, head framework sclerotised, appearing as black line under low magnification. Stylet short but robust. Oesophageal glands overlap intestine ventrally. Posterior vulva, one ovary. Tail conoid-rounded. Males rare or absent in some species, more slender than females.

Widespread endoparasitic nematodes, of economic significance in horticultural and some grain crops. *Pratylenchus coffeae* and *P. goodeyi* are damaging species on tropical crops such as bananas. *P. brachyurus* is economically important in sub-tropical regions. *P. penetrans* has a wide host range, but is particularly important on pome fruit. *P. zeae* occurs mainly in tropical and subtropical areas on maize, sorghum and sugar cane. *P. thornei* and *P. neglectus* are common on cereals.

Burrowing nematode (Figures 19 and 20)
Radopholus   Body often straight when relaxed, sometimes ventrally curved. Head rounded-- conoid, head framework sclerotised, stylet short but robust. Oesophageal glands overlap dorsally. Vulva 55-65%, two ovaries. Tail conoid or rounded with hyaline region.

R. similis attacks bananas and plantains and some other crops in many tropical and subtropical regions.

ECTOPARASITIC TYLENCHIDS

Ring nematodes (Figures 21, 22 and 23)
Nematodes small (less than 1 mm long), but thick in relation to length. Annules prominent. Stylet relatively long, knobs anchor-shaped. Median bulb well developed, larger than basal bulb which abuts intestine. Vulva posterior, one ovary. Lateral field rare in females and juveniles. Males dissimilar to females in appearance, relatively slender, without stylet, oesophagus degenerate, lateral field present.
Generally associated with woody hosts, some species associated with turf, some species confined to indigenous vegetation.

The most frequently occurring plant parasitic genera in this group include;

Criconemella (**syn.** *Macroposthonia*) Generally robust nematodes with moderately long to very long stylet, often appearing rather bluntly rounded at both ends, especially the head end. Lip annules 2-4, smaller than body annules. Lip region with submedian lobes which project forward and are separated from one another. Pseudolips greatly reduced. Vulva open in the common Australian species. Tail usually conoid to rounded. Juveniles with smooth annules.

C. onoense C. ornata and *C. xenoplax* are some of the common species found in Australia.

Discocriconemella   Similar to *Criconemella*, as the posterior edge of the annules is generally smooth. The main difference is the development of the cephalic annule, which appears as a large, anteriorly flattened disc.
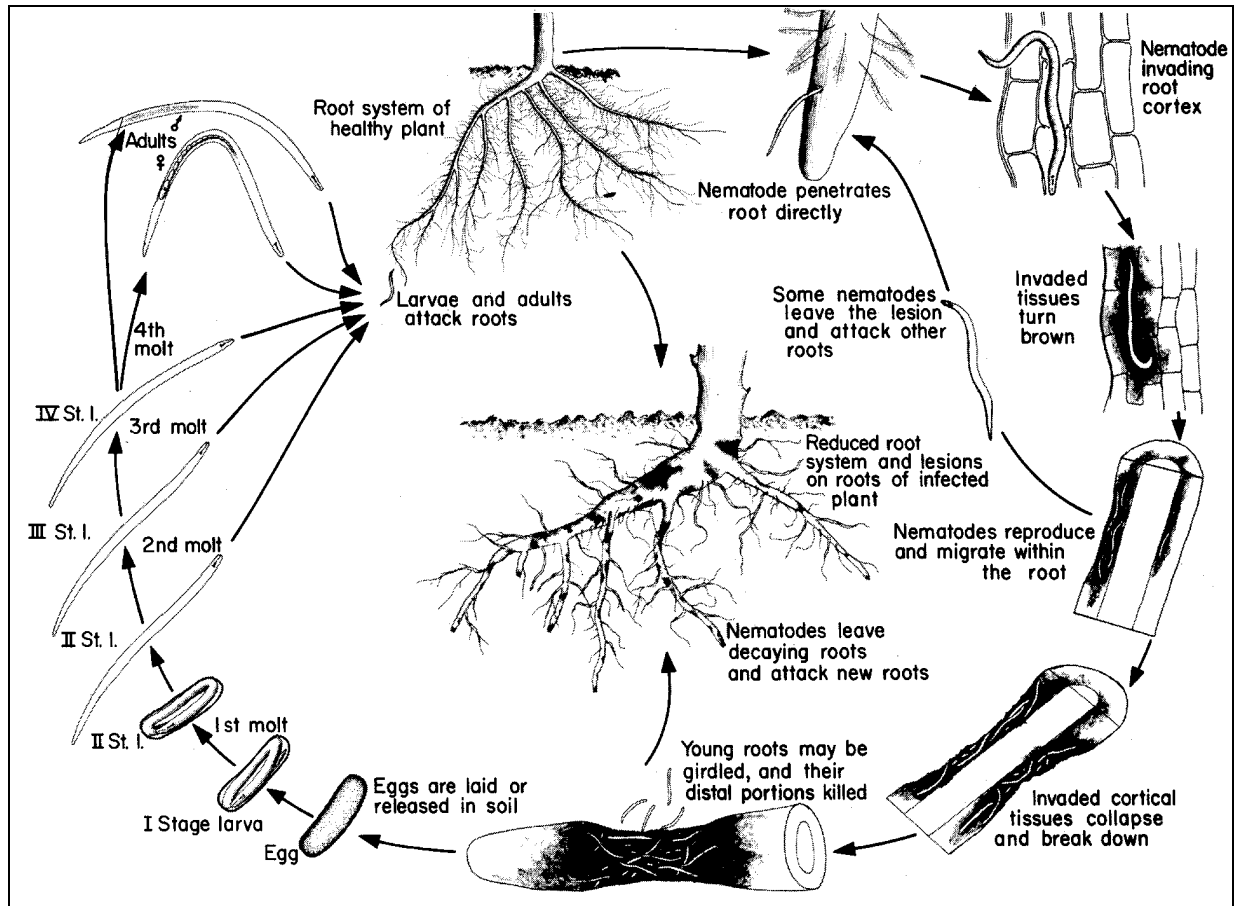
Figure 18. Disease life cycle of the lesion nematode, *Pratylenchus* sp. From "Plant Diseases caused by Nematodes" in *Plant Pathology*, 3rd. edition by George N. Agrios (1987). Reprinted by permission of Academic Press Inc.
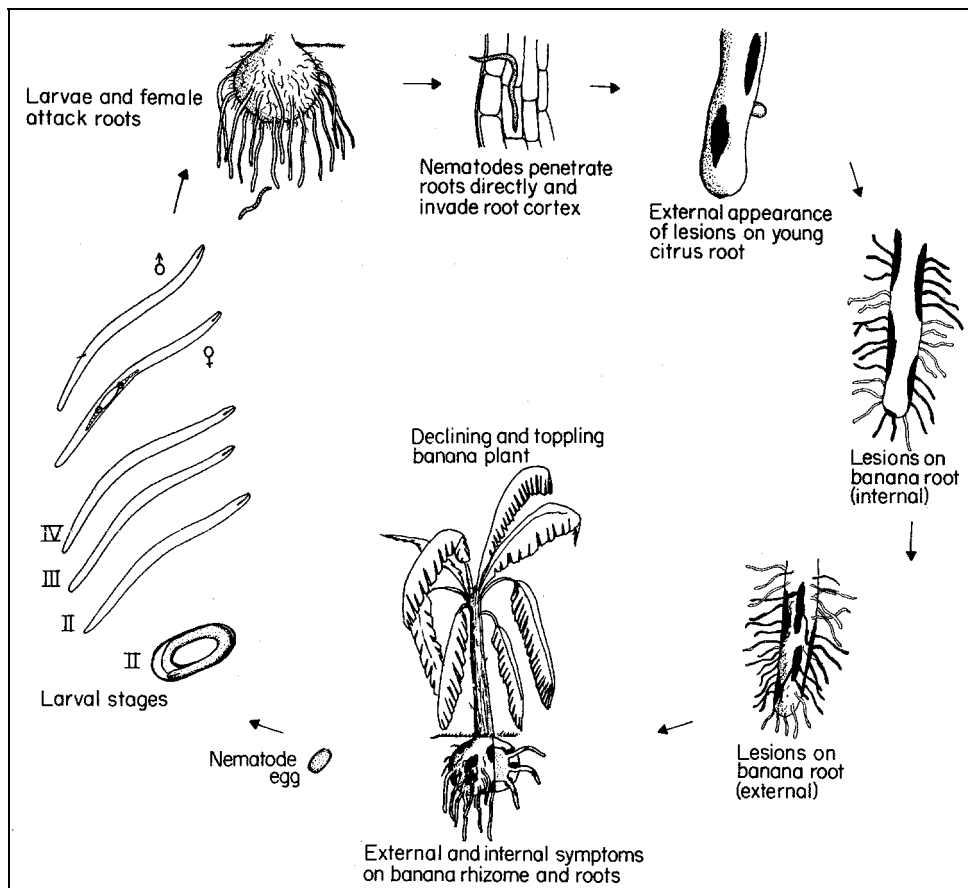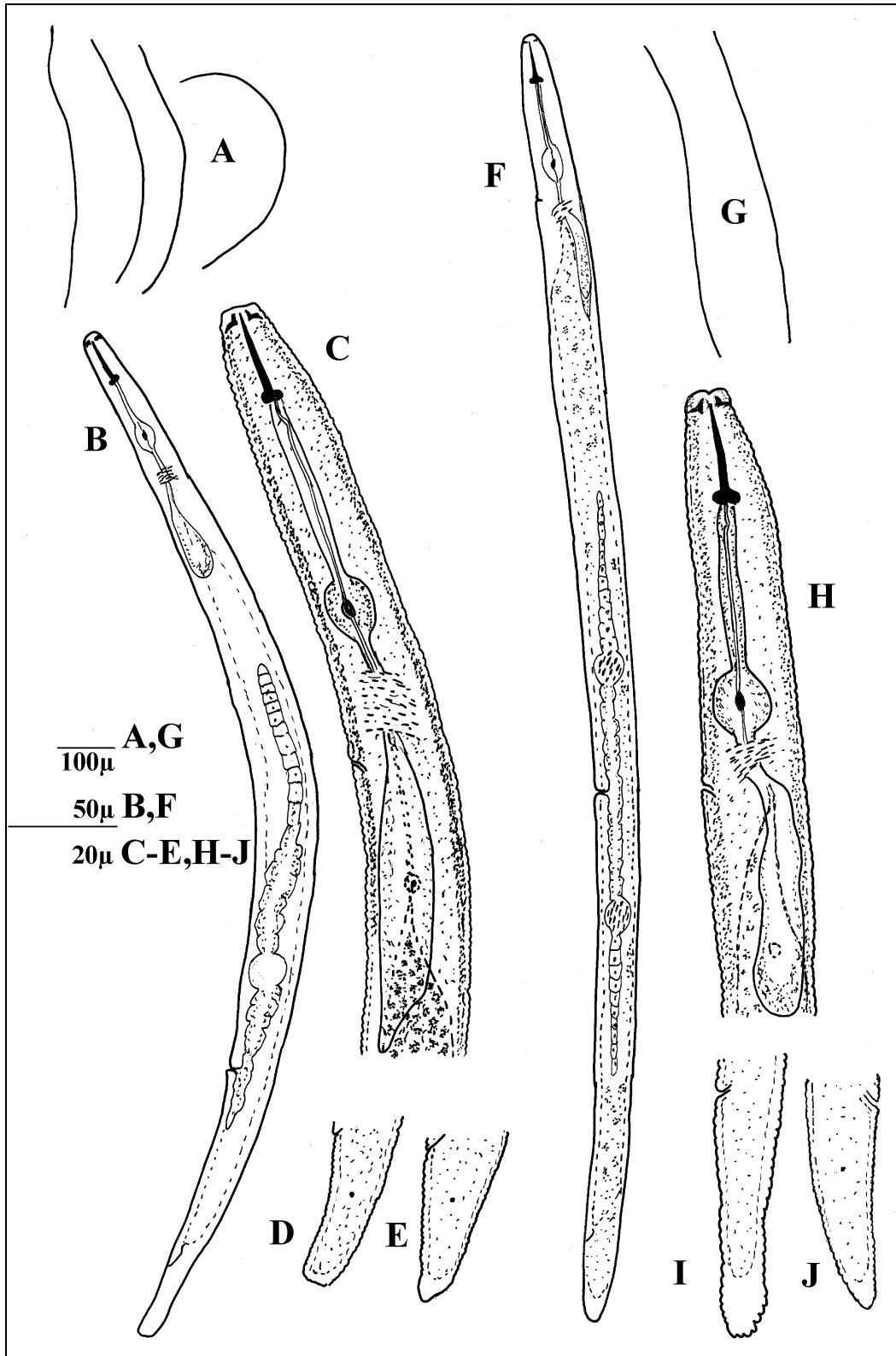
Figure 19. Disease life cycle of the burrowing nematode, *Radopholus similis*. From "Plant Diseases caused by Nematodes" in *Plant Pathology*, 3rd. edition by George N. Agrios (1987). Reprinted by permission of Academic Press Inc.

Figure 20. *Pratylenchus* and *Radopholus*. *Pratylenchus* sp. A, shape when relaxed; B, whole female; C, anterior end of female; D & E, female tails; *Radopholus crenatus* F, whole female; G, shape when relaxed; H, anterior end of female; I & J, female tails.
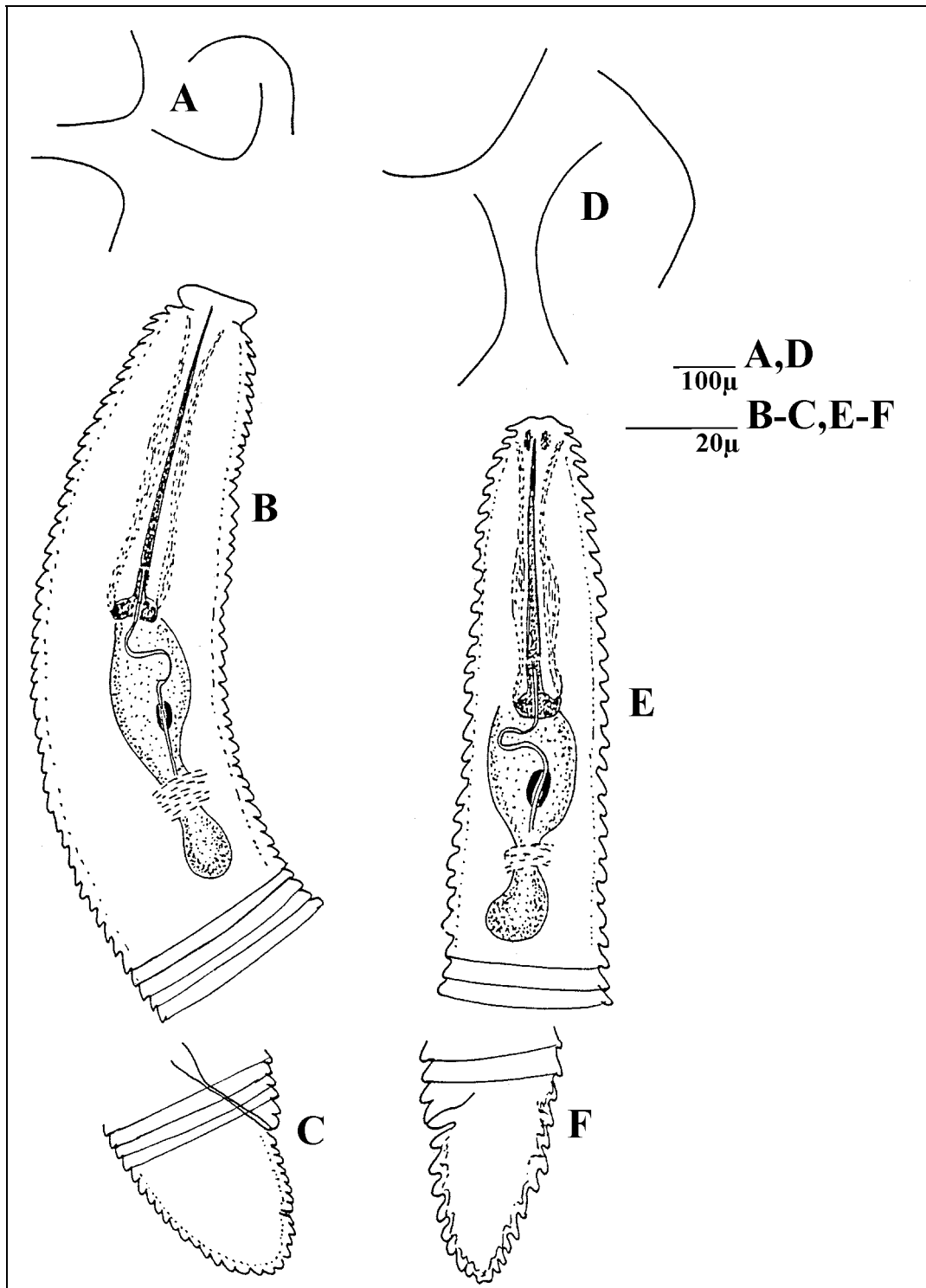
Figure 21. *Discocriconemella* and *Criconema. Discocriconemella limitanea* A, shape when relaxed; B, anterior end of female; C, post-vulval region; *Criconema mutabile* D, shape when relaxed; E, anterior end of female; F, post-vulval region.
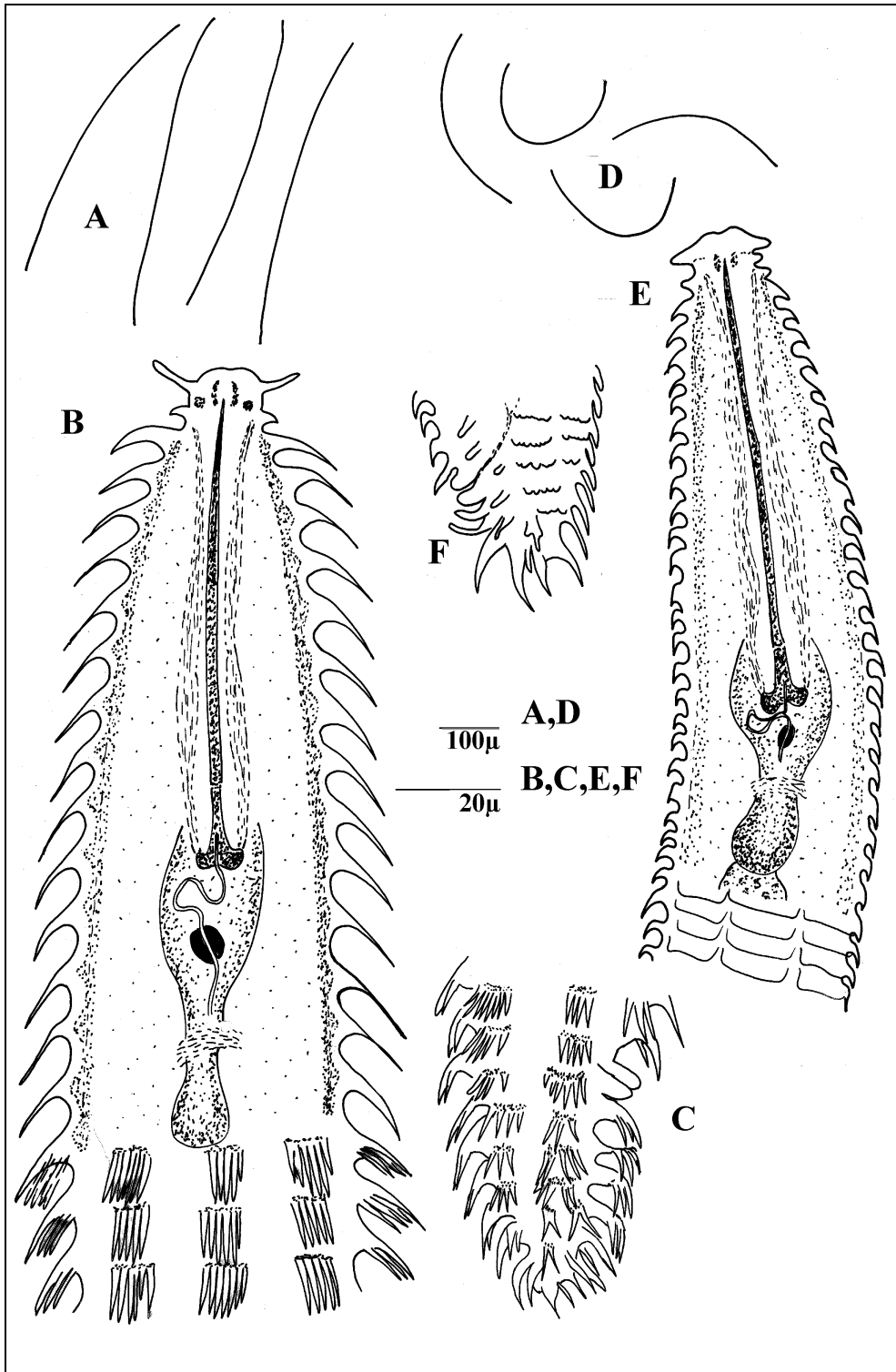
Figure 22. *Ogma (Pateracephalanema). P. pectinatum* A, shape when relaxed; B, anterior end of female; C, post-vulval region; *P. imbricatum* D, shape when relaxed; E, anterior end of female; F, post-vulval region.
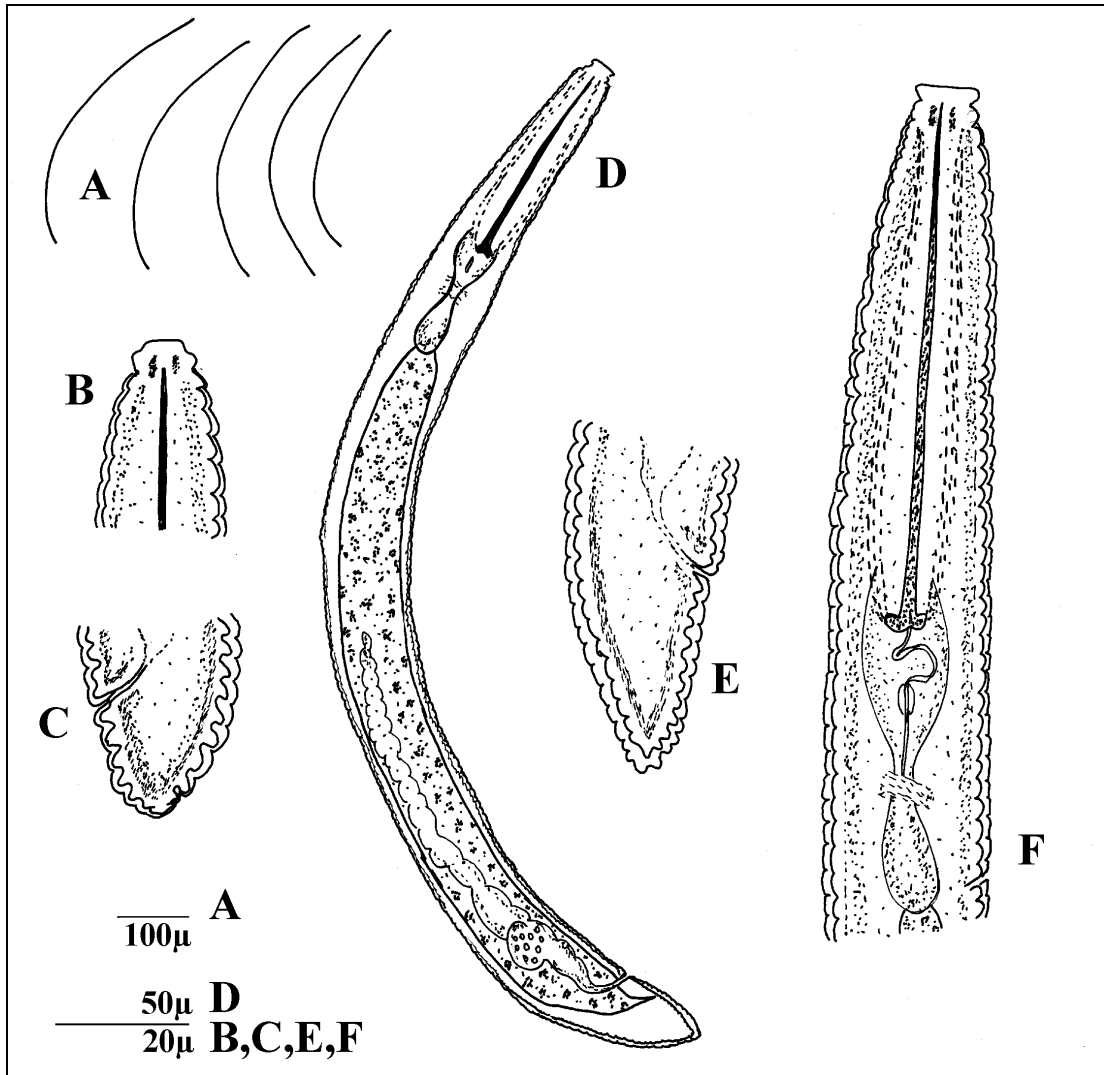
Figure 23. *Hemicriconemoides.* *H. mangiferae* A, shape when relaxed; B, female head; C, post-vulval region; D, whole female; *H. insignis* E, post-vulval region; F, anterior end of female.

Criconema (syn. *Nothocriconema*) Lip annules often larger than and offset from body annules, with 6 pseudolips, submedian lobes absent. Stylet long to very long. Anterior vulval lip usually overhanging vulva. Tail usually conoid to pointed. Juveniles with rows of scales.
C. mutabile is often associated with fruit trees and turf.

Ogma (syn. *Criconema sensu* Hoffmanner & Menzel, syn. *Pateracephalanema* Mehta and Raski) Female often very thick in relation to length. Annules ornamented with scales which often collect soil particles so that nematode appears dark coloured and dirty (all over or in post-vulval region). Scales may somewhat resemble fish scales, or are comb-like, arranged in rows along the body, or alternating, or forming a complete fringe of finger like scales on each annule. Lip annules 1 or 2, often set off from body, may be saucer-like, with smooth margin, crenate or fringed. Juveniles also with scales, which are different to those of corresponding females.

Ogma species are not widely distributed in agricultural situations.

Hemicriconemoides Female with double cuticle, moderately close fitting compared to *Hemicycliophora,* but two cuticles always visible in post-vulval region. Lip region with 6 pseudolips. Stylet long, knobs anchor shaped. Female annules smooth, without scales, lateral lines absent; Juveniles with single cuticle and rows of scales. Male with single cuticle, stylet absent.

H. mangiferae is potentially damaging and frequently associated with mango trees. Several other species of *Hemicriconemoides* occur in Australia but little is known of their pathogenicity.

Sheath nematodes (Figure 24)
Hemicycliophora Nematodes usually 1-2 mm long, with double cuticle, in many species attached to the inner cuticle only at head, vulva and anus. Outer cuticle with lateral field which may be patterned. Lip region continuous or offset. Stylet long to very long, knobs rounded, not anchor-shaped. Median bulb well developed, basal bulb smaller. Vulva posterior, one ovary. Tail pointed to bluntly rounded depending on species. Males with single cuticle but without stylet. Oesophagus degenerate. Juveniles similar to adult females, with double cuticle, no scales.

Widespread genus with many species, often associated with moister soils, occasionally of economic significance, e.g. citrus.

Stunt nematodes (Figure 25A)
Nematodes 0.7 - 1.5 mm long, straight to slightly curved. Stylet varies in length between genera. Oesophageal glands as pyriform basal bulb or overlapping intestine. Vulval epiptygmata often present. Median vulva, two ovaries. Tail conoid-rounded. Small phasmids on tail. Lateral field with 1-7 lateral lines.
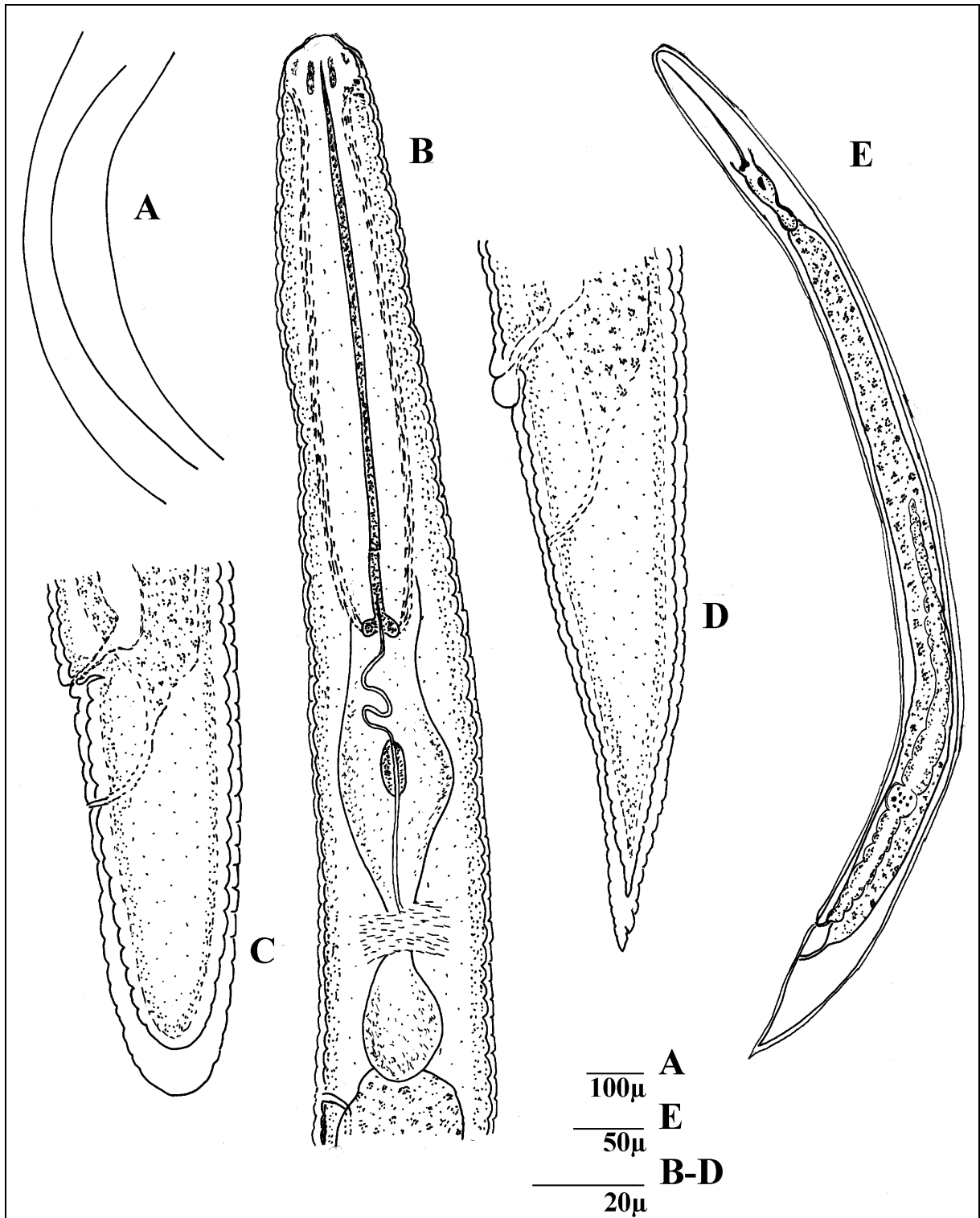
Figure 24. *Hemicycliophora. H. arenaria* A, shape when relaxed; B, anterior end of female; C, post-vulval region; *H. natalensis* D, post-vulval region; E, whole female.
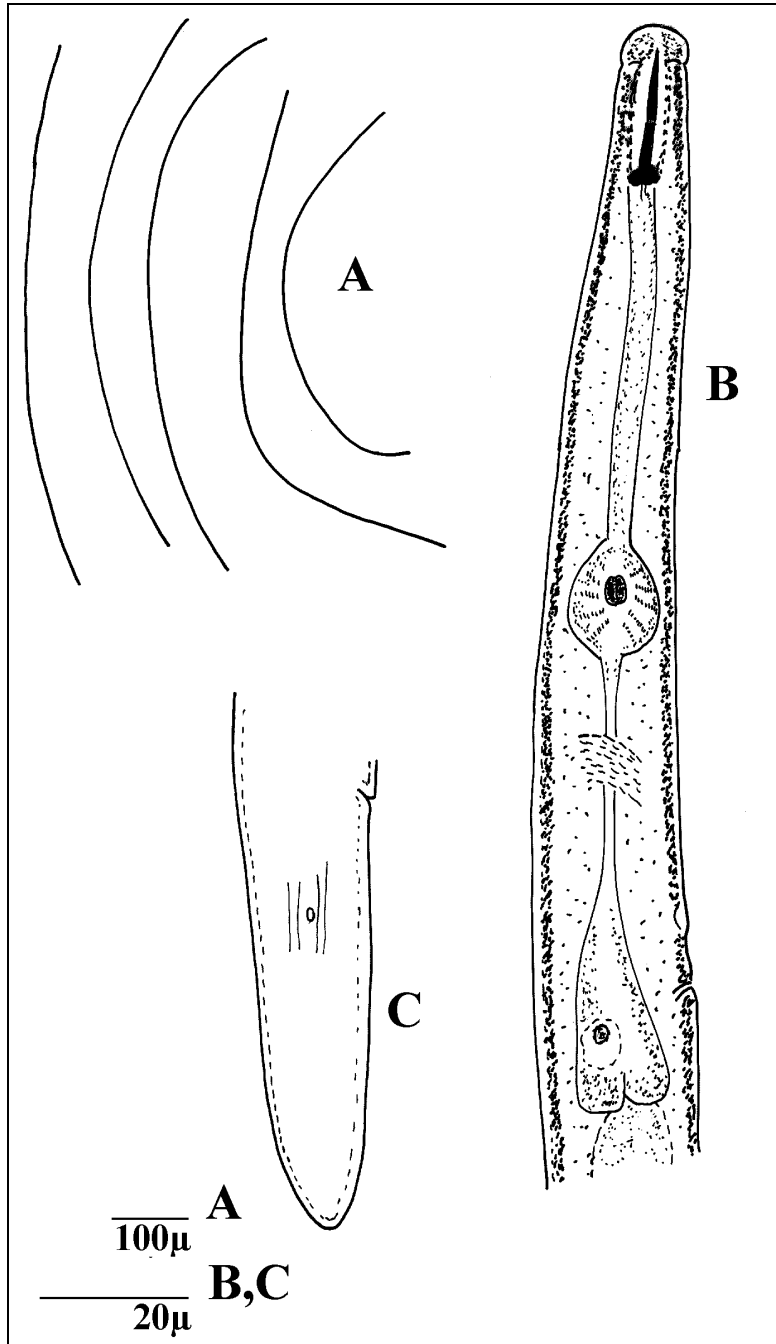
Figure 25A. *Tylenchorhynchus. T. tobari* **A, shape when relaxed; B, anterior end of female; C, female tail.**
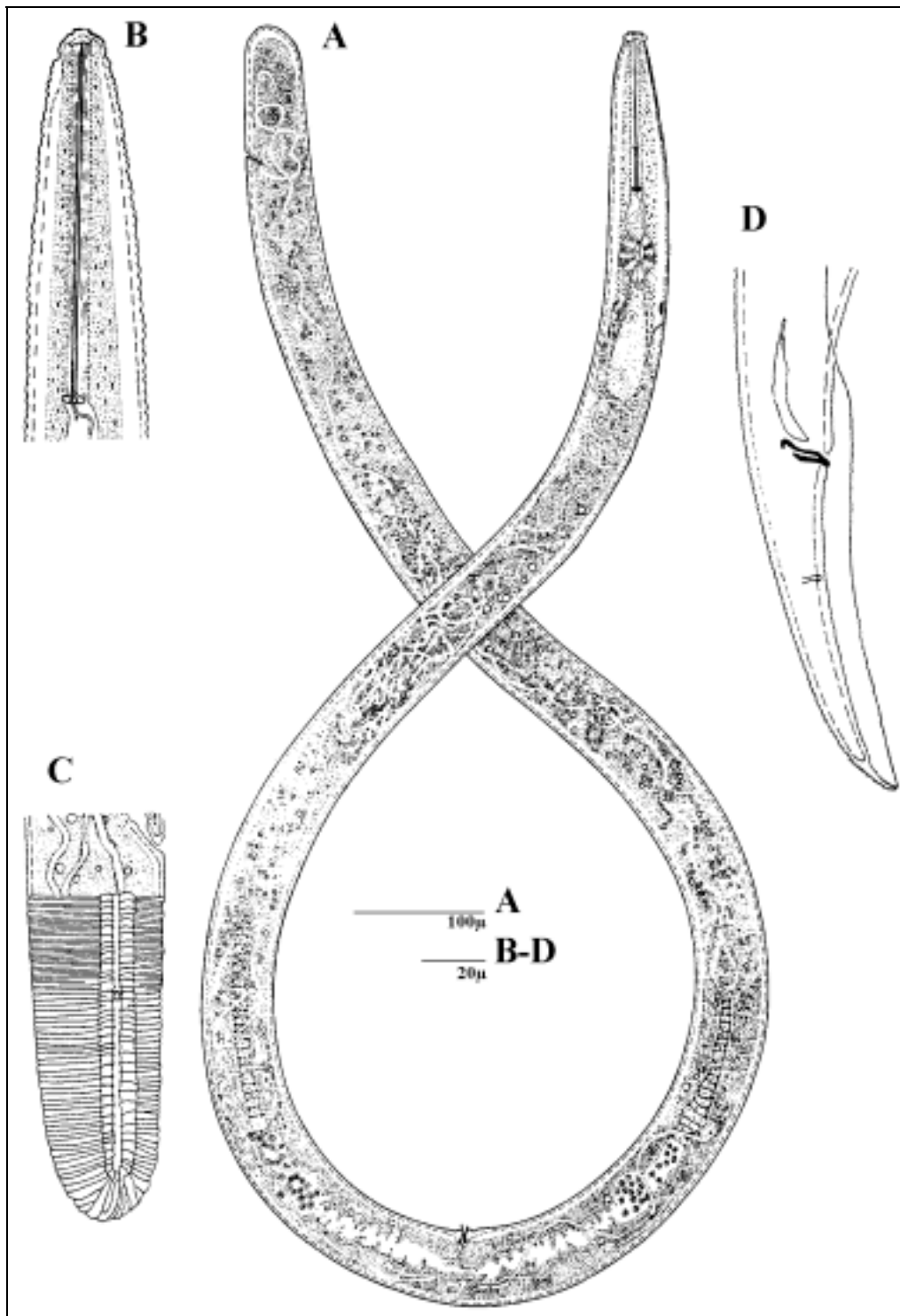
Figure 25B. *Belonolaimus* (*Ibipora*) *lolii* A, female; B, head of female; C, female tail; D, male tail. (From *Nematologica*, volume 25, page 489. Reprinted with permission of E.J. Brill).

Tylenchorhynchus (Figure 25A) Lip region usually set off. Stylet moderately short. Basal bulb not overlapping intestine. Four lateral lines.

Common on grasses, with *T. annulatus* being one of the most widely distributed species.

Merlinius Less than 1 mm long. Lip region continuous, with 6 radial grooves. Stylet less than 20 μm. Six lateral lines. Hypotygma present on posterior lip of the male cloaca.

M. brevidens occurs widely on cereals.

Sting nematodes (Figure 25 B)
Long nematodes with a very long stylet. Oesophageal gland overlapping laterally. Median vulva with two opposing ovaries. Tail variable, generally rounded to conoid.

Belonolaimus (Figure 25B) Stylet 100-160μm long, lateral field with only one groove-like line.

Ibipora (synonymised with *Belonolaimus* by some authors) Stylet 60-100μm long, lateral field with four lines.

Both *Ibipora* and *Belonolaimu*s are most common on turf.

Morulaimus Long slender nematodes, usually over 1 mm, but smaller than *Belonolaimus.* Stylet long to very long, over 50 μm. Four lobes of head poorly separated. Oesophageal glands overlap laterally. Vulva median with epiptygmata. Lateral canals faint. Lateral field with four lines, outer bands areolated. Tail medium to long, terminus more or less rounded.

Genus is restricted to Australia and New Zealand. In Australia it is widely distributed, usually associated with native plants.

Spiral nematodes (Figure 26)
Most of the genera are ectoparasites. When viewed under low power, spiral nematodes are most readily identified as small to medium sized nematodes which are spiral to open C-shaped when relaxed. Cephalic region elevated, framework sclerotised. Stylet moderately robust, relatively short in most species. Oesophageal glands overlap intestine. Median vulva distinct. Tail short, rounded to conoid. Bursa of male enveloping tail. Position and size of phasmids very variable. The most important characters separating genera within this family are the position of oesophageal overlap and size and position of phasmids.

Helicotylenchus Spiral or C-shaped when relaxed. Oesophageal glands less clear in fixed specimens than in other hoplolaimids, longest overlap is ventral. Tail conoid, may have digitate terminus. Phasmids near to anus, more or less opposite, pore-like, only visible using oil immersion lens. Juveniles may be confused with *Paratylenchus* when viewed under low magnification.
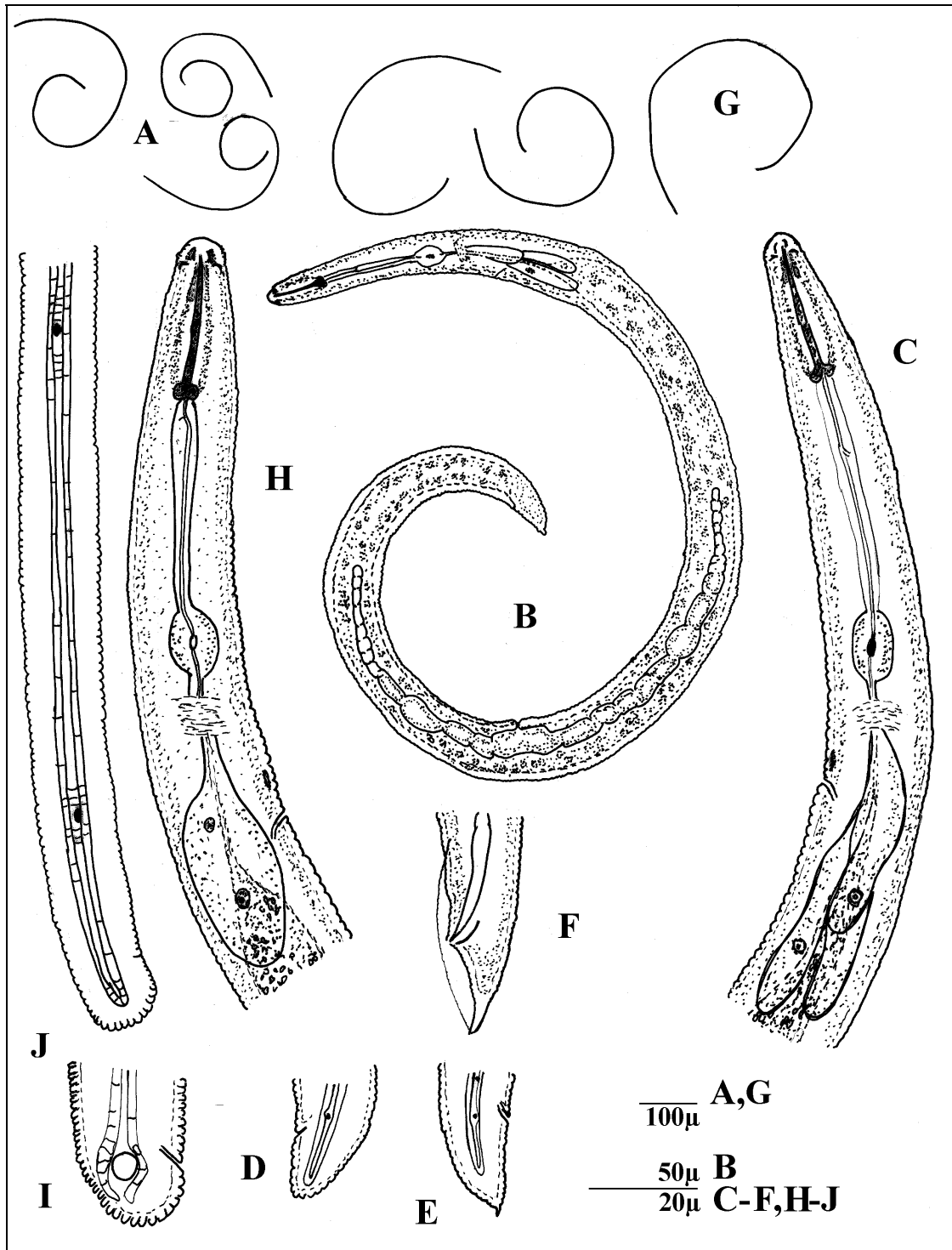
Figure 26. *Helicotylenchus, Scutellonema* and *Hoplolaimus Helicotylenchus* sp. A, shape when relaxed, B, whole female; C, anterior end of female; D & E, female tails; F, male tail. *Scutellonema brachyurum*. G, shape when relaxed; H, anterior end of female; I, female tail. *Hoplolaimus* sp. J, posterior region showing lateral lines and phasmids.

Widespread genus occurring in most parts of the world. *H. dihystera* is common but is not generally considered to be economically important. *H. multicinctus* causes economic damage to bananas.

Scutellonema Widespread genus, spiral or open C when relaxed. Oesophageal glands overlap dorsally, usually distinctly visible. Tail often rounded or conoid-rounded. Phasmids large to very large, close to anus, more or less opposite, slightly yellowish.

S. brachyurum is widely distributed and may be economically damaging on some crops.

Rotylenchus Spiral to open C when relaxed, usually larger than *Helicotylenchus.* Lip region well developed, often striated. Stylet robust. Oesophageal glands overlap intestine dorsally. Tail rounded to conoid. Phasmids pore-like, near to anus, more or less opposite, visible only with oil immersion objective.

Often associated with horticultural crops, but population levels required to cause crop losses are unclear. *R. brevicaudatus* is one of the most common species in Australia.

Hoplolaimus Nematodes 1-2 mm long ventrally curved to open C shaped, but not usually spiral when relaxed. Lip region set off, with distinct striations and strongly sclerotised. Stylet very well developed, with tulip-shaped knobs. Oesophageal glands overlap dorsally and laterally. Phasmids large, usually pre and post vulval, or both post-vulval, but not opposite or near anus. Not as common in Australia as other spiral nematodes.

Pin nematodes (Figure 27)
Nematodes very small, spiral to open C when relaxed. Median bulb very well developed, basal bulb smaller, not overlapping intestine. Vulva posterior, body tapering after vulva. Tail conoid to pointed. Male usually without stylet, oesophagus reduced. May be confused with juvenile stages of spiral nematodes.

Pin nematodes sometimes occur in high numbers but are usually not of economic significance.

Paratylenchus Characters as above. Stylet relatively short, less than 30μm. Fourth stage and male usually without stylet.

Gracilacus Synonymised with *Paratylenchus* by some taxonomic authorities. Stylet long to very long, over 40μm. All stages have a stylet, except the male.
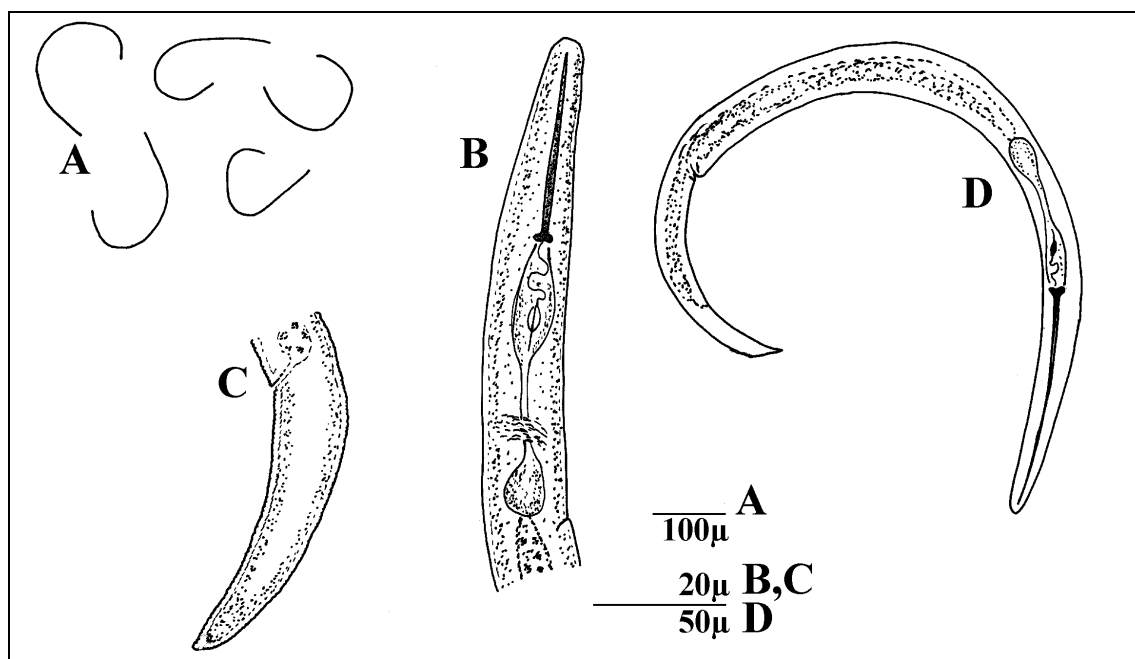
Figure 27. *Paratylenchus* and *Gracilacus. Paratylenchus* **A, shape when relaxed; B, anterior end of female; C, post-vulval region of female.** *Gracilacus* **D, whole female.**

FUNGAL-FEEDING TYLENCHIDS

***Tylenchus* and related genera** (Figure 28) Nematodes usually small, slender, with short, slender stylet, pyriform basal bulb abutting intestine, posterior vulva, one ovary and long to very long filiform tail. Sometimes confused with juveniles of other genera such as *Ditylenchus, Meloidogyne,* etc. The older generic name "*Tylenchus*" is still often used to cover several similar genera of nematodes, e.g. *Cephalenchus, Coslenchus, Psilenchus, Filenchus, Aglenchus, Basiria, Boleodorus and Malenchus.*

These ectoparasitic nematodes are commonly found in soil. They feed on root hairs or fungal hyphae and are not economically important.

ABOVE-GROUND NEMATODES

Leaf and bud nematodes (Figures 29 and 30)
Aphelenchoides Nematodes slender, 0.8-1.5 mm long. Lip region set off, stylet small, with or without small knobs. Median bulb large and conspicuous, usually visible under low magnification. Oesophageal glands overlap intestine dorsally. Vulva posterior. Tail tapered, usually with one or more mucro on the tip. Male without bursa, spicules rose-thorn shaped. Male tail ventrally bent when relaxed.

Pathogenic species are widely distributed. *A. fragariae* is most frequently recorded on ferns and other foliage plants. *A. composticola* is a pathogen of cultivated mushrooms. *A. ritzemabosi* attacks chrysanthemums and foliage plants. *A. besseyi* causes white tip disease of rice and occurs widely on strawberry.

Stem and bulb nematodes (Figures 31 and 32)
Ditylenchus Slender nematodes 1-1.5 mm long, fast moving in water, slightly ventrally curved when relaxed. Continuous lip region, stylet very small, with knobs. Basal bulb a thin elastic sac containing oesophageal glands abutting or overlapping intestine. Posterior vulva, with post-vulval sac. Tail pointed.

Ditylenchus is mainly found in foliar parts of plants. When found in soil samples, it may be confused with *Tylenchus*. However, the latter genus differs in being smaller, having a longer filiform tail and pyriform oesophageal glands.

D. dipsaci is economically important on bulbs, legumes such as broad beans, onions, garlic, potatoes and oats. *D. destructor* attacks potatoes and sweet potatoes.

Seed and leaf gall nematodes (Figures 33 and 34)
Anguina Female ventrally coiled, 1-1½ spirals, 1-2.7 mm long, body very thick or stout. Stylet very small. Oesophageal glands enlarged, overlapping intestine. Ovary reflexed, filling body cavity, vulva posterior. Tail pointed. Males C-shaped, not as stout as females.

The genus is taxonomically difficult at species level due to similar morphology. Causes seed galls on grasses and wheat. In Australia, *A. funesta* is associated with annual ryegrass toxicity through its role as a vector of toxin-producing bacteria. *A. tritici* is not found often in Australia, but is still a problem on wheat and barley in some parts of Asia (Figure 35).
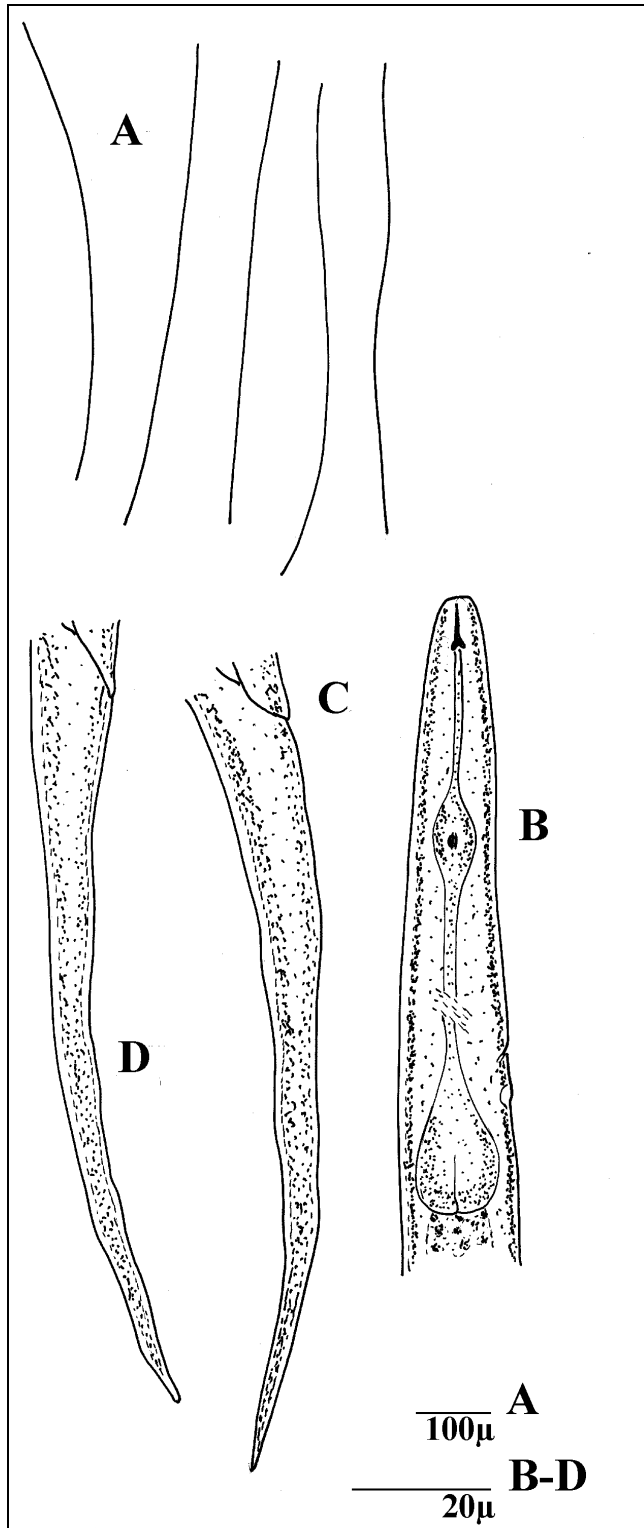
Figure 28.   *Filenchus. Filenchus* **sp. A, shape when relaxed; B, anterior end of female; C & D, femaile tails.**
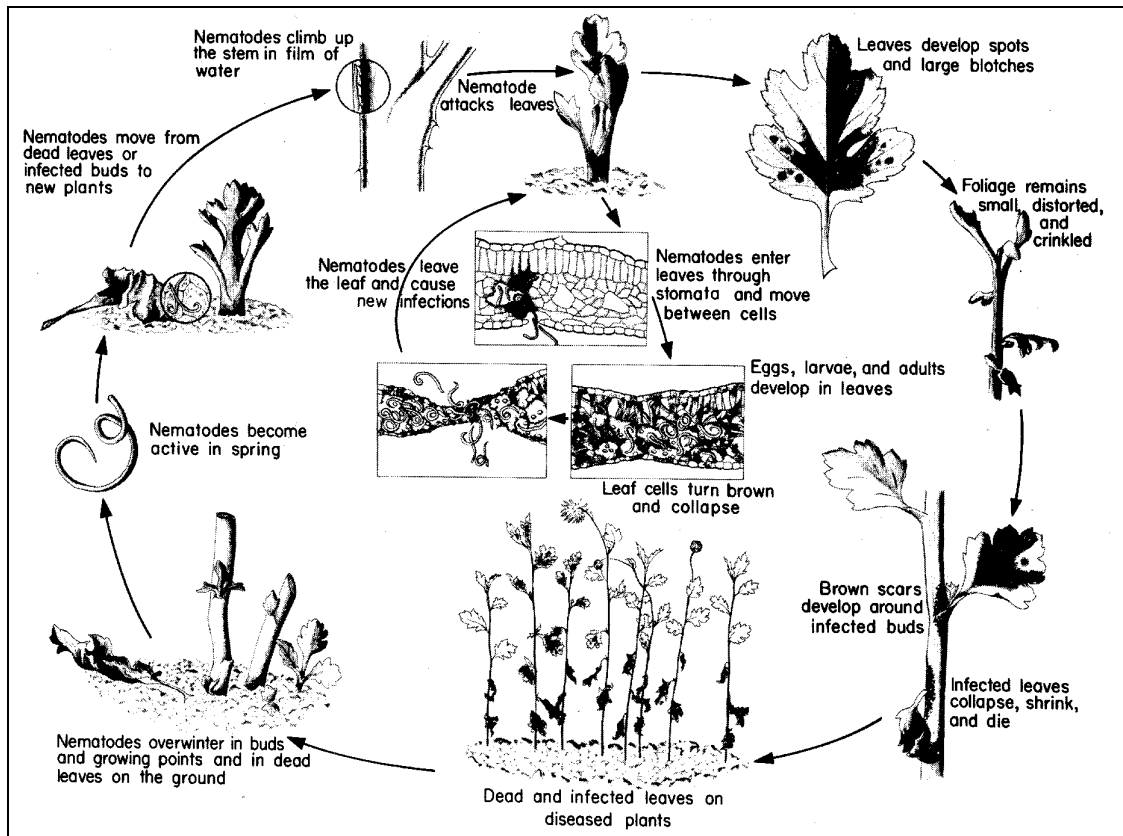
Figure 29. Disease life cycle of the foliar (chrysanthemum) nematode, *Aphelenchoides ritzemabosi.* From "Plant Diseases caused by Nematodes" in *Plant Pathology*, 3rd. edition by George N. Agrios (1987). Reprinted by permission of Academic Press Inc.
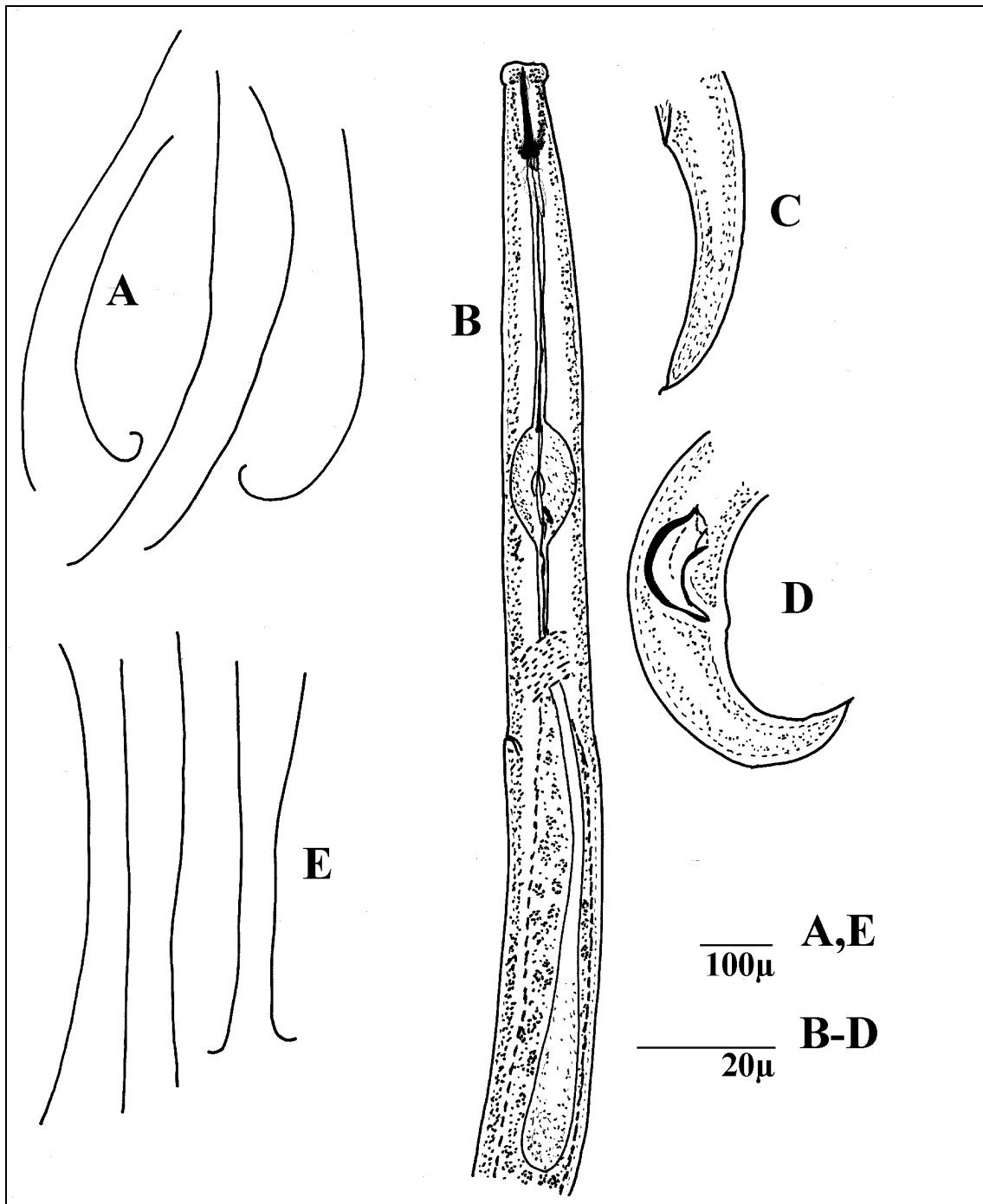
Figure 30.   *Aphelenchoides. Aphelenchoides ritzema-bosi* **A, shape when relaxed; B, anterior end of female; C, female tail; D, male tail;** *A. fragariae* **E, shape when relaxed.**
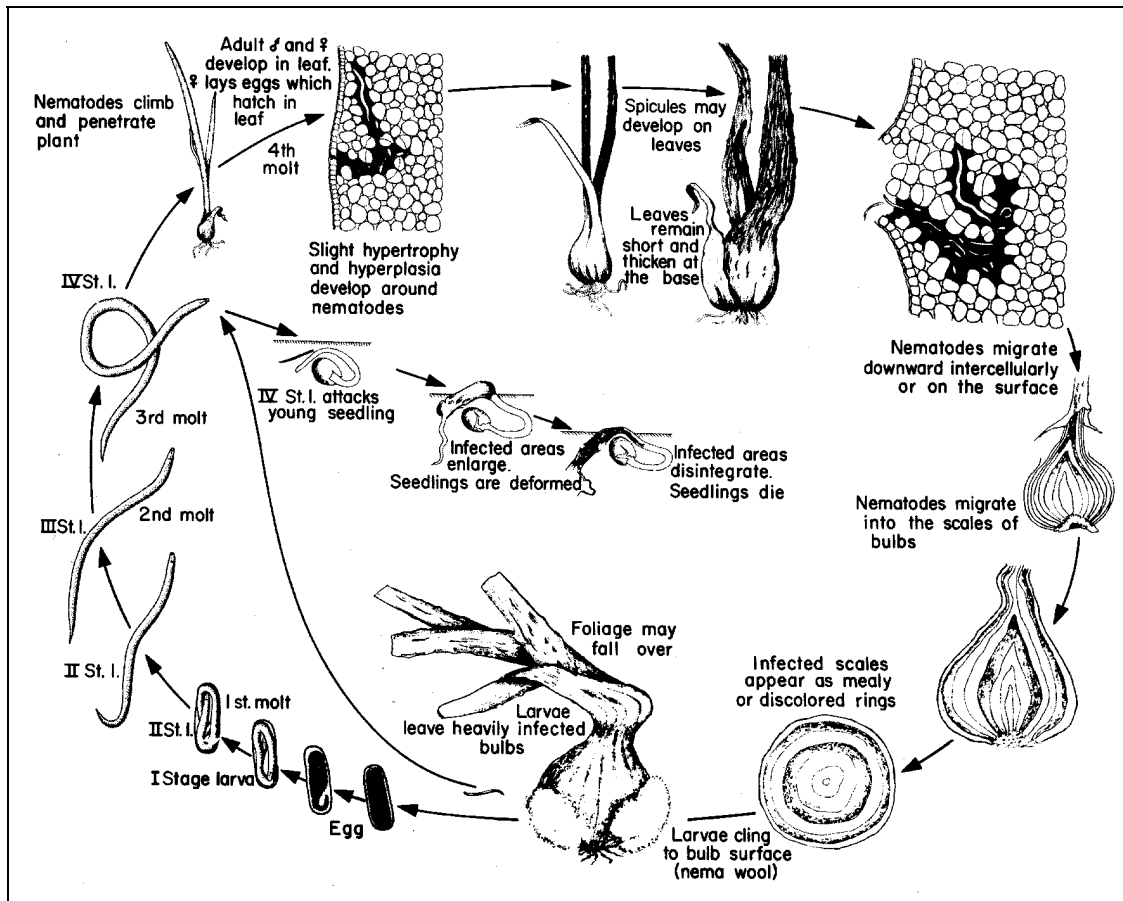
Figure 31. Disease life cycle of the stem and bulb nematode, *Ditylenchus dipsaci*. From "Plant Diseases caused by Nematodes" in *Plant Pathology*, 3rd. edition by George N. Agrios (1987). Reprinted by permission of Academic Press Inc.

Figure 32.   *Ditylenchus. Ditylenchus dipsaci* **A, shape when relaxed; B, anterior end of female; C, female tail.**

Figure 33. Disease life cycle of wheat seed gall caused by *Anguina tritici*. From "Plant Diseases caused by Nematodes" in Plant Pathology, 3rd. edition by George N. Agrios (1987). Reprinted by permission of Academic Press Inc.
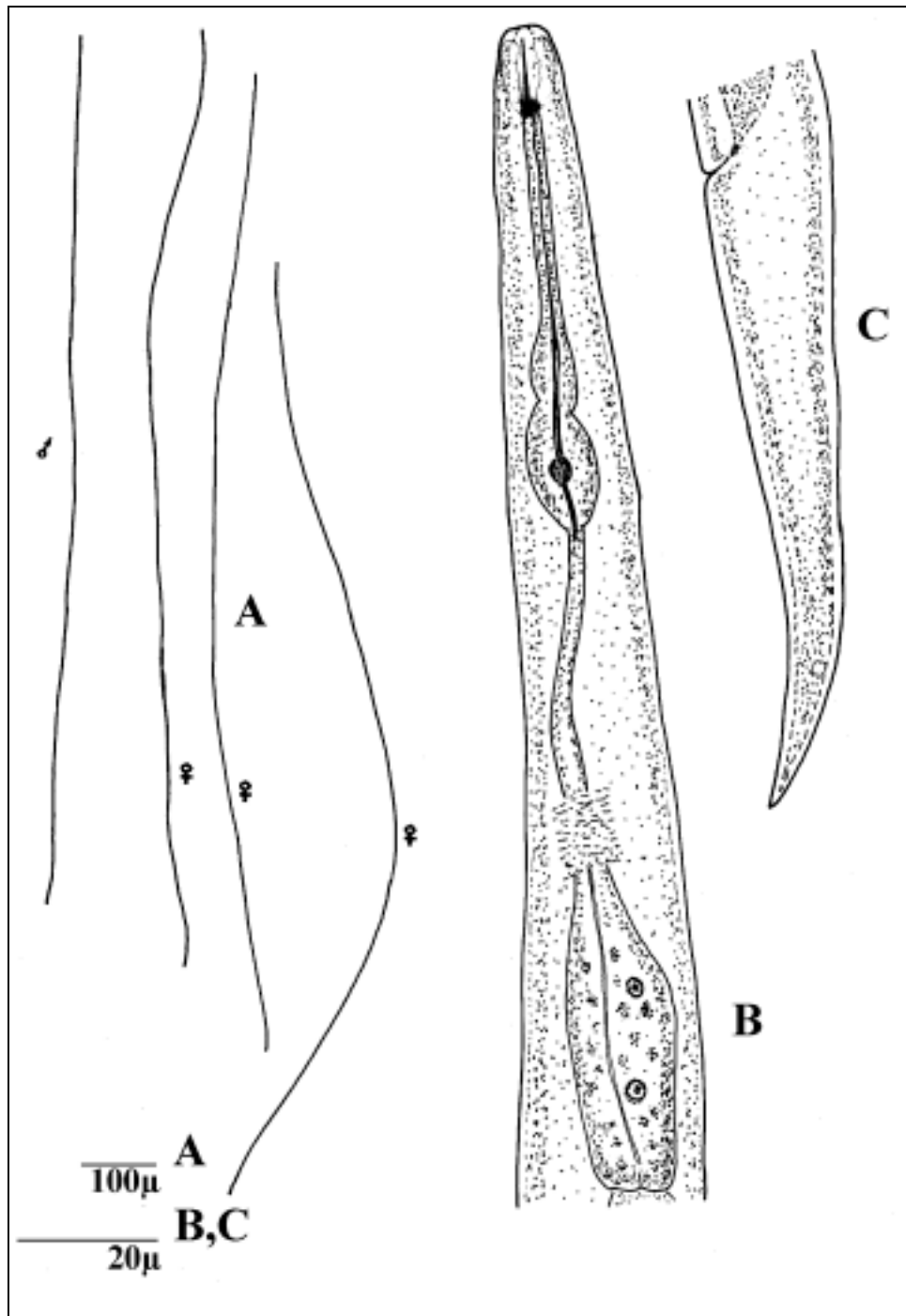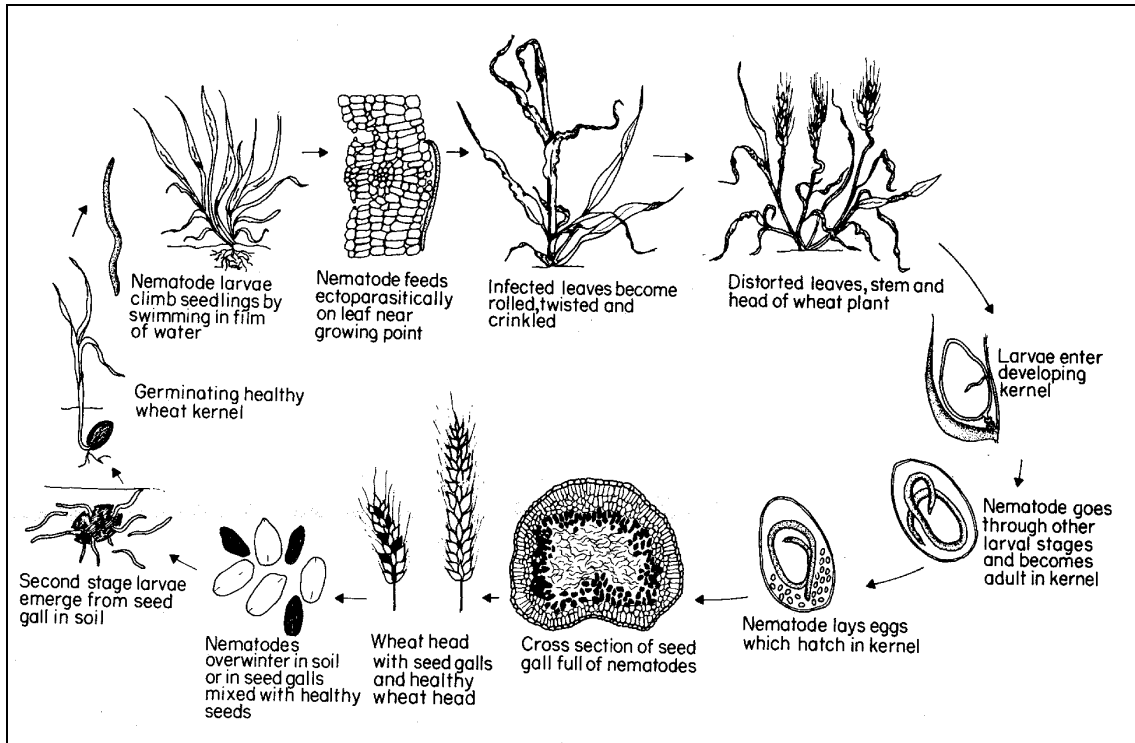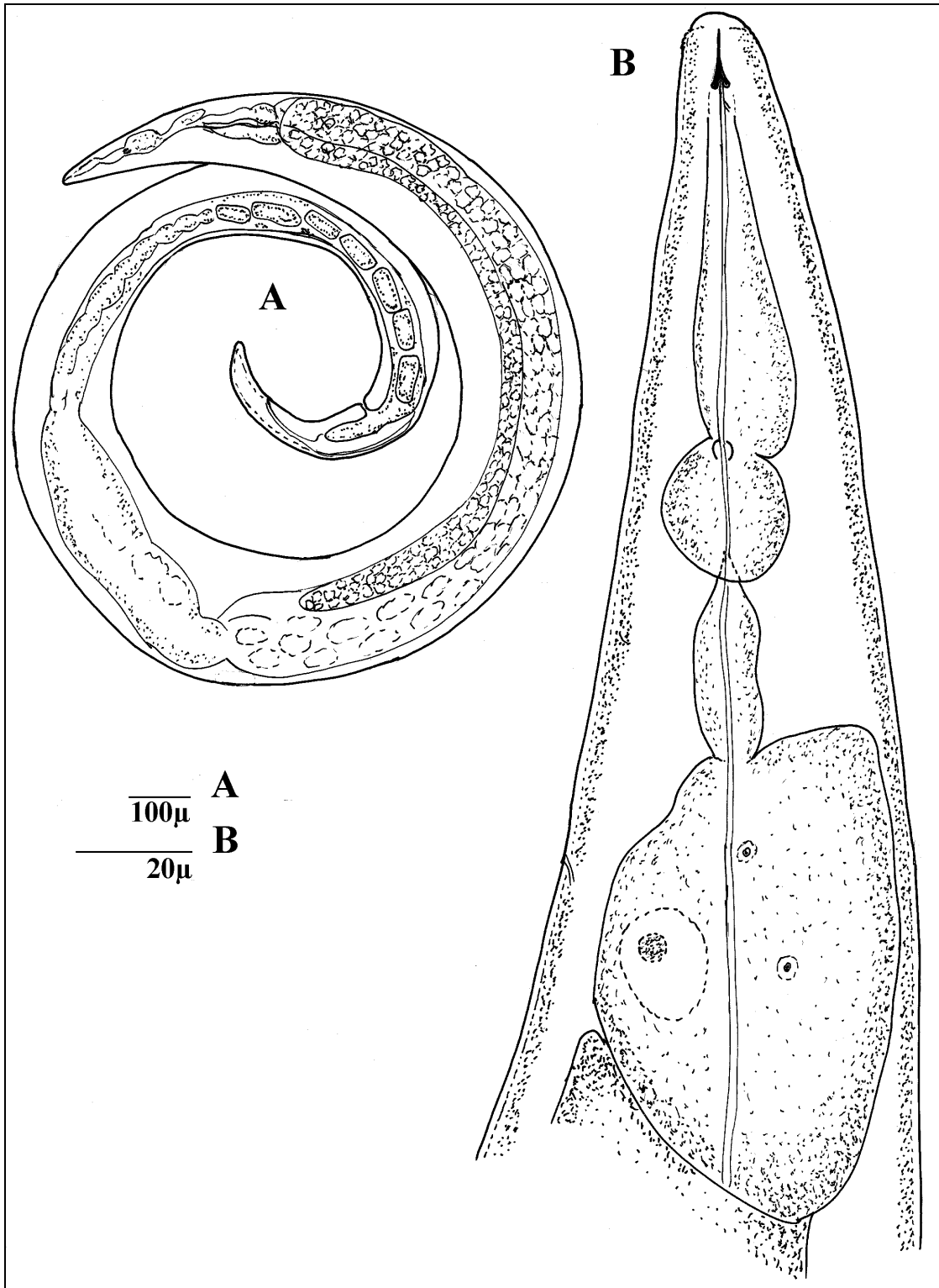
Figure 34. *Anguina. Anguina tritici* **A, shape of female when relaxed; B, anterior end of female.**

Figure 35.   Seed galls caused by A, *Anguina tritici* on wheat and B, *A. funesta* on annual ryegrass. Seeds on the left of each pair are healthy, those on the right are diseased. (Photograph kindly supplied by Dr. I. Riley)

PLANT-PARASITIC DORYLAIMIDS

Unlike the tylenchids, the dorylaimids lack a median bulb and generally have a cylindrical oesophagus. Most soil samples contain dorylaimids and a few of the genera are plant parasites

Dagger nematodes (Figure 36)
Xiphinema Nematodes are long to very long and slender. Ventrally curved to C-shaped in adults, but some juveniles may be straight. Cuticle smooth. Amphids large, pouch or stirrup-like in shape. Stylet very long, in two parts: an anterior odontostylet and a posterior odontophore with well-developed posterior flanges. Lip region continuous or set off, not sclerotised. Oesophagus cylindrical. Guiding ring posteriorly situated around the odontostylet. Vulva anterior or median, one or two ovaries. Tail variable in shape and length depending on species. Juveniles with replacement odontostylet.

Cosmopolitan in both temperate and tropical climates mostly on woody hosts. Crops on which *Xiphinema* can be damaging include rice, fruit trees, grapevines and *Pinus*. A number of species are virus vectors.

Needle nematodes (Figure 36).
Paralongidorus Similar general morphology to *Xiphinema*. Lip region continuous or set off. Nematodes may be very long (to about 10 mm). Flanges not present at base of odontophore. Guiding ring situated anteriorly around the odontostylet. Vulva usually median, two ovaries. Tail short and more or less rounded. Not known to be virus vectors.

Mainly distributed in warmer climates. Generally have a wide host range. Several species are associated with natural vegetation and are widespread throughout mainland Australia. *P. australis* damages rice in north Queensland.

Stubby root nematodes (Figures 37 and 38)
Paratrichodorus Nematodes rather plump, up to 2 mm long, cigar shaped, blunt at both ends. Female body straight. Cuticle thick and smooth. Stylet dorsally curved. Oesophagus expanded posteriorly with lobe-like glands overlapping intestine. Vulva small, median and inconspicuous. Lateral body pores not usually present near vulva. Female anus almost terminal. Caudal pores often present. Male tail ventrally curved. Spicules almost straight, bursa present but often inconspicuous and close to body outline in lateral view. Males rare or absent in several species.

Cosmopolitan. Common in warmer climates and the tropics. *P. minor* is widespread and is particularly common on grasses.
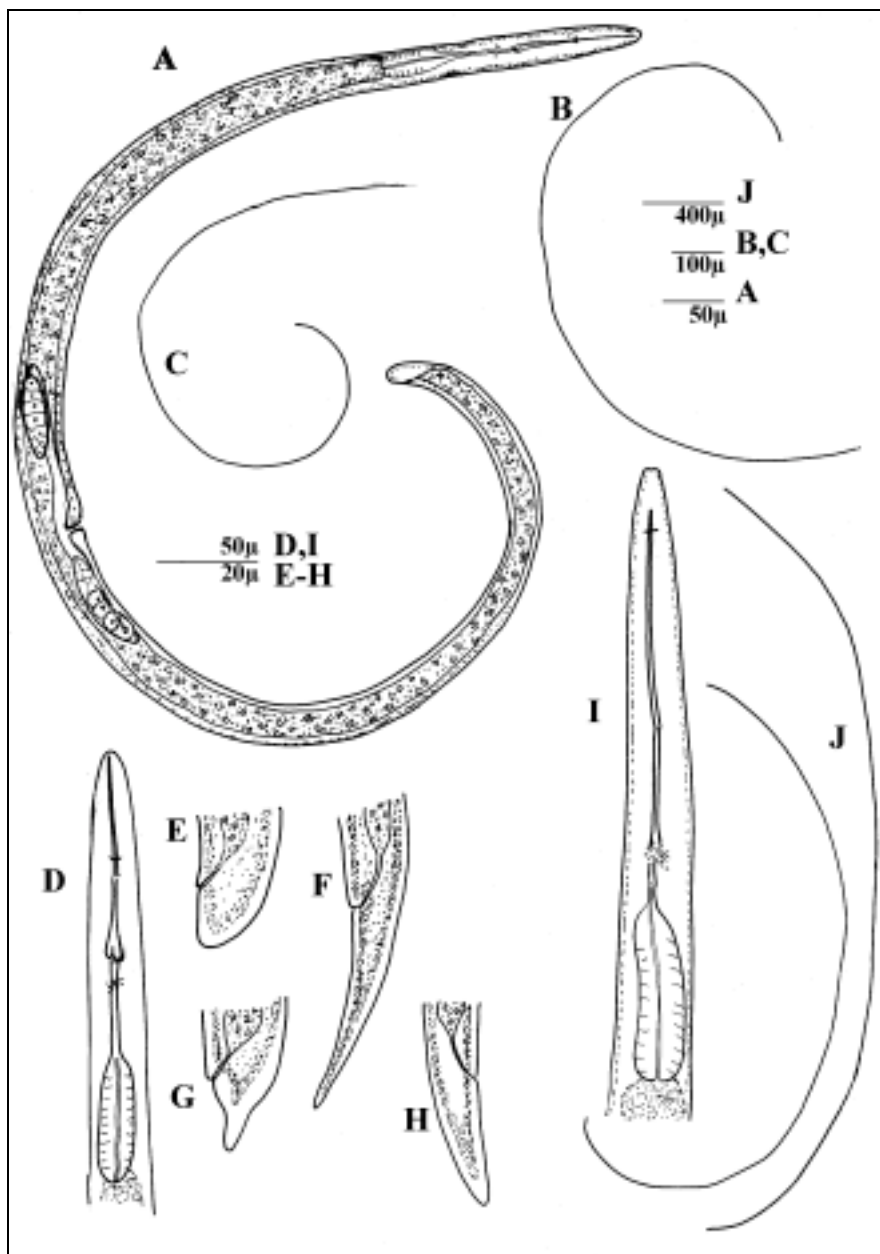
Figure 36. *Xiphinema and Paralongidorus. Xiphinema americanum* **A, whole female; B & C, shape when relaxed; D, anterior end of female;** *Xiphinema* **female tails E,** *X. brevicolle*; **F,** *X. insigne*; **G,** *X. brasiliense*; **H,** *X. radicicola*; *Paralongidorus eucalypti* **I, anterior end of female; J, shape when relaxed.**

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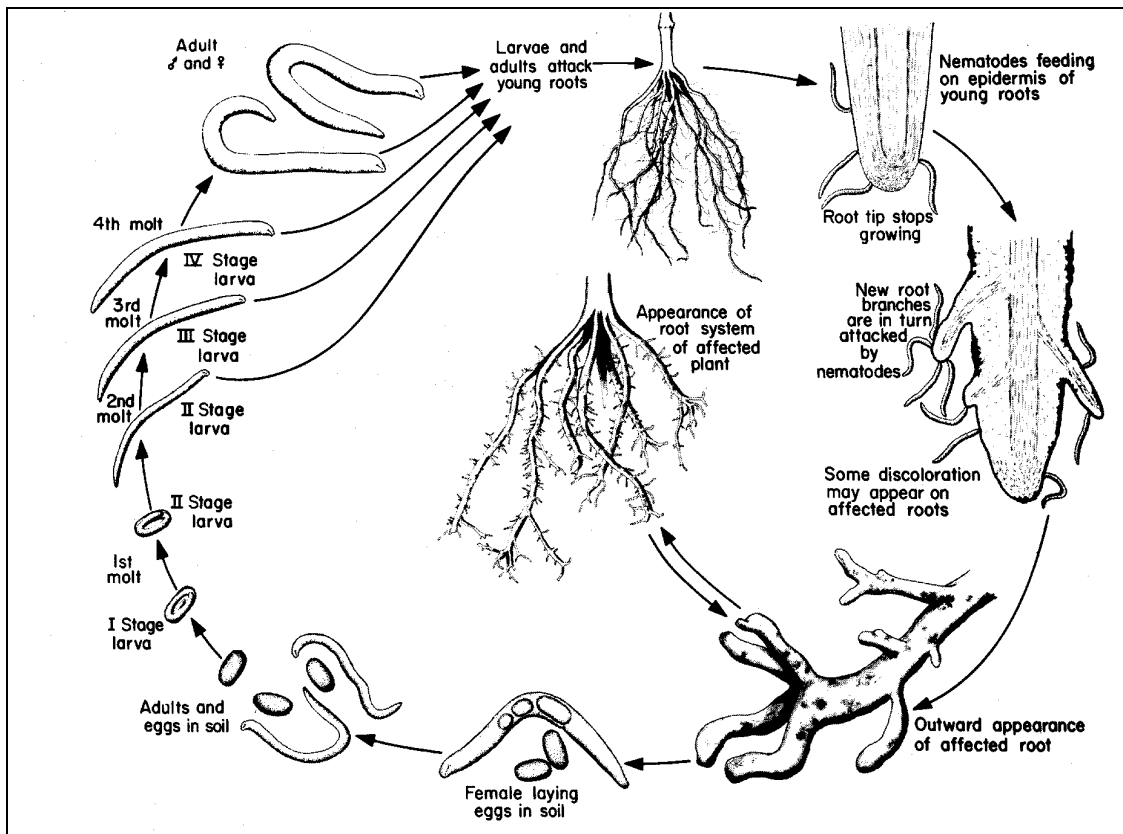**Figure 37.** Disease life cycle of the stubby-root nematode *Paratrichodorus minor*. From "Plant Diseases caused by Nematodes" in *Plant Pathology*, 3rd. edition by George N. Agrios (1987). Reprinted by permission of Academic Press Inc.
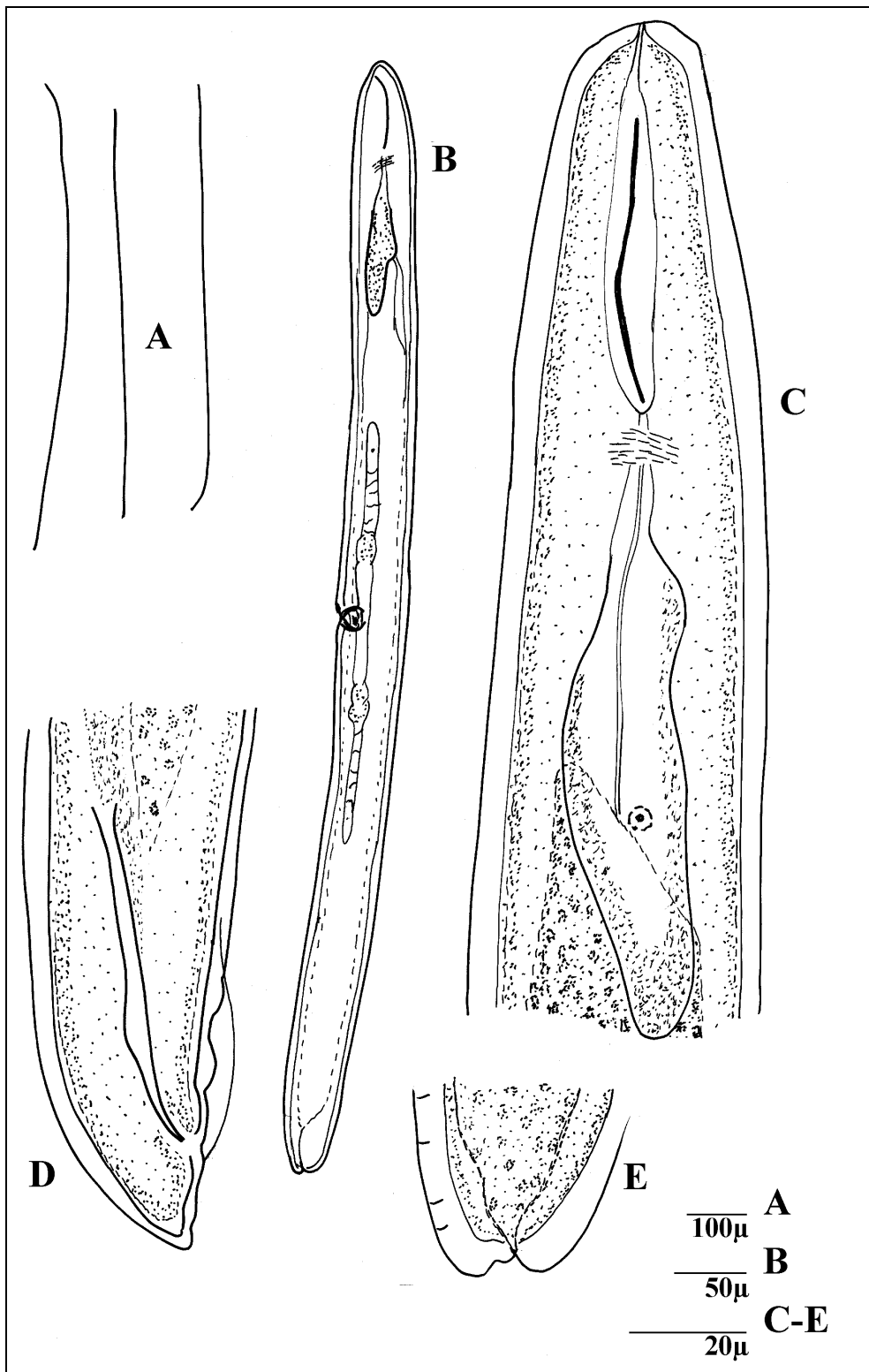
Figure 38. *Paratrichodorus. Paratrichodorus lobatus* **A, shape when relaxed; B, whole female; C, anterior end of female; D, posterior end of male; E, female tail.**

6.2.6 Species identification

There are many diagnostic situations where it is important that nematodes are identified to species level. Quarantine services and clean planting material schemes, for example, are based around freedom from certain nematodes and because they have legislative backing, any nematodes that are present must be identified correctly. When strategies such as crop rotation and cultivar resistance are employed as control measures, identification of nematodes to species level is essential, as host plant resistance is often species-specific.

The ability to differentiate various species of nematodes is a specialist skill which requires appropriate taxonomic training together with years of practical experience. Since it is a skill which many trained nematologists never acquire, laboratories offering diagnostic services will need to develop a working relationship with a nematode taxonomist who is able to confirm species identifications and provide taxonomic help when it is required. For many important groups of nematodes, the taxonomy is so complex that it may be necessary to utilise a taxonomist with expertise in that particular group. Such expertise is best located through a knowledge of the nematological literature or through listings of specialist interests in the membership directories of the world's major nematology societies. In Australia and New Zealand, the nematode taxonomists listed in Appendix 2 will provide taxonomic advice and may be prepared to identify specimens to species.

If nematodes are to be sent to a taxonomist for species confirmation, suspensions of nematodes are concentrated and nematodes are killed with a hot fixative. One common method is to boil a fixative (8 ml formalin, 92 ml distilled water) and pour one volume of the hot fixative into the same quantity of the water containing the nematode specimens. The water dilutes the fixative to the correct concentration (4% formalin). Nematodes can be stored in this fixative and dispatched for examination. In situations where suitable technical expertise is available, nematodes can be hand-picked from the fixative and mounted on slides using procedures described in Table 15. Slides can then be packaged and forwarded to a taxonomist.

Table 15.   Preparation of temporary mounts of nematodes for taxonomic examination.

1. A tube 1.5 cm in diameter is heated in a flame, dipped in paraffin wax and applied to the centre of the glass slide. This forms a wax ring which later serves as a seal and support for the cover glass. The amount of wax should be varied according to the thickness of the nematodes being mounted, with the width and height of the ring being greater for larger nematodes.
2. A small drop of fixative is placed in the centre of the ring. Nematodes previously killed with heat are transferred into the drop using a suitable handling needle and pressed down so that they rest on the glass slide.
3. A cover slip is flamed to remove moisture and lowered onto the wax ring. If the cover slip does not contact the drop of fixative, it should be gently pressed downwards until it does.
4. The wax is melted slowly from one side with a flame as this allows any air bubbles that are formed to escape.
5. When the wax has solidified, remove surplus wax and seal the coverslip with a good quality nail varnish (e.g. Cutex).

6.2.7 Aids to identification of nematode species

Taxonomic keys are available for all economically important genera of plant-parasitic nematodes and these keys are indispensable when nematodes are being identified at a species level. A list of references which contain species keys is provided in the reference section of this book (see section 9.9).

Because root-knot and cyst nematodes are of major economic importance in Australia and New Zealand, specific identification is often required. The following techniques are particularly useful.

Meloidogyne
Perineal patterns Cuticular markings in the posterior region of *Meloidogyne* females form a fingerprint pattern which is useful for identification at a species level. The area around the vulva and anus is known as the perineal pattern and the arrangement of marks or lines on the cuticle (Figure 39) is a useful diagnostic character. Procedures for preparing perineal patterns are described in Table 16.
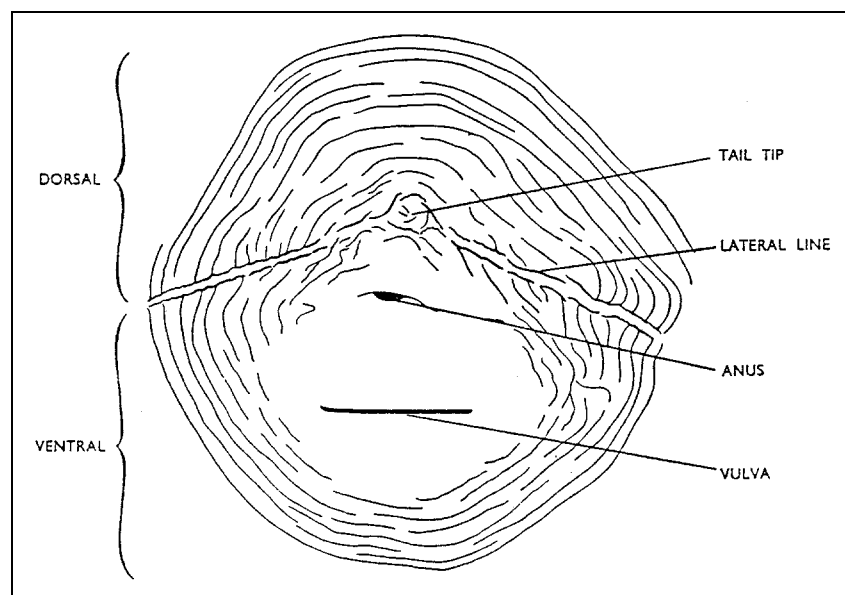

Figure 39.   **Diagram of the perineal pattern of a *Meloidogyne* female, showing features used in identification.**

Table 16.   Preparation of perineal patterns of *Meloidogyne* species.

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1. Intact *Meloidogyne* females are placed in 45% lactic acid on a perspex slide and the posterior end is cut off with an optical scalpel.

2. Body tissues are removed by lightly brushing the inner surface of the cuticle with a slightly flexible bristle.

3. When all tissues are removed, the cuticle is transferred to a drop of glycerin where it is carefully trimmed so that it is only slightly larger than the perineal pattern.

4. The piece of cuticle with the perineal pattern is transferred to a drop of glycerin on a microscope slide. A coverslip is applied and sealed with nail varnish.

Since there is some variation in perineal patterns between individuals of the same species, at least five and probably more individuals should be examined when identifying *Meloidogyne* to species level. The differences between species (Figure 40) will not seem obvious to a novice and it may take years of experience before perineal patterns can be used with any degree of confidence. Species identifications determined by perineal patterns should only be regarded as tentative and further confirmation is required using the morphology of juveniles, the differential host test (see below), or an appropriate molecular test.

North Carolina Differential Host Test   This test provides a simple and practical method of identifying the four major species of *Meloidogyne*. It is based on responses to a series of six differential host plants and is fairly reliable, provided identifications are verified with morphological characters such as perineal patterns. A detailed explanation of the test is given by Hartman and Sasser (1985) and the major steps involved are outlined in Table 17.
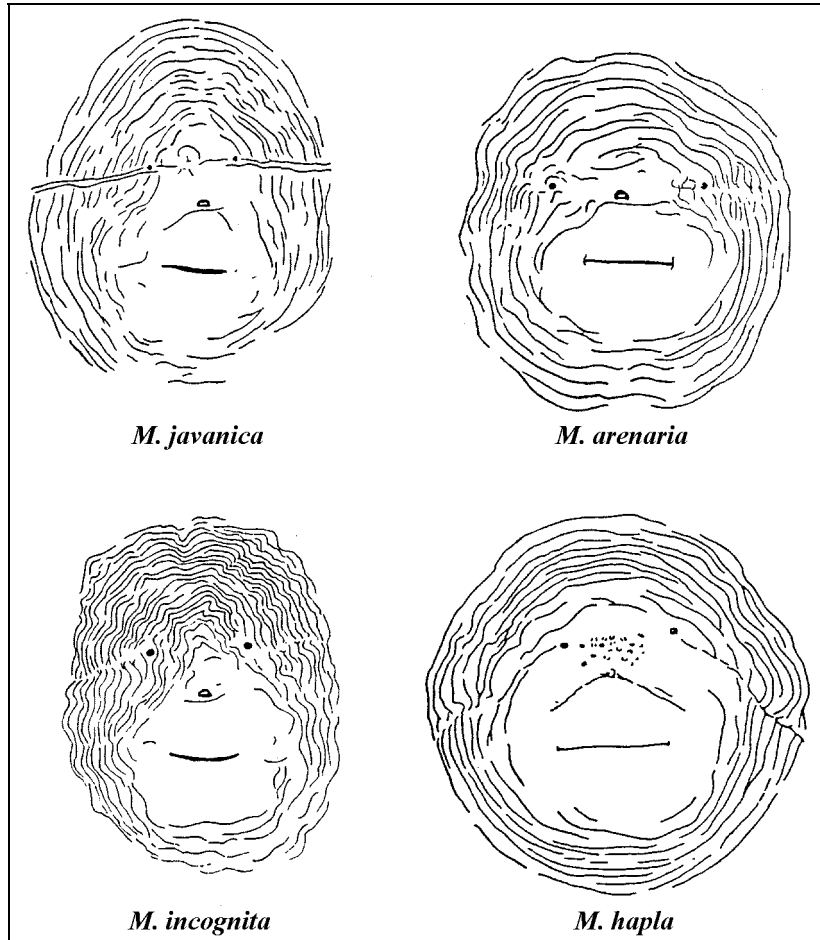
Figure 40. Perineal patterns of the four most important *Meloidogyne* species.

Table 17.   **The basic procedures used in the host differential test for distinguishing species of *Meloidogyne*.**

1. Populations of unknown *Meloidogyne* species are established by inoculating potted tomato seedlings in sterilised soil with either egg masses or infected root fragments. Alternatively, tomato seedlings may be potted into nematode infested field soil. Care should be taken to avoid contamination with nematodes from greenhouse benches and other pots.

2. When egg masses are mature (usually 45-60 days after planting), eggs can be retrieved from nematode-infested roots for use as inoculum. Roots are immersed in 0.5% NaOCl solution and shaken vigorously for 3 minutes. The solution is then poured through a 75-150μm sieve nested on a 25-38μm sieve. Rinsing with tap water washes eggs through the upper sieve. Eggs collected on the lower sieve should be washed for a further two minutes to remove any residual bleach before being washed into a measuring cylinder.

3. An aliquot of the suspension is taken and the concentration of the eggs determined. Eggs (5,000) are then inoculated onto three replicate seedlings of tomato cv. Rutgers; tobacco cv. NC95; pepper cv. Early California Wonder; peanut cv. Florunner; Watermelon cv. Charleston Grey and cotton cv. Deltapine. Seedlings should have been established previously in sterilised soil in 10 cm pots and should be at the 2-4 leaf stage.

4. After about 60 days at 24-30°C, roots are washed from the pots and stained with a solution of Phloxine B (15 mg/litre of water) for 15-20 minutes to make it easier to observe and count egg masses with the naked eye.

5. Tests in which there are less than 100 egg masses on the susceptible tomato should be repeated. Plants with an average of less than 10 egg masses are designated resistant (-). Host plants are those where nematode reproduction is moderate to high (i.e. >10 egg masses), and they are considered susceptible (+). A preliminary indication of the species can be obtained by reference to Table 18.

Table 18.   **Usual response of four common *Meloidogyne* species and their races to the North Carolina Differential Host Test.**

Meloidogyne species and physiological races	Differential host plants					
	Cotton cv. Deltapine 61	Tobacco cv. NC95	Pepper cv. California Wonder	Watermelon cv. Charleston Grey.	Peanut cv. Florunner	Tomato cv. Rutgers
M.incognita						
Race 1	-	-	+	+	-	+
Race 2	-	+	+	+	-	+
Race 3	+	-	+	+	-	+
Race 4	+	+	+	+	-	+
M.arenaria						
Race 1	-	+	+	+	+	+
Race 2	-	+	-	+	-	+
M. javanica	+	+	-	+	-	+
M. hapla	-	+	+	-	+	+

Heterodera and Globodera

All Australasian cyst-forming nematodes are found in two genera, *Heterodera* and *Globodera*. These genera can be separated from other genera of cyst-forming nematodes by the absence of a clear area (fenestration) around the anus of the cyst. In both *Heterodera* and *Globodera*, fenestration is restricted to the vulval area. *Heterodera* differs from *Globodera* in that the vulva is raised on a distinct cone, giving the otherwise more or less pear-shaped cyst and female a lemon-shaped appearance. Fenestration also differs between the two genera (see below).

Globodera Fenestration in *Globodera* takes place all around the vulva, placing the vulva of the cyst in the middle of the transparent area. This transparent area (fenestra) disintegrates in time, leaving nothing to hold the vulva in older cysts. Only a transparent hole remains where the vulva used to be.

Characters of the juveniles identify the species. The potato cyst nematodes are characterised by juveniles with stylets that are between 20 and 24 µm long. In *G. rostochiensis*, the stylet is less than 23µm long and the stylet knobs are rounded or sloping somewhat anteriorly. In *G. pallida*, the stylet is more than 23 µm long and the stylet knobs are anchor shaped or somewhat wider and more flattened.

Heterodera Fenestration in *Heterodera* only takes place along the sides of the vulval slit, parallel to it. When the fenestrae disintegrate, the vulva still remains attached to the cyst through the unfenestrated regions at the ends of the vulval slit. Thus a vulval bridge is formed between the disintegrated fenestrae, and it persists even in fairly old cysts.

Characters of the juveniles, vulval slit and the fenestration are useful for identifying species. *H. avenae* (cereal cyst nematode) has a vulval slit which is less than 20 µm long, whereas the vulval slit is distinctly more than 20 µm in both *H. trifolii* (clover cyst nematode) and *H. schachtii* (sugar beet cyst nematode). *H. trifolii* can be distinguished from *H. schachtii* by the fenestrae of the cyst covering almost the full length of the vulval slit, compared with only about the central half of the vulval slit in *H. schachtii*. The juvenile of *H. trifolii* also has a longer stylet (28 µm versus 26 µm) and a longer tail (>55 µm versus <55µm) than *H. schachtii*.

6.2.8 Molecular aids to nematode identification

Traditional morphological methods of identification have formed the basis of nematode taxonomy for more than 100 years and, for the foreseeable future, they will continue to be important. However, rapid advances in biotechnology have provided taxonomists with a wide range of new tools which may eventually revolutionise nematode identification. These new technologies are already widely used in areas such as medical and veterinary diagnosis, where they have been adopted because of their discriminatory power, low cost and their ease of use and interpretation. Protein electrophoresis and DNA-based technologies are two methods that may eventually be used for the routine identification of nematodes.

Protein electrophoresis

Electrophoretic methods for analysing nematode proteins are used routinely in some nematological laboratories. One of the most widely used systems differentiates the major species of *Meloidogyne* using iso-enzyme phenotypic differences (Fargette 1987 a,b; Esbenshade and Triantaphyllou 1990). Another successful application is the use of isoelectric focusing to discriminate *Globodera rostochiensis* and *G. pallida*. Although of considerable value as a research tool, electrophoretic methods are only likely to be used for advisory purposes in laboratories that handle large numbers of samples, or when samples contain nematodes that are difficult to separate by other means.

DNA-based technologies

Three DNA-based techniques have potential for nematode identification:

Dot-blot assays This test requires that the nematode is fixed in some way to a filter and a DNA probe is added which binds to complementary sequences in the nematode's DNA. This reaction can be visualised by labelling the probe either radioactively or non-radioactively. The test potentially differentiates a 5% difference in sequence and the strength of the binding signal may be used for nematode quantification. Such systems have been developed for several nematode groups including *Globodera rostochiensis* and *G. pallida* (Burrows and Perry 1988) and *Ditylenchus dipsaci* (Palmer *et al.* 1991).

Restriction fragment length polymorphisms (RFLP) This test uses restriction enzymes which recognise and cut particular DNA sequences. When the resultant fragments are run on an electrophoretic gel, they migrate according to their size to create a banding pattern. These patterns may be characteristic of a certain group of nematodes and some can distinguish between variants within species, e.g. in *Meloidogyne* (Curran *et al.* 1986; Hugall *et al.* 1994).

Polymerase chain reaction (PCR) This technique is potentially the most sensitive and useful of those currently available. It amplifies DNA in a certain variable region of the genome to produce millions of copies. The amplified product may be then used in several ways for identification. The product may be different lengths in different nematode groups or, in some groups, amplification may not occur. The products may be further analysed using RFLP's to distinguish additional groupings. The latter combination of techniques has been used in diagnostic tests for *Meloidogyne* (Powers and Harris. 1993; Stanton *et al.* 1997) and *Pratylenchus* (J. Curran pers. comm.).

Molecular diagnostic services

There is considerable commercial interest in the use of molecular tools for diagnosis of plant pathogens, and services in which nematodes are identified and quantified by molecular methods are likely to be made available in the next few years. Such technologies have the potential to reduce the cost of diagnostic tests because laboratories will no longer have to use time-consuming extraction procedures or employ staff with expertise in nematode identification. Whether molecular methods are eventually adopted for routine use with nematodes will depend on their ultimate cost and whether the problems of achieving reliable results with DNA in soil can be solved. The level of quantification that is achievable will also be important because tests will only have predictive value if important plant-parasitic nematodes can be detected at population densities as low as 0.1 nematodes/ g soil.

In future, molecular technologies will undoubtedly play an important role in diagnosing soil-borne pathogens. They are likely to be most useful for bacteria and fungi, which are difficult to quantify with conventional technologies. It remains to be seen whether they will be as useful for nematodes, as conventional quantification techniques are better developed. Even if molecular technologies are eventually used on a large scale, the information presented in this book will still be needed to ensure sampling methods are appropriate and results are interpreted correctly.

7. Interpreting Results of Nematode Assays

In earlier sections of this book, we have demonstrated that reasonably accurate estimates of the population density of important plant-parasitic nematodes can be obtained, provided the following procedures are followed:

- the samples are representative of the area of interest,
- samples are handled carefully and arrive at the laboratory in good condition,
- a quality control system is used in the laboratory, so that extraction efficiencies are known and are kept relatively constant from sample to sample, and
- nematodes are identified correctly

Although a nematode count using appropriate procedures is important for diagnostic purposes, it should not be viewed as the only information needed to make a management decision. Knowledge of the agronomy of the crop, historical information on nematode population densities, details of responses to nematicides in previous crops, observations of symptoms and their distribution in the current crop and knowledge of the root diseases and other limiting factors that are present should all be used in interpreting the result. A certain number of nematodes may cause problems in one situation, but may be relatively unimportant in another environment or under a different crop management system.

Because so many factors (e.g. soil texture, moisture and temperature, crop cultivar, standard of crop management) can influence the relationship between nematode population density and yield, there will always be some doubt about the economic threshold in each particular situation. An agronomist with broad experience of the crop at a local level is therefore invaluable in helping to interpret the results of nematode analyses. Such a person will be able to assess the likely importance of nematodes relative to other factors that could affect crop production.

Another important point is that nematode monitoring data become increasingly valuable after an area has been sampled for a number of years. Thus in the early stages of a monitoring program, a low pre-plant nematode count may result in a decision to treat with a nematicide. However, if previous monitoring had indicated that nematode populations in that field generally remained low or that responses to nematicides had not been obtained previously, a decision to avoid using a nematicide could be made with more confidence.

Estimates of economic damage thresholds for nematodes on various crops are derived by measuring responses to nematicides (or nematode-resistant cultivars) in plots with different densities of nematodes. Experiments of this type are published in the nematological literature and these results have been collated and are summarised in Table 19. Although the specific information may not be relevant to all environments and crop management systems in Australia and New Zealand, it is presented as a broad guide to the number of nematodes usually required to cause damage on particular crops. Unfortunately, data for some crop / nematode combinations are unavailable, which highlights the need for further nematological research on some crops.

Comments in the table reinforce the point that numerous factors influence the extent of the damage caused by a particular density of nematodes. This may seem to hamper the use of nematode counts for making reliable predictions. However, experience has shown that this is not necessarily the case. In regions with little variation in soil texture and climate, for instance, these thresholds can be refined through experimentation to the point where they are quite accurate and therefore very useful. Thus the hazard indexes presented in this book should be viewed as a guide. Further research will invariably improve their usefulness at the local level.

Important notes about Table 19

1. Some laboratories extract nematodes from a specific weight rather than volume of soil. A volume of 200 ml soil is usually equivalent to a weight of 200-225g soil. The actual conversion factor depends on soil moisture and soil type.

2. The data in the table are intended to reflect actual nematode densities. Thus to use this table, nematodes must be extracted using a technique with a known extraction efficiency. Nematode counts are then corrected for extraction efficiency and the corrected count is compared with the thresholds listed in the table.

3. The likely hazard due to nematodes is indicated as a hazard index.

Low: There is little likelihood of a measurable reduction in yield due to nematodes.
Moderate: There is some chance of losses due to nematodes, particularly when environmental conditions are favourable to nematodes or unfavorable to the crop.
High: Large reductions in yield (i.e. >20%) due to nematodes are likely. The extent of losses will depend on factors such as climate, biotic and abiotic components of the soil environment and the standard of crop management.

4. For annual crops, pre-plant nematode densities are presented.

5. Unless otherwise indicated, nematode population densities in established plantings and hazard to the current crop are presented for perennial crops. Hazard indexes for replant situations have not been included. However, because the root systems of replants are limited in size, damage thresholds are generally lower in replant situations than on established crops.

6. Anyone using the data in this table to predict the likely extent of nematode damage should act conservatively until confirmatory data are available at a local or regional level. Nematode control is not as predicable as fertiliser or herbicide application. Therefore, nematode populations which normally cause little damage should not be considered unimportant until this is confirmed by experience.

Table 19.　Estimates of the likely hazard from various nematode population densities on a range of horticultural and field crops

Note: Nematode data in this table reflect the <u>actual</u> number of nematodes present in the sample (i.e. counts have been corrected for extraction efficiency)

Crop	Nematode	Hazard index			Unit	Comments
		Low	**Moderate**	**High**		
Apple	Lesion *Pratylenchus penetrans*	<50	50-300	>300	nematodes/ 200 ml soil	Rootstock tolerance, soil texture and climate affect damage thresholds. Other *Pratylenchus* species (e.g. *P. jordanensis*) can also attack apple.
Banana	Burrowing *Radopholus similis*	0-80	80-175	>175	nematodes /200 ml soil	The disease index is related to the number of burrowing nematodes in roots and can be used to estimate the level of nematode damage in a crop. The proportion of the root cortex occupied by lesions is rated as follows: no lesions =0; 1-25% =1; 26-50% =3; 51-75% =5; 76-100% =7. Disease index = [100 x sum of root ratings] / [7 x total number of roots]
		0-1	1-3	>3	nematodes/g root	
		>0-15	15-35	>35	Disease index	
Carrot	Root-knot *Meloidogyne* spp.	0	1-20	>20	nematodes/ 200 ml soil	Since carrots deformed by root-knot nematode are rejected in the market, a zero tolerance should be assumed until more definitive local information is available. Populations as high as 60 nematodes / 200 ml soil cause little damage in some situations. Intensive sampling and appropriate extraction procedures are necessary to detect low nematode population densities prior to planting. Other nematodes (e.g. *Pratylenchus*) also cause damage to carrots.
Cereals (Wheat)	Cyst *Heterodera avenae*	1-5	5-10	>10	eggs/g soil	Soil type, fertility and rainfall can affect the population ranges for the three hazard categories. Equivalent nematode densities are likely to cause larger yield losses in sandy soils or low rainfall areas compared to areas of high fertility and rainfall.
	Lesion *Pratylenchus thornei* *P. neglectus*	<50 <5	50-150 5-20	>150 >20	nematodes/ g soil	Data have been obtained from limited trial work in South Australia. Yield losses of at least 10% can be expected once the high hazard index is reached. Losses are greater in sandy soils and areas of marginal rainfall.
(Oats)	Stem *Ditylenchus dipsaci*	<2	2-50	>50	nematodes /200 ml soil	Susceptible varieties can suffer heavy losses but resistant varieties are not damaged.
Capsicum	Root-knot *Meloidogyne* spp.	<10	10-60	>60	nematodes /200 ml soil	The species of root-knot nematode may be important as some cultivars are resistant to certain species, particularly *M. javanica*.

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Table 19. continued.

Crop	Nematode	Hazard index			Unit	Comments
		Low	**Moderate**	**High**		
Citrus	Citrus *Tylenchulus semipenetrans*	<2000 <100	2000-20000 100-1000	>20000 >1000	nematodes /200 ml soil females/g root	Since soil moisture influences egg hatch and the survival of juveniles and males, counts of females on roots may be more useful than counts of nematodes extracted from soil. Tree health and rootstock should be taken into account when interpreting infestation levels. Trees with poor root systems or trees in decline can no longer support high nematode populations.
Cucurbits (zucchini, rockmelon, watermelon, squash, cucumber)	Root-knot *Meloidogyne* spp.	<5	5-10	>100	nematodes/ 200 ml soil	The extent of losses due to nematodes varies with the crop, cultivar, soil texture, climate and the standard of crop management. Short-term crops such as zucchini have a higher damage threshold than other cucurbits.
Faba beans	Stem *Ditylenchus dipsaci*	<200	200-1000	>1000	nematodes/ 200 ml soil	The crop is relatively tolerant, but heavy yield losses can occur in wet years.
Ginger	Root-knot *Meloidogyne spp.*	0	1-20	>20	nematodes/ 200 ml soil	Ginger is highly susceptible to root-knot nematode. Late-harvest ginger can be severely affected because of its long growing season. Ideally, root-knot nematode should be undetectable at planting, but this is of little value if planting material is infested with nematodes.
Grape	Root-knot *Meloidogyne* spp Lesion *Pratylenchus* spp Citrus *Tylenchulus semipenetrans*	<40 <40 <100	40-400 40-400 100-800	>400 >400 >800	nematodes/ 200 ml soil	Because nematode populations fluctuate during the year, these threshold levels may need adjustment according to whether vines are sampled while dormant or during the growing season. Soil texture, cultivar, climate and the availability of irrigation are the main factors influencing the extent of crop losses caused by nematodes.
Peas	Stem *Ditylenchus dipsaci*	<100	100-600	>600	nematodes/ 200 ml soil	Losses are greater at lower nematode densities when conditions are wet during autumn.
Pineapple	Root-knot *Meloidogyne javanica*	0 <5	1-10 5-100	>10 >100	nematodes/ 200 ml soil at planting nematodes/ 200 ml soil at 12-15 months	Intensive sampling and efficient extraction techniques are needed to detect the low nematode populations often present at planting. General crop health, severity of symptoms, presence of other root diseases and responses of previous crops to nematicides should be taken into consideration when interpreting the results of samples from established crops.

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Table 19   continued.

Crop	Nematode	Hazard index			Unit	Comments
		Low	**Moderate**	**High**		
Potato	Root-knot *Meloidogyne hapla* Cyst *Globodera rostochiensis*	<20 <1	20-200 1-40	>200 >40	nematodes/ 200 ml soil. eggs/g soil	*M. hapla* is largely restricted to cool climates. Other *Meloidogyne* spp. are important in warm climates, but their threshold levels are likely to be lower than those presented for *M. hapla*. Soil texture, soil moisture and the yield potential of the site will influence losses caused by nematodes on potato.
Stonefruit (peach, almond)	Root-knot *Meloidogyne* spp.	<20	20-400	>400	nematodes/ 200 ml soil	Soil moisture, soil texture, nutritional status, tree age, tree size and rootstock will influence the damage threshold.
Sugarcane	Root-knot *Meloidogyne* spp Lesion *Pratylenchus zeae* Stubby root *Paratrichodorus minor*	<100 <100 <100	100-1000 100-1000 100-500	>1000 >1000 >500	nematodes/ 200 ml soil	The hazard from nematodes varies with the clay content of soil. The nematode densities needed to cause economic damage are lower in soils with <15% clay than in heavier soils. In addition to root-knot and lesion nematodes, as many as four other species of plant-parasitic nematodes are usually present in soil samples from sugarcane. Their effects are likely to be additive.
Tobacco	Root-knot nematode *Meloidogyne* spp.	<2	2-40	>40	nematodes/ 200 ml soil	Tobacco is both susceptible to and intolerant of root-knot nematode. Crop losses can occur at low nematode densities.
Tomato	Root-knot nematode *Meloidogyne* spp	<2	2-40	>40	nematodes/ 200 ml soil	Lower populations cause damage in sandy soils than in heavy soils. Crops in which fruit develops and ripens during the cooler months of the year can tolerate a higher nematode population than crops harvested in summer. Good management of irrigation and nutrition will also increase damage thresholds.
Turf	A complex of nematodes, including sting (*Belonolaimus lolii*), lesion (*Pratylenchus zeae*), stubby root (*Paratrichodorus* spp.), dagger (*Xiphinema* spp.) and various ring and spiral nematodes	<50	50-200	>200	nematodes/ 200 ml soil	Lack of experimental work and the complexity of the nematode fauna mean that damage thresholds for nematodes on turf are not well defined. Also, factors such as grass species, mowing height, nutritional status, soil compaction and presence of other root pathogens influence the extent of losses from nematodes.

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8.   Diagnosing Nematodes of Quarantine Importance

The isolation of Australia and New Zealand from the rest of the world and the relatively strict quarantine regulations in both countries means that they are not plagued by many of the nematode pests that are important elsewhere. Since new nematode pests are most likely to be detected in diagnostic samples, people involved in diagnostic services should be aware of the range of nematodes that could be introduced and need to be continually vigilant with regard to quarantine issues.

The plant nematodes recorded in Australia are listed by McLeod *et al*. (1994). It is apparent from this list that only a small proportion of the described species of plant-parasitic nematodes have been found in Australia. Some of the absent exotic species are associated with minor crops or are restricted in their distribution by climate and are never likely to become important pests in Australia. Others have the potential to cause economic damage and some of these are listed in Table 20. The list has no official status with quarantine authorities, but it does indicate a number of potentially important nematode pests that occur overseas. Although the list contains a diverse range of nematodes, it is not comprehensive. A detailed pest risk assessment would probably identify further potentially important species.

When suspected new nematode introductions are found in diagnostic samples, their identity should be confirmed (see section 6.2.6), voucher specimens should deposited in an appropriate reference collection and quarantine authorities should be notified. The nematode taxonomists listed in Appendix 2 will be able to provide advice on procedures. New records of nematodes and new host/nematode combinations must also be documented so that reliable records of nematode distribution within the country can be maintained.

In addition to detecting newly introduced nematodes, diagnostic services may become involved in quarantine issues through their role in assessing samples of produce destined for overseas export markets. As international trade increases, there will be an increasing demand from overseas quarantine authorities to ensure that agricultural products (e.g. tubers, rhizomes, rooted plants), are free of specific nematodes. If protocols for collecting and processing samples are set by the importing country, these must be followed by the diagnostic service. In the absence of specific protocols, the level of risk should be assessed and appropriate assessment procedures developed.

Table 20.   Plant-parasitic nematodes of major quarantine significance in Australia.

Pest	Important host plants	Main areas of occurrence
Nematodes that are absent from Australia		
Bursaphelenchus xylophilus	Pinus spp.	USA, Japan
Ditylenchus angustus	Rice	South-east Asia
Globodera pallida	Potato	Widespread
Heterodera cajani	Pigeonpea, cowpea	India
Heterodera ciceri	Chickpea	Turkey, Spain
Heterodera glycines	Soybean	USA, China
Heterodera goettingiana	Pea	Mediterranean, north Africa, west Asia
Heterodera latipons	Grasses	Mediterranean
Heterodera zeae	Maize	USA, Egypt, south Asia
Hirschmaniella spp.	Rice	south-east Asia
Meloidogyne altieri	Cereals and grain legumes	Mediterranean
Meloidogyne chitwoodi	Potato	USA
Meloidogyne naasi	Cereals	Europe, USA
Nacobbus abberans	Numerous hosts	South America
Radopholus citrophilus	Citrus	USA (Florida)
Races of economically important species that are absent from Australia		
Ditylenchus dipsaci (giant race)	Faba beans	Mediterranean region
Ditylenchus dipsaci (wheat race)	Wheat	Central & western Europe
Ditylenchus dipsaci (European races)	Numerous hosts	Europe
Heterodera avenae (several pathotypes)	Wheat, oats, barley	Europe
Meloidogyne incognita (races 3 and 4)	Numerous hosts	Widespread
Meloidogyne arenaria (race 1)	Peanut	Widespread
Nematodes that are restricted to certain regions of Australia		
Anguina tritici	Wheat	Occasionally recorded in WA
Aphelenchoides besseyi	Rice	Not recorded from rice-growing region of NSW
Globodera rostochiensis	Potato	Limited to quarantine areas in Victoria and WA
Xiphinema index	Grapevine	Restricted by *Phylloxera* quarantines to parts of the Rutherglen area, Victoria

There are situations in both Australia and New Zealand where a nematode pest has been introduced, but its distribution remains restricted. In such cases, quarantine measures are enforced to prevent the further spread of the new pest. Diagnostic services may then become involved in surveys to define the extent of existing outbreaks and to detect new outbreaks. When plants or soil are sampled in such circumstances, the primary aim is usually to detect a specific nematode rather than determine nematode population densities. Sampling must be intensive enough to satisfy quarantine authorities that there is a high probability that low infestations of the pest will be detected. Although precision increases with the intensity of

sampling, the gains from more intensive sampling must always be balanced against the extra cost involved. One way to minimise costs is to develop sampling regimes which reflect risk. For any crop/nematode situation, the risk factors are usually known (e.g. the number of previous susceptible crops, length of rotations, soil texture, proximity to a known infested area), and risk levels can be defined. High risk areas would then be sampled more intensively or more frequently than areas of moderate risk, and low risk areas may not be sampled at all.

When developing sampling protocols for quarantine purposes, the issues involved will vary according to the crop and the nematode. The detection of potato cyst nematode (PCN) in potato crops provides an example of the range of factors that need to be considered.

1. New outbreaks of PCN develop from introduced cysts. Since it takes many years for an initial infestation to build up to a population level where crop symptoms become noticeable, the initial spread of PCN goes on unnoticed. Thus the nematode may already be well dispersed before stunted patches are noticed in a crop.
2. During the build-up phase, nematodes hatch from cysts and infect nearby plants. The next generation of cysts is then spread by cultivation, usually in a lens-shaped area of infestation, with the long axis of the lens in the direction of cultivation. These lenses increase in size as cropping continues and new lenses develop, so that the nematode usually has a patchy distribution when a new outbreak is first discovered.
3. The low nematode populations present in new outbreaks of PCN and its aggregated distribution makes it difficult to detect PCN in quarantine situations. There are about 2 million litres of soil per hectare to a plough depth of 20 cm. When populations are low, there is every chance that the nematode will be missed if a single 500 ml sample is collected. For chances of detection to reach 95-99%, the population density would have to be greater than 12 million nematodes per hectare. Thus, low populations of PCN often remain undetected, even with the most sensitive survey procedures.
4. The probability of detecting PCN in fields with low levels of infestation can be calculated. For example, if there is a 55 m^2 lens of infestation in each 1/3 hectare of land and the population level in each lens is 50 cysts/kg, the probability of detecting PCN by soil sampling is as follows:
    - 23% if 120 cores are collected on a 5m x 5m grid from each 1/3 ha and a 500g sample is processed.
    - 10% if 50 cores are collected on a 20m x 20m grid across a 2 ha field and a 500g sample is processed.
5. Because of the limitations involved in detecting low populations of PCN, sampling strategies must be established which maximise chances of detecting new outbreaks of the nematode. This can be achieved by varying the intensity of sampling according to:
    - distance from known infested sites.
    - cropping history (particularly the number of susceptible potato crops).
    - whether potatoes are used for seed, ware potatoes or processing.

A plant test (often referred to as a "fork test") can also be used to maximise chances of detection. Since potato plants act as bait for PCN, examining roots for the presence of developing PCN females is more efficient in detecting and locating foci of infection than soil sampling. Such tests, however, can only be carried out at certain times of the year and are only useful in light soils.

Quarantine authorities should be consulted with regard to the assessed level of risk for a particular situation. In Australia, the following protocols have been used for PCN.

High risk sites Collect 10 ml soil on a 5 m x 5 m grid (i.e. 400 probes from each ha, giving a total sample of about 4 kg). Process this soil as 8 x 500 g sub-samples, with each sub-sample representing a separate identifiable area of the field. Where conditions allow, fork test on a 10 m x 10 m grid (i.e. 100 plants/ ha).

Medium-low risk sites Collect 10 ml soil on a 20 m x 20 m grid (i.e. 25 probes from each ha) and process a 500 g sample. Where conditions allow, fork test on a 20 m x 20 m grid (i.e. 25 plants from each ha).

When samples are being processed from quarantine areas, diagnostic laboratories must ensure that nematodes are not spread from the laboratory to new areas. Soil and plant material must be handled within a closed system and should not be discarded until it has been autoclaved or treated with a general biocide such as formalin.

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Appendix 1
Diagnostic laboratories in Australia which provide nematological services

Queensland
Biological Crop Protection Pty. Ltd.
3601 Moggill Road, Moggill, Qld., 4070.
Contact: Dr. Graham Stirling
Ph. 07 3202 7419, Fax. 07 3202 8033, Mobile 0412 083 489

Queensland Department of Primary Industries
Meiers Road, Indooroopilly, Qld., 4068
Contact: Dr. Julie Stanton
Ph. 07 3896 9574, Fax. 07 3896 9533

Victoria
Institute for Horticultural Development
Private Bag 15
South Eastern Mail Centre, Vic 3176
Contact: Mrs Lila Nambiar
Ph. 03 9810 1546, Fax. 03 9800 3521

South Australia
South Australian Research and Development Institute
Plant Research Centre
GPO Box 397
Adelaide, SA, 5001
Contact: Dr. Greg Walker
Ph. 08 8303 9355, Fax. 08 8303 9323

Western Australia
Agriculture WA
South Perth WA 6151
Contact:: Dr Ian Riley
Ph. 08 9368 3263 Fax: 08 9367 2625

Appendix 2

Persons and Organisations Providing Specialist Services in Nematode Taxonomy and Identification

The following persons are prepared to identify nematode specimens on a fee-for-service basis.

Dr. Mike Hodda
CSIRO Division of Entomology
GPO Box 1700
Canberra ACT 2601
Ph. 02 6246 4371 Fax. 02 6246 4000

Dr W.M. Wouts
Landcare Research
Mt. Albert Research Centre
Private Bag 92170
Auckland New Zealand.
Ph. 09 849 3660 Fax. 09 849 7093

The International Institute of Parasitology in the United Kingdom also provides an authoritative nematode identification service which is backed by an extensive reference collection. Details of charges and advice on collection, preservation and dispatch of specimens is available on request.

Contact: Dr. D.J. Hunt
395a Hatfield Road, St. Albans
Herts, AL4 OXU, U.K.
Ph. +44 1727 833 151, Fax: +44 1727 868 721

Appendix 3
Glossary of Taxonomy Terms

Adenophorea - one of the two classes of Phylum Nematoda. Contains the dorylaimids, some of which (in Longidoridae and Trichodoridae) are plant parasitic nematodes.

Amphidelphic - having two ovaries, generally one extending towards the tail and one towards the head.

Amphids - the largest chemosensory organs in nematodes, paired, open laterally in the head region. Visible as pores or slits, very variable in shape.

Annules - regular transverse grooves in the surface of cuticle, may give nematode a segmented appearance.

aphelenchid type of oesophagus - having a narrow procorpus with a large median bulb followed by a narrow tube extending to the intestine. There is no basal bulb, and the three oesophageal glands lie outside the oesophagus proper.

arcuate - having a curved shape.

areolated - cuticle divided into small spaces or areolations

basal bulb (= terminal bulb) - an enlargement of the oesophagus, muscular or glandular, at the base of the oesophagus.

bullae - blister-like protuberances inside the cyst near the vulval fenestra of some Heteroderidae.

bursa (= caudal alae) - a pair of fan-like cuticular structures on each side of the cloaca of males, in extreme cases forming a membranous fan which may encircle the tail tip. Used to grasp the female during copulation.

cardia - muscular structure at the base of the oesophagus opening into the intestine.

caudal - pertaining to the tail.

cephalic - pertaining to the head region.

cephalic framework - a rigid supporting structure for muscle attachment which provides form to the head and acts as a stylet guide.

clavate - club-shaped, broadening towards apex.

cloaca - a common opening for the intestinal tract and the reproductive system in the male.

conoid - cone-shaped.

crenate - having a scalloped margin.

criconematid type of oesophagus - corpus and metacorpus fused to form a single enlargement; basal bulb smaller than metacorpus.

cuticle - noncellular external covering of nematodes, shed at moulting. Has a layered structure, is largely composed of collagen. Also lines the stoma, oesophagus, vagina, terminal excretory/secretory duct, and rectum. The stylet and cephalic framework are made of cuticle.

cyst - body wall of female Heteroderidae after it becomes brown, forming a protective shell for eggs following the death of the female.

deirids - paired, pore-like organs located in the lateral fields, near the nerve ring.

didelphic -with two ovaries.

digitate - finger-like.

distal - furthest from middle of body or point of origin or attachment of a structure.

dorsal - the back surface (i.e. opposite to ventral).

dorsal oesophageal gland - gland associated with the oesophagus and overlapping the intestine in a dorsal position.

dorylaimid type of oesophagus - has the shape of a long-necked bottle, thin at anterior and expanding towards the intestine, usually with a straight base.

ectoparasite - a nematode feeding on a plant from the outside.

endoparasite - a nematode living within plant tissue and feeding on plant cells.

epidermis (= hypodermis) - the outer cylindrical layer of epithelial cells which secretes the cuticle.

epiptygmata - vulval flaps.

excretory pore - the exterior opening of the excretory system, generally located on the ventral side of the body near the base of the oesophagus.

fasciculi - intestinal cell inclusions, visible as tubular structures, throughout intestinal region.

fenestra - window, transparent spot; in Heteroderidae a thin walled transparent region of the vulval cone which may break down to form a hatching pore.

filiform - thread-like; long, tapering gradually to a very fine point.

flange - a rib, rim or expansion for strength, guiding or attachment.

furcate - forked.

fusiform - spindle-shaped, tapering towards each end.

gubernaculum - a grooved cuticular structure, found in males, acts as a guide for the spicules during their extrusion.

guiding ring - of a stylet - the anterior end of the guiding sheath of the stylet which is muscular or cuticularised and thus appears denser than surrounding tissue.

hemizonid - nerve commissure which is highly refractive in nature and appears lens-shaped, extends ventrally from lateral cord to lateral cord.

hexaradiate - six-sided, arranged in a circle. Describes the arrangement of the labial lips.

hyaline - glass-like, transparent.

hypoptygma - posterior vulval flap, or flap at cloacal opening.

isthmus - the portion of oesophagus between the median bulb and the terminal bulb.

labial annule - see labial disc.

labial disc - the more or less circular form of cuticle about the oral opening, terminated posteriorly by the first transverse striation of the cuticle.

labium - lip.

lanceolate - spear-shaped.

lateral canals - tubular-like structures that extend throughout the intestinal area.

lateral field - longitudinal ridges of cuticle that overly the lateral thickenings (cords) of the epidermis and run almost the length of the nematode.

lateral lines - grooves between the ridges of the lateral field (which appear as lines under the microscope).

lateral guiding pieces - cuticularised structures which guide the spicule.

lips - cuticular structures surrounding the mouth opening

monodelphic - with one ovary.

moult - periodic shedding of the cuticle, mainly to permit growth.

mucro - abrupt point or process which terminates tail.

mucron - a small knob-like ending on a terminus.

mucronate - ending abruptly in a point.

mycophagous - feeding on fungi.

nerve ring - the circum-oesophageal commissure, contains the cell bodies of neurons distributed around it. Formed by the dorsal and ventral connections between the lateral ganglia.

odontophore - posterior part of spear of dorylaimids.

odontostyle - anterior part of spear of dorylaimids.

oesophagus - portion of the alimentary canal between the buccal cavity and the anterior portion of the intestine. Sometimes referred to as the pharynx.

offset - standing off from, having a clearly delineated break.

papilla - minute, soft cuticular projection with a sensory function.

perineal pattern - the pattern formed by cuticular folds and annules around the tail, anus, phasmids and vulva, particularly in *Meloidogyne* females.

perineum - region of cuticle between the anus and the vulva.

pharynx - see oesophagus

phasmid (= scutella) - chemosensory organ, usually paired, often found near or on tail.

post-vulval sac - a rudimentary extension of the posterior uterus which functions as a spermatheca in some nematodes.

procorpus - anterior part of the corpus (oesophagus) which has a cylindrical form.

prodelphic - having a single ovary anterior to the vulva.

proximal - nearest to middle of body or to point of origin or attachment of a structure.

pseudolips - fused lips.

pyriform - pear shaped.

replacement stylet - the new odontostyl formed during moulting of juvenile dorylaimids, 'stored' in the oesophageal wall.

reticulate - having markings like a net.

retrorse - in a backward or downward direction.

saccate - sac shaped.

sclerotisation - cuticularisation of, e.g., the labial region. Usually used to imply that the area in question has a greater or lesser than usual deposition of cuticle, seen as more or less dense or refractive.

secretory/excretory pore - a ventral opening in the cuticle by which waste and secretory products of the secretory/excretory system are passed to exterior. Joined to secretory/excretory cell by a cuticle-lined duct.

Secernentea - one of the two classes of Phylum Nematoda. Contains the tylenchids and aphelenchids and most plant parasitic nematodes.

sedentary - remaining in one position in the host.

seminal vesicle - the organ in which sperms are stored in the male reproductive system.

sheath - a cuticular covering enclosing body of a nematode, e.g. *Hemicycliophora*.

sinuate - snake-like.

spathulate - flat paddle-shaped.

spatulate - spoon-shaped; broad and rounded at apex, narrowing towards base.

spermatheca - an enlarged portion of the female gonad between the oviduct and the uterus, functioning in the storage of sperm.

spicules - cuticular pieces, usually two but may be fused, found in the cloaca and inserted into the female's vagina during copulation.

stoma - the mouth cavity. A hollow tube in bacterial feeding nematodes; contains the stylet or spear in plant parasitic forms.

striae - small grooves, minute banding.

stylet (or spear) - a cuticular, hollow, needle-like structure found in the stoma of plant-parasitic, fungal feeding and some predatory nematodes. Used to pierce the host cell and ingest nutrients.

stylet knobs - three cuticular swellings, arranged in triradiate fashion at the base of the stylet. In lateral view, only two knobs can be seen.

terminal cone - terminal region of the female body in the Heteroderidae.

tesselate - chequered; patterned like a mosaic.

torulose - knobby, having knob-like swellings.

truncate - having a squared end.

tuberculate - small, non-sensory cuticular protruberances near the vulva.

tylenchid type of oesophagus - having a narrow procorpus, a strongly formed median bulb, a narrow isthmus, and either a glandular terminal bulb or the oesophageal glands overlap the intestine.

uncinate - hooked.

underbridge - in some species of *Heterodera,* the vulval slit persists crossing the fenestra as a bridge forming two semifenestrae.

vas deferens - a slender duct in the male which conveys sperm from the testis. Unites posteriorly with the rectum to form the cloaca.

vagina - a canal lined with cuticle, that connects the uterus or uteri to the exterior of the female.

ventral - the front surface (i.e. the surface on which the excretory pore, vulva and anus are located).

vermiform - having a worm shape.

vulva - exterior opening of the female's reproductive system. Generally appears as a transverse slit on the ventral portion of the nematode.

vulval position (V) - position of the vulva expressed as a percentage of the total length.

V = distance of vulva from anterior end x 100 ÷ body length